PROGRAMME AND ABSTRACT BOOK

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General Information

CONFERENCE VENUE

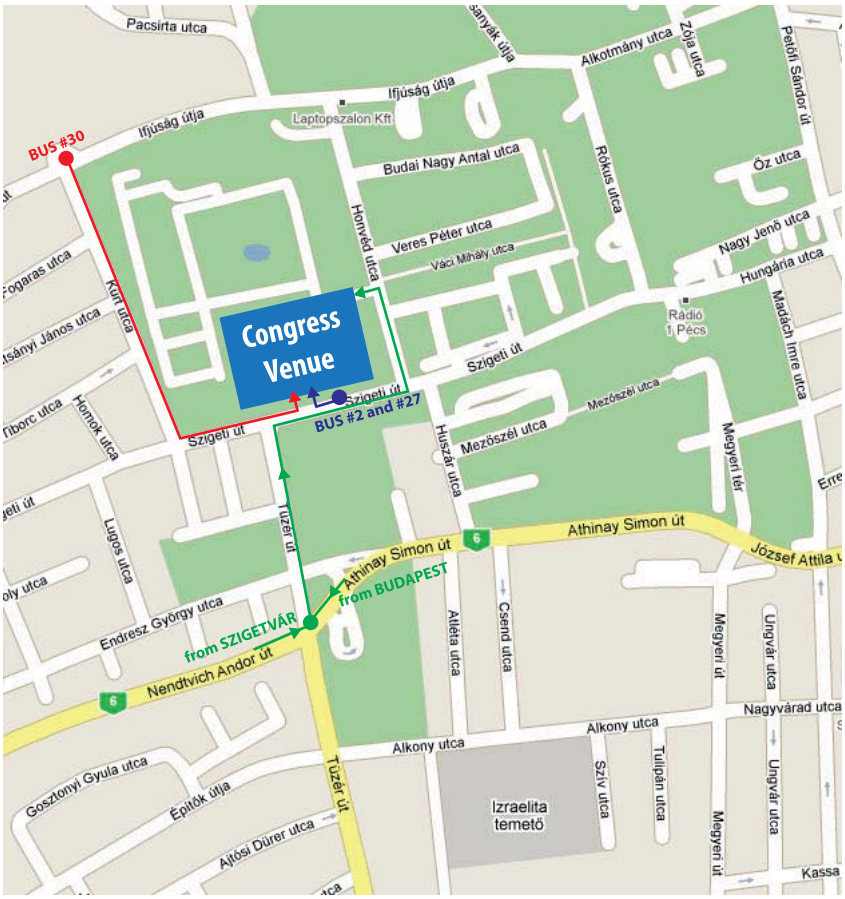
The conference will be organized in one place, in the Central Building of the Medical School, University of Pécs.
Addresse: Pécs, Szigeti út 12.

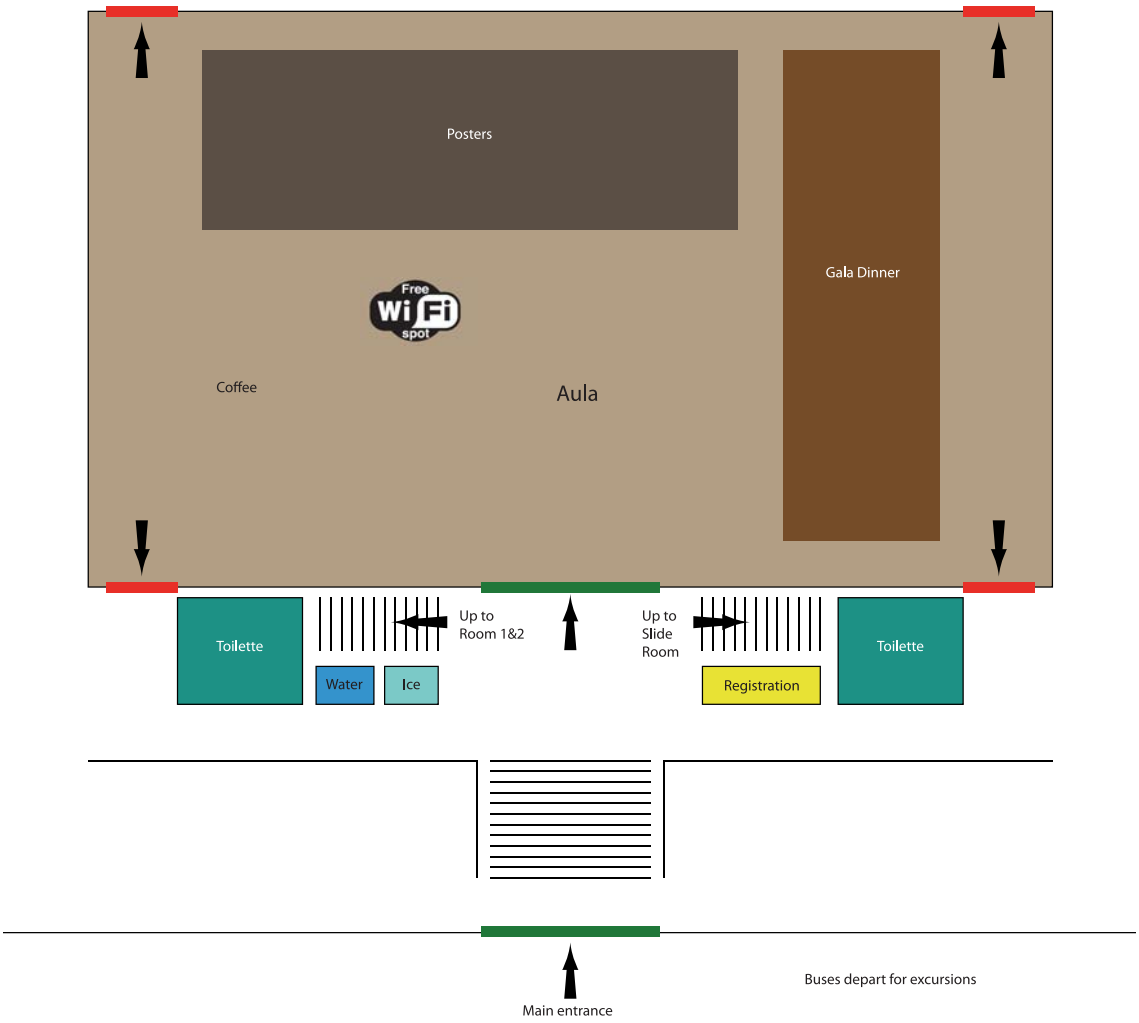
HOW TO FIND THE LOCATION OF THE CONFERENCE

By bus
Buses No. 2 and 27 stop in front of the building (Stop: "Egyetemváros", i.e., "University District", blue line in the map below). From the Railways Station, the easiest way to come here - beside taking a taxi - is to ride bus No. 30 all the way till its other terminal then walk straight south (downhill) about 200 m on Kürt street along the western border of the Campus (red line on the map).

By car
From Budapest, the easiest is to take the newly built freeway No. 6 (M6-M60, Pécs) till its end. Then follow the sign to the city center until you cross National Road #6. Turn left and keep riding until its crossing with Tüzér street. There turn right (north, uphill) and soon you will find yourself just in front of the Central Building. Entering the Campus by car (thin green line) is restricted and also it is very hard to find parking place. Around the Campus there are pay-parking places of very limited number. The best is to find your hotel, park there, and use public transportation or taxi to the place of the Conference. If you have special reason to park in the area of the Campus, contact the secretary of the LOC for help.

By TAXI
You may hail taxis in the street, but it will probably be cheaper to order a taxi by phone from the hotel reception. We recommend VOLAN TAXI: +36 72 333 333 (english speaking)





The lectures and symposia will be held in the Lecture Halls No. I., and II., on the 1st floor (loft, West wing). They open from the gallery of the Aula. The Slide Room will be located in the vicinity of the Lecture Halls. The posters will be exhibited in the Aula. Conference buffet (sandwich-box) will be located in the lounge of the Aula. Free, high speed WiFi Internet will be available for the participants in the conference area.

REGISTRATION AND INFORMATION DESK

Registration will take place at the conference venue in the lounge of Aula .
The registration desk will have the following opening hours:

Tuesday, August 31	14:00–19:00
Wednesday, Sept 1	08:00–18:00
Thursday, Sept 2	08:00–14:00
Friday, Sept 3	08:00–18:00
Saturday, Sept	08:00–13:00

Messages can be left on the CECE message board next to the registration desk. Participants are asked to check the message board routinelz for mail, notes and telephone messages. The telephone number for on/site information is **+36 20 454 1918.**

TECHNICAL INFORMATION TO SPEAKERS

PRESENTATION FORMS

Plenary lectures – 45 minute reviews of invited speakers.
State-of-the-art lectures – 30 minute reviews of invited lecturers in the topic of the symposium
Oral presentations – 10 minute (+5 min. discussion) in the topic of the symposium, and

The presentations are requested to be handed in on a pen drive (if possible). They should be compatible with Microsoft Office 2007 (Windows version).
Available codecs for multimedia presentations:

VIDEO codec: DivX, Xvid, MPEG-1, MPEG-2, h264, x264, WMV-9;
AUDIO codec: MP3, AAC, AC3, WAV, WMA, PCM;
CONTAINER: .avi, .mpg, .mp4, .mkv, .wmv

Please save all files of your presentations in the same folder of your pendrive. In case of video or voice files, please attach them externally to ensure the best quality. In the control files, please use relative links (no absolute paths).
Please make sure that your presentation is handed in in time, at the Slide Room (Small board-room, 1st floor). The best to do it is right after your registration, or at least 10 minutes before the beginning of the actual session. In the Slide Room you have possibilities to try your presentation and also to make last minute corrections.

POSTER PRESENTATIONS

Location: Aula. Posters should be placed from Tuesday 18:00, but at latest before the start of the session (i.e. Wednesday 14:00). Posters should be removed by Friday 15:00. For the duration of the session (Wednesday 14.00–15.30), authors should be standing in front of their poster and should be prepared for presenting their results for interested attendants. For the best two presenters, ESCE poster prize will be given (500 EUR each) during the closing ceremony, Saturday 13:00. The selection will be based on scientific value and quality of presentation.
Poster prize jury members:
Angela Lange, University of Toronto
Elisabeth Eppler, University of Zürich
Dora Reglődi, University of Pécs

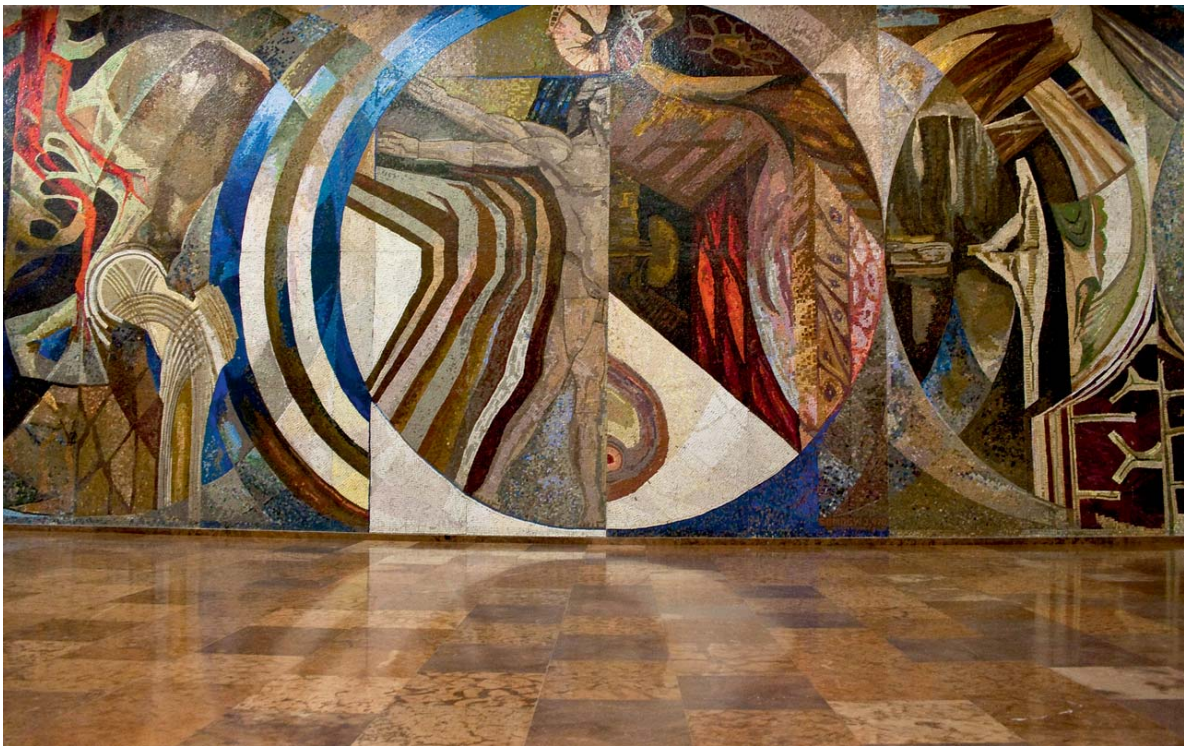
LUNCHES

Lunch (cold snacks, sandwiches and soft drink) is provided as part of the Registration fee and served between 13:00–14:00 in the lounge of Aula.

SPECIAL EVENTS

OPEN DISCUSSION FORUM

Friday, September 3, 14:00–15:20, *Lecture Hall No. I.*
Discussion groups will be organized around the key themes of CECE25 like reproduction, evolution, osmoregulation, environmental disruptors, neuropeptides, steroid hormone mode of action, insect hormones, etc. Plenary lecturers and state-of-the-art lecturers will lead these discussions. The focus of the discussions will be laid on: what will be the next big breakthrough in the particular area. The forum could provide the next meeting organizer with ideas for symposia and plenary speakers. With other words, the open discussions could be series of small group conversations about what participates want to see in the 2012 conference.
The moderators of the session are *Bob Dores, Geoff Coast, Ian Orchard* and *Valér Csernus.*



CECE2010 CONGRESS PHOTO

Friday, September 3, 11:30 in the *Aula,* during the coffee break.

ESCE BUSINESS MEETING

Wednesday, September 1, 17:30–18:30, *Lecture Hall No. I.*

ESCE COUNCIL LUNCH

Wednesday, September 1, 13:00–14:00, *Great board room* (located in the vicinity of the Lecture Halls, 1st floor).

GCE EDITORIAL BREAKFAST

Wednesday, September 1, 7:30–9:00, *Great board room* (located in the vicinity of the Lecture Halls, 1st floor).

SOCIAL PROGRAMS

WELCOME RECEPTION

Tuesday, August 31, 19:30 at the Conference Venue, in the *Gallery,* 1st floor, immediately after the Opening Ceremony and the first Plenary Lecture.

General Information

GALA DINNER

Wednesday, September 1, 19:30 in the *Bartók Room of Hotel Palatinus.* Music accompanied dinner in the Ballroom of one of the most beautiful Hotel in Pécs, built in Art Noveau style.

CECE BANQUET

Friday, September 3, 19:30 - banquet dinner with traditional Hungarian folk dance and music in the *Aula* of the Conference Venue. The evening ends with music and dancing, where you can also accompany our dancers.

EXCURSION TO BIKAL *(Experience the Renaissance)*

Thursday, September 2, 14:00. Travel through time and spend a day in the Renaissance era! You can live like our ancestors at the Land of the Renaissance in Bikal. After getting acquainted with the village, experiencing the architecture and crafts of the age, and trying out pottery, basketry and farriery, the program closes with a real medieval feast. Journey by bus, departing at 14:00 from the parking of Central Building. Casual clothing is recommended.

EXCURSION TO VILLÁNY *(Grape harvest program at the Wunderlich Winery)*

Thursday, September 2, 14:00. Spend an afternoon in the city of grape and wine and taste the finest wines of Villány! The funny harvest competition ends with visiting the wine-cellars and a dinner with music, where you can taste delicacies of the region. Journey by bus, departing at 14:00 from the parking of Central Building. Casual clothing is recommended.

EXCURSION TO ORFŰ *(Riding program)*

Thursday, September 2, 14:00. We will visit a guest house for horseback and carriage rides. The relaxation will be crowned by pálinka (famous Hungarian brandy) and a dinner that presents characteristic dishes of the area. Journey by bus, departing at 14:00 from the parking of Central Building. Casual clothing is recommended.

ACCOMPANIED PERSONS' PROGRAMS

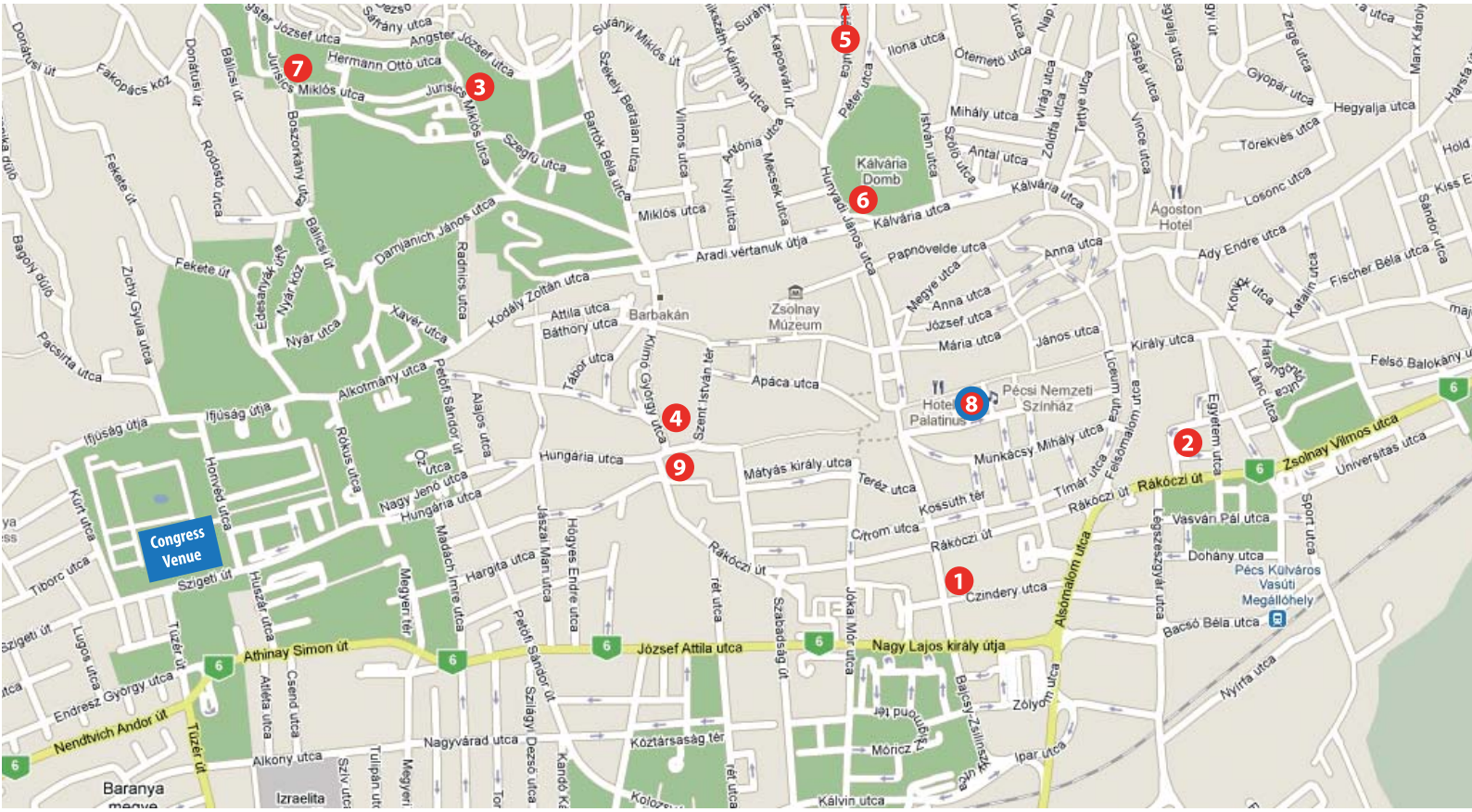
The Welcome Reception, Gala Dinner, Sightseeing Tours and Excursions are integral parts of the Conference Programme for accompanying persons. In addition you can choose from various optional programs.
Wednesday, September 1 – sightseeing tour in the morning in the inner city of Pécs with English-speaking guide.
Friday, September 3 – visiting one of the art exhibitions of Pécs 2010 European Capital of Culture.
Departure: Hotel Palatinus lounge, 10:00 a.m.

ACCOMODATION

LUGGAGE TRANSFER

On the last day of the conference (September 4), participants will be provided free luggage transfer between the participating hotels* and the conference venue. The shuttle buses to Budapest airport will leave from the conference place.

If you would like to use this possibility, please leave your luggage with a LOC member at the reception of your Hotel, on 4th Sept. between 8:00 and 11:00 a.m. You can pick up your luggage at the registration desk at the Conference venue between 12:00–13:30.



***Accomodating hotels**

[1] *Hotel Centrál* (7622 Pécs, Bajcsy-Zsilinszky street 7., Phone: +36 72 525-602)
[2] *Hotel Corso* (7626 Pécs, Koller street 8., Phone: +36 72 421-900)
[3] *Hotel Hunyor* (7624 Pécs, Jurisics M. street 16., Phone: +36 72 512-640)
[4] *Kafka Fogadó* (7624 Pécs, Alkotmány street 8., Phone: +36 72 512 500)
[5] *Hotel Kikelet* (7635 Pécs, Károlyi M. street 1., Phone: +36 72 512-900)
[6] *Hotel Millennium* (7625 Pécs, Kálvária street 58., Phone: +36 72 512-222)
[7] *MTA PAB Székház* (7624 Pécs, Jurisics M. street 44., Phone: +36 72 512-622)
[8] *Hotel Palatinus* (7621 Pécs, Király street 5., Phone: +36 72 889-400) – *venue of the Gala Dinner*
[9] *Hotel Pátria* (7622 Pécs, Rákóczi street 3., Phone: +36 72 889-500)

USEFUL HINTS

BADGES AND TICKETS
All participants are requested to wear their badges throughout the scientific programs. Those registered for the Welcome Reception and the Social Programs should bring their tickets, as it is a precondition for participation.

BANKING AND EXCHANGE FACILITIES
Banks are generally open Monday–Friday 9:00–17:00. Exchange offices are found throughout town, they are usually open till late evening on weekdays and also on Saturdays and Sundays. ATM machines are also found everywhere, including the main building of the University (venue of the conference), near the entrance.

CREDIT CARDS
All major international credit cards are accepted in most shops and restaurants.

COFFEE
During the coffee breaks, coffee with snacks will be served in the Aula.

ECC 2010
Pécs is the European Capital of Culture in 2010. Please find the actual program booklet in your conference package.

ELECTRICITY
The electrical current in Hungary is 220V 50 Hz. Standard continental European outlets are used. Most hotels also provide 110V outlets for shavers.

INSURANCE
The congress organizers cannot accept any liability for personal injuries sustained, or for loss or damage to property belonging to congress participants (or their accompanying persons), either during or as a result of the congress. The registration fee does not include insurance.

SIGHTSEEING TOURS
Free for accompanying guests. For others, tickets can be bought at the registration desk for 20 Euros.

SMOKING
Smoking is not allowed inside the building.

WATER
Water machines can be found in the lounge of Aula and on the gallery.

USEFUL INFORMATION
PCongress Ltd. emphasizes green life and protecting our environment. Therefore we observe the concept of a "green conference" in a great extent. We place out containers for used batteries, paper, CD/DVD disc and plastic, which will be recycled after the conference. Printed matters are produced by digital technology, produced only in the needed amount.
We also intend to add the "electronic voucher" to our profile, which will decrease the amount of paper used, by settling the account management electronically.
Please look for these signs:

	Tuesday, 31st August	Wednesday, 1st September		Thursday, 2nd September		Friday, 3rd September		Saturday, 4th September	
9:00		**9:00-9:50 Plenary: Manuel Tena-Sempere**		**9:00-9:50 Plenary: Xavier Bellés**		**9:00-9:50 Plenary: Christopher Secombes**		**SOA5_5 Lange**	**SOA10 Kloas**
9:30								**SOA5_6 Mita**	10.1 Gutierrez
									10.2 Hasunuma
10:00		**SOA1_1 Kah**	**SOA2 Davies**	**SOA5_1 Favrel**	6.1 Herichova	**SOA8 van Kemenade**	**SOA9_1 Janssen**	5.13 Meyering-Vos	10.3 Benato
					6.2 Zeman			5.14 Palstra	10.8 Boerjan
10:30		1.1 Cowan	2.1 Orchard	5.1 Badisco	6.3 Kommedal	8.1 Iwamuro	9.1 Rafaeli	5.16 Vandersmissen	10.5 Heijlen
		1.2 Chianese	2.2 Dow	5.2 Link	6.4 Bódis	8.2 Teles	9.2 Fonagy		10.6 Rodriguez
11:00		1.3 Ocampo-Daza	2.3 Gade	5.3 Cacciola	6.5 McStay	8.3 Vraskou	9.3 Tiu		10.7 Navarro
		11:15-11:45 COFFEE BREAK						11:30-12:00 COFFEE BREAK	
11:45		**SOA1_2 Dores**	2.4 Marchal	**SOA5_2 Lindemans**	7.1 Tamás	8.4 Eppler	**SOA9_2 Kiss**		
12:00			2.5 Verleyen		7.2 Harvey	8.5 Link		**12:00-12:50 Plenary: Shinobu Yasuo**	
		1.4 Liang	2.6 Smagghe	5.4 Castro	7.3 Wilhelm	8.6 Eppler	9.4 Bendena		
12:30		1.5 Horváth		5.5 Chand			9.5 Marco		
		1.6 Verlinden		5.6 Crespo			9.6 Lovejoy		
13:00		1.7 Acher		5.7 Urbatzka			9.7 Gade	Closing Ceremony	
		13:00-14:00 LUNCH							
14:00		14:00-15:20 POSTER SESSION		EXCURSIONS		14:00-15:20 OPEN DISCUSSION FORUM			
15:30		**SOA3 Shioda**	4.1 Larhammar			**SOA5_3 Swevers**	**SOA9_3 Kemenes**		
			4.2 Sass						
16:00		3.1 Meyering-Vos	4.3 Lippai			**SOA-5_4 Carnevali**	9.8 Tan		
		3.2 Carnevali	4.4 Löw				9.9 Pirger		
16:30		3.3 Morooka	4.5 Juhász			5.8 Habibi	9.10 Kodrik		
		3.4 Nagata	**SOA4 Vellai**			5.9 Moya	9.11 Bartu		
17:00		3.5 vanHoef				5.10 Marín	9.12 Isaac		
		3.6 Gardi				5.15 Zanuy	9.13 Vleugels		
17:30		17:30-18:30 ESCE business meeting					9.14 Gade		
18:00	Opening Ceremony **18:20-19:20 Plenary: Maria Malagon**								
19:00									
19:30	Welcome Reception	Gala Dinner				Conference Banquet			

Conference Overview

TUESDAY, AUGUST 31

18:00–18:20 **Opening Ceremony** – *Lecture Hall I.*
18:20–19:20 **Opening Plenary Lecture** – *Lecture Hall I.*
19:30–21:30 **Welcome Reception** – *Gallery*

WEDNESDAY, SEPTEMBER 1

9:00– 9:50 **Plenary Lecture** – *Lecture Hall I.*
10:00–13:15 **Symposium 1** – *Lecture Hall I.* Polypeptides and their Receptors, including Kisspeptins and GPR54: Co-Evolution, Biosynthesis, and Signal Transduction *(organized by: Robert M. Dores, USA and Manuel Carrillo, Spain)*
Symposium 2 – *Lecture Hall II.* Neuroendocrinology of Insects: advances through genomics and proteomics *(organized by: Ian Orchard, Canada)*
11:15–11:45 **Refreshment break** – *Aula*
13:00–14:00 **Lunch** – *Aula*
14:00–15:20 **Poster Session** – *Aula*
15:00–15:30 **Refreshment break** – *Aula*
15:30–17:30 **Symposium 3** – *Lecture Hall I.* Regulation of Food Intake in Invertebrates and Vertebrates *(organized by: Seiji Shioda, Japan and Mauro Vallarino, Italy)*
Symposium 4 – *Lecture Hall II.* Insulin-like growth factor (IGF) Signalling and Ageing *(organized by: Miklos Sass, Hungary)*
19:30–23:00 **Gala Dinner** – *Hotel Palatinus*

THURSDAY, SEPTEMBER 2

9:00– 9:50 **Plenary Lecture** – *Lecture Hall I.*
10:00–13:15 **Symposium 5_1** – *Lecture Hall I.* Reproductive Endocrinology *(organized by: Jozef Vanden Broeck, Belgium)*
Symposium 6 and 7 – *Lecture Hall II.* Circadian Rhythm *(organized by: Horst-Werner Korf, Germany)* and Avian Endocrinology *(Rita Jozsa, Hungary)*
11:15–11:45 **Refreshment break** – *Aula*
13:00–14:00 **Lunch** – *Aula*
14:00–19:30 **Excursions**

FRIDAY, SEPTEMBER 3

9:00– 9:50 **Plenary Lecture** – *Lecture Hall I.*
10:00–13:15 **Symposium 8** – *Lecture Hall I.* Neuroendocrine-Immune Interactions *(organized by: Elisabeth Eppler, Switzerland)*
Symposium 9_1 – *Lecture Hall II.* Invertebrate Neuropeptides and Peptide Hormones: key players in the regulation of physiological processes and behavior *(organized by: Tibor Kiss, Hungary, Gerd Gade, South Africa, William Bendena, Canada and George Kemenes, United Kingdom)*
11:15–11:45 **Refreshment break** – *Aula*
13:00–14:00 **Lunch** – *Aula*
14:00–15:20 **Open Discussion Forum** – *Aula*
15:00–15:30 **Refreshment break** – *Aula*
15:30–17:30 **Symposium 5_2** – *Lecture Hall I.* Reproductive Endocrinology *(organized by: Jozef Vanden Broeck, Belgium)*
Symposium 9_2 – *Lecture Hall II.* Invertebrate Neuropeptides and Peptide Hormones: key players in the regulation of physiological processes and behavior *(organized by: Tibor Kiss, Hungary, Gerd Gade, South Africa, William Bendena, Canada and George Kemenes, United Kingdom)*
19:30–23:00 **CECE Banquet** – *Aula*

SATURDAY, SEPTEMBER 4

9:00–11:30 **Symposium 5_3** – *Lecture Hall I.* Reproductive Endocrinology *(Jozef Vanden Broeck, Belgium)*
Symposium 10 – *Lecture Hall II.* Comparative Developmental Biology *(organized by: Werner Kloas, Germany)*
11:30–12:00 **Refreshment break** – *Aula*
12:00–12:50 **Closing Plenary Lecture** – *Lecture Hall I.*
13:00–13:30 **Closing Ceremony** – *Lecture Hall I.*

Detailed Programme

Tuesday, 31st August

18:00– Opening Ceremony *(Lecture Hall 1 and 2)*

18:20–19:20 **M. Malagon** (Spain): In search for new players of the regulated secretory pathway: lessons from amphibians. Chair: Larhammar

19:30– Welcome reception *(Gallery of Aula)*

Wednesday, 1st September

09:00–09:50 **Manuel Tena-Sempere** (Spain): Lessons from kisspeptin physiology: from fish to mammals. Chair: M. Carrillo

HALL 1

1. Polypeptides and their Receptors, including Kisspeptins and GPR54: Co-Evolution, Biosynthesis, and Signal Transduction.
(Chair: R. M. Dores)
(Chair: M. Carrillo)

10:00–10:30 ***SOA1_1*** **O. Kah** (France): Kiss systems in non-mammalian vertebrates with special emphasis on fishes

10:30–10:45 ***O01_01*** **M. Cowan** (United Kingdom): Kisspeptin and seasonal control of reproduction in European sea bass (Dicentrarchus labrax) and Atlantic cod (Gadus morhua).

10:45–11:00 ***O01_02*** **R. Chianese** (Italy): Cloning and characterization of GPR54 in the testis of the anuran amphibian, Rana esculenta, during the annual reproductive cycle

11:00–11:15 ***O01_03*** **D. Ocampo-Daza** (Sweden): The oxytocin/vasopressin receptor family has at least five members in gnathostomes

11:45–12:15 ***SOA1_2*** **R. M. Dores** (USA): Structure-Function Analysis of Endoproteolytic Cleavage by PC2: Site-directed Mutagenesis Studies on the α-MSH Cleavage Site in Silurana tropicalis POMC

12:15–12:30 ***O01_04*** **L. Liang** (USA): Understanding the Ligand Selectivity Features of the Melanocortin 2 Receptor

12:30–12:45 ***O01_05*** **G. Horváth** (Hungary): Conserved antiapoptotic effects of pituitary adenylate cyclase activating polypeptide – from mollusks to humans

12:45–13:00 ***O01_06*** **H. Verlinden** (Belgium): The role of octopamine in insects and the cloning, phylogenetic relationship and distribution pattern of three new putative octopamine GPCR's in the desert locust

13:00–13:15 ***O01_07*** **R. Acher** (France): Structural neurohypophysial hormone-receptor coevolution through the animal kingdom

HALL 2

2. Neuroendocrinology of Insects: advances through genomics and proteomics
(Chair: I. Orchard)

10:00–10:30 ***SOA2*** **S. Davies** (United Kingdom): Mechanism and function of the Drosophila capa receptor

10:30–10:45 ***O02_01*** **I. Orchard** (Canada): Identification of the elusive peptidergic diuretic hormone in the kissing bug, Rhodnius prolixus: a CRF-related peptide

10:45–11:00 ***O02_02*** **J. A. Dow** (United Kingdom): The insect Malpighian tubule: it does more than we expected, so its endocrine control is more sophisticated than we thought

11:00–11:15 ***O02_03*** **G. Gäde** (South Africa): What does the information from recent insect genome projects tell us about AKH precursors and the mature peptides?

11:15–11:45 Coffee break

11:45–12:00 ***O02_04*** **E. Marchal** (Belgium): Final steps in JH biosynthesis in the corpora allata of the desert locust, Schistocerca gregaria

12:00–12:15 ***O02_05*** **P. Verleyen** (Belgium): Microarray analysis of the physiology of reproductive and non-reproductive honeybee workers

12:15–12:30 ***O02_06*** **G. Smagghe** (Belgium): The ecdysone receptor in the hemimetabolous pea aphid: cloning, protein structure modeling and functional analysis by RNAi

13:00–14:00 Lunch

14:00–15:20 Poster Session *(Aula)*

HALL 1

3. Regulation of Food Intake in Invertebrates and Vertebrates
(Chairs: S. Shioda, M. Vallarino)

15:30–16:00 ***SOA3*** **S. Shioda** (Japan): Feeding regulation and energy metabolism in the brain

16:00–16:15 ***O03_01*** **M. Meyering-Vos** (Germany): Food intake, digestive enzyme activity and contraction of the gut is regulated by sulfakinins from the cricket Gryllus bimaculatus

16:15–16:30 ***O03_02*** **O. Carnevali** (Italy): The central role of melatonin in Danio rerio appetite regulation

16:30–16:45 ***O03_03*** **N. Morooka** (Japan): Structure determination of O-linked carbohydrate moiety of HemaP-like peptide, a novel feeding-modulating peptide from the sweet potato hornworm, Agrius convolvuli

16:45–17:00 ***O03_04*** **S. Nagata** (Japan): Effects of peptide hormones on feeding initiation and termination in the larvae of the silkworm, Bombyx mori

17:00–17:15 ***O03_05*** **V. van Hoef** (Belgium): Serine protease inhibitors and their role in regulating the activity of digestive enzymes in insects.

17:15–17:30 ***O03_06*** **J. Gardi** (Hungary): Energy homeostasis regulatory peptides in hibernating grizzly bears

17:30–18:30 ESCE Business Meeting

HALL 2

4. Insulin-like growth factor (IGF) Signalling and Ageing
(Chair: M. Sass)

15:30–15:45 ***O04_01*** **D. Larhammar** (Sweden): Evolution of the insulin-like growth factor binding protein (IGFBP) family in vertebrates

15:45–16:00 ***O04_02*** **M. Sass** (Hungary): Hormonal regulation of developmental autophagy and ageing by IGF-1 pathway in insects

16:00–16:15 ***O04_03*** **M. Lippai** (Hungary): The Role of AMPK in the regulation of autophagy and lifespan

16:15–16:30 ***O04_04*** **P. Löw** (Hungary): The role of ubiquitin-proteasome system in ageing

16:30–06:45 ***O04_05*** **G. Juhász** (Hungary): Regulation of lifespan through the Insulin/ PI3K/TOR/autophagy pathway

16:45–17:15 ***SOA4*** **T. Vellai** (Hungary): Hormonal regulation of aging

19:30– Gala Dinner *(Hotel Palatinus – see map on page 9)*

Thursday, 2nd September

09:00–09:50 **X. Bellés** (Spain): The endocrine regulation of insect metamorphosis and the emerging role of microRNAs. Chair: G. Coast

HALL 1

5. Reproductive Endocrinology
(Chairs: J. Vanden Broeck, P. Favrel)

10:00–10:30 ***SOA5_1*** **P. Favrel** (France): Genomic approaches of regulation of reproduction in the Pacific oyster

10:30–10:45 ***O05_01*** **L. Badisco** (Belgium): Insulin-related peptide and neuroparsins in locust reproductive physiology

10:45–11:00 ***O05_02*** **K. Link** (Switzerland): Insulin-like growth factor-3 (IGF-3) in male and female gonads of the tilapia: development and regulation by growth hormone (GH) and 17α-ethinylestradiol (EE2)

11:00–11:15 ***O05_03*** **G. Cacciola** (Italy): Cannabinoid receptor 1 influences chromatin remodeling in mouse spermatids by affecting content of transition protein 2 mRNA and histone displacement

11:15–11:45 Coffee break

11:45–12:15 ***SOA5_2*** **M. Lindemans** (Belgium): Characterization of the gonadotropin-releasing hormone receptor in C. elegans

12:15–12:30 ***O05_04*** **F. Castro** (Portugal): Ancestral bilaterian retinoic acid signalling: characterization of the Retinoic Acid Receptor (RAR) from the lophotrochozoan mollusc Nucella lapillus

12:30–12:45 ***O05_05*** **D. Chand** (Canada): Gonadal characterization of the evolutionary conserved teneurin-1 protein from Caenorhabditis elegans to mouse: A case for the newly elucidated Teneurin C-terminal Associated Peptide (TCAP) in the mouse testis

12:45–13:00 ***O05_06*** **D. Crespo** (Spain): Involvement of trout TNFα in LH-induced preparatory events for ovulation

13:00–13:15 ***O05_07*** **R. Urbatzka** (Portugal): Evaluation of steroidogenic gene expression in adult zebrafish (Danio rerio) exposed to environmental levels of xenoestrogens found in Douro River estuary, Portugal

HALL 2

6. Circadian Rhythm & Avian Endocrinology
(Chairs: R. Jozsa)

10:00–10:15 ***O06_01*** **I. Hereichova** (Slovak Republic): Expression of clock and metabolic genes in the liver and heart of rats exposed to lighting rotation schedule with phase delays

10:15–10:30 ***O06_02*** **M. Zeman** (Slovak Republic): Circadian system in birds develops earlier than in mammals

10:30–10:45 ***O06_03*** **S. Kommedal** (Hungary): An insight into the development of the circadian clock in the chicken pineal model

10:45–11:00 ***O06_04*** **G. Bódis** (Hungary): Clock mRNA expression patterns in the chick pineal gland under experimental jet lag

11:00–11:15 ***O06_05*** **E. McStay** (United Kingdom): Biological Clocks in the Atlantic Salmon Pineal Organ

11:45–12:00 ***O07_01*** **A. Tamás** (Hungary): Pituitary adenylate cyclase activating polypeptide (PACAP) protects chicken inner ear cells against H2O2-induced oxidative stress

12:00–12:15 ***O07_02*** **S. Harvey** (Canada): Growth hormone in the chick retina: an autocrine/paracrine neurotrophic factor

12:15–12:30 ***O07_03*** **M. Wilhelm** (Hungary): Neuro-immun interactions in the dove brain

13:00–14:00 Lunch

14:00–19:30 Excursions *(see details on page 8)*

Journey by bus, departing at 14:00 from the parking of Central Building.
Casual clothing is recommended for all three programs.

BIKAL – Experience the Renaissance
Travel through time and spend a day in the Renaissance era! You can live like our ancestors at the Land of the Renaissance in Bikal. After getting acquainted with the village, experiencing the architecture and crafts of the age, and trying out pottery, basketry and smithery, the program closes with a real medieval feast.

VILLÁNY – Harvest program at the Wunderlich Winery
Spend an afternoon in the city of grape and wine and taste the finest wines of Villány!
The funny harvest competition ends with visiting the wine-cellars and a dinner with music, where you can taste delicacies of the region.

ORFŰ – Riding program
We will visit a guest house for horseback and carriage rides. The relaxation will be crowned by palinka (famous Hungarian brandy) and a dinner that presents characteristic dishes of the area.

Friday, 3rd September

09:00–09:50 **C. Secombes** (United Kingdom): How far have we got with elucidating the cytokine network in fish? Chair: E. Eppler

HALL 1

8. Neuroendocrine-Immune Interactions
(Chair: E. Eppler)

10:00–10:30 *SOA8* **I. van Kemenade** (The Netherlands): Neuroendocrine-immune interaction: Differential regulation of phagocyte activity in fish by neuroendocrine factors

10:30–10:45 *O08_01* **S. Iwamuro** (Japan): Frog antimicrobial peptide genes: Gene expression in the Harderian gland and thyroid-hormone dependency of gene transcription

10:45–11:00 *O08_02* **M. Teles** (Spain): Elevated plasma cortisol induces organ-specific changes in glucocorticoid receptor expression and in the innate immune response in Sparus aurata

11:00–11:15 *O08_03* **Y. Vraskou** (Spain): Cytokine regulation of glucose entry into the skeletal muscle of the rainbow trout (Oncorhynchus mykiss): role of tumour necrosis factor alpha (TNFα)

11:15–11:45 Coffee break

11:45–12:00 *O08_04* **E. Eppler** (Switzerland): Growth hormone (GH) acts on the GH/IGF-system in adult tilapia liver and immune organs

12:00–12:15 *O08_05* **K. Link** (Switzerland): Seawater and freshwater challenges effects on osmoregulatory organs in black-chinned tilapia (Sarotherodon melanotheron heudelotii)

12:15–12:30 *O08_06* **E. Eppler** (Switzerland): Insulin-like growth factor-I mRNA and peptide are distinctly confined to subtypes of macrophages, antigen-presenting cells, lymphocytes and HEV cells in non-neoplastic human lymph node

HALL 2

9. Invertebrate Neuropeptides and Peptide Hormones: key players in the regulation of physiological processes and behavior
(Chairs: T. Kiss, G. Gäde)

10:00–10:30 *SOA9_1* **T. Janssen** (Belgium): Neuropeptidergic signaling systems in nematodes

10:30–10:45 *O09_01* **A. Rafaeli** (Israel): Developmental Regulation of the Pheromone Biosynthesis Activating Neuropeptide-Receptor (PBAN-R)

10:45–11:00 *O09_02* **A. Fónagy** (Hungary): Studies of sex pheromone production under neuroendocrine control by analytical and morphological means in the oriental armyworm, Pseudaletia separata, Walker (Lepidoptera: Noctuidae)

11:00–11:15 *O09_03* **S. Tiu** (Canada): Molecular cloning and characterization of retinoid X receptor and ecdysone receptor from the lobster, Homarus americanus

11:45–12:15 *SOA9_2* **T. Kiss** (Hungary): Diversity and abundance: the basic properties of neuropeptide action in molluscs

12:15–12:30 *O09_04* **W. Bendena** (Canada): C. elegans allatostatin-like receptors

12:30–12:45 *O09_05* **H. Marco** (South Africa): Moult regulation in the South African spiny lobster, Jasus lalandii

12:45–13:00 *O09_06* **D. A. Lovejoy** (Canada): New Members of the Corticotropin-Releasing Factor (CRF) Family from the Tunicates, Ciona intestinalis and Ciona savignii, and from the Holocephalan, Callorhinchus milii

13:00–13:15 *O09_07* **G. Gäde** (South Africa): A novel AKH decapeptide from a South African saucer bug: how does the structural and functional information compare to other aquatic Heteroptera?

13:00–14:00 Lunch

14:00–15:20 Open discussion forum

HALL 1

5. Reproductive Endocrinology
(Chairs: J. Vanden Broeck, O. Carnevali)

15:30–16:00 *SOA5_3* **L. Swevers** (Greece): Induction of the transition from vitellogenesis to choriogenesis by decline in ecdysone signalling in the ovary of the silkmoth, Bombyx mori: a possible regulatory role for the orphan nuclear receptor BmE75C

16:00–16:30 *SOA5_4* **O. Carnevali** (Italy): Melatonin controls reproduction in Danio rerio

16:30–16:45 *O05_08* **H. Habibi** (Canada): Thyroid hormone and reproduction in goldfish

16:45–17:00 *O05_09* **M. Moya** (Spain): Changes in gonadal development in sea bass (Dicentrarchus labrax) following intramuscular injection of an FSH single-chain-expression plasmid

17:00–17:15 *O05_10* **R. Marin-Juez** (Spain): Analysis of gene expression in the Senegalese sole (Solea senegalensis) testis

17:15–17:30 *O05_15* **S. Zanuy** (Spain): Development of a specific enzyme-linked immunosorbent assay (ELISA) for determining FSH levels in sea bass (Dicentrarchus labrax), using recombinant gonadotropins

HALL 2

9. Invertebrate Neuropeptides and Peptide Hormones: key players in the regulation of physiological processes and behavior
(Chairs: S. Tobe, A. Fonagy)

15:30–16:00 *SOA9_3* **G. Kemenes** (United Kingdom): Evolutionarily conserved molecular mechanisms of learning: A novel role for a molluscan homologue of the vertebrate pituitary adenylate cyclase activating polypeptide (PACAP) in the rapid formation of long-term associative memory in Lymnaea

16:00–16:15 *O09_08* **L. A. Tan** (Canada): Newly elucidated vertebrate peptides play a role in the regulation of stress and neural plasticity: the role of the teneurin C-terminal associated peptides (TCAPs)

16:15–16:30 *O09_09* **Zs. Pirger** (Hungary): Mass spectrometric analysis of seasonal-dependent changes of neuropeptide profile in the snail, helix pomatia

16:30–16:45 *O09_10* **D. Kodrik** (Czech Republic): Adipokinetic peptides increase effectivity of insecticides

16:45–17:00 *O09_11* **I. Bartu** (Czech Republic): Spectrum of diacylglycerols and fatty acids mobilized by adipokinetic hormones

17:00–17:15 *O09_12* **R. Isaac** (United Kingdom): Drosophila male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female

17:15–17:30 *O09_13* **R. Vleugels** (Belgium): Study of two putative 5-HT G protein-coupled receptors in the desert locust (Schistocerca gregaria)

17:30–17:45 *O09_14* **G. Gäde** (South Africa): Beetles are an excellent source for novel members of the adipokinetic peptide family

19:30– Banquet *(Aula)*

Saturday, 4th September

HALL 1

5. Reproductive Endocrinology
(Chairs: J. Vanden Broeck, A. Lange)

09:00–09:30 ***SOA5_5*** **A. Lange** (Canada): Coordination of spermathecal muscle activity with oviposition in Locusta migratoria

09:30–10:00 ***SOA5_6*** **M. Mita** (Japan): Hormonal action of a relaxin-like gonad-stimulating substance (GSS) in starfish, Asterina pectinifera

10:00–10:15 ***O05_13*** **M. Meyering-Vos** (Germany): Silencing of allatoregulating neuropeptide genes affects the fertility of Spodoptera frugiperda (Lepidoptera, Noctuidae)

10:15–10:30 ***O05_14*** **A. Palstra** (Spain): Deep RNA sequencing of red and white skeletal muscle in response to exercise in rainbow trout

10:30–10:45 ***O05_16*** **H. P. Vandersmissen** (Belgium): Myoinhibiting peptides as ancestral ligands for the Drosophila sex peptide receptor

HALL 2

10. Comparative Developmental Biology
(Chairs: W. Kloas, J. Gutierrez)

09:00–09:30 ***SOA10*** **W. Kloas** (Germany): Amphibians as models in comparative developmental biology covering metamorphosis and sexual differentiation

09:30–09:45 ***O10_01*** **J. Gutierrez** (Spain): Differential effects of GH and IGF-I in sea bream cultured myocytes

09:45–10:00 ***O10_02*** **I. Hasunuma** (Japan): Molecular cloning and expression analysis of bullfrog soluble form-like prolactin receptor

10:00–10:15 ***O10_03*** **F. Benato** (Italy): Possible mechanisms of developmental alterations in zebrafish embryogenesis by morpholino knockdown of the glucocorticoid receptor

10:15–10:30 ***O10_08*** **B. Boerjan** (Belgium): Gel based proteomics without a genome: an EST based approach in Schistocerca gregaria

10:30–10:45 ***O10_05*** **M. Heijlen** (Belgium): Antisense Morpholino-mediated knockdown of type 3 iodothyronine deiodinase in the embryonic zebrafish (Danio rerio)

10:45–11:00 ***O10_06*** **M. A. Rodriguez Diaz** (Spain): Comparative analysis of several neurochemical marker sin the trout developing hipothalamus-hypophysial system, with special attention to the pituitary

11:00–11:15 ***O10_07*** **I. Navarro** (Spain): Regulation of LXR, its target genes and fatty acid transporters by insulin, growth hormone and tumour necrosis factor-α in rainbow trout myocytes (Oncorhynchus mykiss)

11:15–11:45 Coffee break

12:00–12:50 **S. Yasou** (Japan): Multiple messengers from the hypophysial pars tuberalis: their functions and signaling pathways.

13:00– Closing Ceremony, Poster Awards

Posters

1. POLYPEPTIDES AND THEIR RECEPTORS, INCLUDING KISSPEPTINS AND GPR54: CO-EVOLUTION, BIOSYNTHESIS, AND SIGNAL TRANSDUCTION

P01_01 Neuroanatomical characterization of the kisspeptin systems in the brain of european sea bass (D.labrax)

Sebastian Escobar Aguirre1*, Arianna Servili2, Alicia Felip1, Felipe Espigares1, Ana Gómez1, Silvia Zanuy1, Manuel Carrillo1, Olivier Kah2

1 Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Castellón, Spain; 2 Neurogenesis and Estrogens, UMR CNRS 6026, IFR 140, University of Rennes 1, France

P01_02 Identification of neuropeptide Y-related receptors potentially involved in the coordination of reproduction and energy balance in the Pacific oyster Crassostrea gigas

Laetitia Bigot*, Charlotte Corporeau, Marie-Pierre Dubos, Pierre Boudry, Pascal Favrel.

University of Caen Lower Normandy, UMR M100 IFREMER, Physiologie et Ecophysiologie des Mollusques Marins, Esplanade de la paix 14032 Caen Cedex, France / Centre de Brest, BP 70, 29280 Plouzané, France

P01_03 Changes in mRNA levels of receptors for TRH and dopamine in the bullfrog pituitary during metamorphosis

Masaki Nakano1*, Atsuko Minagawa1, Itaru Hasunuma2, Kazutoshi Yamamoto2, Sakae Kikuyama2, Takeo Machida1, Tetsuya Kobayashi1

1 Division of Life Science, Graduate School of Science and Engineering, Saitama University, Saitama, Japan

2 Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, Tokyo, Japan

P01_04 Evolution of Melanocortin Receptors: Studies on the genome of the Elephant Shark, Callorhinchus milii

Christina Reinick*, Liang Liang, Robert M. Dores

University of Denver, USA

P01_05 Neuroanatomical localization of kiss ligands and receptors in zebrafish brain

Arianna Servili1*, Yann Le Page1, Jae Young Seong2, Jérome Leprince3, Hubert Vaudry3, Olivier Kah1

1 UMR CNRS 6026, University of Rennes 1, Beaulieu Campus, Rennes, France; 2 Laboratory of G Protein-Coupled Receptors, Graduate School of Medicine, Korea University, Seoul, Republic of Korea; 3 INSERM U982, Neuronal and Neuroendocrine Differentiation and Communication, European Institute for Peptide Research (IFRMP 23), University of Rouen, Mont-Saint-Aignan, France

2. NEUROENDOCRINOLOGY OF INSECTS: ADVANCES THROUGH GENOMICS AND PROTEOMICS

P02_01 Analysis of the hemimetabolous pea aphid genome for nuclear receptors involved in the ecdysone signaling cascade

Guy Smagghe1*,Olivier Christiaens1, Masatoshi Iga1, Rodrigo A. Velarde2, Pierre Rougé3

1 Laboratory of Agrozoology, Department of Crop Protection, Ghent University, Belgium; 2 Department of Biology, Wake Forest University, Winston-Salem, USA; 3 Faculté des Sciences Pharmaceutiques, Université de Toulouse, UMR 152 IRD-Université Paul Sabatier, Toulouse, France

P02_02 Quillaja saponaria saponin is causing an anti-ecdysteroid action in insect cells that may be explained by cytotoxicity and permeation

Guy Smagghe1*, Ellen De Geyter1,2, Danny Geelen1,Thomas Soin1, Luc Swevers3

1 Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

2 Department of Plant Production, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

3 Institute of Biology, National Centre of Scientific Research 'Demokritos', Athens, Greece

P02_03 Comparing activity of non-steroidal ecdysone agonists between dipteran and lepidopteran insects using cell-based EcR reporter assays

Guy Smagghe1*, Thomas Soin1, Georgia Kotzia2, Kostas Iatrou2, Colin R. Janssen1, Pierre Rougé3, Toshiyuki Harada4, Yoshiaki Nakagawa4, Luc Swevers2

1 Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium; 2 Insect Molecular Genetics and Biotechnology, Institute of Biology, National Centre for Scientific Research "Demokritos", Aghia Paraskevi Attikis, Athens, Greece; 3 Surfaces Cellulaires et Signalisation chez les Végétaux, UMR Université Paul Sabatier CNRS 5546, Castanet Tolosan, France; 4 Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

P02_04 The ecdysone receptor in a neuropteran (Chrysoperla carnea) and dermapteran insect (Forficula auricularia) used in biological control: sequencing and structural modeling

Guy Smagghe1*, Moises Zotti1,2, Olivier Christiaens1, Pierre Rougé3

1 Laboratory of Agrozoology, Department of Crop Protection, Ghent University, 9000 Ghent, Belgium; 2 Federal University of Pelotas (UFPel), Faculty of Agronomy "Eliseu Maciel" (FAEM), Fitossanitary Department, Laboratory of selectivity of pesticides on natural enemies, Box mail 354, zip code: 96010-900, Pelotas - RS, Brazil; 3 Université de Toulouse, UMR 152 IRD-Université Paul Sabatier, Faculté des Sciences Pharmaceutiques, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France

3. REGULATION OF FOOD INTAKE IN INVERTEBRATES AND VERTEBRATES

P03_01 Impairment of food intake and lipid metabolism by DEHP in zebrafish

Beatrice Migliarini*, Chiara Carla Piccinetti, Andrea Martella, Francesca Maradonna, Luca Tosti, Oliana Carnevali

Department of Marine Sciences, Polytechnic University of Marche, Ancona, Italy

4. INSULIN-LIKE GROWTH FACTOR (IGF) SIGNALLING AND AGEING

P04_01 Insights into the mechanisms involved in the exercise-enhanced white muscle growth in adult zebrafish Danio rerio

Mireia Rovira*, Arjan P. Palstra, Josep V. Planas

Departament of Physiology, Faculty of Biology, University of Barcelona and Universitat de Barcelona and Institut de Biomedicina de la Universitat de Barcelona (IBUB), Spain

5. REPRODUCTIVE ENDOCRINOLOGY

P05_01 The probiotic Lactobacillus rhamnosus, increase fecundity in Danio rerio

Oliana Carnevali*, Giorgia Gioacchini

Department of Marine Sciences, Polytechnic University of Marche, Ancona, Italy

P05_02 Endocrine regulation of gonad maturation of pre-pubertal sea bass (Dicentrarchus labrax L.) kept under different light regimes and steroid treatments

Manuel A. Carrillo1*, Alicia Felip1, Gregorio Molés1, Ozlem Yilmaz2, José Miguel Cerdá-Reverter1, Silvia Zanuy1

1 Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Castellón, Spain.; 2 Faculty of Fisheries, Akdeniz University, Antalya, Turkey

P05_03 Establishment and characterization of a primary culture of ovarian follicular cells for sea bass (Dicentrarchus labrax)

Berta Crespo*, Silvia Zanuy Ana Gómez

Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Castellón, Spain.

P05_04 Is the function of bank vole seminal vesicles affected by of 4-tert-octylphenol?

Jerzy Galas*, Ma³gorzata Kotula-Balak, Anna Hejmej, Jolanta Sadowska and Barbara Biliñska

Department of Endocrinology and Tissue Culture, Institute of Zoology, Jagiellonian University, Krakow, Poland

P05_05 Levels of vitellogenin-inhibiitng hormone (VIH) in hemolymph in relation to molting in the whiteleg shrimp, Litopenaeus vannamei

Bong Jung Kang1*, T. Okutsu1, J. Shinji2, S. Jasmani1, V. Jayasankar1, M.N. Wilder1

1 Japan International Research Center for Agricultural Sciences, Tokyo, Japan; 2 Graduate School of Agricultural and Life Sciences, the University of Tokyo, Japan

P05_06 Presence of pituitary adenylate cyclase activating polypeptide in human body fluids

Peter Kiss1*, Krisztian Lammel1, Reka Brubel1, Dora Reglodi1, Andrea Tamas1, Andrea Lubics1, , Akos Varnagy2, Miklos Koppan2, Jozsef Bodis2, Zsolt Biro3, Endre Czeiter4, Peter Bukovics4, Andras Buki4, Samuel Komoly5, Laszlo Mark6

1 Department of Anatomy, University of Pecs, Hungary; 2 Department of Obstetrics and Gynecology, University of Pecs, Hungary; 3 Department of Ophthalmology, University of Pecs, Hungary; 4 Department of Neurosurgery, University of Pecs, Hungary; 5 Department of Neurology, University of Pecs, Hungary; 6 Department of Biochemistry and Medical Chemistry, University of Pecs, Hungary

P05_07 Involvement of cAMP/PKA and MAP kinase pathways on steroid synthesis stimulated by FSH in sea bass (Dicentrarchus labrax)

Maria Jose Mazon Moya *, Silvia Zanuy, Ana Gomez

Department of Fish Physiology and Biotechnology, Instituto de Acuicultura de Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Castellón, Spain.

P05_08 Presence of pituitary adenylate cyclase activating polypeptide in the milk of domestic animals and humans

Dora Reglodi1*, Andrea Tamas1, Levente Czegledi2, Balint Szalontai3, Rita Borzsei4, Terez Bagoly4, Katalin Csanaky1, Eszter Banki1, Peter Kiss1, Gabriella Horvath1, Edit Szauer1, Jozsef Nemeth5, Laszlo Mark6, Reka Brubel1, Zsuzsanna Helyes4

1 Department of Anatomy, University of Pecs, Hungary; 2 Institute of Animal Sciences, Centre of Agricultural Sciences and Engineering, University of Debrecen, Hungary; 3 Department of Plant Physiology, University of Pecs, Hungary; 4 Department of Pharmacology and Pharmacotherapeutics, University of Pecs, Hungary; 5 Department of Pharmacology and Pharmacotherapeutics, University of Debrecen, Hungary; 6 Department of Biochemistry and Medical Chemistry, University of Pecs, Hungary

P05_09 Effect of Largagtil on Physiology of Reproduction

Shariati Mehrdad*, Ghavami Mehrnoush

Islamic Azad University, Kazeroun Branch, Kazeroun, Iran

P05_10 Seasonal mRNA expression of urate oxidase in brown trout liver and analysis of transcriptional regulation by different hormones

Ralph Urbatzka1, L. Filipe C. Castro1, Maria J. Rocha1,2, Alexandre Lobo-da-Cunha1,3, Rogério A.F. Monteiro1,3 and Eduardo Rocha1,3

1 Interdisciplinary Centre for Marine and Environmental Research (CIIMAR), CIMAR Associate Laboratory, University of Porto, Portugal; 2 Superior Institute of Health Sciences-North (ISCS-N), Porto, Portugal; 3 Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal

6. CIRCADIAN RHYTHM

P06_01 Photoperiod effects on the expression of type II Iodothyronine deiodianse in Atlantic salmon parr

Andrew Davie*, Giulia Micallef, Elsbeth McStay, Herve Migaud

Institute of Aquaculture, University of Stirling, Stirling, UK

P06_02 The effects of PACAP on the signaling pathways and cell survival in chicken pineal cells is dependent on the circadian rhythm

Gabriella Horvath1*, Dora Reglodi1, Balazs Opper1, Reka Brubel1, Andrea Tamas1, Peter Kiss1, Gabor Toth2, Valer Csernus1, Boglarka Racz3

1 Department of Anatomy, University of Pecs, Hungary; 2 Department of Medical Chemistry, University of Szeged, Hungary; 3 Department of Biochemistry and Medical Chemistry, University of Pecs, Hungary

P06_03 Expression of the circadian clock gene clock in human melanoma skin biopsy

Zsuzsanna Lengyel1*, András D. Nagy2, Valér Csernus2, Zita Battyáni1

1 Department of Dermatology, Faculty of Medicine, University of Pécs, Hungary; 2 Department of Anatomy, Faculty of Medicine, University of Pécs, Hungary

7. AVIAN ENDOCRINOLOGY

P07_01 Seasonal changes in courtship behavior, testicular development and in hypothalamic aromatase immunoreactivity in male free-living European starlings (Sturnus vulgaris)

Ottó Pintér *, Péter Péczely, Dóra Zelena

1 Department of Behavioural Neurobiology, Institute of Experimental Medicine of the Hungarian Acadamy of Sciences, Budapest, Hungary; 2 Laboratory of Reproductive Biology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, Hungary

9. INVERTEBRATE NEUROPEPTIDES AND PEPTIDE HORMONES: KEY PLAYERS IN THE REGULATION OF PHYSIOLOGICAL PROCESSES AND BEHAVIOR

P09_01 Structural identification and possible functional determination of the pea aphid, Acyrthosiphon pisum adipokinetic hormone

Pavel Jedlicka, Petr Simek*
Laboratory of Analytical Biochemistry, Biology Centre, Academy of Sciences of the Czech Republic, Èeské Budìjovice, Czech Republic

P09_02 The foraging gene of the desert locust

Ronit Kornfein*, C. Lucas, R. M. Chakaborty-Chatterjee, J. Schonfeld, N. Geva1, M.B. Sokolowski2, A. Ayali1
Department of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

P09_03 Transfer function analysis reveals that trout pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) decrease baroreflex sensitivity in trout

Frédéric Lancien1*, Nagi Mimassi1, J. Michael Conlon2, Jean-Claude Le Mével1
1 Université Européenne de Bretagne, Université de Brest; INSERM U650, Laboratoire de Neurophysiologie, IFR 148 ScInBioS, CS 93837, 29238 Brest Cedex 3; CHU de Brest, France; 2 Department of Biochemistry, Faculty of Medicine and Health Sciences, United Arab Emirates University, 17666 Al Ain, United Arab Emirates

P09_04 Cardiovascular and ventilatory actions of trout neuropeptide Y and peptide YY in trout

Ali Al Arab1*, Frédéric Lancien1, Nagi Mimassi1, J. Michael Conlon2, Jean-Claude Le Mével1
1 Université Européenne de Bretagne, Université de Brest; INSERM U650, Laboratoire de Neurophysiologie, IFR 148 ScInBioS, CS 93837, 29238 Brest Cedex 3; CHU de Brest, France; 2 Department of Biochemistry, Faculty of Medicine and Health Sciences, United Arab Emirates University, 17666 Al Ain, United Arab Emirates

P09_05 Brain extirpation stimulate PACAP expression in the central nervous system of the earthworm

Andrea Lubics1, B. Horváth2, Ildiko Somogyi2, Dora Gunszt2, Akos Boros2, Edit Pollak2, Jozsef Nemeth3, Dora Reglodi1, Laszlo Molnar2
1 Department of Anatomy, University of Pecs, Hungary; 2 Department of General Zoology, University of Pecs, Hungary; 3 Department of Pharmacology and Pharmacotherapy, University of Debrecen, Hungary

P09_06 Effects of crustacean hyperglycemic hormone (CHH) on carbohydrate metabolism-related enzymes in the kuruma prawn, Marsupenaeus japonicus

Chiaki Nagai1*, Hideaki Mabashi-Asazuma2, Shinji Nagata1, Hiromichi Nagasawa1
1:Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan
2:Department of Molecular Biology, the University of Wyoming, USA

P09_07 LC/MS analysis of peptide hormones and their processing in the larval and adult Drosophila melanogaster midgut

Wencke Reiher1*, Jörg Kahnt2, Stefan Baumeister3, Christian Wegener1
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P09_08 Examination of the role of crustacean hyperglycemic hormone in stress response using the whiteleg shrimp, Litopenaeus vannamei

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P09_09 Antioxidant effect of adipokinetic neuropeptides in Spodoptera littoralis

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P09-10 Intracellular calcium signaling is essential for coelomocyte activation in earthworm

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10. COMPARATIVE DEVELOPMENTAL BIOLOGY

P10_01 Characterization of growth hormone in green iguana (Iguana iguana)

Jose Avila Mendoza*, Martha Carranza Salas, Maricela Luna Munoz, Carlos Aramburo de la Hoz
Instituto de Neurobilogia, Universidad Nacional Autonoma de Mexico

P10_02 Insulin and IGF-I effects on the proliferation of an osteoblast primary culture from seabream (Sparus aurata)

Encarnación Capilla*, Laura Acerete, Isabel Navarro and Joaquim Gutiérrez
Department of Physiology, Faculty of Biology, University of Barcelona, Barcelona Spain

P10_03 The knockdown of the maternal estrogen receptor-beta2 mRNA affects embryo transcript contents and larval development in zebrafish

Andrea Celeghin, Benato Francesca, Pikulkaew Surachai, Colombo Lorenzo, Dalla Valle Luisa
Department of Biology, University of Padua, Padua, Italy

P10_04 The deubiquitinating enzyme mUBPy in the brain and sensory organs of mouse during embryonic development

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P10_05 Identification of PINK1 in the brain, eye and ear of mouse during embryonic development

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P10_06 Expression of PINK1 in the zebrafish D. rerio during development

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P10_07 Molecular cloning and characterization of Dmc1, a meiosis-specific gene, from the whiteleg shrimp, Litopenaeus vannamei

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P10_09 The brown shrimp (Crangon crangon L.) ecdysone receptor complex: cloning, structural modeling of the ligand-binding domain and functional expression in an EcR-deficient Drosophila cell line

Guy Smagghe5*, Yves Verhaegen1,2,3, Koen Parmentier2, Luc Swevers4, Pierre Rougé5, Thomas Soin1, Wim De Coen3, Kris Cooreman2
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P10_10 The RXR receptor: a target of endocrine disruption in the brown shrimp (Crangon crangon L.)?

Guy Smagghe1, Yves Verhaegen1,2,3, Ellen Renders1, Koen Parmentier2, Luc Swevers4, Pierre Rougé5, Wim De Coen3, Kris Cooreman2
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P10_11 Potential of de novo ecdysteroid biosynthesis in the testis of Spodoptera littoralis

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P10_12 High-throughput screening of ecdysone agonists using a dipteran-specific EcR reporter assay

Luc Swevers*, Thomas Soin, Luc Swevers, Georgia Kotzia, Kostas Iatrou, Colin R. Janssen, Pierre Rougé, Toshiyuki Harada, Yoshiaki Nakagawa, Guy Smagghe

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P10_13 Relationship between larval-pupal metamorphosis and gene expression of insulin-like peptide and insulin receptor in Spodoptera littoralis

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P10_14 Teneurin C-terminal Associated Peptides (TCAPs): a conserved peptide system in metazoans that regulate cell growth and metabolism

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Plenary lecturers

PL1 In search for new players of the regulated secretory pathway: lessons from amphibians

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The regulated secretory pathway, a hallmark of neuroendocrine cells, is an intricate, multi-step process that involves generation of transport carriers, sorting and packaging of specific cargo proteins, delivery of transport carriers to the plasma membrane, and membrane fusion in response to specific extracellular stimuli. Neuroendocrine cells tightly control particular stages of this process by synthesizing a wide variety of regulatory proteins with specific functions within the regulated secretory pathway. Given the complexity of this system, it is difficult to find appropriate cellular models wherein to investigate the multiple components of the secretory process in a physiologically relevant, experimentally manipulable setting. In this scenario, the intermediate lobe (IL) of the amphibian pituitary appears as a powerful model to understand different aspects of the regulated secretory pathway. Thus, IL is composed of a single endocrine cell type, alpha-melanocyte stimulating hormone (α-MSH)-producing melanotropes, a fact that greatly facilitates its study. Furthermore, the melanotrope population of the amphibian IL is composed of two distinct melanotrope cell subtypes exhibiting opposite morphophysiological phenotypes of hypo- and hypersecretory cells. In this presentation, we show how the comparative genomic analysis of these two cell subtypes has contributed to the identification and molecular characterization of novel players in the regulated secretory pathway, Rab18 and Neuroendocrine long coiled-coil proteins (NECCs) and to unveil unexpected functions for these proteins in regulating intracellular traffic events. We will discuss the relevance of these proteins as useful markers for reliably determining the general secretory status in an endocrine gland, as well as valuable new tools to further investigate this complex process under both physiological and pathological conditions.

PL2 Lessons from kisspeptin physiology: from fish to mammals

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Kisspeptins, the peptide products of the Kiss1 gene, were initially identified in mammalian vertebrates as ligands of the G protein-coupled receptor 54 (GPR54; also termed Kiss1R) with ability to suppress tumor metastasis. In late 2003, the indispensable role of kisspeptins in the control of reproductive function was disclosed by the seminal observations that humans and mice carrying inactivating mutations of GPR54 suffer absence of puberty and hypogonadotropic hypogonadism. Since then, numerous experimental studies, conducted initially in mammalian species, have substantiated the function of kisspeptins as essential players in the physiologic regulation of key aspects of reproductive maturation and function, which are likely to include: (i) the timing of puberty onset; (ii) the process of brain sexual differentiation; (iii) the dynamic control of gonadotropin secretion via stimulation of GnRH neurons; (iv) the transmission of the negative feedback effects of sex steroids; (v) the mediation of the positive feedback effects of estrogen and, hence, the generation of the preovulatory surge of gonadotropins; (vi) the metabolic regulation of fertility; and (vii) the control of reproductive function by environmental and photoperiodic cues. Of important note, while studies about kisspeptins in non-mammals appeared initially to lag behind, significant efforts have been put recently to define the genomic organization and functional characteristics of Kiss1/kisspeptins and GPR54 in different non-mammalian species, including fish, reptiles and amphibians. Indeed, these analyses have not only substantiated the conserved, essential roles of kisspeptins in the control of reproduction, but have also disclosed intriguing evolutionary aspects of kisspeptins and their receptor. In this context, it is anticipated that comparative endocrinology approaches will be of great help to fuel further studies on the molecular regulation and physiological roles of kisspeptins, thus aiding to unveil the biology of this fascinating system as indispensable regulator of the reproductive axis in a wide diversity of animal species.

PL3 The endocrine regulation of insect metamorphosis and the emerging role of microRNAs

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Insect are classified into two groups according to the metamorphosis mode: the hemimetabolans, which hatch as nymphs with a morphology similar to that of the adult and grow progressively until the adult stage; and the holometabolans, which hatch as a larva with a morphology different from that of the adult, then grow through successive molts until the last larval instar, then to the pupae and to the adult.
In the 1930 decade, the experiments of Vincent B. Wigglesworth already showed that the endocrine regulation of insect metamorphosis is based on molting hormones, which induce the successive molts, and juvenile hormones which maintain the juvenile character of them. Later, a number of transcription factors have been reported as mediators of the above hormones, as well as a number of target genes that codify for proteins giving the characteristic shape and behaviour of a juvenile or an adult stage. Most of the information available, however, refers to the most derived holometabolan species, especially to the fruitfly Drosophila melanogaster, whereas data on the less modified hemimetabolan species are scarce.
Working on the hemimetabolan Blattella germanica (the German cockroach), we have recently found that microRNAs, which are RNAs of ca. 22 nucleotides that play a generally repressing action on mRNA stability and translation, have a key role in metamorphosis. With RNA interference (RNAi), we silenced the expression of dicer-1, a ribonuclease that mediates the maturation of microRNAs. When dicer-1 was silenced in the last nymphal instar, the production of microRNAs was impaired and the cockroaches, instead of molting to the adult stage as controls did, they transformed into gigantic supernumerary nymphs (Gomez-Orte & Belles, PNAS 106: 21678-21682, 2009). These results demonstrated that microRNAs are essential in insect metamorphosis, at least in hemimetabolans.
The hypothesis emerging is that particular microRNAs would repress the expression of genes giving nymphal characters in the molt to adult. To test it, we are now working on a number of microRNAs that could be candidates to play such a role, studying their pattern of expression and the influence of the molting hormone and the juvenile hormone on their abundance. Experiments of silencing of those microRNA that might play a role in metamorphosis, studies on the effect of them on predicted targets, and building of networks representing the interaction microRNA/targets in pre-metamorphic and metamorphic stages are also included in our project. In the end, we hope that the results obtained will illuminate the endocrine mechanisms that allowed the evolutionary transition from the ancestral hemimetaboly to the derived holometaboly.

PL4 How far have we got with elucidating the cytokine network in fish?

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The repertoire of cytokines present in fish is becoming more apparent following the sequencing of the genome of a number of fish species. On the one hand, a complex cytokine network is clearly present, with many genes clear homologues of those known in higher vertebrates. On the other hand, some genes have less clear homology and may be the result of gene duplication events within fish groups. Curiously, some genes well known in higher vertebrates are not apparent, as seen with some of the Th2 cytokines (IL-5, IL-9, IL-13) and others in the same locus (IL-3, GM-CSF). Such data confirm that many cytokine genes were present in early vertebrates prior to the fish-tetrapod divergence, and have expanded independently in different vertebrate groups since that time. This talk will review some of the key cytokine genes discovered in fish to date, outlining what is known about their bioactivity where possible, and highlighting any differences to known mammalian genes.

PL5 Multiple messengers from the hypophysial pars tuberalis: their functions and signaling pathways

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In temperate zones, the reproductive physiology of most vertebrates is controlled by changes in photoperiod. In recent years, thyrotropin produced in the pars tuberalis (PT) has been shown to play an essential role in regulation of gonadal axis via a retrograde pathway from the PT to the hypothalamus. However, the factors of the anterograde pathway through which the PT affects the hormonal secretion from the hypophysial pars distalis (PD) have not been identified for many decades. Several data suggest the involvement of endocannabinoids (e.g. anandamide and 2-arachidonoylglycerol, 2-AG) and their specific receptors (CB1 and CB2) in neuroendocrine regulation, although it remained unclear where and how endocannabinoids elicit their effects. Recently, we found in Syrian hamsters that the PT expresses enzymes involved in endocannabinoid synthesis and degradation and that the PD expresses cannabinoid receptor 1 (CB1). Synthesis of 2-AG was upregulated under long days and 2-AG stimulates prolactin secretion from the PD in the in the presence of forskolin, suggesting that 2-AG plays an important role in the photoperiodic regulation of hypophysial hormones. Most recently, we have identified an endocannabinoid system also in the PT and PD of human. These data suggest that endocannabinoids are important anterograde signals from the PT to the PD, and the functional role of endocannabinoids within this pathway will be discussed.
Supported by LOEWE Lipid Signaling Forschungszentrum Frankfurt, Alfred und Gertrud Kassel-Stiftung.

State of the Art

1. Polypeptides and their Receptors, including Kisspeptins and GPR54: Co-Evolution, Biosynthesis, and Signal Transduction

SOA1_1 Kiss systems in non-mammalian vertebrates with special emphasis on fishes

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Although kisspeptins are new comers in the field of reproductive biology, they have attracted considerable attention as potential central players in the integration of internal and external factors and key actors for the timing of puberty. Recent phylogenetical analyses provided evidence that the number of kiss genes and kiss receptors varies from one class of vertebrate to the other. According to these studies (Lee et al., 2009, Endocrinology 50: 2837-46) modern mammals have only one KISS gene, monotrems have two, birds would have none, reptiles have one, amphibians have three and fishes have two KISS genes. Similarly, the number of genes encoding GPR54 receptors also varies from one class to the other. To date, data on the organization of kiss systems in relation to GPR54 receptors are very limited in non-mammalian vertebrates. This state-of-the-art lecture will summarize the current knowledge on the organization and potential functions of kisspeptins in non-mammalian vertebrates with special emphasis on new data obtained in fishes, namely zebrafish. In this species, recent studies have allowed obtaining a detailed vision of the organization of the KISS1 and KISS2 systems in fish and the relationships between these two systems and the GPR54 receptors. Furthermore, the potential interactions between Kiss neurons and GnRH neurons will be discussed as well as the relationships with leptin receptors and the estrogen receptors.
Supported by the EU Project LIFECYCLE (FP7-222719) and the NEMO project

SOA1_2 Structure-Function Analysis of Endoproteolytic Cleavage by PC2: Site-directed Mutagenesis Studies on the α-MSH Cleavage Site in Silurana tropicalis POMC

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The posttranslational processing of many prohormone precursors requires endoproteolytic cleavage at paired basic amino acid residues, such as the KR↓site that is located N-terminal to the α-MSH sequence in POMC of the amphibian, Silurana tropicalis. Cleavage at this site in the intermediate pituitary is performed by the endoprotease, Prohormone Convertase 2 (PC2). This cleavage site is preceded by the amino acid motif, 137-RQENKR↓-142. In order to determine which amino acids in this motif are required for recognition by PC2, alanine substitutions were made in this region of S. tropicalis POMC. The mutant construct genes were separately expressed in mouse αTC1.9 cells. Pilot studies indicated that expression of the wild-type POMC construct in this cell line yielded the same end-products as the intermediate pituitary of S. tropicalis. Moreover, biochemical analysis of the mutant constructs expressed in α-TC1 cells indicated that only alanine substitutions at K141 and R142 blocked endoproteolytic cleavage at this site. These experiments indicated that neither the R137 residue nor the apparent beta-turn produced by the RQEN motif is needed for endoproteolytic cleavage at this site. These observations raise new questions with respect to the perceived substrate selectivity of PC2 that will be discussed in this presentation.
This research was supported by the Ira E. Cutler Endowment (University of Denver).

2. Neuroendocrinology of Insects: advances through genomics and proteomics

SOA2 Mechanism and function of the Drosophila capa receptor

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The D. melanogaster capability gene encodes capa-1, -2 and -3 neuropeptides. Capa-1/-2 are diuretic in the Malpighian tubules via elevation of intracellular calcium ([Ca2+]i) and nitric oxide/cGMP signaling (Dow and Davies 2003). The Malpighian tubules of insects, equivalent to vertebrate kidney and liver (Beyenbach, Skaer et al.) regulate fluid and ion homeostasis, and detoxification. Thus, diuretic peptides and their cognate receptors are of some interest.

The capa receptor (capaR) is a GPCR identified in Drosophila (gene CG14575) via analysis of the PRXamide peptide receptor family (Iversen, Cazzamali et al. 2002; Park, Kim et al. 2002). CapaR has also been identified in Anopheles (Pollock, McGettigan et al. 2004; Olsen, Cazzamali et al. 2007), and most recently, in R. prolixus (Paluzzi, Park et al. 2010), where capa is anti-diuretic.

To precisely determine the characteristics of capa ligand-receptor [Ca2+]i responses, Drosophila S2 cells and intact Malpighian tubules were used to assess the affinities of capa peptides for capaR, which we show are very similar between these systems. EC50 values of stimulated [Ca2+]i responses in capaR-transfected S2 cells, capa-1: 3.06 nM; capa-2: 4.32 nM vs intact tubule, capa-1: 5 nM; capa-2: 3.02 nM. Furthermore, cell surface biotinylation experiments and desensitisation/resensitisation of the calcium response in S2 cells and in intact Malpighian tubules, show that the Drosophila capaR behaves as a canonical GPCR in vivo, whose mechanism of action also involves b-arrestin

Expression mapping of endogenous capaR using a novel capaR-promoter-specific GAL4 line shows that as predicted, capaR is strongly expressed in larval/adult Malpighian tubules. However, capaR is also expressed in other epithelia and in neuronal cells. Manipulation of capaR expression levels in only the tubule principal cells using the c42 GAL4 driver results in direct modulation of capa-induced calcium and fluid-transport rates. capaR RNAi significantly decreases capa-1-stimulated [Ca2+]i and fluid transport rates; whilst targeted over-expression of capaR to only tubule principal cells increases [Ca2+]i. Thus, modulation of expression of this single GPCR in a cell-specific manner affects epithelial function in vivo. Deletion at the capaR locus resulting in 75% knock-down in capaR tubule gene expression results in lethality of capaR deletion homozygotes. Deletion of the capaR locus is embryonic lethal; lethality is partially rescued by one copy of the capaR gene. A putative human orthologue of capa, neuromedinU, induces [Ca2+]i increases in capaRtransfected S2 cells and in native tubules. This [Ca2+]i rise is physiologically relevant, as neuromedin also stimulates fluid transport in intact Malpighian tubules.

Thus, the capa/capaR signaling system may have important parallels within the human endocrine system. Study of insect systems, whilst of value in themselves, may also reveal putative novel regulation of vertebrate physiology.

3. Regulation of Food Intake in Invertebrates and Vertebrates

SOA3 Feeding regulation and energy metabolism in the brain

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Novel neuropeptides of G-protein coupled receptor (GPCR) ligands are shown to be localized in brain and play a range of physiological functions including feeding regulation and energy metabolism. Here, I will describe the distribution and localization of these new GPCR ligands identified recently and to review their involvement in neuronal networks, particularly feeding regulation and energy metabolism in the brain. This presentation concerns some novel GPCR ligands of feeding and energy metabolismregulating neuropeptides such as orexin, ghrelin, galanin-like peptide (GALP) and neuropeptide W (NPW), such as those studied by our group and other collaborating groups, and neuronal interactions among these and other well known neuropeptides such as neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (alpha-MSH) in the hypothalamus. Cross-talk among several of these neuropeptides-containing neurons in the hypothalamus plays a crucial role in determining feeding states as well as feeding behavior. I will show some structural and functional characteristics of these recently discovered neuropeptides and summarize the known interactions between these different kind of feeding regulating neurons and leptin-targeting neurons in the hypothalamus. Moreover, I will show a new strategy for analyzing the neural circuit of these feeding- and energy metabolism-regulating GPCR ligands-containing neurons in the brain by use of transgenic model mice. Finally, I will present our recent results of GALP which are involved in regulation of feeding as well as energy homeostasis and body temperature. In addition, I will show our hot data of intranasal infusion of GALP to decrease body weight and locomotor activity in normal and obese mice. Research in this field will serve a very important role of clarifying neurologically-based causes for appetite dysfunctions and life-style diseases and it may help to establish and to lead new therapies for people and/or patients who are suffering from such conditions.

4. Insulin-like growth factor (IGF) Signalling and Ageing

SOA4 Hormonal regulation of aging

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Understanding how the aging process in regulated is a fundamental and fascinating problem in biology, with significant medical and economical implications. Accumulating data indicate that insulin/IGF-1 (insulin-like growth factor-1) signaling, which is a highly conserved hormonal system, plays a pivotal role in controlling - in addition to development and metabolism - aging in divergent animal phyla. For example, mutations that decrease or eliminate the activity of the IGF-1 receptor or type I phosphatidyl inositol-3 kinase (PI3K) can double the natural lifespan of worms, flies and mice. The lifespan-extending effect of compromised insulin/IGF-1 signaling is mediated by the Forkhead transcription factor FoxO, a downstream component of the pathway. In the absence of IGF-1 receptor or PI3K activity, the cytoplasmic form of FoxO becomes dephosphorylated and translocated into the nucleus, where it transcriptionally modulates their target genes modulating the rate at which cells and tissues age. Genetic evidence shows in the nematode Caenorhabditis elegans that FoxO regulates the activity of TOR (kinase target of rapamycin), which in turn acts as a negative regulator of autophagy (the process of cellular self-eating in eukaryotes, during which parts of the cytoplasm are delivered into lysosomes for degradation). Thus, insulin/IGF-1 signaling modulates the aging process eventually in an autophagy-dependent manner. Furthermore, the effects of other distinct longevity pathways, including the mitochondrial respiratory chain, nutrient-sensing pathway and TOR, Ras or TGF-β signaling, are also mediated by autophagy genes in this organism. Together, upon hormonal signals, autophagy may operate as a central regulatory mechanism of animal aging.

SOA5_1 Genomic approaches of regulation of reproduction in the Pacific oyster

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Despite the major commercial and ecological importance of some species, mollusks are like a majority of Lophotrochozoan species still poorly documented with respects to genomics data and functional assignments. Because the Pacific oyster Crassostrea gigas has a worldwide distribution and presents the world highest annual production of any aquatic organism, it has become an attractive species for genome-related research activities focusing on physiological mechanisms of economically important traits. As a result a number of international programs for the development of C. gigas genomic resources are currently in progress. Recently our group generated a C. gigas specific EST database: the "Gigasdatabase" containing up to 30000 unigenes (1) To investigate the molecular bases of summer mortality, a phenomenon which has become a major concern to oyster aquaculture worldwide, a genome-wide expression profiling of resistant (R) and susceptible (S) oyster families was conducted using a cDNA microarray. With the characterization of differentially expressed genes encoding endocrine /paracrine components in the gonad, this approach clearly highlighted the regulation of reproduction and its allocation as one of the main pathways that operate differentially. Based on these examples, we illustrate how the genomic data available in C gigas can be used to explore the endocrinology of reproduction or associated processes of this species and in a comparative context to assess the influence of evolution on both structure and function of endocrine components.
(1) BMC genomics 2009, 10:341

SOA5_2 Characterization of the gonadotropin-releasing hormone receptor in C. elegans

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In mammals, key hormones of the hypothalamic-pituitary-gonadal axis control reproduction. The hypothalamic hormone gonadotropin-releasing hormone (GnRH) is a neuropeptide that stimulates the release of gonadotropins from the anterior pituitary. The existence of a putative functional equivalent of this reproduction axis in protostomian invertebrates has been a matter of debate. In this study, the ligand for the GnRH receptor in the soil nematode Caenorhabditis elegans (Ce-GnRHR) was found using a bioinformatic approach. The peptide and its precursor are reminiscent of both insect adipokinetic hormones (AKH) and of GnRH-preprohormone precursors from tunicates and higher vertebrates. We cloned the AKH-GnRH-like preprohormone and the Ce-GnRHR and expressed the GPCR in HEK293T cells upon characterization. The Ce-GnRHR was activated by the C. elegans AKH-GnRH-like peptide as well as by Drosophila AKH and other nematode AKH-GnRHs that we found in EST databases. Analogous to insect AKH receptor and vertebrate GnRH receptor signaling, Ce-AKH-GnRH activated its receptor through a Gαq protein with Ca2+ as second messenger. Gene silencing of Ce-GnRHR or Ce-AKH-GnRH or both resulted in a delay in the egg laying process. This is comparable to a delay in puberty in mammals lacking a normal dose of GnRH peptide or with a mutated GnRH precursor or receptor gene.
In addition, the Ce-AKH-GnRH precursor is expressed in a pair of head neurons during all C. elegans life stages, and particularly in the fourth larval stage - just before adulthood – also in neuronal projections at the vulva. The present data support the view that the AKH-GnRH signaling system probably arose very early in metazoan evolution and that its role in reproduction might have been developed prior to the divergence of Protostomians and Deuterostomians.

SOA5_3 Induction of the transition from vitellogenesis to choriogenesis by decline in ecdysone signalling in the ovary of the silkmoth, Bombyx mori: a possible regulatory role for the orphan nuclear receptor BmE75C

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In the silkmoth, Bombyx mori, oogenesis is initiated in the ovarian follicles at high titers of 20-hydroxyecdysone during pupal and pharate adult development. Studies using the ecdysone agonist tebufenozide have shown that the transition from vitellogenesis to choriogenesis requires down-regulation of ecdysone signalling. Expression studies and analysis of binding sites in promoter regions have lead to the identification of several putative regulatory factors in this pathway, including the orphan nuclear receptor BmE75 and the chorion gene regulator BmGATAβ. Yeast two-hybrid screens using BmE75C as bait were carried out to identify interacting partners and led to the isolation of BmCAP, a putative adaptor protein containing three SH3 domains at its C-terminus that is implicated in cytoskeleton organization, cell adhesion and insulin signalling in other developmental systems. Interestingly, BmCAP encodes multiple mRNA isoforms that can be processed in different protein isoforms. Localization studies indicate the presence of the large isoform, BmCAP-A, at the junctions of the cells of the follicular epithelium as well as at their apical surface during late choriogenesis, indicating functional roles in cell adhesion as well as in secretion of the chorion proteins and the sculpting of the egg shell surface. While a role for BmCAP-A in the modulation of the function of BmE75C was not found, an involvement for such a role may seem more likely for the short isoform, BmCAP-B. Our studies provide a better understanding of the regulation of oogenesis in Bombyx and lead to new insights in the functioning of the ecdysone regulatory pathway.

SOA5_4 Role of melatonin on the regulation of ovarian function: new findings in Danio rerio

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Melatonin is involved in a number of activities including reproduction acting on hypothalamus, pituitary and gonads. The mechanism by which melatonin acts on the reproductive axis is still unknown although this hormone does not appear to have a direct influence on GnRH neurons. The nature of relationship between pineal melatonin and reproductive physiology remains controversial; in mammals the administration of melatonin may induce or inhibit reproduction depending on the species reproductive strategy: melatonin inhibits long day summer phenotype and induces a short day winter phenotype reproduction. In these latter species, melatonin is referred to as being progonadotrophic. Conflicting conclusions on the role of melatonin in the neuroendocrine system has been also found in teleost; administration of melatonin decreases LHβ and FSHβ secretion in the ell and inhibits reproduction in catfish, trout, etc…while LHβ is induced in kroacker, sea bass and carp. Recently, the antioxidant activity of melatonin has been found in the mammalian oocyte evidencing the beneficial effects of this hormone on reproductive physiology by improving oocyte quality. In zebrafish, we have demonstrated a clear positive role of melatonin in follicle growth and maturation. The large variability of the impact of melatonin on brain, pituitary and on gonadal factors observed among vertebrates will be discussed.

SOA5_5 Coordination of spermathecal muscle activity with oviposition in Locusta migratoria

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The spermatheca, oviducts and oviposition digging muscles are under neural and neuromodulatory control of a variety of neuroactive chemicals, including biogenic amines and neuropeptides. Some of these neuroactive chemicals are released following appropriate neural activity and help coordinate the activity of these reproductive tissues such that eggs are fertilized and deposited at appropriate times. Electrophysiological recordings were conducted to determine the control of spermathecal contractions during oviposition of interrupted egg-laying locusts. Following transection of the central nervous system below the metathoracic ganglion, rhythmic patterned bursting was observed in the nerve, N2B2 that innervates the spermatheca. Subsequent transections at more posterior regions of the ventral nerve cord revealed more robust rhythmic bursting in N2B2. This rhythmic bursting pattern was found to be coordinated with bursting in the ventral opener nerve that innervates the ventral opener muscle. This muscle controls the ventral ovipositor valves. Electromyographic recordings from the spermathecal muscle and ventral opener muscle confirmed rhythmic bursting resulted in an increase in muscle activity with observable muscle contractions. Taken together, the results indicate that there is likely a central pattern generator (CPG) that is regulated by descending inhibition that controls the spermathecal muscle activity. This CPG appears to be localized within the VIIth and VIIIth abdominal ganglia, and was found to integrate with the CPG that regulates oviposition digging in locusts. These results provide further insight into the intricate coordination and control of reproductive tissues underlying reproductive behaviours in locusts.
This study was supported by NSERC.

SOA5_6 Hormonal action of a relaxin-like gonad-stimulating substance (GSS) in starfish, Asterina pectinifera

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Gonad-stimulating substance (GSS) of starfish is the only known invertebrate peptide hormone responsible for final gamete maturation, rendering it functionally analogous to gonadotropins in the vertebrates. GSS stimulates ovary to induce oocyte maturation by producing maturation-inducing hormone, 1-methyladenine (1-MeAde) in the ovarian follicle cells. Recently, we purified GSS of starfish, Asterina pectinifera, from radial nerves and identified the chemical structure as a heterodimer composed of two different peptides (A- and B-chain) with disulfide cross-linkages. According to phylogenetic analyses, starfish GSS belongs to the insulin/insulin-like growth factor (IGF)/relaxin superfamily and, more precisely, to the subclass of a relaxin-like peptide. In this study, we examined de novo synthesis and hormonal actions of GSS. The cDNA of GSS encoded a preprohormone sequence with a C-peptide between the A- and B-chains. High levels of mRNA expression and activity of GSS were detected in the radial nerves and nerve rings. Further, the chemically synthesized GSS could stimulate ovarian follicle cells to produce 1-MeAde through an increase in cyclic AMP. According to competitive experiments using radioiodinated and radioinert GSS, highly specific bindings were observed in the membrane fraction of follicle cells, suggesting that GSS receptors are distributed on the follicle cell membrane. Additionally, alpha subunit of stimulatory type of G-protein was immunologically detected in the membrane fraction. These findings strongly suggest that upon secretion from nervous tissues, GSS interacts with its receptor on the follicle cell surface to activate G-proteins and adenylyl cyclase and induce 1-MeAde production.

SOA6 Endocannabinoids in the rodent circadian system

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Introduction: The cannabinoid receptor type 1 (CB1) and its endogenous ligands, the endocannabinoids (EC) play an important role in the regulation of food intake and energy balance. The coordinated daily rhythms in food intake and metabolism are controlled by an endogenous rhythm generator residing in the suprachiasmatic nucleus of the hypothalamus (SCN). Individual SCN neurons represent autonomous oscillators with a molecular clockwork that is composed of transcriptional/translational feedback loops of clock genes. Rhythmic hormonal and neuronal output from the SCN drives daily rhythms in behaviour and physiology. Interestingly, rhythmic clock gene expression has been shown in many brain regions known to express CB1 receptors and to be involved in maintenance of metabolic homeostasis. However, little is known about the impact of the EC system on the molecular clockwork and, vice versa, the impact of the molecular clockwork on the EC system. Objective: To investigate the interactions between the molecular clockwork and the EC system, we analyzed (1) distribution of CB1 and of key enzymes in EC metabolism (NAPE-PLD and FAAH) in different hypothalamic regions by immunohistochemistry in mice with a disturbed molecular clockwork (PER1-/-) and the corresponding wildtype (WT) and (2) the spontaneous locomotor activity rhythms of CB1-deficient (CB1-/-) mice and the corresponding wildtype. Results: (1) In the WT SCN we found circadian rhythms in CB1-, NAPE-PLD- and FAAH-immunoreactivity (Ir). These rhythms were significantly altered in the SCN of PER1-/- mice. In the median eminence (ME) of both genotypes, there were no circadian rhythms in CB1-, NAPE-PL- or FAAH-Ir. However, NAPE-PLD-Ir was significantly elevated in PER1-/- mice as compared to WT. This suggests that the molecular clockwork in the ME affects NAPE-PLD synthesis. (2) Both, WT and CB1-/- mice showed high locomotor activity during the dark phase and low locomotor activity during the dark phase. However, after a 6 h phase delay of the photoperiod, CB1-/- mice entrained significantly faster to the new phase. Conclusion: Our data suggest that the molecular clockwork affects the EC system and the EC system affects the endogenous rhythm generator.
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SOA8 Neuroendocrine-immune interaction: Differential regulation of phagocyte activity in fish by neuroendocrine factors

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Coping with physical, chemical and biological disturbances depends on an extensive repertoire of physiological, endocrinological and immunological responses. Fish provide intriguing models to study bi-directional interaction between the neuroendocrine and the immune systems.

Macrophages and granulocytes are the main actors in the first and rapid innate immune response. They are resident in different organs and are moreover rapidly recruited and activated upon infection. They act in response to recognition of pathogen associated molecular patterns (PAMPs) via a repertoire of surface and intracellular receptors by inducing a plethora of defense reactions aiming to eradicate the pathogen. Subsequent production of inflammatory mediators stimulates other leukocytes required to develop an adaptive and specific antibody response. The type of phagocyte reaction will therefore depend on their differentiation state, specific receptor repertoire and their specific location. Apart from these pathogen induced responses, immune reactivity may be modulated by neuroendocrine factors. Over the last years we extensively studied changes in carp stress axis activity and the effect of its end-products on the immune system in an acute stress paradigm. We focus on specific neuroendocrine receptors on leukocytes and their effect on crucial phagocyte activities. We performed identification and functional analyses of different glucocorticoid, opioid and adrenergic receptors on carp phagocytes. Results show that their ligands of neuroendocrine origin may have substantial impact on specific macrophage functions in a differential way. Inflammatory and microbicidal responses fight pathogens but can be detrimental to the host tissue. Neuroendocrine modulation may regulate inflammation to reach an optimum defense while preventing excessive host cell damage.

9. Invertebrate Neuropeptides and Peptide Hormones: key players in the regulation of physiological processes and behavior

SOA9_1 Neuropeptidergic signaling systems in nematodes

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Despite the general knowledge and repeated predictions of peptide G protein-coupled receptors following the elucidation of the C. elegans genome in 1998, only a limited number have been deorphanized so far. This was attributed to the apparent lack of co-evolution between (neuro)peptides and their cognate receptors. To resolve this issue, we have used an in silico genomic data mining tool to identify the real putative peptide GPCRs in the C. elegans genome and we then made a well considered selection of orphan peptide GPCRs. To maximize our chances of a successful deorphanization we adopted a combined reverse pharmacology approach. In this way, we have successfully uncovered four C. elegans neuropeptide signaling systems that support the theory of receptor-ligand co-evolution. All four systems are extremely well conserved within nematodes and show a high degree of similarity with their vertebrate and arthropod counterparts. Our data indicate that these four neuropeptide signaling systems have been well conserved during the course of evolution and that they were already well established prior to the divergence of protostomes and deuterostomes.

SOA9_2 Diversity and abundance: the basic properties of neuropeptide action in molluscs

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Neuropeptides, the most diverse signaling molecules, are responsible for regulating a variety of cellular and behavioral processes in all vertebrate and invertebrate animals. Peptide signals play a role in information processing that is fundamentally different from that of conventional neurotransmitters. Neuropeptides may act as neurotransmitters or neuromodulators and released at the synaptic or non-synaptic sites. Others may be neurohormones controlling developmental processes, specific behaviors and be involved in the mechanisms of learning and memory storage. Co-transmission within peptide families, across peptide families, and between peptide and non-peptide signaling molecules, is a common incidence, which ensures the great versatility of their action. How these tasks are fulfilled when multiple neuropeptides are released becomes an important issue of the recent peptide research. Although our knowledge concerning the physiological and behavioral roles most of the neuropeptides isolated from mollusks is incomplete, here we attempt to provide some general principles in answer to the questions delineated above.

SOA9_3 Evolutionarily conserved molecular mechanisms of learning: A novel role for a molluscan homologue of the vertebrate pituitary adenylate cyclase activating polypeptide (PACAP) in the rapid formation of long-term associative memory in Lymnaea

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The last common ancestor of snails and humans lived more than 600 million years ago so it is not surprising that there are obvious differences in body design and behaviour. There is however a remarkable level of conservation between the molecular mechanisms underlying associative learning in snail and man, indicating that these mechanisms emerged early in coelomate evolution. Here I will first review the fundamental molecular mechanisms of memory function and dysfunction that are shared between snails and humans. Prime examples of such evolutionarily conserved mechanisms are the learning-induced activation of the same types of transcription factors (e.g., CREB, C/EBP) and key kinase enzymes (e.g., PKA, CaMKII) in both the molluscan and the human brain; or how some molecules (e.g., amyloid peptides) wreck havoc on learning and memory in both snails and humans. An exciting discovery that we have made recently is that a molluscan homologue of the vertebrate pituitary adenylate cyclase activating polypeptide (PACAP) is expressed in the Lymnaea brain, where it is both necessary and instructive for the rapid formation of long-term associative memory after food-reward classical conditioning. Thus it seems that ancestral PACAP evolved as a mediator of memory formation in invertebrates and later acquired a function in hormonal regulation in vertebrates while also retaining its role in learning and memory.

SOA10 Amphibians as models in comparative developmental biology covering metamorphosis and sexual differentiation

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Biology of amphibians in general is characterized by dramatic changes during larval development shifting from a fully aquatic into semiterrestrial life style. The complex processes associated with these obvious changes affecting morphology and physiology are named metamorphosis and thyroid hormones (TH) are the primary morphogen regulators. Amphibians are the classical models for developmental biology covering metamorphosis as well as sexual differentiation, the latter one being achieved also during larval development. Ontogeny and regulation of the thyroid system is one of the major issues concerning amphibian development. To characterize the endocrine mechanisms regulating thyroid gland activity in Anuran tadpoles gene expression profiles were determined during spontaneous metamorphosis of Xenopus laevis. The results revealed elevated expression of thyroidal markers such as slc5a5, tpo, TSH-R, and sar1a mRNAs at late prometamorphic and climax stages. Thyroidal expression of dio2 and dio3 but not dio1 demonstrated a strong up-regulation at late metamorphic stages. Conversely, expression of the DNA replication markers mcm2 and pcna declined at climax stages. The existence of functional feedback mechanisms of thyroid axis was assessed at premetamorphic stages by treatments using T4 and perchlorate indicating that functional feedback signalling regulating thyroid activity is already present during premetamorphosis. The role of the gonadotropins, luteinising hormone (LH) and follicle stimulating hormone (FSH), for reproductive biology in amphibians is well established but information about the functional development of the hypothalamus-pituitary-gonad (HPG)-axis during larval ontogeny is scarce. Expression profiles of hypophyseal LH and FSH and of their corresponding gonadal receptors, LH-R and FSH-R, during sexual differentiation of Xenopus laevis revealed a significant elevation of LH and FSH expression at late premetamorphic stage. LH mRNA increased during prometamorphic stages while the corresponding LH-R slowly increased during development having highest levels during metamorphic climax. On the contrary, FSH mRNA expression was only slightly raised during ontogeny while the corresponding FSH-R mRNA was considerably increased at prometamorphosis and furthermore during metamorphic climax. These results suggest that LH and LH-R expression might be involved in initial maturation events of gametes, especially in males, while the gradually increase of FSH-R mRNA was correlated with the advancing processes of gamete maturation in both sexes providing evidence that the hypothalamus-pituitary-gonad axis evolves already at early stages of ontogeny and sexual differentiation. Thus the endocrine mechanisms triggering metamorphosis via thyroid system and sexual differentiation via the HPG-axis develop already quite early at premetamorphic stages and demonstrate that amphibians provide a very sensitive model for developmental biology.

Oral communications

1. Polypeptides and their Receptors, including Kisspeptins and GPR54: Co-Evolution, Biosynthesis, and Signal Transduction

O01_01 Kisspeptin and seasonal control of reproduction in European sea bass (Dicentrarchus labrax) and Atlantic cod (Gadus morhua)

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Kisspeptins have been shown to act as key factors in mammalian puberty onset and the regulation of reproductive function. The role of the kisspeptin system appears to be conserved in fish and studies have suggested the involvement of kisspeptins in the regulation of gonadotropin release and onset of puberty. However its role in the neuroendocrine regulation of reproduction in fish still needs to be further confirmed especially in repeat spawners. Recent findings in sea bass reported two kisspeptin forms namely Kiss-1 (sbKiss-1) and Kiss-2 (sbKiss-2). We therefore developed and validated novel molecular assays to measure the expression of kisspeptin related genes in two commercially important marine teleost species. First, in repeat spawning male European sea bass, Dicentrarchus labrax, the expression of kiss1, kiss2 and kissr4 were characterised by QPCR in a population of maturing fish along with GnRH1, FSHβ and LHβ expression and levels of testosterone as well as gonadal development over a full reproductive cycle. Results clearly showed seasonal profiles in expression of all genes (except GnRH1) as well as sex steroids in the blood. However, a lack of correlation between Kiss and GnRH1 expression as well as the mismatch between Kiss and the onset of gonadotropin surge contrast with mammalian data and requires us to rethink the proposed role of Kisspeptins in fish. Then, the expression of kiss2 and kissr4 along with sex steroids were characterised in a population of male and female Atlantic cod, Gadus morhua, first time spawners (exposed to simulated natural photoperiod, SNP) and a population of non-maturing individuals (maturation inhibited by exposure to constant light). However, once again the kisspeptin genes did not appear to be directly involved in the initiation of the reproductive cycle. These results open interesting new avenues to unravel the role of kisspeptins in teleost reproductive physiology.

O01_02 Cloning and characterization of GPR54 in the testis of the anuran amphibian, Rana esculenta, during the annual reproductive cycle

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Kisspeptins belong to the RF-amide family of peptides; a unique precursor is post-translationally cleaved to produce a 54-amino acid protein, also known as kisspeptin-54 or KISS-1 or metastin, a cancer metastasis suppressor. Three shorter peptides, kisspeptin-10, -13 and -14, are suggested degradation products of kisspeptin-54. KISS-1 binds and activates GPR54, a G protein-coupled receptor.
KISS-1/GPR54 system also represents a novel key player in the regulation of reproduction. Mutations in kiss-1/GPR54 genes cause hypogonadotropic hypogonadism, with delayed sexual maturation, low levels of gonadal sex steroids, lack of spermatogenesis and ovulation.
This system is widely expressed in the brain, pituitary, and in several peripheral tissues, gonads included. The primary site of action of the system is the gonadotropic axis. In fact, it stimulates luteinizing hormone (LH) secretion in many species, through a direct activity upon gonadotropin-releasing hormone (GnRH)-secreting neurons. Furthermore, KISS-1/GPR54 system is evolutionary conserved from fish to mammals, humans included. Therefore, to investigate the direct involvement of kisspeptin system in the spermatogenetic process, we have cloned a fragment of GPR54 cDNA (503bp) from the testis of the anuran amphibian, Rana esculenta. GPR54 expression levels have also been analysed in frog tissues (brain, spinal cord, testis, ovary, muscle, kidney) and in the testis during the annual reproductive cycle. Finally, GPR54 mRNA has been localized in the testis by in situ hybridization.
These data suggest that kisspeptins/GPR54 might act as intratesticular autocrine/paracrine signal in reproductive functions.

O01_03 The oxytocin/vasopressin receptor family has at least five members in gnathostomes

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The vertebrate oxytocin and vasopressin receptors mediate a variety of functions, including the regulation of water balance, reproduction and blood pressure. In mammals four members of this family of GPCRs have been identified and studied, three that respond to vasopressin (VP) named V1A, V1B and V2, and one that is activated by oxytocin (OT), called the OT receptor. The corresponding receptors have been identified in chicken and a few amphibians but received different names. Until recently only V1-type receptors have been described in several species of teleost fishes. In order to deduce the evolutionary relationships within the family we have identified family members in a wide selection of vertebrate genomes and combined thorough phylogenetic analyses with data on chromosomal locations relative to neighboring gene families. We can report the existence of five distinct ancestral gnathostome receptor subtypes in the OT/VP receptor family: V1A, V1B, V2A, V2B and OT receptors. The identification of distinct V2A and V2B receptors has not been previously recognized. We have found these two subtypes in all examined teleost genomes and conclude that the V2A-type is orthologous to mammalian V2 receptors whereas the V2B-type is orthologous to avian V2 receptors. Thus this analysis reveals unprecedented complexity in the gnathostome repertoire of OT/VP receptors. Teleosts in particular show greater diversity than previously thought with up to eight receptor family members. This provides a means of classifying OT/VPfamily receptors and opens potentially rewarding research avenues into the endocrine functions of fishes, in particular the regulation of water balance.

O01_04 Understanding the Ligand Selectivity Features of the Melanocortin 2 Receptor

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The melanocortin receptors (MCRs) are a family GPCRs that serve as the cognate receptors for the melanocortin-related peptides (ACTH, α-MSH, β-MSH, γ-MSH, δ-MSH). The unifying feature of the melanocortin-related peptides is the presence of the core amino acid motif, HFRW. This motif is absolutely required for activation of the MCRs. Given this background, it is surprising that ACTH can activate all five of the mammalian MCRs, but α-MSH can only activate MC1R, MC3R, MC4R, and MC5R, but does not bind to nor does this ligand activate MC2R. Since the HFRW motif is present at positions 6 through 9 of both mammalian α-MSH and ACTH, residues at positions 14 to 24 in the sequence of ACTH may be the source of the unique ligand selectivity of the MC2 receptor. In this study the human MC2R gene was transfected into CHO cells and the ability of analogs of human ACTH (1-24) [SYSMEHFRWGKPVGKKRRPVKVYP] to induce a cAMP response in these cells was ascertained. An analog in which residues 14 to 19 were replaced with alanine residues had no biological activity. Furthermore, this analog could not inhibit the activation of MC2R by ACTH(1-24). However, N-terminally truncated analogs of ACTH(1-24) could block the activation of MC2R to varying degrees of efficacy, yet were incapable of activating the receptor when incubated with the CHO cells along. These data suggest that the activation of mammalian MC2R may involve two binding sites on the receptor.
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O01_05 Conserved antiapoptotic effects of pituitary adenylate cyclase activating polypeptide -from mollusks to humans

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide with highly conserved structure, showing only a few amino acid differences in vertebrate species and being identical in all mammalian species. This conserved structure implies that PACAP plays a role in basic biological processes. One such potential conserved function is the anti-apoptotic effect of the peptide. Numerous studies have shown that PACAP is a strong anti-apoptotic peptide in various neuronal and many other cell lines. We have also demonstrated this action in cells derived from different species. In this presentation we show some examples for the anti-apoptotic effects of PACAP in an invertebrate molluscan species (Helix pomatia), chickens and humans. We have demonstrated that PACAP decreases dopamine-induced apoptosis in salivary gland cells of the snail Helix pomatia, which demonstrates, for the first time, that PACAP has anti-apoptotic effects in an invertebrate species. Other examples are also presented: PACAP showed survival-promoting effect against oxidative stress in chicken inner ear hair cells and pinealocytes. In mammals, similar survival enhancing actions of PACAP were observed: it prevents rat cardiomyocytes, renal cells and mouse endothelial cells against H2O2-induced cell death. Furthermore, it has the same protective effect in human kidney cells. In summary, the anti-apoptotic effect of PACAP could be observed from invertebrates to humans.

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O01_06 The role of octopamine in insects and the cloning, phylogenetic relationship and distribution pattern of three new putative octopamine GPCR's in the desert locust

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The biogenic amine octopamine functions as a neuromodulator, neurotransmitter and neurohormone in insect nervous systems. It plays a prominent role in modulating multiple physiological and behavioural processes in insects. Octopamine exerts its effects by binding to specific proteins that belong to the superfamily of GPCR's. We picked up three partial sequences of putative octopamine receptors in S. gregaria (SgOctαR1, SgOctαR2 and SgOctβR) using degenerated primers and investigated their transcript levels in males and females of both phases and during the transition between long-term solitarious and gregarious locusts. The transcript levels of the SgOctαR's are the highest in the CNS, whereas those of SgOctβR are the highest in the flight muscles, followed by the CNS. Both SgOctαR1 and SgOctβR show higher transcript levels in long-term gregarious locusts compared to solitarious ones. The rise of SgOctβR transcript levels already appears during the first four hours of gregarisation, in which also the behavioural changes take place.

O01_07 Structural neurohypophysial hormone-receptor coevolution through the animal kingdom

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Most physiological functions can now be described as spatiotemporal networks of cascades of protein machineries in which specific protein-protein recognition depends upon respective conformations. Progress in the determination of protein conformations through X-ray crystallography and computational homology modelling allow to trace hormone-receptor coevolutionary lineages. Up to date 13 neuropeptides from some 80 vertebrate species and 6 invertebrate homologous peptides have chemically been characterized evoking the existence of an ancestral gene antedating the animal divergence, 700 million years ago. All these peptides possess nine residues, a disulfide bridge in position 1-6, two hydrophobic residues in positions 2, 3, two polar residues in position 4, 5, a Pro in position 7 and a C-terminal glycinamide, suggesting similar foldings and conformations. They derive through specific processing from homologous precursors (110-150 residue long) in which there are linked to a 7-disulfide bridge-containing "neurophysin" domain. All encoding genes have 3 exons displaying different evolutionary rates. In vertebrates, vasotocin is the single peptide found in the most primitive Cyclostomes, but in all higher classes two similar peptides exist, suggesting a duplication of the vasotocin gene before the rise of bony fishes. On the basis of structure and activity, two paralog evolutionary lineages have been traced, a basic vasopressin line: vasotocin (non-mammals)-Arg/Lys vasopressin (mammals) and a neutral oxytocin line: isotocin (bony fishes), mesotocin (non-mammalian land vertebrates) oxytocin (placental mammals). Evolution operates essentially by punctual substitutions in positions 3, 4 and 8 through neutral or selective mechanisms. Neurophysin domains, encoded by the last 2 exons, show particular subdomain evolutionary rates.
Three types of vasopressin receptors V1a, V1b, V2 and one type of oxytocin receptor have been identified in mammals. They trigger particular signal transducing cascades through heterotrimeric G proteins (Gq or Gs) and control distinct physiological functions, namely vascular tone, corticotropin secretagogue, antidiuresis and milk ejection, respectively. Corresponding evolutionary lineages exist across vertebrates. All belong to the superfamily of 7-transmembrane G protein-coupled receptors. Crystal structures of two prototypes, rhodopsin (Palczewski, 2000) and β2-adrenoreceptor (Kobilka, 2007) are known, allowing computational homology modeling for vasopressin receptors. Speculative localization of ligand- and G protein-binding sites suggest an allosteric functioning of the signaling machinery due to conformational flexibility.
From a unique ancestral vasotocin receptor AVT-R in Cyclostomes, three successive gene duplications led to the four vasopressin Vla, V1b, V2 and oxytocin receptors in mammals. Co-evolution of hormones and receptors has required multiple structural adaptations in each other through genetic mechanisms.

2. Neuroendocrinology of Insects: advances through genomics and proteomics

O02_01 Identification of the elusive peptidergic diuretic hormone in the kissing bug, Rhodnius prolixus: a CRF-related peptide

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Probing a host and ingestion of a blood meal in the kissing bug Rhodnius prolixus, results in a cascade of tightly integrated physiological and endocrinological events. The massive blood meal is pumped into the anterior mid-gut where excess water and salts are absorbed into the haemolymph and then eliminated by diuresis via the Malpighian tubules and hindgut.
Serotonin is a diuretic hormone in R. prolixus, but there is ample evidence for the presence of a peptidergic diuretic hormone. The identity of this peptide has remained elusive, despite a number of attempts to isolate and characterize it. Here, we have employed molecular techniques and mass spectrometry to sequence the peptidergic diuretic hormone, and find it is a member of the CRF-related family of insect peptides. We have determined the distribution of this Rhopr DH using in situ hybridization and immunohistochemistry and confirm its presence in neurosecretory cells in the central nervous system. Rhopr DH has potent biological activity on anterior midgut and Malpighian tubules.

O02_02 The insect Malpighian tubule: it does more than we expected, so its endocrine control is more sophisticated than we thought

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Classically, textbooks describe the insect renal (Malpighian) tubule in terms of fluid secretion, and its control in terms of diuretic (and more recently antidiuretic) peptides. Recently, we have used post-genomic resources combined with experimentation to extend our view of the functional repertoire of the tubule. As well as fluid secretion and ionic homeostasis, the tubule is a major immune tissue, and also a major site for detoxification and excretion of toxins, such as insecticides. It also plays a role in response to several stress modalities. These roles are distributed through several genetically-distinct regions and cell types. The neuroendocrine control of these processes thus has the potential for sophistication and subtlety beyond the familiar fluid secretion modality.
Here, we draw on recent data from our group that suggests quite unexpected roles for known neuropeptides in communicating messages from other tissues to the tubule, allowing it to integrate a range of inputs to provide the optimum response for organismal health. Interestingly, tubule function, and potentially its control, differs between the left (posterior) and right (anterior) tubules, and between male and female tubules.

O02_03 What does the information from recent insect genome projects tell us about AKH precursors and the mature peptides?

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A neuropeptide, generically called the adipokinetic hormone (AKH), is produced in retrocerebral glands of insects and is one of the most abundant peptide hormones in these organs. In the past, primary sequence information of these peptide hormones has been useful to corroborate or suggest phylogenetic relationships, but this has limitations since AKHs are mostly octa- or decapeptides. Using information from a longer sequence, such as from the AKH precursor, could be more conclusive for comparative purposes. To date, a number of insect genomes have been sequenced and the information is publicly available. From these genomic databases, one could deduce the sequence information of AKH precursors, as well as the encoded mature AKH peptide. The precursor sequence information could be used in a phylogenetic context, while there are many methods for determining unequivocally whether the encoded AKH is, indeed, expressed and whether post-translationally modified forms are present within the organism. This paper is aimed as a short report on information that can be obtained on AKHs from the various insect genome projects, and will present examples of different strategies in which this information can be deployed.
Supported financially by the National Research Foundation and UCT Block Grants.

O02_04 Final steps in JH biosynthesis in the corpora allata of the desert locust, Schistocerca gregaria

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Post-embryonic development in insects is highly dependent on two hormones: the steroid molting hormone 20-hydroxyecdysone and the sesquiterpenoid, juvenile hormone (JH). While regular peaks in the ecdysteroid titer trigger each molt, JH determines the nature of the developmental transition. Its presence causes the insect to undergo a larval-larval molt. When the JH titer drops at the end of the last larval stage, its absence causes the insect to molt into its final adult form.. Next to its role in insect development and metamorphosis, JH also influences other major processes during insect life such as aging, diapause, caste differentiation, phenotypic plasticity and reproduction. In insects JH is produced in the corpora allata (CA), a pair of small endocrine glands near the brain. Its biosynthetic pathway can be divided into two phases. The first phase comprises the well-conserved mevalonate pathway. Using endogenous acetyl-CoA, farnesyl-pyrophosphate (FPP) is formed. The second phase is arthropod-specific and involves the conversion of FPP to the functional hormone. The last steps in this arthropod-specific phase involve the epoxidation and esterification of farnesoic acid. The order, in which these reactions occur, appears to be species-dependent. We will focus on these last enzymatic steps in the biosynthesis of JH in an orthopteran insect, the desert locust, Schistocerca gregaria.

O02_05 Microarray analysis of the physiology of reproductive and non-reproductive honeybee workers

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The success of a honeybee colony depends largely on the reproductive altruism of the worker bees, which usually remain sterile, and even help their mother queen to reproduce. At present, little is known about the genomic basis of this spectacular form of altruism. In this study, we compared gene expression patterns among 16 laying and 16 non-laying 18-day old individual honeybee workers using a third generation microarray of whole-body RNA extracts. Honeybee workers were paint-marked between 0 and 24 hours after hatching and reintroduced into their original colonies from which the queens were removed. The two colonies were placed in a natural environment during the entire experiment. At day 18, marked bees were randomly taken and their ovary development was checked. Eight laying and non-laying worker bees from each colony were analysed. Our results demonstrate that there were massive differences in the gene expression between these two sets of workers, with a total of 1284 genes being differentially expressed (BH corrected p-value<0.05), of which 871 being up-regulated in fertile workers and 413 being up-regulated in sterile workers. GO enrichment analysis demonstrated that ca. one quarter of all the GO-terms enriched in egg-laying workers were linked to oogenesis, mitosis or meiosis, whereas the GO-terms enriched in non-laying workers were associated with wing muscle contraction, metabolism and flight behavior. Interestingly, these results therefore suggest that non-laying workers were foraging, whereas laying workers of the same age were not, in accord with theoretical predictions that reproductive workers should tend to carry out less work and less risky tasks inside the colony. In addition, we also discovered several genes of interest, such as Apis mellifera odorant receptor 156 which was nearly 2-fold up-regulated in sterile workers and farnesyl pyrophosphate synthase (involved in juvenile hormone synthesis), odorant binding proteins 9 and 7 and chemosensory protein 5, which were up-regulated in reproductive workers. Several of these genes of interest are situated within the region of previously identified QTLs linked to differences in worker reproductive capacity in honeybees. Overall, our results provide unprecedented insight into the detailed physiology of non-reproductive honeybee workers.

O02_06 The ecdysone receptor in the hemimetabolous pea aphid: cloning, protein structure modeling and functional analysis by RNAi

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Ecdysteroids are the key insect hormone in insect growth and development, and they manifest their activity via interaction with the nuclear receptor complex of ecdysone receptor (EcR) and the Retinoid X Receptor (RXR). These two nuclear receptors form the start of the ecdysteroid cascade. In this project we report on the full length coding sequence of EcR and RXR in the pea aphid Acyrthosiphon pisum. The converted amino acid sequences were compared with those of other insects and we examined the phylogenetic relationships of EcR and RXR genes in hemimetabolous and also holometabolous and ametabolous insects belonging to the orders of Hemiptera, Orthoptera, Lepidoptera, Diptera, Hymenoptera, Coleoptera, Collembola, and also Crustaceans within the phylum of Arthropoda. In addition we used the EcR sequence to make an initial in silico 3D model of the ligand-binding pocket docked with ecdysteroid hormone. This model exhibited the typical canonical structural scaffold with 12 α-helices associated with a short hairpin of two antiparallel β-strands. Upon docking, 20E was located in the hormone-binding groove, supporting the hypothesis that EcR has a role in 20E signaling. In a last step we investigated the phenotypes that can result from silencing of EcR in RNAi experiments. These data support the advent of Acyrthosiphon1 as a model allowing a better understanding of insect growth and development.

1 The International Aphid Genomics Consortium. 2010. Genome sequence of the pea aphid Acyrthosiphon pisum. PLoS Biology 8: e1000313.

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3. Regulation of Food Intake in Invertebrates and Vertebrates

O03_01 Food intake, digestive enzyme activity and contraction of the gut is regulated by sulfakinins from the cricket Gryllus bimaculatus

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In vertebrates, a variety of mechanisms are involved in the regulation of food intake and energy balance, and recent developments in insect research indicate that the endocrine regulation of these processes in invertebrates is at least as complex.
Sulfakinins (SK) are neuropeptides structurally displaying sequence homology with the hormonally active portion of the vertebrate gastrin/CCK peptide family. In Gryllus bimaculatus two sulfakinins (I, II) are encoded on the gene and the peptides could be identified in brain extracts from adult females by MALDI-TOF analysis. In Gryllus bimaculatus after in vivo degradation of the specific sulfakinin mRNAs by the RNA interference method, the expression is reduced and this leads to a drastic increase in the food-intake indicating the inhibitory bioactivity of the peptides. Synthetic SK I peptide has a stimulatory effect on the release of α-amylase activity in the ventriculus, whereas SK II stimulates the release of this enzyme in the caecum of unfed crickets. Additionally, the sulfakinins stimulate the release of proteases into the caecum. SK I stimulates trypsin release and in a similar manner as gastrin stimulates biosynthesis in vertebrates. SK II increases most specifically the released aminopeptidase activity. Finally, SK I stimulates the myoactivity of the crop, wheras SK II has no effect.
This work was supported by the DFG Me 2034/1-3

O03_02 The central role of melatonin in Danio rerio appetite regulation

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Melatonin is the hormonal mediator of photoperiodic information to the central nervous system in vertebrates and allows the regulation of energy homeostasis through the establishment of a proper balance between energy intake and energy expenditure. The aim of this study was to evaluate the role of melatonin in the regulation of feeding behavior in the zebrafish Danio rerio. For this purpose, the effect of two different melatonin doses (100 nM and 1ìM) administered for ten days via water to zebrafish adults was evaluated at both physiological and molecular level and the effect of melatonin was considered in relation to the most prominent systems involved in appetite regulation. The melatonin control of food intake by the modulation of leptin, MC4R, ghrelin, NPY and CB1 gene expression was evaluated.
The results obtained indicate that melatonin significantly reduces food intake and the reduction is in agreement with the changes observed at the molecular level. A significant increase in genes codifying for molecules involved in feeding inhibition, such as leptin and MC4R, and a significant reduction in the major orexigenic signals including ghrelin, NPY and CB1 was demonstrated.
These results support the idea that melatonin falls fully into the complex network of signals that regulate food intake and therefore this hormone plays a key role in central appetite regulation.

O03_03 Structure determination of O-linked carbohydrate moiety of HemaP-like peptide, a novel feeding-modulating peptide from the sweet potato hornworm, Agrius convolvuli

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We have recently identified a novel feeding-modulating peptide, HemaP (hemolymph major anionic peptide) from the silkworm, Bombyx mori (in preparation). HemaP consists of 62 amino acid residues, and is predominantly produced by the fat body. Injection of HemaP into Bombyx larvae induces foraging behaviors such as nibbling and ingesting. Datebase search indicated that HemaP is an unknown peptidyl factor and is conserved among lepidopteran insects.
To characterize the HemaP-like peptide from other lepidopteran insects, we tried to purify it from larval hemolymph of Agrius convolvuli, taxonomically closely related to the tobacco hornworm, Manduca sexta. Finally, we isolated two distinct HemaP-like peptides and designated those two peptides p6692 and p6894 according to their molecular masses. N-terminal amino acid sequence and ESI MS/MS analyses of p6692 and p6894 revealed that they had the same amino acid sequence but were different in mass by 202, corresponding to that of N-acetylhexosamine and that the N-acetylhexosamine was linked to Thr5. To determine the structure of the carbohydrate moiety, p6894 was hydrolysed in TFA, followed by labeling with 2-aminobenzoic acid (2AA). The resulting 2AA derivative was identified as 2AA-GalNAc by the elution time on reversed-phase HPLC. Since p6894 was recognized by GSLI, a lectin, specific for a-Gal and a-GalNAc, we concluded that p6894 linked a-GalNAc. Although Agrius hemolymph had two isoforms of HemaP-like peptides with or without a carbohydrate moiety, which was not the case in Bombyx HemaP, they both showed almost comparable activity of inducing foraging behaviors in Agrius larvae.

O03_04 Effects of peptide hormones on feeding initiation and termination in the larvae of the silkworm, Bombyx mori

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Phytophagous insects do not always feed, even though they reside on their host plants. In the larvae of the silkworm, Bombyx mori, the regularly occurring feeding pattern is observed. It has been proposed that such feeding cycles were modulated by the sequential effects by positively and negatively regulating factors including peptidyl factors.
To elucidate the mechanisms of regulation of feeding behavioral cycle, especially that of feeding motivation, we established a bioassay for the activity of feeding initiation by observing the first bite after sample injection in starved Bombyx larvae. The biological activity was evaluated by measuring the duration until the first bite. To test the several biologically active peptides on feeding behavior, we synthesized chemically those peptides including neuropeptide hormones, which were then subjected to the feeding motivation bioassay. As a result, injection of synthetic allatostatin and myosupressin into starved Bombyx larvae delayed the first bite significantly compared with the vehicle-injected larvae, indicating that these peptides are candidates for feeding terminating factors. By contrast, injection of short neuropeptide F (sNPF) into starved larvae resulted in the significantly shortened duration to the first bite, indicating that sNPF might be a candidate for a feeding initiating factor. These results suggest that a number of peptidyl factors are involved in the regulation of insect feeding behaviors. To understand the scenarios of the regulatory order by peptidyl factors, further studies on quantification of those peptides during the feeding cycle are required.

O03_05 Serine protease inhibitors and their role in regulating the activity of digestive enzymes in insects

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Serine proteases (SP) can be found in almost every multicellular organism as they play crucial roles in regulating a multitude of physiological processes like inflammation, development, immune response, food digestion and many more. The activity of these proteases has to be controlled very tightly since they are capable of inflicting severe damage to cell and tissue. One of the most important ways the activity of these SP is regulated is by using protease inhibitors (PI), like those of the Kazal-type family. These inhibitors share a characteristic cystein residue pattern which forms three disulfide bridges giving rise to a conserved 3D-conformation stabilizing the binding loop. The P1-residue located on this binding loop determines the specificity of an inhibitor. So far, over 89 Kazal-type inhibitors have been identified in vertebrates, invertebrates, plants and bacteria. An important member of this family is 'pancreatic secretory trypsin inhibitor' (or PSTI) which can be found in many vertebrates. PSTI is expressed in the acinar cells of the pancreas and in epithelial cells throughout the gastrointestinal tract. It is packaged in the pancreatic granules, and is secreted with trypsinogen and the inactive precursors of the other digestive enzymes in the pancreatic juice. As for its function, it is thought to prevent the premature activation of the proenzymes in the pancreas itself. Since trypsin is the first step in the activation cascade of all the other digestive enzymes, inhibiting prematurely activated trypsinogen should stop the other zymogens becoming active. This hypothesis is backed by numerous publications establishing a link between mutations in the gene coding for PSTI and diseases such as pancreatitis and pancreatic cancer which have a pathology that resembles autophagy or 'self-digestion'. Insects, lacking a pancreas, produce digestive enzymes in the columnar cells of the midgut and caeca. As in vertebrates, enzymes are produced as precursors that need to be activated in a cascade-like manner at the moment they are needed. So, also in these organisms there is a need for a regulatory mechanism to avoid excessive damage from prematurely activated digestive enzymes. We discovered a possible homologue of PSTI (LmPSTI) in an EST-database of the locust Locusta migratoria and performed a biochemical characterization of this inhibitor. After cloning and localizing LmPSTI, it was produced in a bacterial expression system and tested in vitro for its specificity. Moreover, the effect of a dsRNA-mediated knockdown of LmPSTI was studied.

O03_06 Energy homeostasis regulatory peptides in hibernating grizzly bears

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O0Many mammals undergo an unique state of energy conservation in response to harsh climatic conditions. Grizzly bears (Ursus arctos horribilis) do not eat, drink, urinate, or defecate and demonstrate minimal activity during their annual 4-6 months hibernation period. The aim of our study was to determine the potential role of ghrelin, leptin, obestatin, and neuropeptide-Y (NPY) in the regulation of energy homeostasis during hibernation in grizzly bears. Blood samples were collected during the active summer, early-hibernation (first 2 months of hibernation, n = 10 for each period) and the late-hibernation periods (4 months after the beginning of hibernation, n = 4). Radioimmunoassays were used to measure plasma hormone levels. We found that plasma ghrelin concentrations were significantly lower during hibernation than during the summer. Obestatin and NPY levels did not change between pre-hibernation and hibernation. Plasma leptin tended to decrease during late-hibernation relative to active and early-hibernation periods. Ghrelin is involved in the short-and long-term regulation of energy balance by stimulating food intake, reducing lipolysis, and suppressing energy expenditure. High ghrelin levels may help enhancing the accumulation of fat during pre-hibernation, while the lower ghrelin concentrations during hibernation period may increase lipolysis and give the animals a satiety signal. Same pattern of ghrelin levels were found in the racoon dog which capable of sleeping through the winter. The falling leptin levels at the end of the hibernation period could be important in energy preservation. Our results provide evidence that ghrelin and leptin could play a role in the regulation of energy homeostasis during hibernation.

4. Insulin-like growth factor (IGF) Signalling and Ageing

O04_01 Evolution of the insulin-like growth factor binding protein (IGFBP) family in vertebrates

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The IGFBPs are made from six distinct genes in placental mammals and several more in teleost fishes. Due to uneven rates of evolution in the different parts of the IGFBP proteins, it has been difficult to resolve their evolutionary history. Opposing views have favoured either block (chromosome) or serial duplications. We have combined sequence-based phylogenies with data on chromosomal locations in a large number of species and arrive at the following duplication scenario which is supported by the phylogenies of four adjacent gene families: An ancestral vertebrate IGFBP gene underwent a local gene duplication resulting in a gene pair. Subsequently, the two basal vertebrate tetraploidizations quadrupled this pair, after which one gene was lost from two of the pairs. This resulted in the six genes found in placental mammals. Teleost fishes have undergone a third tetraploidization that doubled the IGFBP repertoire to twelve members whereupon differential losses occurred in different lineages. The five sequenced teleost genomes retain 9-11 of the IGFBP genes. The aminoterminal domain is involved in IGF binding and has 12 perfectly conserved cysteines except in mammalian IGFBP-6, which has ten. Teleost fish IGFBP-6 has lost four additional cysteines, leaving only six, implying important structural differences. Interestingly, IGFBP-4 seems to have been lost independently in opossum and birds. The duplicates of IGFBP-1, 2 and 5 have been retained in all five sequenced teleost genomes suggesting important roles for these duplicates. The great ages for the IGFBP genes strongly suggest that they evolved distinct functions in early vertebrate evolution.

O04_02 Hormonal regulation of developmental autophagy and ageing by IGF-1 pathway in insects

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Our work has focused on the hormonal and genetic regulation of autophagy in insects. Insect metamorphosis represents an excellent model system to study the molecular mechanisms involved in elimination of larval cell types, tissues, and even organs by autophagy.
Detailed descriptions of morphological events in larval organs during insect metamorphosis have proved that autophagy is an integral part of postembryonic development. Formation of autophagosomes and autolysosomes in larval fat body cells is temporally and spatially well controlled. This phenomenon has also been observed in all other larval tissues and cell types (e.g. salivary glands, midgut, prothoracic gland, Malpighian tubule, etc.).
The developmental autophagy is under a tight hormonal control in insects. Molting hormone (20-hydroxyecdysone) stimulates while juvenile hormone inhibits it both in vivo and in vitro. 20-hydroxyecdysone-induced autophagy can be inhibited at transcriptional and translational levels. The molting hormone also has a strong influence on the regulation of lysosomal enzyme synthesis and activity. Therefore, developmental autophagy has been considered as one of the mechanisms of programmed cell death, which is enhanced in cells undergoing remodelling in the course of postembryonic development.
The molecular mechanism of hormone-induced autophagy has been also investigated. It was proved by our genetic interaction studies that ecdysone signaling downregulates the PI3K/Akt-TSC1/TSC2-Rheb signaling pathway in vivo, and inhibits by this way the activity of the TOR kinase which is the central regulator of the autophagy. Inhibition of TOR kinase strongly stimulates the autophagic machinery in the larval cell types of insets.

O04_03 The Role of AMPK in the regulation of autophagy and lifespan

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The insulin/insulin-like growth factor (IGF)-1 signaling (IIS) pathway has an evolutionary conserved role in the determination of organismal lifespan. A key enzyme to mediate the longevity effect of reduced IGF-1 signaling (and also of calorie restriction) is the energy sensor AMP-activated protein kinase (AMPK). In order to maintain the cellular energy-balance AMPK switches off energy-consuming anabolic processes while it activates energy-catabolic ones. One of the mechanisms being activated is autophagy, the cellular process of self-producing digestion. The decrease of autophagic activity is observed in almost all cells and tissues as organisms age. Growing evidence supports that the IIS pathway – due to inhibition of AMPK and activation of Tor kinase – inhibits autophagy. Moreover, increased lifespan and vitality evoked by reduced insulin/IGF-1 signaling is the consequence of its positive effect on autophagy.
P-element-induced loss-of-function Drosophila AMPKγ mutants show defects in the normal autophagy process during the late larval stage. We investigated the possible role of AMPK in regulation of autophagy in Drosophila larval fat body and in the regulation of adult lifespan.

O04_04 The role of ubiquitin-proteasome system in ageing

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Two major pathways accomplish regulated protein catabolism in eukaryotic cells: the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal system. The UPS serves as the primary route for degradation for thousands of short-lived proteins and many regulatory proteins and contributes to the degradation of defective proteins. Autophagy, by contrast, is primarily responsible for degrading long-lived proteins and maintaining amino acid pools in chronic starvation.
The UPS and autophagy were long viewed as independent, parallel degradation systems with no point of intersection. By now we know that these degradation pathways share certain substrates and regulatory molecules, and show coordinated and compensatory function. We found that not only the ubiquitinylated proteins can be directed into either degradation systems but proteasome itself has an enzymatic regulatory role in autophagy. Insulin/IGF-1 signalling regulates aging in worms, flies and mammals. The insulin/IGF-1 receptor, through activating a cascade of conserved kinases, inhibits the forkhead transcription factor FoxO. Longevity increases when FoxO becomes activated in response to reduced insulin/IGF-1 signalling. These findings indicate that IGF-I or insulin can reduce protein degradation rapidly by suppressing autophagy via mTOR activation and independently Akt suppressing FOXO transcription, which also inhibits proteasomal degradation through the reduction of atrogin-1 and MuRF1 transcription. We discuss the involvement of the ubiquitin-proteasome pathway of protein degradation at different levels of cellular life in relation with ageing. It appears that altered ubiquitin-proteasome system might be one of the molecular mechanisms of insulin resistance in many pathological situations. Drugs that modulate the SOCS action and/or proteasomal degradation of proteins could become novel agents for the treatment of insulin resistance and Type 2 diabetes. The ubiquitin-proteasome system plays a major role in signal transduction associated with stress and ageing. The understanding of specific proteolytic targeting by E3 ubiquitin-ligases paves the way for a new generation of active molecules that may control particular steps of normal and pathological ageing.

O04_05 Regulation of lifespan through the Insulin/PI3K/TOR/autophagy pathway

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Caloric restriction is a well-known practice to extend lifespan in a variety of model organisms. Reduced nutrient intake decreases growth signaling such as the Insulin/PI3K and TOR (Target Of Rapamycin) pathways. In accordance with that, genetic suppression of these routes results in longevity in worms and flies. Recent findings showed that all these effects are dependent on autophagy, the lysosome-mediated degradation of the cytoplasm. I will present our results on the effect of autophagy manipulation on longevity in Drosophila.

O05_01 Insulin-related peptide and neuroparsins in locust reproductive physiology

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The desert locust (Schistocerca gregaria) displays a fascinating type of phenotypic plasticity. In response to changes in population density the genome of these locusts can be translated in either the solitarious or gregarious phenotype. Locusts in the gregarious phenotype (or 'phase') aggregate in large swarms and migrate over large distances. In this phase the animals are devastating pest insects. An interesting target for pest control strategies is the reproductive process and this fact stimulates fundamental research in locust reproduction. In this context, our research group previously characterized an insulin-related peptide (Scg-IRP) in the desert locust. 'Quantitative real time reverse transcriptase PCR' assays were indicative for a role of Scg-IRP in female locust reproductive physiology. In addition, a neuroparsin was originally identified as an anti-gonadotropic factor in the migratory locust (Locusta migratoria) and was later suggested to be an insulin-binding factor. Subsequently, four neuroparsins (Scg-NP1-4) had also been identified in the desert locust. By means of RNA interference experiments the transcript levels for Scg-IRP and the four Scg-NPs were knocked-down in two separate experiments. The experimental design allowed for a knock-down during the period of sexual maturation and vitellogenesis. Knock-down of Scg-IRP resulted in lowered levels of vitellogenin transcripts and, accordingly, in smaller oocytes in the double-stranded (ds) RNA-treated animals. Oppositely, lowered levels of Scg-NP transcripts resulted in increased levels of vitellogenin transcripts and larger oocytes in the dsRNA-treated animals. The obtained results are indicative that Scg-IRP and Scg-NPs are regulators of the female reproductive event of vitellogenesis, although it is not yet clear whether they directly or indirectly act upon the fat body to regulate this process. Since the fat body has a well-known function as a storage organ for nutritional energy, immediate information about the animal's nutritional status is available from this organ. In addition, insulin is a conserved sensor of nutritional status. A rise in Scg-IRP transcript levels in the fat body had previously been observed in females during a period characterized by extensive growth of oocytes. Therefore we suggest that Scg-IRP regulates the nutrient-dependent process of vitellogenesis by 'sensing' the nutritional status of the animal and perhaps acting as a paracrine substance on the fat body to stimulate vitellogenesis. It should then not be excluded that the Scg-NPs exert their negative influence on female reproduction by interacting with Scg-IRP.

O05_02 Insulin-like growth factor-3 (IGF-3) in male and female gonads of the tilapia: development and regulation by growth hormone (GH) and 17α-ethinylestradiol (EE2)

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Recently, in addition to IGF-1 and IGF-2 the existence of a third form of IGF, termed IGF-3, limited to fishes, to be present only in the gonads and encoded by a separate gene has been reported. However, no further data have been presented on IGF-3. The present study on tilapia (Oreochromis niloticus) uses quantitative real-time PCR specific for tilapia IGF-1 and IGF-3. The organ distribution of IGF-3 mRNA in adult fish and the early ontogeny of IGF-3 in male and female gonads were studied. The potential sensitivity of IGF-3 to GH was revealed by intraperitoneal injections of bream GH using IGF-1 as control gene. The effects of 17α-ethinylestradiol (EE2) exerted after feeding of high EE2 doses and exposure to low environmentally relevant EE2 doses on IGF-3 expression in testis and ovary during early development were determined. Low IGF-3 mRNA expression levels were detected in most organs studied, with the highest extra-gonadal amount in the pituitary. During development, the IGF-3 gene was significantly upregulated in male but downregulated in female gonad. Injections of GH elevated IGF-1 mRNA in male and female liver and ovary. IGF-3 did not respond to GH treatment neither in ovary nor in testis. Both EE2 treatments resulted in significant downregulations of IGF-3 mRNA in testis while ovarian IGF-3 mRNA did not respond. Thus, IGF-3 may be involved in reproduction of fishes most likely in the male gonad only. Whether IGF-3 also has some physiological significance in ovary or other organs should be the topic of further studies.
Work was supported by the Swiss National Science Foundation (projects no. 118165; NRP 50, project 4050-66580).

O05_03 Cannabinoid receptor 1 influences chromatin remodeling in mouse spermatids by affecting content of transition protein 2 mRNA and histone displacement

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Marijuana smokers and animals treated with Δ^9-tetrahydrocannabinol, THC, the principal component of marijuana, show alterations of sperm morphology suggesting a role for cannabinoids in sperm differentiation and/or maturation. Since the cannabinoid receptor 1 (CNR1) activation appears to play a pivotal role in spermiogenesis, the developmental stage where DNA is remodeled, we have hypothesized that CNR1 receptors might also influence chromatin quality in sperm. In this respect, we have used Cnr1 null mutant (Cnr1-/-) mice to study the possible role of endocannabinoids on sperm chromatin during spermiogenesis. We have demonstrated that CNR1 activation regulates chromatin remodeling of spermatids by either increasing Tnp2 levels or enhancing histone displacement. Comparative analysis of WT, Cnr1+/- and Cnr1-/- animals reveals, in the Cnr1+/-, the possible occurrence of haploinsufficiency for Tnp2 turnover control by CNR1, whereas histone displacement is disrupted to a lesser extent. Further, flow cytometry analysis demonstrates that the genetic loss of Cnr1 decreases sperm chromatin quality and is associated with sperm DNA fragmentation. Such damage increases during epididymal transit, from caput to cauda.
Collectively, our results show that the expression/activity of CNR1 controls the physiological alterations of DNA structure during spermiogenic maturation and epididymal transit.
Given the deleterious effects of sperm DNA damage on male fertility, we suggest that the reproductive function of marijuana users may also be impaired by deregulation of the endogenous endocannabinoid system.

O05_04 Ancestral bilaterian retinoic acid signalling: characterization of the Retinoic Acid Receptor (RAR) from the lophotrochozoan mollusc Nucella lapillus

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Retinoic Acid (RA), a vitamin A-derived morphogen, plays a multitude of biological roles, namely during animal embryonic development controlling positional and cellular information; more recently it was found that it triggers meiotic initiation in mammalian embryonic ovaries and probably in postnatal testes. The Retinoic Acid Receptor (RAR) is central to the mediation of RA signalling, forming a heterodimer with the Retinoid X Receptor (RXR). A fundamental question relates to the evolutionary origin of the RA signalling pathway and of its components. Whilst RXR is found in all major animal lineages including sponges, the presence of RAR outside deuterostomes has remained mysterious. Recent genome mining analyses have argued that a Proto-RAR was present in the common ancestor of protostomes and deuterostomes. Here, we report the cloning and functional characterization of NlRAR, the single RAR in the lophotrochozoan neogastropod mollusk Nucella lapillus. NlRAR was found to be expressed in N. lapillus adults, just like its previously reported heterodimeric partner NlRXR, but clear expression differences between tissues and sexes were registered. In silico analyses indicate the possibility of NlRAR binding to all-trans-RA. In vitro binding assays partially corroborate this finding, but also suggest complex ligand binding patterns. Furthermore, we find that crude retinoid extracts of N. lapillus adults exhibit binding to human RAR, but not to NlRAR. The evolution of the RA signalling pathway in Bilateria is discussed in the context of these findings.
This study was supported by PTDC/MAR/68106/2006 and in part by KAKENHI [21590143] (to J.N.) and ANR-07BLAN-0038 and ANR-09-BLAN-0262-02 (to M.S.).

O05_05 Gonadal characterization of the evolutionary conserved teneurin-1 protein from Caenorhabditis elegans to mouse: A case for the newly elucidated Teneurin C-terminal Associated Peptide (TCAP) in the mouse testis

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The teneurins are a family of four type-II transmembrane glycoproteins that have been implicated as signaling molecules, receptors and transcription cofactors during development in vertebrates. Teneurins are phylogenetically conserved among the metazoans and have been described in Caenorhabditis elegans, Drosophila, zebrafish, chicken and mice. In Caenorhabditis elegans, teneurin-1 plays an important role in gonad development and basement membrane integrity. Teneurins possess a carboxy terminal sequence oriented in their extracellular domain that may be cleaved to generate a 40or 41-amino acid bioactive peptide known as TCAP. In adult mouse testis, immunoreactive TCAP-1 was detected in spermatogonia, spermatocytes, spermatids and flagella of developing spermatozoa. Confocal microscopy indicates that TCAP-1 expression is cytosolic and strongest in spermatogonia. In order to identify potential receptor binding sites, testis slices treated with FITC-labeled TCAP-1, showed specific binding to spermatids. To determine whether TCAP is expressed independently from the teneurins, the cellular localization of TCAP-1 and teneurin-1 in F9 testicular carcinoma cells were examined by immunofluorescent microscopy. These studies localized teneurin-1 to the plasma membrane, while intense expression of TCAP-1 was observed in the cytosol. Some co-localization was observed at the plasma membrane. High Performance Liquid Chromatography and immunoblotting suggests the existence of an extended 14.8kDa TCAP-form, consistent with a cleavage site encoded within the last exon of the teneurin gene. Our data provides novel evidence of TCAP-1 expression in areas of the testes distinct from those known to express teneurin, and shows that TCAP-1 is both structurally and functionally distinct from the larger teneurin proteins.

O05_06 Involvement of trout TNFα in LH-induced preparatory events for ovulation

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Ovulation is a multistep process triggered by luteinizing hormone (LH), initiating a cascade of events that result in follicle rupture and oocyte release from the ovary. A large body of evidence indicates that ovulation is an inflammatory-like process in mammals. In this study, we tested the hypothesis that the pro-inflammatory cytokine tumor necrosis factor α (TNFα) could affect ovarian function and, specifically, mediate the effects of LH during the preovulatory period in the brown trout (Salmo trutta). We examined the in vitro effects of coho salmon LH (sLH) and showed that it significantly increased follicle contraction and that this effect was blocked by indomethacin (a prostaglandin synthesis inhibitor) and TAPI-1 (an inhibitor of TACE/ADAM17 that blocks TNFα secretion). Furthermore, sLH increased the expression of tnfα, tace/adam17 and prostaglandin synthases 1 and 2 (cox-1 and cox-2), as well as the production of prostaglandin F2α (PGF2α) into the culture medium. In order to further study the possible involvement of TNFα as a mediator of sLH in the trout ovary, we incubated preovulatory follicles in the presence of recombinant trout TNFα (rtTNFα). First, rtTNFα caused a significant increase in follicle contraction and indomethacin blocked this effect, suggesting a possible involvement of prostaglandins in rtTNFα action. Second, rtTNFα stimulated the expression of cox-1 and cox-2. Third, rtTNFα stimulated the production of PGF2α. In view of these results we propose that TNFα is a potential mediator of the effects of sLH in the ovulatory process in trout through its stimulation of the production of PGF2α.

O05_07 Evaluation of steroidogenic gene expression in adult zebrafish (Danio rerio) exposed to environmental levels of xenoestrogens found in Douro River estuary, Portugal

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Endocrine disrupting compounds (EDCs) can elicit biological responses mimicking those caused by sex steroids, potentially interfering with reproductive physiology. Since in natural environments fish populations are exposed too many potential EDCs, the main objective of the present study was to understand if and how an environmental relevant mixture of estrogenic EDCs can disrupt the normal sex steroidogenesis in fish. For this purpose, adult zebrafish of both sexes were exposed during 21 days to an environmental mixture (MIX) of eleven EDCs we previously measured in Douro River estuary. A 100 ng/L ethynylestradiol positive control (EE2) was added. Real-time PCR was used to analyze mRNA expression in males and females of steroid acute regulatory protein (StAR), aromatase (AroB, cyp19a2) in brain, vitellogenin 1 (Vtg1) expression in liver and StAR, 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), 20β-hydroxysteroid dehydrogenase (20β-HSD), and aromatase (AroG, cyp19a1) in gonads. Vtg1 mRNA expression was dramatically increased in males in both treatment groups confirming the experimental setup of the exposure and the estrogenicity of the mixture of EDC at concentrations observed in the Douro River estuary. Interestingly, analyzed steroidogenic genes in gonads and brain of males were less affected by estrogenic exposure, with the exception of StAR in the EE2 group. In contrast, StAR and 17β-HSD1 mRNA were decreased in the female EE2 group, while AroG was diminished in EE2 and MIX treatments. Overall, the study revealed a sex-specific gene expression pattern, which may indicate different mechanisms of estrogenic disturbance in males and females.
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O05_08 Thyroid hormone and reproduction in goldfish

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As in many vertebrates, fish reproduction is regulated by environmental cues such as photoperiod and temperature. During reproductive season a significant amount of metabolic energy is diverted to development of ovary and testis through precise regulation of brain-pituitary-peripheral axis by a number of key hormones, including pituitary gonadotropins and gonadal steroids. Since thyroid hormones are important regulator of metabolism and development in vertebrates, we investigated the possibility that thyroid hormones may mediate a change from gonadotropic to somatotropic state. Multiple experiments were performed across the four reproductive seasons to test the effects of triiodothyronine (T3) on estrogen and androgen receptors in the liver, testis and ovary as well as secretion of gonadal steroids and expression of pituitary glycoprotein hormone subunits (TSHβ, LHβ & FSHβ) and aromatase. Previous studies suggested that thyroid hormones influence reproduction in vertebrates, but little information is available on the mechanisms. In the present study, we test the hypothesis that thyroid hormones regulate pituitary hormone and gonadal steroid production in fish. The results demonstrate that thyroid hormone, (T3) regulates estrogen level as well as estrogen receptor subtypes (ER alpha, beta-1 and beta-II) in the testis and ovary of goldfish. We also observed that T3 influence pituitary gonadotropin and gonadal aromatase gene expression in goldfish. Collectively, it appears that T3 acts to diminish gonadotropic response by influencing hormone production as well as decreasing sensitivity to estrogen by down-regulating the estrogen receptor subtypes. We propose that thyroid hormones play a role in switching energy expenditure from reproductive to growth in goldfish. Funded by NSERC grants to HRH.

O05_09 Changes in gonadal development in sea bass (Dicentrarchus labrax) following intramuscular injection of an FSH single-chain- expression plasmid

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The gonadotropin hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are central players in the control of vertebrate reproduction. In fish, the knowledge on their functions is still too scarce to assign differential roles to these hormones. In the specific case of the FSH, information is very limited in fish species other than salmonids. To undertake functional studies of FSH in sea bass we aimed to evaluate the potential of intramuscular injection of an FSH encoding plasmid as means of hormone delivery in vivo. Immature sea bass males were injected with an expression plasmid containing a scFSH coding gene (pscFSH), or with empty plasmid as control (pControl). Plasmid injections effects were monitored by changes in plasma FSH levels. The biological activity of the FSH produced by fish muscle cells was proved by evaluating plasma 11-ketotestosterone (11-Kt) levels and gonadal histology. Expression changes for LH receptor, Anti-Müllerian Hormone (AMH) and synaptonemal complex protein 3 (SCP3) genes, were also evaluated in gonads by real-time PCR. Increments in plasma FSH levels were detected in the pscFSH injected group 15 days after first injection and lasted until day 23. Up to day 15, plasma levels of 11-Kt were significantly higher in pscFSH group respect to controls. Changes in gonad structure were already observed at day 15, when a significant increase in the percentage of mitosis was observed. mRNA levels of the meiotic cell marker SCP3 rose at day 15, in the pscFSH group, reaching a peak 23 days after the first injection. This increase in SCP3 expression was concordant with the histology, as the pscFSH group showed more advanced germ cells at day 23, while the control group remained immature. The raise of circulating FSH was accompanied by an elevation of LHR transcripts at day 15 and 23 and by a decreasing tendency in AMH gene expression. These findings support the putative role of FSH as the initial signal of spermatogenesis in sea bass, mediating steroid synthesis and germ cell proliferation. Additionally we demonstrate the utility of gene delivery for hormone therapy in fish.
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O05_10 Analysis of gene expression in the Senegalese sole (Solea senegalensis) testis

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The Senegalese sole (Solea senegalensis) has recently become one of the main target species for the aquaculture industry in southern Europe. Owning to poor gamete quality and production in F1 males, an accurate knowledge of spermatogenesis and its hormonal regulation is needed. To further understand this process, through our participation in a large-scale expressed sequence tag (EST) project in this species, we identified several ESTs corresponding to key genes involved in the production and action of testicular steroids: Steroidogenic Acute Regulatory protein (StAR) related, 3?-hydroxysteroid dehydrogenase (3β-HSD), 17β-HSD, 20β-HSD and progesterone nuclear receptor (PR). Full length cDNA sequences were obtained for StAR-related, 3β-HSD, 17β-HSD and 20β-HSD. Furthermore, the full length cDNA sequence of StAR was obtained by conventional cloning. Our study also aimed at describing the transcriptomic changes taking place in the sole testis in response to a gonadotropic stimulation by in vivo administration of human chorionic gonadotropin (hCG) . Interestingly, hCG treatment increased the expression of StAR, StAR-related, 3β-HSD, 17β-HSD, 20β-HSD and PR. Gene expression analysis by microarray, using an oligo-based platform recently developed for this species, identified close to 100 genes differentially expressed in the sole testis in response to hCG. Amongst them, genes involved in steroid synthesis (e.g. StAR related) and progestin action (e.g. progesterone receptor) as well as genes involved in primordial germ cell and spermatogonial proliferation (e.g. gonadal soma-derived growth factor) appeared to be induced by the treatment. This approach evidenced an important transcriptional regulation of testicular function by gonadotropic stimulation in this species.

O05_11 Stannius corpuscles and serum calcium in the indian freshwater fish, notopterus notopterus

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The histology, histochemistry,biochemistry and electron microscopic studies of corpuscles of Stannius and serum calcium has been studied in the freshwater fish, Notopterus notopterus. A pair of corpuscles of Stannius are present embedded in the anterior dorsal region of the posterior kidney. The cellular organization of the CS consists of three types of cells identified based on the ultrastructural cellular components.The type-I cells are predominant cell type exhibit charectoristics stenniocalcin hormone secreting cell.The molecular weight of the secretory product of CS is having 41kDa identified by SDS-PAGE analysis. Under different experimental conditions such as calcium rich and deficient water exposure,CS extract and some steroid hormone administration results in changes of CS cells indicating calcium is a factor for CS activity particularly type-I cells. The serum calcium increased under calcium rich water exposed fish which decreases only after 15 days whereas serum calcium decreases in 10 days after CS extract administration indicating stimulation of CS is not immediate. The corpuscles of Stannius cells are active during breeding period indicating parallel increase with the growth of the gonads. Concurrently, the serum calcium also increases markedly until spawning. This increase is mainly accounted for calcium bound to proteins (vitellogenin). Hence, it indicates that activation of corpuscles of Stannius during breeding period is a response to elevated calcium levels that accompany gonadal maturation in the fish, Notopterus notopterus.

O05_12 Effect of photoperiod and salinity on the ovary of Glossogobius giuris

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Glossogobius giuris a bottom dweller is widely distributed in fresh water ponds and tanks around Bangalore. Maturity cycles are evaluated in relation to photoperiod, temperature, rainfall and physico - chemical factors in water. In the natural habitat, ovarian recrudescence is observed to be directly related to increased photoperiod and higher temperatures (March and April), while spawning occurs when there is a decrease in photoperiod and fall in temperature (September). This is evident from the increase in GSI and ova diameter. To understand the reproductive modulation, the fish were exposed to various photoperiod regimes (8L ±16D, 12D ±12D and 0L± 24D) and salinity (10 to 30%) for 7 to 14 days during different phases of the reproductive cycle. Long photoperiod (12L ± 12D) stimulates the formation of yolky oocytes in the ovary. While, short photoperiods (8L ± 16D) caused retardation in growth and proliferation of oocytes and formation of atretic follicles. However, total darkness (0L ± 24D) induced ovarian recrudescence by inhibiting vitelogenesis and causing artesia of all yolky oocytes. This was more significant in 10% saline treated fish indicating photoperiod and salinity to play an important role in maturation and spawning. This probably is due to impairment of hypothalamo hypophysial axis activity.

O05_13 Silencing of allatoregulating neuropeptide genes affects the fertility of Spodoptera frugiperda (Lepidoptera, Noctuidae)

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Allatostatins (AS) and allatotropins (AT) are neuropeptides that inhibit or stimulate the biosynthesis of JH in the corpora allata, respectively. In the moth Spodoptera frugiperda, three types of AS (A, B, C) are expressed and two of AT (1, 2), grouped by structural features. The widespread expression of these genes in various tissues corroborate their multifunctional role.
After RNA interference (RNAi) in vivo, triggered by abdominal injection or feeding of dsRNA, the transcript level of the various allatoregulating neuropeptides was reduced in the brain for at least 4-6 days. AS and AT silencing in adult females increased or decreased the titer of the various JH isoforms in the hemolymph, depending on the age. As a result, oviposition was altered.
During mating of the moths, various substances, including JH, are transferred from the male to the female with the sperm. In the controls a high titer of JH was measured in the male accessory reproductive gland (MARG) prior to copulation, whereas in the female bursa copulatrix (BC) no JH was detected. After mating, MARG were depleted of JH, while the BC of the females contained high amounts of JH. Silencing of the AS and AT genes in male altered the amount of JH isoforms present in these organs. The modified JH titer was accompanied by a strong decrease in egg production and oviposition of these females that paired with AS-C and AT-2 silenced males, whereas the effect of AT-1 knockdown was the reverse.

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O05_14 Deep RNA sequencing of red and white skeletal muscle in response to exercise in rainbow trout

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Next-generation sequencing (NGS) technologies are able to provide an unprecedented view of the transcriptome. In this study, we have used the Solexa/Illumina GA2 sequencing system to provide an in-depth view of the transcriptome of red and white skeletal muscle of exercised and non-exercised rainbow trout with the specific objective to identify novel expressed genes and quantify transcriptomic effects of exercise. Pubertal autumn-spawning seawater-raised female rainbow trout (Oncorhynchus mykiss) were rested (n=10) or swum (n=10) for 1176 km at 0.75 body-lengths per second in a 6,000-L swim-flume under natural conditions for 40 days.
Red and white muscle RNA of exercised and non-exercised fish (4 lanes) was sequenced and resulted in approximately 15 million reads per lane that after de novo assembly yielded 149,159 red muscle contigs and 118,572 white muscle contigs. Most contigs were annotated by blasting against salmonid ESTs and the zebrafish Danio rerio genome.
Among large contigs (≥ 500 bp) in red muscle, 51 were up-regulated at a fold change (fc) of ≥ 2 and 118 were down-regulated at fc ≤ 0.5 in read-per-kilo-base-of-exon-model (RPKM) values of swimmers vs. resters. In white muscle, 29 large contigs were up-regulated at fc ≥ 2 and 71 were down-regulated at fc ≤ 0.5 in swimmers vs. resters. At this moment we are validating results by Q-PCR.
This study will greatly contribute to the understanding of red and white muscle functioning in general, and specifically during long-term salmonid reproductive migration, and will allow future discovery of essential genes regulating these processes.

O05_15 Determining the contamination rates of cosmetics to environmental hormones

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Background and purpose: Environmental hormones are substances which can compete with natural hormone receptors and disrupt endocrine systems and cause many diseases ranging from hormone dependent cancers to infertility and so on.
Material and Methods: Cosmetics industry is one of the important industries in many countries and many products originate from synthetic and chemical ingredients. In order to test these cosmetics' products contamination with environmental hormones, this study conducted to look at different cosmetic products in Iranian market. In this study all cosmetic creams, aromatics and soaps were studied to determine the number of ingredients with environmental hormones' activity.
Results: From overall 935 ingredients in 200 creams, 100 perfumes and 30 soaps; 721 ingredients found in creams, 43 in soap and 168 in perfumes and sprays. 56.9% of these ingredients were environmental disrupting chemicals (63.9 in creams, 46.5 in soaps, 21.9 in perfumes) and 43% were not
Conclusions: The results showed at least half of cosmetic products in Iranian markets are contaminated with environmental hormone pollutants. Regarding the widespread usage of cosmetics in Iran (with a sharp increase in this trend) show that many people are being exposed to environmental hormone pollutats and urgent action should be taken into account to limit the exposure of population to those potentially dangerous compounds.

O05_16 Myoinhibiting peptides as ancestral ligands for the Drosophila sex peptide receptor

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Upon mating, female Drosophila melanogaster undergo striking behavioural changes induced by sperm-carried accessory gland proteins, such as sex peptide (SP). The G-protein coupled sex peptide receptor (SPR) plays a pivotal role in this process and is found in the female fly's genital tract as well as its nervous system. We report on the activation of SPR by an additional group of evolutionary conserved agonists, the myoinhibiting peptides (MIPs). Furthermore, we have identified structural determinants responsible for SPR's activation by both MIPs and SP. Given the restricted expression of SP (only found in the male's reproductive system) and since SPR expression is not limited to the adult female's nervous and reproductive tissues but occurs throughout embryonic and larval stages as well as in the male nervous system, MIPs could be more general ligands for SPR. In this light it is also highly plausible that the strong conservation of MIPs constrains the evolutionary rate of insect SPRs. These receptors indeed prove to be remarkably well conserved in comparison to the high rate of evolution witnessed in genes encoding accessory gland proteins.

O05_17 Reproductive and spawning activity of the fish, Myst-us cavasius (Ham) in the Bhadra reservoir of Western Ghats of India

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The influence of factors such as photoperid, temperature and seasonal rainfall has been studied on the gonadal growth and spwaning activity of the fish, Mystus cavasius from Bhadra reservoir of western Ghats in the southern region of India. The correlation between environmental factors and reproductive events indicate that the fish Mystus cavasius does not depend much on the micro level changes in the natural photoperiod for timing its annual reproductive cycle, whereas increase in the temperature during the month of March to April triggers gametogenic events in both male and female fish. Heavy rainfall starts in the second week of June. Hence during the month of July the ovary contains oocytes at female occurs during August to September-October. While in males it occurs September - October. This indicates that female spawns their gametes earlier to male to synchronies fertilization for prorogation and better survival of the species in the reservoir.

O05_18 Development of a specific enzyme-linked immunosorbent assay (ELISA) for determining FSH levels in sea bass (Dicentrarchus labrax), using recombinant gonadotropins

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The follicle-stimulating hormone (FSH) is a key gonadotropin for control of reproduction in vertebrates. FSH is a heterodimeric glycoprotein secreted by the pituitary gland, formed by the non-covalent association of two subunits (alpha-beta) where beta-subunit confers hormone specificity. Since late 1980´s, gonadotropins have been isolated and characterized in several fish species but the development of specific immunoassays for FSH has been accomplished only for a few ones. The present study reports the production of recombinant sea bass FSH-beta in the methylotrophic yeast Pichia pastoris and the development of a specific and homologous competitive ELISA for FSH determination in plasma and pituitary. The coding sequence of the mature sea bass FSH-beta was inserted in-frame into the pPIC9K expression vector, which was used to transform the host strain GS115 by electroporation. After large-scale production and purification of recombinant FSH-beta, this was used to generate polyclonal primary antibodies by rabbit immunization. Sea bass FSH dimer produced in our laboratory in a baculovirus expression system was used both as coating and standard curve. Wells were coated with FSH (0.5 mg/well), and the final concentration of the antisera was 1:8000. Validation of this assay showed a high degree of parallelism between the standard curve and serially diluted plasma and pituitary samples of sea bass but not with others perciforms. The sensitivity of the assay was 1.59 ng/ml [Bo-2SD] and the intra- and inter-assays coefficients of variation of 2.12% (n=10) and 5.44% (n=16) (Bi/Bo 45%), respectively. Plasma FSH levels of males and females sea bass were measured during one reproductive cycle using this ELISA. In females, plasma FSH levels increased during vitellogenesis and decreased in the maturation-ovulation stage, whereas in males increased during late-spermatogenesis (stage IV). In conclusion, a sensitive and accurate immunoassay for analysis of FSH in biological samples of sea bass has been developed. This assay represents a valuable tool to investigate the role of this hormone in the reproductive endocrinology of this species.
Research supported by MICINN (AGL2005-0079, AGL200604672 and CSD2007-00002 / Aquagenomics)

O06_01 Expression of clock and metabolic genes in the liver and heart of rats exposed to lighting rotation schedule with phase delays

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Clock genes represent molecular basis of the circadian molecular oscillator. Single cell oscillators are hierarchically organized into the multilevel circadian system. Peripheral oscillators in tissues and organs are responsive to many internal and external synchronizing cues in a tissue specific manner. Therefore under conditions of deregulated synchronizing signals circadian system looses its hierarchical structure and its components run independently. This contributes to general desynchrony of biological rhythms inside the organism and can issue in initiation or worsening of diseases. Similar situation occurs during shift work with a rotating light:dark (LD) cycle. In our experiment male Wistar rats were used to evaluate effect of LD cycle mimicking the rotating shift-work with a 8h phase delay every second day. Expression of clock genes per2 and bmal1 and clock controlled metabolic genes rev-erbα, pparα and pdk4 was analyzed in the liver and heart of rats by real time PCR. Control Wistar rats were exposed to the regular LD cycle 12:12. The experimental group was exposed to the LD regimen with 8h phase delays during period of 10 weeks. Sampling was performed in 4h intervals during 24h cycle. Clock gene expression in the heart and liver of experimental rats was rhythmic and phase delayed by 8-9 hrs compared to control. Expression of metabolic genes was influenced more in the liver than in the heart. Results indicate that frequent shifts of LD cycle may interfere with functioning of peripheral oscillators and control of lipid metabolism.
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O06_02 Circadian system in birds develops earlier than in mammals

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In contrast to mammalian species the avian circadian system develops during the embryonic period. The aim of our research was to analyze ontogeny of circadian melatonin rhythmicity and rhythms in expression of clock genes in peripheral structures in chick embryo. We measured pineal melatonin concentrations and clock gene expression under light (L): dark (D) and temperature cycles and their responsiveness to light pulses in chicks and chick embryo. Melatonin was measured by RIA and gene expression by real time PCR. Pineal melatonin concentrations and expression of Per2 and E4bp4 were rhythmic in embryos incubated under LD conditions but the rhythm of E4bp4 mRNA did not persist in constant darkness. The amplitude of Per2 mRNA expression was dumped under constant conditions. Per2 expression and melatonin synthesis were light responsive only during the subjective day. Prenatally we did not observe daily rhythms in Per2 and Bmal1 expression in peripheral tissues but distinct rhythms were found in 4-day old chicks. Our data demonstrate that in the chick embryo the central part of circadian system develops during the last third of incubation with a prompt development of peripheral oscillations after hatching. The early development of circadian system in chicken reflects their precocial developmental strategy and diurnal activity in comparison with altricial and nocturnal mammals studied till now.
Study was supported by grants APVV-0214-07, VEGA 1/0365/10

O06_03 An insight into the development of the circadian clock in the chicken pineal model

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The chicken pineal clock is an excellent model to investigate circadian rhythms, as it can be influenced by the environment in vitro. The rhythm of clock gene expression develops in vivo under constant darkness (DD) at embryonic day 17 (ED17). To assess if the in vivo environment is responsible for starting the clock at this stage, we monitored the 24 h mRNA patterns of cry1 and clock clock genes in vitro at various developmental stages. Since PACAP is a key neuromodulator in synchronizing the mammalian suprachiasmatic clock (SCN), we examined its effects on the expression of clock genes in the chicken pineal gland.
Eggs of white Leghorn chickens were incubated from ED0 under DD. Pineal glands were placed in a multichannel perifusion system on ED13, 14 or 17 for 3 days in DD. In a similar ED17 experiment, the glands were treated after 30h with 10nM PACAP-38 for 1h. Glands were removed from the perifusion chambers from the second day every 4h (n=3 at each time point). Changes in mRNA contents were determined using semiquantitative RT-PCR and statistical analysis.
The expression of clock genes showed episodic alterations at each developmental stage. However, the in vitro patterns matched those of in vivo only on ED17. Both clock and cry1 mRNA contents were altered within 2h after exposure to PACAP.
The clock may start even before ED17 within the chicken pineal gland. From ED17, pineal oscillatory units may be synchroized by PACAP in vivo, similarly to that seen in the mammalian SCN.

O06_04 Clock mRNA expression patterns in the chick pineal gland under experimental jet lag

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Circadian biological clocks help organisms to anticipate changes in the environment occuring on a regular 24-hour rhythmic basis. If the daily pattern of environmental exposure such as light/dark periodicity becomes unusual (e.g. jet lag), it may perturb many physiological processes by resetting the circadian clock.
Shift-workers have a higher risk for metabolic syndrome, a condition which developes also in clock -/- mice. To collect data on the transcriptional changes of clock gene under unusual light/dark conditions, we examined the 24h mRNA expression patterns of clock in the chicken pineal model exposed acutely to reversed light/dark conditions.
White Leghorn chickens were kept under 14h light/10h dark control environment (lights on at 6:00). In our in vivo experiments, chickens were placed under reversed light/dark conditions (lights on at 20:00), where pineal glands were collected every 4 hours (n=3). We carried out in vitro experiments in our perifusion system: chicken pineal glands were placed in 6 chambers (n=3 glands/chamber), which were then collected every 4 hours beginning at 18:00 next day. For mRNA measurements, we optimized a semiquantitative RT-PCR method.
Under control LD conditions, clock expression peaked at 2:00 in vivo and at 22:00 in vitro. Compared to control, under in vivo reversed light/dark conditions, we measured between 22:00 and 6:00 higher mRNA contents in the first cycle, but lower in the second cycle. Under in vitro reversed light/dark conditions, we detected between 10:00 and 14:00 higher mRNA contents if compared to control data.
Night-time peaks of clock mRNA amounts in the control group suggest a darkness-related activation of the clock gene. This is supported by our data collected under reversed light/dark conditions: in vivo the nighttime activation is diminished under illumination, and in vitro a daytime activation is seen under darkness. We have found differences in the 24h mRNA patterns between the first and the second reversed cycles in vivo, but also between in vivo and in vitro data. Both suggest that unexpected night-time illumination may rapidly reset the regulation of clock transcription via neurohumoral signals and signalling pathways which may be different from those working under normal, entrained conditions.

O06_05 Biological Clocks in the Atlantic Salmon Pineal Organ

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To date the majority of work on biological clocks in teleosts has focused on the zebrafish model. However, very little attention has focused on clocks in seasonal temperate fish species such as the Atlantic salmon (Salmo salar). In order to unravel some of the mechanisms underling seasonality in the Atlantic salmon we have investigated whether daily patterns of gene expression in representative clock genes (Clock and per2) vary under different seasonal photoperiods, Long day (16L:8D), 12L:12D and Short day (8L:16D), in the brain, pectoral fin and liver of salmon parr. Results showed there to be distinct rhythmicity in the expression of Per2 and Clock in the brain and the liver however the tissues displayed a differential response to seasonal photoperiod both in terms of the presence and phase of the rhythms observed. In a second body of work we investigated the presence of clock genes in the salmon pineal. Of all teleosts studied so far, salmonids are the only fish in which pineal melatonin synthesis does not exhibit an endogenous "clock controlled" rhythm however it remains to be seen whether a clock is present in the salmonid pineal. Individual pineal organs were isolated from salmon parr acclimated to 12L:12D and maintained in culture under three different photoperiods (LD, LD /DL switch and DD). Patterns of Clock, Per2 and AANAT2 expression were measured in conjunction with melatonin from individual pineal organs. The results revealed that clock genes are indeed present suggesting that melatonin synthesis has become decoupled from the biological clock.

O07_01 Pituitary adenylate cyclase activating polypeptide (PACAP) protects chicken inner ear cells against H2O2-induced oxidative stress

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic and multifunctional neuropeptide. Numerous studies prove that PACAP has neuroprotective effects in diverse neuronal systems in vitro and in vivo. The involvement of PACAP in visual and olfactory sensory processing has also been documented, but little is known about its effects in the auditory system. The presence of PACAP and its receptor, the specific PAC1 receptor, has been shown in the cochlea and in brain structures involved in auditory pathways. The aim of the present study was to investigate whether PACAP is protective in cochlear oxidative stress-induced cell death, which is known to play a role in several ototoxic insults. Chicken cochlear cells were exposed to 1 mM H2O2, which resulted in a marked reduction of cell viability and a parallel increase of apoptotic and necrotic cells assessed by MTT test, annexin V/propidium iodide flow cytometry and JC-1 apoptosis assay. Co- incubation with 100 nM PACAP increased cell viability and reduced the percentage of apoptotic cells. Furthermore, oxidative stress increased the activation of caspase-3, while simultaneous PACAP treatment reduced it. In summary, our present results demonstrate that PACAP effectively protects cochlear cells against oxidative stress-induced apoptotic cell death.
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O07_02 Growth hormone in the chick retina: an autocrine/paracrine neurotrophic factor

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Although growth hormone (GH) is primarily produced in chicks by pituitary somatotrophs, the GH gene is widely expressed in chick embryos, prior to pituitary differentiation. While pituitary somatotrophs appear ontogenetically at ED (embryonic day) 15 of the 21 d incubation period, GH immunoreactivity and mRNA are abundant in the neural retina by ED 7. This largely reflects the expression of the GH gene in the retinal ganglion cells (RGCs), which also express the GH receptor (GHR) and retinal GH is thus likely to act locally as an autocrine or paracrine factor. The functional importance of retinal GH in early neurogenesis was demonstrated by its in vitro and in vivo immunoneutralization, which increased cell death by caspase- and Akt-dependent mechanisms, whereas exogenous GH promoted in vitro and in vivo cell survival during developmental waves of apoptosis. The siRNA knockdown of retinal GH gene expression in early embryos similarly promoted apoptosis in vitro and in vivo. The knockdown of retinal GH also suppressed the sprouting of RGC neurons and reduced their length. Neurite sprouting and length was, conversely, increased by exogenous GH treatment. A role for endogenous GH in retinal neurogenesis was also indicated by the observation that its expression in RGCs and its presence in the optic fiber layer, from which the optic nerve is derived, is restricted to the period when the retinofugal neurons project to and synapse with visual centers in the brain (at ED 10-12), since RGC GH is not present afterwards. These results clearly demonstrate that GH is an autocrine/paracrine factor regulating RGC cell survival and neural differentiation during early chick embryogenesis and that GH has hitherto unsuspected roles in neural development.
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O07_03 Neuro-immun interactions in the dove brain

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Mast cells (MC) are of hematopoetic origin. Connective tissue type MCs are able to function in IgE dependent and independent fashion, change their phenotype according to the tissue environment. They are able to enter the brain in normal physiological conditions, and move in the compact tissue of neurons. In doves MCs are found only in the medial habenula and their number is changing according to the amount of sex steroids in the body. After a 7day treatment with estrogen, testosterone and DHT, MC number elevated to the numbers found in the habenula after courtship.
MCs are able to synthesize and store a big variety of biologically active compounds, like transmitters, neuromodulators and hormones. They are able to secrete GnRH. With the aid of electronmicroscopy we were able to describe MC-neuron interactions among GnRHpositive MCs and neurons. We have found that not only the number, but the activational state of MCs also changes with the amount of sex steroids. The biggest effect was found in the case of estrogen, 37% of detected MCs were in the fully degranulated state. Unlike in IgEdependent activation, MCs did not show any anaphylactic reactions, only filopodial, granular and vesicular release was visible. Peacemeal degranulation - secretory vesicles budding off swollen and active granules- seems to be a very efficient type of communication between MCs and surrounding neurons. Different types of granular and vesicular transports are seen between MCs and neurons in the medial habenula of doves. Sometimes whole granules are visible in the neuronal cytoplasm, in other cases exocytotic vesicles empty materials of MC origin. Thus MCs might modulate neuronal functions.
The activational state of MCs is changing in higher estrogen/testosterone level, thus with the secretion of neuromodulators they might change the animals sexual and parental behaviour.

O08_01 Frog antimicrobial peptide genes: Gene expression in the Harderian gland and thyroid-hormone dependency of gene transcription

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In vertebrates, eyes and skin are situated at the interface between the organism and its environment and as so require defensive systems to protect against invasion by environmental pathogenic microorganisms. Amphibian skin, a rich source of antimicrobial peptides (AMPs), is known to develop in a thyroid hormone (TH)-dependent manner. Thus, our experiments with frogs were directed 1) to investigate whether the Harderian gland (HG), an orbital gland that provides secretory substances to the eye, is involved in the innate immune system of the host's eyes via AMP production, and 2) to examine the effects of TH on the transcription of amphibian AMP genes. Firstly, employing RT-PCR, cDNAs encoding biosynthetic precursors for the AMPs, temporin-CBa and chensirin-2CBa, were cloned from the bullfrog (Lithobates catesbeianus) HG. By performing in situ hybridization using digoxigenin-labeled preprotemporin-CBa and preprochensirin-2CBa cRNA probes, we identified mRNAs encoding both precursors in the cytoplasm of epithelial cells of the HG. Secondly, we cloned the promoter region of the temporin gene from the liver of Rana ornativentris to investigate the presence of any thyroid hormone (TH)-responsive element (TRE). Although no typical TRE consensus sequence was found, five TRE half-site sequences were present in the gene. In vitro experiments using a reporter gene system revealed that transcription of the preprotemporin gene transfected in mammalian COS7 cells was upregulated by treatment with T3. Mutants with a serially deleted promoter region in the reporter gene system showed that removal of all five TRE half-sites caused inhibition of T3-inducible gene transcription.

O08_02 Elevated plasma cortisol induces organ-specific changes in glucocorticoid receptor expression and in the innate immune response in Sparus aurata

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Cortisol controls a wide variety of biological responses, such as intermediary metabolism, osmoregulation and immune functions. Its action involves the activation of specific intracellular receptors - the glucocorticoid receptors (GR) -that have been previously described in different fish species including the teleost Sparus aurata (gilthead seabream). The present work was designed to assess the effects of high plasma cortisol levels induced by slow-release cortisol implants in the mRNA transcription of the GR in head kidney, spleen, gills and intestine of the gilthead seabream. For that purpose fish were intraperitoneally injected with the implants containing two different concentrations of cortisol (50 or 200 µg/g body weight) to simulate a cortisol secretion after chronic stress, and blood and organs sampled after 7 and 14 days of implantation. Only fish with 200 µg/g implants exhibited a significant rise in the plasma cortisol. On the basis of these results, we evaluated the expression of the GR in the previously mentioned organs of the gilthead seabream injected with 200 µg/g cortisol implants. GR gene expression was up-regulated in head kidney after 7 and 14 days of cortisol implantation, as well as on gills 7 days after cortisol implantation. On the other hand, GR expression was significantly decreased in intestine 14 days after cortisol implantation and unchanged in spleen. These results suggest that increased plasma cortisol induced by a slow-release implant of cortisol mimics the effects of chronic stress and affects the expression of GR in a time and organ-specific manner. Additionally, we determined the effects of high plasma cortisol levels in the haemolytic activity of plasma (complement activity), as a non-specific defence mechanism in teleosts. However, while plasma cortisol levels were generally increased, the tendency of plasma complement was opposite, showing a significant decrease after 14 days implantation with 50 or 200 µg/g body weigh cortisol. These results suggest that high plasma cortisol affects the innate immune response of gilthead seabream and may increase its susceptibility to disease.

O08_03 Cytokine regulation of glucose entry into the skeletal muscle of the rainbow trout (Oncorhynchus mykiss): role of tumour necrosis factor alpha (TNFα)

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Tumour necrosis factor alpha (TNFα) is one of the most studied cytokines for its catabolic effects in target cells during excessive production. TNFα is known to have a direct action on skeletal muscle in mammals. However, little is known regarding the potential effects of cytokines on non-immune tissues, and particularly in skeletal muscle, in fish.
The aim of this study was to investigate the effects of recombinant trout TNFα (rtTNFα), on skeletal muscle function in rainbow trout (Oncorhynchus mykiss). We used a primary cell culture of muscle cells from rainbow trout to show that rtTNFα stimulates glucose uptake in myoblasts and myotubes at concentrations that do not affect the viability of the cells and requiring de novo protein synthesis, as shown by the impairment of rtTNFα-stimulated glucose uptake by cycloheximide. With the use of specific inhibitors we show that rtTNFα-stimulated glucose uptake is mediated by the p38-MAPK, NF-kB and JNK pathways . Additionally, we provide evidence that the stimulatory effects of rtTNFα on glucose uptake in trout skeletal muscle cells may be caused, at least in part, by an increase in the amount of GLUT4 at the plasma membrane. Incubation of trout muscle cells with conditioned medium from LPS-stimulated trout macrophages stimulated glucose uptake.
Our results indicate that recombinant as well as native trout TNFα directly stimulates glucose uptake in trout muscle cells and provides evidence, for the first time in non mammalian vertebrates, for a potential regulatory role of TNFα in skeletal muscle function.

O08_04 Growth hormone (GH) acts on the GH/IGF-system in adult tilapia liver and immune organs

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In mammals, the GH/IGF-I and the immune system have been shown to interact. The stimulatory significance of GH for the immune system is indicated by the presence of the GH-receptor (GHR) and insulin-like growth factor (IGF)-I. However, little is known on the local regulation of the IGFs and the GHR in immune organs. There is evidence for interactions also in bony fish since the GHR, IGF-I and IGF-II are also found in different fish species including the tilapia. Immune regulation is important for aquaculture and wild-life fish and thus plays an increasing role in basic science and aquaculture research. To investigate the local regulation of IGF-I, IGF-II and GHR by GH adult tilapia (Oreochromis niloticus) were challenged with intraperitoneal GH injections. The expression levels of hepatic GHR and IGF-I and IGF-I serum levels were quantified. Hepatic GHR mRNA was downregulated and IGF-I mRNA elevated as well as IGF-I serum levels in the GH-injected fish. IGF-I gene expression in spleen and head kidney was raised, in the latter even stronger than in liver. In the spleen, in addition to IGF-I also IGF-II mRNA was strongly elevated. Thus, head kidney and spleen reacted differently to GH treatment indicating different roles of GH and the IGFs in growth, differentiation and sustainment in the immune system of the tilapia. Whether this also has implications for immune response demands for further studies using bacteria and other pathogens.
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O08_05 Seawater and freshwater challenges effects on osmoregulatory organs in black-chinned tilapia (Sarotherodon melanotheron heudelotii)

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Contradictory studies suggest IGF-I in fish liver and gills is involved in osmoregulation, but little or nothing is known about its role in the kidney, intestine and other central organ systems nor about IGF-II's role in any organ.
Methods: Tilapia (Sarotherodon melanotheron heudelotii) were transferred from freshwater (FW) to seawater (SW) for one week and retransferred to FW for another week. At 4 hours, 1, 2, 3 days and 1 week after SW-transfer and FW-retransfer IGF-I, IGF-II, growth hormone receptor (GHR1) mRNA were measured by real-time PCR.
Results: Hepatic IGF-I, IGF-II and GHR1 mRNA were downregulated in parallel after SW-transfer, recovered and were again downregulated after FW-retransfer. In gills, IGF-I, IGF-II and GHR1 were upregulated synchronously after SW-transfer and, partially also after FW-retransfer. The renal genes were downregulated after SW-transfer and partially upregulated after FWretransfer. Persisting upregulation in intestinal IGF-I mRNA occurred after FW-retransfer. Currently, these genes are under further investigation in order to evaluate the auto/paracrine role of IGF-I and IGF-II in other relevant organs.
Conclusion: Thus, endocrine and auto/paracrine IGF-I and IGF-II seem to be involved in fish osmoregulation in an organ-specific manner.
Supported by the Swiss National Foundation (project nos. 111028, 118165).

O08_06 Insulin-like growth factor-I mRNA and peptide are distinctly confined to subtypes of macrophages, antigen-presenting cells, lymphocytes and HEV cells in non-neoplastic human lymph node

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IGF-I is a potent hormone that stimulates growth and differentiation and inhibits apoptosis in numerous tissues. Preliminary evidence suggests that IGF-I exerts differentiating, mitogenic and restoring activities also in the immune system. The present study investigates the cellular sites of IGF-I mRNA and peptide on archival human lymph node samples by routine staining, in situ hybridisation with an IGF-I probe, and immunohistochemistry with antisera specific for human IGF-I and CD3 (T-lymphocytes), CD20 (B-lymphocytes), CD68 (macrophages), CD21 (follicular dendritic cells, DC), S100 (interdigitating DC) and podoplanin (fibroblastic reticular cells). Numerous cells within the B- and T-cell compartments showed IGFI gene and peptide expression the majority of which was identified as macrophages. Solitary follicular DC exhibited IGF-I mRNA and peptide. B-lymphocytes did not contain IGF-I immunoreactive material, while few T-lymphocytes did. Furthermore, IGF-I mRNA and peptide-expressing cells were identified as high endothelial venule (HEV) cells. From this we conclude that the main task of IGF-I in human non-neoplastic lymph node may be autocrine and paracrine regulation of differentiation, stimulation and survival of lymphocytes, antigen-presenting cells and macrophages and differentiation and maintenance of HEV cells, and that the role for IGF-I in formation and sustaining of lymph node structures is more important than thought so far.

O09_01 Developmental Regulation of the Pheromone Biosynthesis Activating Neuropeptide-Receptor (PBAN-R)

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Sex pheromone production in Helicoverpa armigera is regulated by the timely release of a pheromone biosynthesis activating neuropeptide (PBAN). PBAN is produced by neurosecretory cells of the subesophageal ganglion, released into the hemolymph and binds to a G-protein coupled receptor in the pheromone glands of female moths where it activates sex pheromone production only in newly emerged and adult females. The PBAN receptor (PBAN-R) protein and its transcript were also demonstrated in neural tissues of adult females and the transcript was also detected and quantified in the aedaegi of the male, which is a tissue homologous to the female pheromone gland. We demonstrate temporal differential expression levels of the PBAN-R in females and males, reaching peak levels at a critical period of 5 hours post-eclosion. The role of juvenile hormone (JH) in mating and regulation of sex pheromone production in moth species has been under controversy. Previous studies implied a possible regulatory role for JH. We herein demonstrate1 that PBAN-R expression levels increase normally even when females are decapitated or head-ligated before peak transcript levels are reached. Furthermore, sex pheromone production can be induced by PBAN in such decapitated females. These results indicate that up-regulation, at this critical time, is not dependent on JH originating from the head. Conversely, JH injected in vivo at this critical period significantly inhibits PBAN-R transcript levels. These results comply with previous evidence2 which demonstrated that the JH analog, fenoxycarb inhibited both binding of PBAN to its receptor and levels of sex-pheromone production in adult female moths.

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O09_02 Studies of sex pheromone production under neuroendocrine control by analytical and morphological means in the oriental armyworm, Pseudaletia separata, Walker (Lepidoptera: Noctuidae)

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Most moths have species-specific sex pheromone blends produced by females in the modified epidermal pheromone gland (PG) cells generally located between the 8-9th abdominal segments. The C10-C18 (un)saturated acyclic aliphatic compounds with limited functional groups (aldehyde, alcohol, acetate ester) are de novo synthesized from acetyl-CoA through 16 or 18:Acyl, converted by desaturation and/or by chain shortening followed by reductive modifications of the carbonyl carbon. The biosynthesis is often regulated by the pheromone biosynthesis activating neuropeptide (PBAN) acting either in, or prior to fatty acid synthesis or regulates the final fatty acyl reduction step.
In P. separata, 16-OH and Z11-16:OH, along with16:Ac and Z11-16:Ac constitute the blend. Information about the species' life span, calling behavior, daily fluctuation of pheromone production and its hormonal regulation, PG and cells' morphology, precise location was limited. We aimed to strengthen or disclose that lipid (droplets formed from triacylglycerides; TAGs) reservoirs are present or not as sources of intermediates necessary for pheromonogenesis, and to clarify which step is regulated by PBAN.
Sampling was performed for pheromone titer measurements daily (16:8;L:D) at 2h intervals in the scotophase (D) and after the end of D, at 2nd-4thh of photophase. Production and ratio of blend stabilizes on the 2nd day till 6-7th, most constant in late D. Females show calling behavior from the first full D, since pheromone production is a condition of feeding. Ovipositor is protruded downwards ('smearing' behavior) indicating that PG involves not only the intersegmental membrane, but a part of 9th abdominal segment. Our observation that the intersegmental membrane of 8-9th segment (A part) and the dorso-lateral part of the 9th segment (B part) both take part in the pheromone production were justified by several means. B part is encountering for 35-40% of total production under natural conditions. Fluorescence microscopy studies of trimmed PGs using double

staining (Nile Red & Hoechst) support these findings and the dynamics of cell size and shape is in correlation with their activity, but lipid accumulation of precursors in form of TAGs were not found. This underlines that PBAN stimulates de novo biosynthesis of 16:Acyl precursors, without any substantial depositions before fatty acyl reduction. In vivo PBAN injections (3x5pmol, 2h intervals) into decapitated females stimulated pheromone production in both A, B parts. Blend analyses showed that initial phase of de novo fatty acid synthesis is stimulated, but desaturation step does not follow proportionally, thus more saturated fatty acid is converted to the final OH and Ac. Similarly, the in vitro studies revealed that the ratio of saturated component is always higher than that of unsaturated ones (vs. natural blend) when 2 days old 16-18h decapitated females' PGs were incubated for 6h in presence of 2mM malonyl-CoA and 0.5 µM PBAN.

O09_03 Molecular cloning and characterization of retinoid X receptor and ecdysone receptor from the lobster, Homarus americanus

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Two cDNAs encoding retinoid X receptor (HaRXR) and ecdysone receptor (HaEcR) were cloned and sequenced from the lobster Homarus americanus by PCR cloning techniques. The amino acid sequences of both HaRXR and HaEcR are highly homologous with those of other crustacean and insect, and also showed moderate homology to those of vertebrates. Both HaRXR and HaEcR show highest gene expression in mandibular organ (MO) in both adult male and female lobster, and showed a different expression pattern in different regions of MO. Farnesoic acid (FA) and methyl farnesoate (MF) biosynthesis rate are different in different regions of MO, which is coincided to the gene expression profile of HaRXR in MO. Different regions of MO were treated by 20-hydroxyecdysone (20-HE) and semi-qualitative PCR was performed to study the effect of 20-HE on HaRXR and HaEcR gene expression. Opposite to the stimulatory effect of 20-HE on HaRXR and HaEcR in hepatopancreas and ovary, 20-HE has an inhibitory effect on HaRXR and HaEcR in MO. Furthermore, 20-HE also inhibits FA and MF biosynthesis in MO. Hence, 20-HE may play an important role in MF metabolism, and regulating expression of different genes by binding of RXR/EcR in crustacean.

O09_04 C. elegans allatostatin-like receptors

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Neuropeptides and their action on receptors are involved in regulating various behaviours of many organisms. With the arrival of sequenced invertebrate genomes many new invertebrate neuropeptides as well as their G-protein coupled receptors (GPCRs) have been identified. In Drosophila melanogaster GPCR expression studies, generally in mammalian cells, have matched neuropeptides to their cognate receptor(s). Apart from the matching of receptor with ligand, very little is known about signal transduction pathways or how activation of the receptor may regulate animals physiology or behaviour.
Allatostatins are a family of neuropeptides found in invertebrates that share a conserved C-terminal-sequence of Tyr/PheXaaPheGlyLeu-NH2. In insects, allatostatin functions vary contributing to several physiological mechanisms such as inhibition of juvenile hormone biosynthesis, inhibition of muscle contraction, myoendorine regulation, neuromodulation, and regulation of enzymatic activities. Two allatostatin receptors were first identified in Drosophila (Dar1 and Dar2) and orthologs have been identified in a variety of other invertebrates. The role of each receptor to a particular phyisological function or behaviour is unknown.
As a comparative approach to understanding the contribution of allatostatin receptors to behaviour(s) we identified two allatostatin/galanin-like GPCRs in the nematode C. elegans. A deletion mutant of a C.elegans receptor that resembles Drosophila Dar-1 lost roaming behaviour with increased pivoting which impairs their ability to travel long distances on food. Animals with a mutant receptor gain fat. In the absence of food the mutant has normal foraging/roaming behaviour. A deletion mutant of a second C.elegans receptor that resembles Drosophila Dar-2 appears to have foraging, metabolic alterations and reproductive defects.
With these observations it will be of interest to address whether allatostatin-like receptors regulate foraging/roaming behaviour in other invertebrates.

O09_05 Moult regulation in the South African spiny lobster, Jasus lalandii

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Jasus lalandii is a cold water spiny lobster species that occurs off the western shore of South Africa and which does not have a terminal moult; hence, growth continues in adulthood, concurrently with reproductive activities. During the moult cycle, steroid hormones (ecdysteroids) are produced by paired thoracic glands called the Y-organs (YOs). The titre of ecdysteroids fluctuates in a consistent pattern during a moult cycle, and ecdysis is triggered by a high titre of this steroid hormone. Ecdysteroidogenesis is inhibited by neuropeptide hormones that are produced in the X-organ - sinus gland (XO-SG) system in the eyestalk. These neuropeptides have been identified as moult-inhibiting hormone (MIH) and crustacean hyperglycaemic hormone (cHH); the latter is also essential for intermediate metabolism (elevating glucose concentration in the haemolymph). To understand the control of moulting in J. lalandii, homologous biological assays with HPLC-purified cHH and MIH were carried out to investigate the action of these hormones during intermoult, at different doses, on metabolism (in vivo) and growth (in vitro). Assays with MIH on YOs at different stages of the moult cycles were also performed, as well as investigations into the second messenger pathways involved in regulation of the moult cycle of J. lalandii. I will report on these findings in the spiny lobster and discuss the data in a comparative context.
Funding from the National Research Foundation and UCT is gratefully acknowledged.

O09_06 New Members of the Corticotropin-Releasing Factor (CRF) Family from the Tunicates, Ciona intestinalis and Ciona savignii, and from the Holocephalan, Callorhinchus milii

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The corticotropin-releasing factor (CRF) superfamily of peptides includes the four paralogous vertebrate peptide lineages of CRF, urotensin-1/urocortin/sauvagine, urocortin 2 and urocortin 3, as well as the diuretic hormones and peptides of the arthropods. However, there are considerable sequence differences between the group of vertebrate peptides and those of the arthropods, notably insects. Because of the likely incidence of the formation of paralogous forms within the insects and the great variation in primary structures among these peptides, establishing the structure of the ancestral vertebrate version has not been possible. We screened the genomes of the tunicates, Ciona intestinalis and Ciona savignii, in silico, using the various conserved motifs of both the vertebrate CRF paralogues and the insect diuretic hormone sequences to identify the structure of the Ciona CRF/DH-like peptide genes. A single peptide gene was found in both genomes that possessed motifs reflective of both groups of peptides. These structures suggest a single CRF-like peptide was inherited by vertebrates and possibly chordates. Moreover, the conserved structure of the CRF peptide may have become constrained once it became associated with the regulation of the hypothalamus-pituitary-adrenal/interrenal axis. In contrast, the Callorhinchus milii genome showed the presence of five CRF family members indicating an additional duplication event within the Holocephali.

O09_07 A novel AKH decapeptide from a South African saucer bug: how does the structural and functional information compare to other aquatic Heteroptera?

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Laccocoris spurcus, a saucer bug (Family: Naucoridae, Subfamily: Laccocorinae) contains in its corpora cardiaca a decapeptide AKH, denoted Lacsp-AKH. This peptide represents a novelty: the first decapeptide AKH in Heteroptera. The primary structure of Lacsp-AKH was elucidated by ion trap mass spectrometry as pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-Gly-Gly- amide. In the European naucorid species, Ilyocoris cimicoides (Subfamily: Naucorinae), the octapeptide Anaim-AKH is present. Anaim-AKH is identical to Lacsp-AKH but lacks the two Gly residues at the C-terminus. Anaim-AKH is also found in members of other true water bug families, viz. three species of Notonectidae and Aphelocheirus aestivalis of the family Aphelocheiridae. Our structural data on AKHs, thus, support close relationships between the three families (Naucoridae, Notonectidae and Aphelocheiridae) of the infraorder Nepomorpha. Functionally, Lacsp-AKH increases the lipid concentration in the haemolymph of L. spurcus upon injection in a low dose, and upon active muscular work (swimming) this hormone is apparently released, as indicated by an increase of the circulating lipid concentration after 1 h of exercise.
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O09_08 Newly elucidated vertebrate peptides play a role in the regulation of stress and neural plasticity: the role of the teneurin C-terminal associated peptides (TCAPs)

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The teneurins are a family of four large glycoproteins that were originally discovered in Drosophila as the ten-m or odz genes which serve as segmentation genes. Mutations in the ten-m/odz genes resulted in embryonic lethality, indicating an important function in development. Homologous genes were later identified in vertebrates such as the chicken and mouse. The 31st exon of the teneurin gene encodes the C-terminus of the protein and contains a peptide-like sequence that is flanked by cleavage and amidation motifs. Four such peptide-like sequences were identified, with each peptide corresponding to the C-terminus of each of the teneurin proteins, and these were named the teneurin C-terminal associated peptides (TCAPs). One of these peptides, TCAP-1, is bioactive in vitro, modulating neurite outgrowth and conferring neuroprotective effects on immortalized neurons.
TCAP-1 mRNA has been found in the limbic system and hypothalamus of the brain, which suggests that TCAP-1 has a role in behaviour. Intracerebral injections of TCAP-1 into rats attenuated corticotropin-releasing factor (CRF)-induced expression of the immediate-early gene, c-Fos, in the hippocampus, amygdala, medial prefrontal cortex, and dorsal raphe nucleus. In the rat hippocampus, repeated injections of TCAP-1 increased the spine density of CA3 pyramidal neurons but not in CA1 or basolateral amygdala (BLA) neurons. Furthermore, intracerebral injections of TCAP-1 into rats modulated CRF-induced anxiety-like behaviours in several tests of anxiety. These in vivo results indicate that TCAP-1 has a role in modulating synaptic connections by altering dendritic morphology, and is a novel regulator of stress behaviours.

O09_09 Mass spectrometric analysis of seasonal-dependent changes of neuropeptide profile in the snail, Helix pomatia

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Terrestrial snails are able to transform themselves into inactivity ceasing their behavioral activity under unfavorable environmental conditions. In the present study, we report on the activity-dependent changes of the peptide or/and polypeptide profile in the brain and hemolymph of the snail, Helix pomatia, using MALDI TOF and quadrupole mass spectrometry. The present data indicate that the snails respond to low temperature by increasing or decreasing the output of selected peptides. Cumulative mass spectra of the brain and hemolymph revealed numerous peaks predominantly present during the active state (19 and 10 peptides/polypeptides, respectively), while others were observed only during hibernation (11 and 13). However, there were peptides and/or polypeptides or their fragments present irrespective of the activity states (49 and 18). The intensity of fourteen peaks that correspond to previously identified neuropeptides varied in the brain of active snails compared to those of hibernating animals. Among those the intensity of eight peptides increased significantly in active animals while in hibernated animals the intensity of another six peptides increased significantly. A new peptide or peptide fragment at m/z 1110.7 was identified in a brain of the snail with the following suggested amino acid sequence: GSGASGSMPATTS. This peptide was found to be more abundant in active animals because the intensity of the peptide was significantly higher compared to hibernating animals. In summary, our results revealed substantial differences in the peptide/polypeptide profile of the brain and hemolymph of active and hibernating snails suggesting a possible contribution of peptides in the process of hibernation.
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O09_10 Adipokinetic peptides increase effectivity of insecticides

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Adipokinetic hormones (AKHs) are insect neuropetides controlling stress situations including those elicited by insecticide treatments. The effect of Pyrap-AKH on the mortality of the firebug Pyrrhocoris apterus treated by the insecticides with different mode of action including those eliciting the oxidative stress was studied. Co-application of the insecticides with 80 pmol Pyrap-AKH induced a significant increase in the bug mortality compared to the insecticide alone. Injection of the insecticides elicited significant increase of the AKH level in CNS and the haemolymph, which indicates an immediate involvement of AKH in the stress response. The enhanced effect of the insecticides by AKH treatment probably results from the stimulatory role on bug metabolism: the carbon dioxide production was increased significantly both after the insecticide treatment (vs. control) and after AKH plus insecticide co-treatment (vs. insecticide treatment alone). The AKH elevation of metabolism could intensify the action of insecticides by their faster penetration into tissues and by stimulation of metabolic turnover that results in enhancing of the insecticide-elicited mortality. A role of AKH in the oxidative stress pathways was studied as well.
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O09_11 Spectrum of diacylglycerols and fatty acids mobilized by adipokinetic hormones

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Insect energy metabolism, predominantly activation of lipids, is controlled by adipokinetic hormones (AKH). The diacylglycerols (DG) molecular species and their fatty acids (FA) composition were investigated by electrospray mass spectrometry (ESI-MS) in haemolymph of Locusta migratoria and Pyrrhocoris apterus after application of corresponding AKHs. Mobilization of DGs from fat body after injection of AKH was not uniform. AKHs mobilized the DGs selectively with preference of those possessing the C18 and C16 FAs, especially 18:1 in L. migratoria and 18:2 in P. apterus. The fat body FAs with carbon chain longer than 18 did not participate in the mobilization. In L. migratoria Locmi-AKH-I preferred release of DGs with unsaturated FAs, while AKH-II and AKH-III stimulated DG release with saturated FAs. In P. apterus significant quantitative differences between Pyrap-AKH-I and Peram-CAH-II were also shown. The metabolically active C16 and C18 FAs were preferentially absorbed from the linden seeds and accumulated in the FB. Heterologous assays suggested that activation of DGs and/or FAs was rather species- than hormone-specific.
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O09_12 Drosophila male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female

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Quiescence or a sleep-like state is a common and important feature of the daily lives of animals from both invertebrate and vertebrate taxa, suggesting that sleep appeared early in animal evolution. Recently, Drosophila melanogaster has been shown to be a relevant and powerful model for the genetic analysis of sleep behaviour. The sleep architecture of D. melanogaster is sexually dimorphic with females sleeping much less than males during daytime, presumably because reproductive success requires greater foraging activity by the female as well as the search for egg-laying sites. However, this loss of sleep and increase in locomotor activity will heighten the risk for the female from environmental and predator hazards. In this study, we show that virgin females can minimize this risk by behaving like males with an extended afternoon "siesta". Copulation results in the female losing 70% of daytime sleep and becoming more active. This behaviour lasts for at least eight days after copulation and is abolished if the mating males lack sex peptide (SP), normally present in the seminal fluid. Our results suggest that SP is the molecular switch that promotes wakefulness in the post-mated female, a change of behaviour compatible with increased foraging and egg-laying activity. The stress resulting from SP-dependent sleep deprivation might be an important contribution to the toxic side-effects of male accessory gland products that are known to reduce lifespan in post-mated females.
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O09_13 Study of two putative 5-HT G protein-coupled receptors in the desert locust (Schistocerca gregaria)

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The desert locust, Schistocerca gregaria, shows an extreme, density dependent phenotypic plasticity between an innocent solitary and a swarming gregarious phase. The phase transition is a multilayered process with remarkable changes in several aspects of the physiology and the morphology of the locusts. However the first thing that changes is their behaviour. Within two to four hours of crowding, behaviour switches from a strong reciprocal repulsion in solitary locusts to coherent aggregation and more active behaviour in gregarious ones. Phase transition is the reason why desert locusts make such huge, devastating swarms and the whole multi-stage process thus depends upon the simple behavioural choice of avoiding each other or grouping together.
Anstey et al. (2009) showed that serotonin (5-hydroxytryptamine (5-HT)) is both sufficient and necessary for induction of the behavioural gregarization in the desert locust and thus is a crucial factor in the gregarization process. Our research focusses on the 5-HT receptors that are important in mediating the 5-HT signal during the gregarization process. We successfully picked up fragments of two putative 5-HT G-protein-coupled receptors in the desert locust and investigated their expression patterns. We also investigated the role of these receptors in the behavioural gregarization using RNA interference.

O09_14 Beetles are an excellent source for novel members of the adipokinetic peptide family

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With more than 300 000 described species, Coleoptera is the most species-rich and diverse order of the class Insecta. Taking this fact together with the knowledge about the structural biodiversity of adipokinetic peptides (AKHs) in insects, it is not surprising that we found AKHs with novel primary sequences in representatives of two families, Scarabaeidae (25 000 species world wide) and Coccinellidae (4 500 species). The corpora cardiaca (CC) of the flightless dung beetle Circellium bacchus contain two AKHs: one is identical to an AKH found previously in other scarabaeid beetles and is denoted Scade-CC-I (pGlu-Phe-Asn-Tyr-Ser-Pro-Asp-Trp amide); the other peptide which we name Cirba-AKH is novel and has the sequence pGlu-Phe-Asn-Phe-Ser-Ala-Gly-Trp amide, thus, as other scarabaeid AKHs not Ile/Leu/Val at position 2 but an aromatic amino acid, Phe.
The CC of the invasive, multi-coloured Asian harlequin ladybird beetle, Harmonia axyridis, who has very recently also invaded South Africa, contain the novel octapeptide, denoted Harax-AKH: pGlu-Ile-Asn-Tyr-Ser-Thr-Gly-Trp amide. Surprisingly, both peptides, Cirba-AKH and Harax-AKH, are structurally quite close "relatives" of locust adipokinetic hormones: Locmi-AKH-II: pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp amide; Schgr-AKH-II: pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp amide.
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O10_01 Differential effects of GH and IGF-I in sea bream cultured myocytes

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Growth hormone (GH) and insulin like growth factors (IGFs) exert an important role on muscle growth regulation including proliferation, differentiation and metabolism. These effects are general on vertebrates although information in fish muscle is less available.
We have previously utilized trout and sea bream myocytes in culture (Castillo et al., 2002 and Montserrat et al., 2007) to investigate insulin and IGFs effects on growth, metabolism and receptor signal transduction in fish muscle (Codina et al., 2008). The technique is useful because it permits to investigate myocyte response to specific treatments avoiding the effects of the endocrine systemic regulation.
We have investigated the effects of GH in comparison to IGF-I in this same cell model. By western blot analysis we have measured GH stimulation on Akt phosphorylation. No differences were observed in AKT response at early days of culture development (day 2). However, a dose response of GH action was detected at the myotube stage indicating an activation of this signalling pathway. These results are in agreement with the effects of IGF-I on AKT, where we demonstrated that phosphorylation response was maintained through the myocyte culture or even higher effect was obtained when muscle cells were differentiated.
We have also studied the stimulatory effects of GH and IGF-I on muscle proliferation, measured as the presence of Proliferating Cell Nuclear Antigen (PCNA) positive cells quantified by immunocitochemistry. Preliminary data demonstrate that both IGF-I and GH stimulate myocytes proliferation. However, in all conditions tested, IGF-I shows higher effects than GH on PCNA activation. These results can help to understand the differential effects of these endocrine factors as well as the independent role of IGF-I in muscle growth regulation.
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O10_02 Molecular cloning and expression analysis of bullfrog soluble form-like prolactin receptor

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Actions of PRL are mediated through its receptor, a member of the superfamily of single transmembrane cytokine receptors. Previously, we obtained a cDNA encoding a mammalian long form-like bullfrog (bf) PRL receptor (PRLR). In this study, novel isoform of bfPRLR cDNA was cloned from the tail fin of tadpoles at metamorphic climax. The predicted molecule consisting of 242 amino acids lacked both the transmembrane domain and the intracellular domain. This indicated that it structurally corresponded with a soluble form PRLR reported in mammals. The expression of this bfPRLR isoform mRNA in the organs and tissues of tadpoles at stages XVII and XX and adult frogs was analyzed by RT-PCR. The organ and tissue distribution pattern of this isoform mRNA was similar to that of long form bfPRLR mRNA. To determine the binding characteristics of the soluble form-like bfPRLR isoform, competitive assay was conducted employing 125I-labeled and unlabeled PRL and growth hormone of the bullfrog origin. The specific binding of PRL to the buffer-soluble protein fraction, but not to the membrane fraction, both obtained from the COS7 cells in which the newly identified bfPRLR isoform had been transiently expressed, was observed. This indicates that the bfPRLR isoform exists in a soluble form and not in a membrane-bound form. Likewise, the medium in which the bfPRLR isoform cDNA-transfected COS7 cells had been incubated was revealed to possess an ability to specifically bind PRL, suggesting that this isoform protein was released from COS7 cells into the medium.

O10_03 Possible mechanisms of developmental alterations in zebrafish embryogenesis by morpholino knockdown of the glucocorticoid receptor

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In zebrafish, eggs contain both cortisol deposited from the maternal circulation and the maternal mRNA for the glucocorticoid receptor (gr mRNA) which, during the cleavage period, is transferred from the ooplasm inside the initial blastomeres. This transcript is available for translation till 8 hours post-fertilization (hpf), as found by qRT-PCR, is no longer detectable by whole-mount in situ hybridization (WMISH) at 9 hpf and is replaced by zygotic gr mRNA from 12 hpf onwards with mainly cephalic and caudal localizations. A reverse genetic approach was applied by morpholino knockdown of translation (ATGMO) of both maternal and zygotic gr transcripts, and by missplicing morpholino (splicMO) to block post-transcriptionally the zygotic transcript alone. ATGMO (but not splicMO) treatment increased apoptosis in developing embryos and produced cephalic and caudal malformations in embryos and 5-dpf larvae. These effects were rescued by co-treatment of morphant embryos with rainbow trout gr2 mRNA. Pangenomic microarray analysis revealed that 114 and 37 highly expressed transcripts were up- and down-regulated, respectively, by maternal GR protein deficiency in 5-hpf embryos. Similar alterations were found at 10 hpf. These effects were confirmed by RT-PCR of 4 up- (casp8, centaurin-a1, grp1 and igf2a) and 2 down-regulated transcripts (mcm6 and anxA2b) evaluated at 1, 2, 4, 8, 12, 24 and 48 hpf. As the contents of transcripts were modified already at 4 hpf, it seems that the lack of GR affects both ways the molecular machinery for the degradation of maternal mRNAs. However, also the corresponding zygotic transcripts were similarly affected at later times (12-48 hpf), although their sites of expression were not changed, as shown by WMISH. In silico analysis evidenced GRE sequences in the promoters of the encoding genes, except in centaurin-a1. Interestingly, in the igf2a promoter, none of the 15 CpG sites of a CpG island were methylated at 8 hpf, when zygotic transcription starts but, in 6-dpf larvae, three sites were methylated in controls but not in morphants. These results indicate that gr mRNA could be involved in the epigenetic programming of zebrafish embryogenesis.

O10_05 Antisense Morpholino-mediated knockdown of type 3 iodothyronine deiodinase in the embryonic zebrafish (Danio rerio)

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Although the pivotal role of thyroid hormones (THs) in vertebrate development has been widely documented, little information is available on their role in early embryogenesis. Early teleost embryos rely on the maternal TH deposit in the egg yolk, consisting predominantly of T4. In adult zebrafish, as in most other vertebrates, a tight control of intracellular T3 levels is achieved by the combined action of the three types of iodothyronine deiodinases (D1-D3). Recently, our team investigated the importance of the two Ds capable of T4 to T3 conversion, D1 and D2, during early embryogenesis in zebrafish. This research showed that D2 is the major contributor to TH activation in developing zebrafish embryos (1). We have now inhibited the expression of the TH inactivating enzyme, D3, by antisense morpholino-mediated knockdown. To estimate the impact of D3 knockdown on the rate of embryonic development, three morphological indices were measured: otic vesicle length, tail length and pigmentation index. The increase in otic vesicle length and the decrease in tail length and pigmentation index were all indicative of an aberrant development. Additional morphological abnormalities observed in some embryos were pericardial edema, flattening of the head, curved tail and an overall decrease in size. To control for a possible induction of a p53-dependent cell death pathway, which is the major off-targeting effect of morpholinos, we repeated the experiments with concurrent knockdown of p53. Since this did not rescue the phenotype, we can conclude that the observed defects are indeed D3-dependent. Our present results indicate that next to intracellular TH activation also local inactivation by D3 is essential for normal early development in zebrafish embryos.

(1) Walpita, C.N., A.D. Crawford, and V.M. Darras, Combined antisense knockdown of type 1 and type 2 iodothyronine deiodinases disrupts embryonic development in zebrafish (Danio rerio). Gen Comp Endocrinol, 2009. 166(1): p. 134-41.

O10_06 Comparative analysis of several neurochemical marker sin the trout developing hipothalamus-hypophysial system, with special attention to the pituitary

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It is well known that in adult teleost fishes, as in other vertebrates, the activity of the pituitary gland is controlled by numerous neuroactive substances that are synthesized by specific neuronal populations and reach the pituitary. However, lesser is known about the control of the brain on the pituitary gland during the transition from larval to juvenile period, in which drastic changes in its life cycle occurs (e.g. feeding, mobility, growth).Therefore, by using immunohistochemical techniques we have studied the expression pattern of galanin (Gal), Met-enkephalin and glutamate acid decarboxylase (GAD, the synthesis enzyme of GABA) in the hypothalamus-hypophysial system of alevins and juveniles of brown trout (Salmo trutta fario). We have also analyzed the expression of Pax6, a transcription factor involved in the pituitary development, and of a proliferation marker (PCNA, proliferating cell nuclear antigen), from early developmental stages.Scarce Gal-immunoreactive (ir) fibres coursed in the neurohypophysis of alevins while in early juveniles, these fibres were abundantly observed in the shallow interdigitations of the neural lobe penetrating the proximal pars distalis (PPD) of the adenohypophysis, and in the neurohypophysis, which was not interdigitated with the pars intermedia. At these developmental stages, Gal-ir perikarya were present in the preoptic nucleus, lateral tuberal nucleus, and lateral and posterior recess nuclei (Rodríguez et al., 2003, JCN 465:263). In late alevins and early juveniles, numerous Met-enkephalin-ir cells were observed in all divisions of the adenohypohysis, and scarce fibers were present in the interdigitations of the neural lobe with the PPD; a strong labelling was observed in cells and fibres around the preoptic recess, in the lateral tuberal nucleus, and lateral and posterior recess nuclei. At those developmental stages, a heavy GAD expression was present throughout the preoptic area, anterior and posterior hypothalamus and numerous GAD-ir fibres coursed through the neural lobe of the hypophysis. In early embryos, a strong Pax6 expression was detected in the Rathke´s pouch that forms the adenohypophysis, and numerous PCNA-ir cells were also observed in the same location. The Pax6 expression continued in the adenohypophysis of early alevins, but decreased in later stages. PCNA-ir cells were abundant in the neurohypohysis of late embryos and early alevins, but their density in the adenohypophysis decreased through development. These data suggest that both neuropeptides (galanin, Met-enkephalin) and a classic neurotransmitter (GABA) may be implicated in the control of the pituitary gland acting in the synthesis and/or release of hormones in larvae and juveniles trout. On the other hand, the observed Pax6 and PCNA expression patterns indicate that Pax6 could be related with pituitary differentiation and adenohypophyseal cell types determination.
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O10_07 Regulation of LXR, its target genes and fatty acid transporters by insulin, growth hormone and tumour necrosis factor-α in rainbow trout myocytes (Oncorhynchus mykiss)

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There is increasing evidence that correct dietary energy regulation, specifically good lipid homeostasis, may be crucial in maintaining the health and quality of farmed fish. However, in the last years, few studies have focused in the endocrine control of fish lipid metabolism and the molecular mechanisms underlying it. The transcription factor, liver X receptor (LXR), has recently been described in salmonids, and some of its target genes, related to lipid metabolism, have been characterized in vitro in trout myocytes. This study characterizes LXR and fatty acid (FA) uptake in trout myocytes analyzing the LXR target gene and FA transporters expression, the gene profile during myocyte development, and the LXR response to hormones. Furthermore, the endocrine regulation of FA transporters (FATP1 and CD36) mRNA expression and the FA uptake is also analyzed in rainbow trout myotubes. The differentiated myocytes were incubated with insulin and growth hormone (GH) for 3h, 6h and 18h, and with tumour necrosis factor-α (TNFα) treatments for 24h. Samples were also obtained in various development stages of cell differentiation to study the evolution of gene expression.
The present study demonstrates that LXR, peroxisome proliferator-activated transporter-α (PPARα,) and ATP-binding cassette transporter A1 (ABCA1) expression is regulated by both insulin and GH, and that insulin stimulates fatty acid synthase (FAS) and PPARα, while GH stimulates lipoprotein lipase (LPL) expression. Regarding the FA transporters, insulin decreased FATP1 and increased CD36 mRNA levels while GH decreased FATP1 in trout myotubes. Insulin and insulin growth factor-I (IGF-I) stimulated FA uptake (measured as 14C-Oleic acid uptake) in these cells. TNFα, did not modulate LXR gene transcription but increased CD36 expression in trout myotubes.
These results suggest that LXR may play a lipogenic role through insulin stimulation and a tendency to promote anabolic effects through GH on trout myocytes. We show, for the first time in fish, that insulin, among others, is an important regulator of the FA transporters FATP1 and CD36 mRNA in vitro. Besides, we also shown that insulin and IGF-I increase FA uptake the through the PI3K/Akt signaling pathway. These findings can contribute to the better understanding of the control of lipid metabolism in fish muscle.
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O10_08 Gel based proteomics without a genome: an EST based approach in Schistocerca gregaria

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The enigmatic phenomenon of phase polyphenism in the desert locust (Schistocerca gregaria) has been studied in many ways. Both behavioural and morphological studies have been performed over the years. Each yielded specific insights in this biological mechanism that has enormous consequences from a humanitarian as well as an economical point of view.
The lack of genomic information in Schistocerca hampers the application of rapidly evolving state of the art technology to locust studies. For example, differential proteomics could be used to analyze differences at the proteome level in both the solitary and gregarious phase, hereby revealing the players that are taking part in phase polyphenism and transition. Without genomic information however, it is difficult to analyse proteomics data in a straightforward manner by means of mass spectrometric approaches.
This study provides an approach to identify protein spots from gel based proteomics studies on the prothoracic glands of the desert locust. The prothoracic gland takes part in ecdysteroid production which is necessary for insects to molt. However, there are clear differences in the morphological structure and presence of the glands in both phases, which make this an interesting tissue to study in the frame of phase polyphenism. We combine the classical identification workflow of tryptic digests with a bioinformatics software package (Peaks studio, Bioinformatics Solutions inc.). Together with partial genomic information in the format of an Schistocerca EST-library and the DE NOVO sequencing of tryptic peptides we have been able to identify a significant part of visualised proteins and hereby provide a first step to implement modern proteomics technologies in locust research.

Abstracts

P01_01 Neuroanatomical characterization of the kisspeptin systems in the brain of european sea bass (D.labrax)

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Kisspeptins are a family of peptides encoded by the KISS1 gene, in mammals, and kiss1 and kiss2 in fish. They were first identified by their capacity to inhibit tumor metastasis through the receptor GPR54, also termed KISSR. Nowadays, it is largely demonstrated that the kiss system plays an essential role in the neuroendocrine control of puberty and reproduction in several mammalian species, stimulating GnRH secretion at the hypothalamus. In the sea bass, an economically-important marine teleost, kiss1 and kiss2 coding sequences and those of their cognate receptors have been recently identified. Thus, this is the first study aimed to elucidate the neuroanatomical distribution of the cells expressing the two kiss genes and their receptors in adult sea bass brain by in situ hybridization. Our findings indicate the mediobasal hypothalamus, notably the nucleus of the lateral recess, as an important area of expression of both kiss1 and kiss2 genes. Furthermore, the kiss1expressing cells are also located at the level of the habenular region. Interestingly, the kisspeptin receptors-expressing cells are mostly placed in the same regions as the corresponding ligands. These results are in line with those obtained in the other fish species investigated so far. In conclusion, this work represents the first neuroanatomical analysis of the kisspeptin systems in the sea bass brain and suggests a putative involvement of the kisspeptins in the control of reproduction in this species.

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P01_02 Identification of neuropeptide Y-related receptors potentially involved in the coordination of reproduction and energy balance in the Pacific oyster Crassostrea gigas

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The pacific oyster Crassostrea gigas exhibits an annual cycle of reproduction. The regulation of this cycle requires the integration of multiple outdoor signals leading to the secretion of (neuro)hormones, such as the neuropeptide Y (NPY), which is involved in the coordination of energy flows in relation with food intake and reproduction in various animal models. As most neuropeptide hormones, the neuropeptide Y binds to receptors of the G protein-coupled receptor (GPCR) family. Screening of the "GigasDatabase" (1) containing up to 30 000 independent sequences from C. gigas resulted in the retrieval of 5 GPCRs related to neuropeptide Y receptors. Studies of these receptor genes by qRT-PCR showed they are mostly expressed in the gonad, the digestive gland and the visceral ganglia. We also investigated the expression of these receptors in the ganglia of fed and fasted animals and found that three of the five receptors examined were over-expressed in fasted oysters. This pattern of expression is in agreement with the potential implication of NPY/receptor couples in the coordination of energy balance in this organism. Since phylogenetic analyses indicate that the various oyster NPY-related receptors display distinct degrees of divergence with functionally characterized NPY receptors from other species, it remains to determine whether the NPY-related neuropeptide already identified in C. gigas actually binds to these receptors. To address this issue, a reverse pharmacology approach is underway.

(1) BMC genomics 2009, 10:341

P01_03 Changes in mRNA levels of receptors for TRH and dopamine in the bullfrog pituitary during metamorphosis

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The hypothalamic regulation of the release of PRL from the pituitary of amphibians, inhibitory control and stimulatory one appear to be exerted mainly by dopamine and thyrotropin-releasing hormone (TRH), respectively. In the bullfrog larvae, plasma PRL concentrations are relatively low during premetamorphosis and prometamorphosis, rise gradually through early and mid-climax, and reach a maximum at the beginning of late-climax stage. Recently, we cloned three distinct cDNAs for isoforms of D2 dopamine receptor (D2R). We also showed that the inhibitory effect of dopamine on the release of PRL from the bullfrog pituitary gland is mediated through D2R, although it is not clear which of the isoforms mediates the action of dopamine on the PRL cells. In this experiment, the changes in mRNA levels of receptors for TRH and dopamine in the bullfrog pars distalis during metamorphosis were investigated. For this purpose, isolation of cDNAs encoding TRH receptors (TRHRs) from the bullfrog brain was attempted. As a result, three distinct cDNAs for TRHRs were obtained and the putative three TRHR subtypes were designated as fTRHR1, fTRHR2 and fTRHR3. RT-PCR analysis revealed that the fTRHR3 mRNA expression was gradually elevated from prometamorphic stages toward climactic stages, whereas both mRNAs for fTRHR1 and fTRHR2 were scarcely expressed throughout metamorphosis. On the other hand, mRNAs for three D2R isoforms were invariably expressed, but no obvious differences were noted in their expression pattern throughout the metamorphic period. Possible involvement of fTRHR3 in the elevation of PRL levels during metamorphic climax was suggested.

P01-04 Evolution of Melanocortin Receptors: Studies on the genome of the Elephant Shark, Callorhinchus milii

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Based on the 2R Hypothesis the chordate genome has undergone at least two genome duplication events over the past 500 million years. In this scenario if one copy of a gene were present in the ancestral protochordate lineage, then two copies of that gene could have been present in the genome of jawless vertebrates, and four copies of that gene my be present in extant gnathostomes. When this hypothesis is applied to the Melanocortin Receptor (MCR) gene family, two MCR genes have been found in the lamprey genome, and usually five MCR genes are found in the genomes of tetrapods and some bony fish. Since the MC2R gene and the MC5R gene appear to be the result of a gene duplication, the 2R hypothesis would appear to explain the radiation of this gene family in gnathostomes. The apparent exception to this generalization may be the cartilaginous fish. Only three MCR genes have been cloned from the genome of the spiny dogfish. Is this outcome because some MCR genes were missed in these cloning attempts, or have cartilaginous fish lost some MCR genes. The availability of the elephant shark (Callorhinchus milii) genome project makes it possible to evaluate these possibilities. Our search of the genome has revealed the presence of three potential melanocortin genes. These genes were synthesized (GenScript) and transfected into CHO cells. In this study we will present the results of melanocortin ligand activation studies of these putative receptors and discuss the evolutionary implications of these observations.

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P01-05 Neuroanatomical localization of kiss ligands and receptors in zebrafish brain

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Kisspeptins, the products of the KISS1 gene, have recently emerged as key players in the regulation of the reproductive axis in mammals. They stimulate GnRH release at the hypothalamic level through the receptor KISS1R. Due to genome duplication and gene loss events, up to three isoforms of both kisspeptin ligands and receptors have been reported in non-mammalian vertebrates. In teleost fish, which have two kiss and two kiss receptors genes, the anatomical characterization of these systems remains elusive and their interactions with the GnRH systems are not established. This study aimed at defining the anatomical localization of kiss1 and kiss2-expressing cells as well as that of the receptors kiss1r and kiss2r by in situ hybridization in adult zebrafish brain. Our findings show two separated neuronal systems: kiss1 neurons were only localized in the habenular region, whereas kiss 2-expressing cells were mostly observed in the mediobasal hypothalamus. In addition, the organization and projections of kiss1- and kiss2-containing cells were studied by immunohistochemistry using specific antibodies generated against the precursors of the zebrafish kiss isoforms. Lastly, the potential relationships between the kiss and GnRH systems, as well as estrogen and leptin receptors are discussed. Altogether, our results provide new evidence for the involvement of the kiss systems in the modulation of the reproductive axis in zebrafish.
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P02-01 Analysis of the hemimetabolous pea aphid genome for nuclear receptors involved in the ecdysone signaling cascade

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Screening of the Acyrthosiphon pisum genome1 indicates the presence of 19 different nuclear receptors, representing all of the seven known subfamilies (NR0-NR6) in this hemipteran species. After identification and, where necessary, manual corrections, phylogenetic trees were made based on this set of nuclear receptors and those of other species from some of the major insect orders such as Lepidoptera, Diptera, Hymenoptera, Coleoptera, and also Crustaceans. The analysis of sequence data and phylogenetic analysis revealed a strong conservation in the class of Insecta and shows the presence of all major NR members of the ecdysone signaling cascade such as the 'early' genes EcR and E75, the 'early-late' genes HR3, HR4,
HR38 and E78 and also FTZ-F1 which is responsible for passing the signal on to the 'late' genes. This research also showed some differences however towards the holometabolous insects. Three nuclear receptors, the NR1 subfamily member and ecdysone-induced NR HR96, the NR2 subfamily member PNR-like and the NR0 subfamily member Knirps, which have previously been identified in some other insect genomes, were not found in the A. pisum genome.
1 The International Aphid Genomics Consortium. 2010. Genome sequence of the pea aphid Acyrthosiphon pisum. PLoS Biology 8: e1000313.
This work was supported by the Special Research Fund of Ghent University, and the Fund for Scientific Research (FWO-Vlaanderen, Brussels)

P02-02 Quillaja saponaria saponin is causing an anti-ecdysteroid action in insect cells that may be explained by cytotoxicity and permeation

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In this project we studied the cytotoxic effects of the triterpenoid (five-ringed) saponin of the oleanane-glucuronide-type from the soapbark tree Quillaja saponaria. In a first series of experiments, we transfected S2 observed a concentration dependence for the ecdysteroid responsiveness of the cells when these were transfected with an EcR-reporter construct. There was no activation of the EcR-signaling, but we demonstrated a typical loss of ecdysteroid signaling at low concentrations with a respective IC50 of 1.7 µM and 0.68 µM. A concentration-dependent change in cell survival was observed when insect cells of Drosophila melanogaster (S2, embryo) and Bombyx more (Bm5, ovary) were incubated. A loss of 50% of cell survival (LC50) was observed at 5.1 µM and 1.7 µM, respectively, in an MTT bioassay. The cell permeation was also confirmed in a trypan blue assay. To explain the latter anti-ecdysteroid action we investigated be explained by the cytotoxic and permeation action. In addition, it was of interest that saponin effects were counteracted with addition of cholesterol to the cell culture medium. Finally, caspase 3-like measurements showed that Quillaja saponin could not induce nuclear events as apoptosis in treated cells. Our results suggest that the loss of ecdysteroid responsiveness after the addition of Quillaja saponin could thus be result of a cytotoxic permeation action and not by interacting with the EcR.
This work was supported by the Special Research Fund of Ghent University, and the Fund for Scientific Research (FWO-Vlaanderen, Brussels)

P02-03 Comparing activity of non-steroidal ecdysone agonists between dipteran and lepidopteran insects using cell-based EcR reporter assays

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Diacylhydrazine (DAH) analogs have been developed successfully as a new group of insect growth regulators, called ecdysone agonists or molting accelerating compounds. These DAHs have been shown to manifest their toxicity via interaction with the ecdysone receptor (EcR) in susceptible insects as does the natural insect molting hormone 20-hydroxyecdysone (20E). A notable feature is their high activity and specificity, particularly against lepidopteran insects, raising the question whether non-lepidopteran-specific analogues can be isolated. However, for the discovery of ecdysone agonists that target other important insect groups such as Diptera, efficient screening systems that are based on the activation of the EcR are needed.

In this study, we developed a dipteran-specific reporter-based screening system with transfected S2 cells of Drosophila melanogaster to discover and evaluate compounds that have ecdysone agonistic or antagonistic activity. A library of non-steroidal ecdysone agonists containing different mother structures with DAH and other related analogues such as acylaminoketone (AAK) and tetrahydroquinoline (THQ) was tested. None of all tested compounds was as active as 20E. This is in contrast to the very high activity of several DAH and AAK congeners in lepidopteran cells (Bombyx mori-derived Bm5 cells). The latter agrees with a successful docking of a DAH, tebufenozide, in the binding pocket of the lepidopteran EcR (B. mori), while this was not the case with the dipteran EcR (D. melanogaster). Of note was the identification of two THQ compounds with activity in S2 but not in Bm5 cells. Although marked differences in activity exist with respect to the activation of EcR between dipterans and lepidopterans, there exists a positive correlation (R = 0.724) between the pLC50 values in S2 and Bm5 cells. In addition, it was found through protein modeling that a second lobe was present in the ligand binding pocket of lepidopteran BmEcR but was lacking in the dipteran DmEcR protein, suggesting that this difference in structure of the binding pocket is a major factor for preferential activation of the lepidopteran over the dipteran receptors by DAH ligands.

The present study confirmed the marked specificity of DAH and AAK analogues towards EcRs from lepidopteran insects. THQ compounds did not show this specificity, indicating that dipteran-specific ecdysone agonist-based insecticides based on the THQ mother structure can be developed. The differences in activity of ecdysone agonists in dipteran and lepidopteran ecdysone reporter-based screening systems are discussed.

This work was supported by the Special Research Fund of Ghent University, and the Fund for Scientific Research (FWO-Vlaanderen, Brussels)

P02-04 The ecdysone receptor in a neuropteran (Chrysoperla carnea) and dermapteran insect (Forficula auricularia) used in biological control: sequencing and structural modeling

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During the last few decades, the ecdysone receptor has emerged as a possible target site for novel insecticides that act on selective biochemical sites present in specific insect groups. In this project we worked with two insect species that are used as natural enemies in biological control programs in modern agriculture and that belong to two distinct insect orders that have not been investigated in detail so far: namely the green lacewing Chrysoperla carnea (Neuroptera) and the common earwig Forficula auricularia (Dermaptera). We investigated the ligand binding domain (LBD) of their ecdysone receptor and their position in the molecular phylogeny. In second, we constructed a structural model of the LBD and herein we docked ecdysteroid hormone and also the dibenzoylhydrazine-based ecdysone agonist methoxyfenozide, that is used as selective insecticide in IPM to control Lepidopteran pests. The data are discussed in relation to insect specificity, molecular evolution and selective toxicity.

P03-01 Impairment of food intake and lipid metabolism by DEHP in zebrafish

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Toxicological and epidemiological studies have suggested the involvement of different environmental chemicals in increasing metabolic disorders. Evidence points to endocrine disruptors that interfere with cellular biology of the adipose tissue and central hypothalamic-pituitary-gonadal/adrenal axis derailing the pathways involved in homeostatic and metabolic control.
DEHP is an ester of phthalic acid utilized for the production of several plastic products. For this plasticizer, an adverse effect on lipid metabolism has been described in rats, in terms of increase of fatty acid oxidation and lipid mobilization atrophy. Nevertheless, very few information are still available regarding DEHP potential impairment of food intake. Thus, in this study, DEHP capability to induce metabolic disorders was verified by relating its effect on lipid metabolism and food intake in zebrafish.
A number of metabolic regulators critical for lipid metabolism such as PPARa, SREBP and CB1 were analyzed in zebrafish liver by Real time PCR. Food intake was measured and the gene expression of key molecules involved in the central control of appetite (NPY, orexin, CB1, SREBP and leptin) was tested.
The results indicated an estrogenic and adverse effect of DEHP on feeding behaviour which is maybe due to altered levels of signals controlling hepatic de novo fatty acid synthesis. In particular, the lowest dose tested resulted to be the most dangerous thus stressing the importance to focus on the regulation on pollutants emission in the environment.

P04-01 Insights into the mechanisms involved in the exercise-enhanced white muscle growth in adult zebrafish Danio rerio

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Exercise stimulates growth in salmonids but a very limited amount of information is available on the mechanism(s) by which exercise potentiates growth. Recently, we have investigated the potential of adult zebrafish to serve as a model for exercise-induced muscle growth. Therefore the optimal swimming speed at which zebrafish swims most efficient was quantified and applied in a long-term swimming experiment with zebrafish (n=83) swimming for 6h/day for 5 days/week over a 4 week period for comparison with zebrafish (n=83) that rested. For the first time a highly significant exercise-induced growth was demonstrated in adult zebrafish. Zebrafish that swam increased their total body length by 5.6% and body weight by 41.1% as compared to resting fish, comparable or even higher than reported for adult salmonid fish.
The main aim of the present study is to provide insights into the mechanisms involved in exercise-induced muscle growth. White muscle gene expression was examined by investigating the effects of exercise on the expression of marker genes involved in muscle development, proliferation and differentiation under regulation of myogenic factors. The long-term swimming experiment was repeated to raise samples for histological analysis of muscle fiber size, number and area to quantify and qualify changes in muscle development (hypertrophy and/or hyperplasia). We show that zebrafish can be used as a vertebrate exercise model for enhanced growth, with implications in both basic, biomedical and applied sciences, such as aquaculture.

P05-01 The probiotic Lactobacillus rhamnosus, increase fecundity in Danio rerio

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Probiotics has attracted considerable attention in recent years since their ability to modulate immunity but, at present, the possible effects of probiotics on reproduction and on mechanisms controlling oogenesis are still unexplored.
In this study, the effects of ten days treatment with the probiotic Lactobacillus rhamnosus IMC 501, as feed additive, were examined on zebrafish, Danio rerio, ovarian follicle maturation and ovulation. The stimulating role of probiotic on follicles maturation and ovulation process was indicated by the higher number of ovulated eggs in vivo and by the higher GVBD rate obtained by the in vitro maturation assay. The stimulation of oocyte maturation was associated with the increase of LH receptor (LHR), the 20β-hydroxysteroid dehydrogenase (20β-HSD), the CyclinB and the isoform β of the membrane receptor for the Progesterone (mPRβ). Concomitantly, the transcription decrease of genes codifying for local factors preventing oocyte maturation like transforming growth factorβ1 (TGFβ1), growth and differentiation factor9 (GDF9) and bone morphogenetic proteins15 (BMP15), was evidenced. In addition, the cyclooxygenase2A (Cox2A) gene expression, an enzyme involved in prostaglandin biosynthesis, was significantly induced by the probiotic administration.
Concurrently, the embryos vitality was significantly improved in embryo from L. rhamnosus treated females. The stimulatory role of L. rhamnosus in zebrafish follicle maturation, fecundity and egg quality, evidenced in this study indicated the great potency of this feed additive on ovarian physiology and embryo development evidencing the high potentiality of this probiotic on reproduction process improvement.

P05-02 Endocrine regulation of gonad maturation of pre-pubertal sea bass (Dicentrarchus labrax L.) kept under different light regimes and steroid treatments

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It has been shown that continuous light (LL) inhibits gametogenesis and prevents early puberty in male sea bass. Nevertheless, available information on the underlying mechanisms is scarce. The aim of this study was to elucidate the effects of long term administration of sexual steroids to pre-pubertal sea bass exposed either to simulated natural photoperiod (SNP) or to LL and to explore the possible hormonal mechanisms at pituitary and gonadal levels. Fish previously maintained under SNP and LL were implanted with steroid silastic pellets containing 0 (Sham, S0), 100 mg/g of testosterone (T) or 100 mg/g of 4-androsten-3, 11, 17-trione, a precursor of 11 Ketotestoeterone (11KT). The experience started in June and lasted ten months. Sham implanted fish exposed to SNP (control) showed a drastic reduction in type A spermatogonia (SPGA) and the rate of testicle developmental stages I+II (% I+II) at 107 days after implantation (DAI). This coincided with the first significant surge of plasma T and 11KT and follicle-stimulating hormone (FSH) both in plasma and pituitary. By contrary, LL exposed S0 fish had high and constant levels of SPGA and % I+II and constant low plasma levels of 11KT and FSH throughout all the experience. T levels behaved as in controls and exhibited a significant rise at 107 DAI. These results suggest that at the pituitary the effect of LL it could result in an inhibition of the synthesis and release of FSH which in turn, would block the synthesis and release of 11KT by the gonads, supporting the relevant role of this steroid on the regulation of spermatogenesis. Under SNP, T implanted fish displayed high number of SPGA and % I +II and low levels of 11KT and FSH. Gonad remained completely immature throughout the experience suggesting a down regulation of T on FSH synthesis and release. LL powered this effect and thus, T by itself could not reverse the inhibitory effects of LL on maturation. Conversely, 11KT accelerated testicular recrudescence and unblocked the LL inhibitory action. However, the rate of spermiating males (% IV-V) was lower than in controls likely because a weak down regulation effect of 11KT on
1 / 2 2010.06.02. 14:29 FSH at the pituitary level. These results suggest a relevant role of FSH at early stages of gametogenesis mediated by the 11-KT. Granted by EU Q5RS-2002-01801 and MEC AGL200604672

P05-03 Establishment and characterization of a primary culture of ovarian follicular cells for sea bass (Dicentrarchus labrax)

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Follicular somatic cells support oocyte development, where interactions between the germ cells and the surrounding follicular cells are critical for the successful maintenance of the reproductive activity. The absence of immortalized granulosa or theca cell lines in fish led us to develop a primary culture system that enabled in vitro studies of processes that take place within the ovary along the reproductive cycle. For this purpose, we optimized the isolation procedure as well as the culture conditions of sea bass ovarian follicular cells, and evaluated their behaviour at three different physiological temperatures (25ºC, 18ºC and 15ºC). To characterize this primary culture, the steroidogenic capability of the follicular cells was measured periodically along 16 days. For this aim, culture medium was collected every 4th day and levels of both testosterone and estradiol were determined through specific EIAs. Moreover, expression of several genes, typically characteristic of granulosa and theca cells, was tested to ensure that the cellular types present in the culture were those of the follicular layer.
Finally, to evaluate the suitability of this system for gene function studies several transient transfection conditions were tested. As a first qualitative approach, an expression vector containing the green fluorescent protein (GFP) under the control of the human cytomegalovirus promoter (CMV) was introduced into the cells through different transfection reagents: Lipofectamine (Invitrogen Corp), Fugene and Fugene 6 (Roche Diagnostics) and the conventional calcium phosphate method. GFP expression was monitored by means of a fluorescence microscope. The most efficient transfection methods (Fugene 6 and calcium phosphate) were verified by transfection of different plasmids containing the luciferase gene, and subsequent quantification of the luciferase activity. All together our results show that this primary culture could be a good in vitro model to study follicular cell physiology in fish, and a useful conspecific expression system for functional analysis of certain sea bass genes and promoters related with ovarian development.
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P05-04 Is the function of bank vole seminal vesicles affected by of 4-tert-octylphenol?

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In rodents, the secretion of seminal vesicles is important for metabolism, motility and surface properties of sperm. Thus, factors that influence function of the seminal vesicles have the potential to alter male fertility. This study was designed to evaluate the in vivo effects of 4-tert-octylphenol (OP), an estrogenic environmental compound, on the seminal vesicles of adult bank voles, seasonally breeding rodents. Mature males reared under short (6L:18D) and long (18L:6D) light cycles were orally administered with OP (200 mg/kg) or vehicle for 30 or 60 days. The expression and the presence of aromatase, estrogen receptor α (ERα) and androgen receptor (AR) proteins in the seminal vesicles were investigated by means of immunohistochemistry and Western blot analysis, respectively. Steroid hormone concentrations were measured in tissue homogenates using radioimmunological methods. Treatment with OP for 30 days had no discernible effect on seminal vesicle morphology, aromatase and sex steroid receptor expression when compared with controls. In males treated with OP for 60 days the seminal vesicles were significantly reduced in both size and weight. An increase in the stromal volume and reduction or lack of secretions in the lumen was observed as well as the mucosal folds surrounding the luminal areas were lined with cuboidal rather than columnar epithelium. Importantly, in voles kept under short light cycles more marked changes were observed. In the epithelium of OP-treated voles the expression of androgen receptor (AR) was reduced, whereas that of estrogen receptor α (ERα) was increased in comparison to control males. These alterations were also more evident in voles kept in short light cycles, whereas, significantly stronger immunostaining for aromatase was noticed irrespective of the light regime. Quantitative evaluation of the intensity of immunohistochemical staining, expressed as relative optical density (ROD), confirmed qualitative data. Moreover, the results from Western blot analysis of seminal vesicles homogenates confirmed the major observations from immunohistochemistry. Our results revealed that only long-term OP exposure is able to induce morphological alterations in the seminal vesicles of adult bank voles. Chronic OP administration decreased testosterone synthesis in adult males concomitantly with the increase in endogenous estrogen production, and expression of ERα was increased and the expression of AR was down-regulated. Thus, it seems likely that male reproductive tissue abnormalities result from altered androgen-estrogen balance but the exact mechanism needs to be investigated. Finally, it is also concluded that there might be a subtle difference in the sensitivity to OP between the voles kept in different light conditions.
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P05-05 Levels of vitellogenin-inhibiitng hormone (VIH) in hemolymph in relation to molting in the whiteleg shrimp, Litopenaeus vannamei

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In crustaceans, ovarian maturation is regulated by neurohormones according to their reproductive cycles. Vitellogenesisinhibiting hormone (VIH), which acts negatively on reproduction, inhibits vitellogenin (Vg) synthesis, is synthesized at the X-organ/sinus gland complex and secreted into the hemolymph. Along these lines, changes in VIH hemolymph levels provide basic information necessary for understanding reproductive mechanisms in Crustacea. In this study, we aimed to elucidate VIH hemolymph levels in the whiteleg shrimp, Litopenaeus vannamei, in relation to molting. A time-resolved fluoroimmunoassay (TR-FIA) method was developed for VIH using polyclonal antibodies raised against recombinant VIH of L. vannamei in order to measure VIH levels in the hemolymph. Molt stage was checked in sub-adults of L. vannamei and the hemolymph was collected for purposes of analysis. In addition, in order to examine VIH levels in the hemolymph in context of reproductive status, we developed a measurement system for Vg in the hemolymph; levels of Vg in the hemolymph were used as an index of ovarian maturation. Levels of VIH and Vg in L. vannamei hemolymph were then quantified, and their relative dynamics are discussed.

P05-06 Presence of pituitary adenylate cyclase activating polypeptide in human body fluids

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional and pleiotropic neuropeptide. Although PACAP was first described as a hypothalamic peptide influencing the functions of the pituitary gland, the peptide has numerous other effects throughout the nervous system and peripheral organs. Its endocrine actions and effects in reproductive organs are still in focus of research. PACAP plays important roles in spermato- and oogenesis, in implantation and in the development of the nervous system. We have previously shown the presence of PACAP in human serum and milk, but no data are available on the possible occurrence of PACAP in other body fluid samples. The aim of the present study was to determine, by means of mass spectrometry, whether PACAP is present in gynecological and andrological samples, such as amniotic fluid, follicular fluid, seminal plasma and in the utero-vaginal smear. Furthermore, PACAP was also investigated in human aqeous humor, vitreous body, cerebrospinal fluid, human nasal fluid and saliva. Samples were obtained from healthy adult volunteers during routine examinations and/or scheduled surgery. Our results show that PACAP38 is present in the seminal fluid and follicular fluid, but absent in normal cervico-vaginal and amniotic fluids. Furthermore, our results clearly show that PACAP is also present in the cerebrospinal fluid, but the characteristic peak representing PACAP38 was not found in other samples. The functional implications of these results await further investigation.
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P05-07 Involvement of cAMP/PKA and MAP kinase pathways on steroid synthesis stimulated by FSH in sea bass (Dicentrarchus labrax)

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Follicle-stimulating hormone (FSH) is essential to achieve follicular growth. Its action is exerted via interaction with its specific receptor, a transmembrane G-protein-coupled receptor. Activation of G proteins results in the regulation of a complex pattern of gene expression through different signaling cascades. In the present study the involvement of cAMP/PKA and MAP-kinase routes on FSH induced sea bass steroidogenesis was evaluated. Ovarian explants of one year old female sea bass were pre-incubated with different doses of the cAMP/PKA inhibitor Rp-cAMPs or the MAPK inhibitor PD-98059. Next, tissues were stimulated with sea bass recombinant FSH or the cAMP/PKA activator (8Br-cAMPs). After 20 hours culture at 21ºC the levels of estradiol (E2) and testosterone (T) were analyzed in the culture medium. Expression levels of StAR and CYP19A1 were as well evaluated by real-time PCR. Both, FSH and 8Br-cAMPs treatments significantly increased secretion of E2 and T to the culture medium. When the cAMP/PKA inhibitor Rp-cAMPs was added to the medium a marked decrease on T but not E2 levels was found. The MAPK inhibitor, PD-98059, either alone or in the presence of FSH or 8Br-cAMPs, was able to reduce T levels. In addition, E2 levels induced by the activators were also inhibited by PD-98059.
Strong up-regulation effects on the expression of StAR and CYP19A1 were detected after FSH or 8Br-cAMPs exposure. The inhibitor Rp-cAMPs clearly blocked the expression of both genes in cultures treated with 8Br-cAMPs, but had no clear effect on gene expression in FSH treated tissue. These results confirm that FSH in sea bass exerts its action via the cAMP/PKA signaling pathway, but the action of MAP-kinases over the steroidogenic process is also relevant. Further research is necessary to elucidate the relation between the cAMP/PKA and MAP-kinase pathways in the steroidogenic.
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P05-08 Presence of pituitary adenylate cyclase activating polypeptide in the milk of domestic animals and humans

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Milk contains a variety of proteins and peptides that possess biological activity. Several hormones have been described in the milk, including pituitary, hypothalamic, pancreatic, thyroid, adrenal, gonadal and gut hormones. Growth factors, such as growth hormone, insulin-like growth factor, epidermal growth factor, nerve growth factor and transforming growth factor, are important milk components. Bioactive substances in milk may regulate growth and differentiation in various neonatal tissues and also that of the mammary gland itself. Milk-borne growth factors play an especially important role in the maturation of the alimentary tract. Investigation of growth factors in the milk of domestic animals is of utmost importance for their nutritional values and agricultural significance. The aim of the present study was to determine the presence and concentration of pituitary adenylate cyclase activating polypeptide (PACAP), a neurotrophic factor, in the milk of various domestic animal species and humans. Furthermore, the presence of PACAP and PAC1 receptor and their mRNAs were investigated in the mammary glands.
Mass spectrometry analysis showed that PACAP was present in human milk. RIA measurements revealed that PACAP concentrations exceeded those in plasma 5-20-fold. A similar serum/milk ratio was found in the milk of goat, sheep and cow. The levels did not show significant changes within the examined 3 month-period of lactation after birth. PACAP receptor expression was found in the mammary gland biopsies of sheep by immunohistochemistry and PCR. These data show that PACAP is present in the milk of various mammalian species at high concentrations, the physiological implications of which awaits further investigation.
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P05-09 Effect of Largagtil on Physiology of Reproduction

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Background: Largagtil is a serotonin and norepinephrine reuptake inhibitor. Considering the importance of this drug in treating nervous diseases, its side effects are very important on the endocrine axis.
In this research the effect of Largagtil were studied on the concentration of testosterone, FSH and LH level and spermatogenesis.
Materials and Methods: The experiments were done on 40 male Wistar rats that divided to 5 groups of 8. The control group received nothing. The sham group was given distilled water as a solvent. The experimental groups were injected 50, 100 and 150 mg/kg of the drug orally for 21 days. The blood samples were taken at 22th day and the concentration of testosterone, FSH and LH were measured by RIA method. In addition, at the 22th day, the testes were separated and histological changes were studied among experimental, sham and control group. The results were evaluated by using ANOVA and Duncan tests.
Results: The results showed that 150 mg/kg of Largagtil reduced serum testosterone level while it increased FSH and LH levels ($P<0.05$). Histological investigations of the testes showed a decline on spermatogenesis chain in dose of 150 mg/kg.
Conclusions: According to our findings, Largagtil decreases the concentration of testosterone level and the number of spermatogenic cells and increases FSH and LH levels at high doses. Also, it can weaken the function of reproductive activity, probably.

P05-10 Seasonal mRNA expression of urate oxidase in brown trout liver and analysis of transcriptional regulation by different hormones

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Purines are important components needed to synthesize DNA or RNA and emerge vice versa via degradation of both molecules. With catabolism, uric acid formation (primarily in liver) occurs in the cytosol and its degradation may occur in peroxisomes. We have previously shown that peroxisomes change their morphology during the annual reproductive cycle of brown trout (Salmo trutta f. fario), and that enzymes involved in purine catabolism decreased their activity during late vitellogenesis). Herein, seasonal mRNA expression of urate oxidase (uox) was analyzed in liver of adult male and female brown trout using real-time PCR. In females, uox mRNA expression decreased with increasing GSI, and was lowest at advanced vitellogenesis. Results suggested that uox mRNA expression in females could be regulated by estrogens. To investigate the transcriptional regulation of uox, a part of the 5'-UTR promoter region (1254 bp) was isolated by genome walking technique and analyzed in silico for the presence of transcription factor binding sites (TFBS). Observed TFBS include ERE, and also GRE, RXR, PPAR, AhR, ROR, and CREB. Further analyzes revealed that the promoter contained three known promoter modules (GRE + OCT1, GRE + GATA, CREB + NFKB) indicating that a gene regulation will be likely for glucocorticoids (GRE) and for cAMP (CREB). A functional promoter analysis will follow to further our knowledge about potential interference of hormones (estradiol, glucocorticoids or gonadotropins) with purine catabolism.
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P06-01 Photoperiod effects on the expression of type II Iodothyronine deiodianse in Atlantic salmon parr

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Type II iodothronine deiodianse (Dio2) is a member of the selenoprotein family and catalyzes the outer ring deiodination of thyroxine (T4) to produce the bioactive form triiodothyronine (T3). While it is well known that thyroid hormones play an important role in normal development and growth, recent research has also suggested a specific role of Dio2 in mediating the physiological response to seasonal photoperiod signals in temperate vertebrates. Work to date has focused on Aves and Mammals and demonstrated a conserved response to seasonal photoperiod shifts in hypothalamic expression of Dio2. Atlantic salmon is a teleost of great commercial value and significant scientific interest however little is know about the molecular mechanisms regulating the animals seasonal physiological processes like reproduction and smoltification. In this study we have isolated, cloned and sequenced a 2027bp Atlantic salmon partial cDNA that contains a 615 partial cds, coding for a 205 aa protein that had high identity with other teleost Dio2 sequences (>80%ID) which we have named Atlantic salmon Dio2. Expression analysis of whole brain homogenates of salmon parr acclimated for 1 month to either a long day or short day photoperiod demonstrated a consistent diel expression profile of Dio2 under the long day photoperiod. The expression peaked in both cases around sunrise at 05:00 external time. These results suggest that the role played by Dio2 in signalling seasonal state is conserved across vertebrates and as such represent a significant advance in our understanding of the photoneuroendocrine mechanism in teleosts.

P06-02 The effects of PACAP on the signaling pathways and cell survival in chicken pineal cells is dependent on the circadian rhythm

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Pituitary adenylate cyclase activating polypeptide (PACAP) is involved in the regulation of circadian rhythms. In mammals, the brain\\\'s biological clock is the suprachiasmatic nucleus, receiving photic information from the retina through the retinohypothalamic pathway, where PACAP is the main co-transmitter of glutamate. The primary conductor of circadian rhythms of birds is the pineal gland. The presence of PACAP has been demonstrated both in the rat and avian pineal gland, where PACAP stimulates melatonin synthesis. The signaling mechanism, by which PACAP modulates melatonin synthesis and circadian rhythmic functions of the pineal gland is only partially known. The aim of the present study was to investigate the effects of PACAP on the changes of p38 MAPK and 14-3-3 protein in chick pineal cell culture both of which have been shown to participate in the regulation of rhythmic functions. Also, based on the general cytoprotective effects of the peptide, we also examined the possible survival-promoting effects of PACAP on pinealocytes against oxidative stress. Pineal cells were treated with 1, 10 or 100 nM PACAP38 every 4 hours during a 24-hour-period. The phosphorylation of p38 MAPK showed obvious changes during the observed 24 hours, while the level of 14-3-3 protein did not. We found that the lowest used dose of PACAP did not cause any phase alteration in p38 MAPK phosphorylation. 10 nM PACAP induced a 4-hour-long delay, and 100 nM abolished the circadian changes of p38 MAPK phosphorylation. PACAP was not effective on the level of 14-3-3 protein in the early morning hours, and only the highest tested dose (100 nM) could evoke a change in the appearance of 14-3-3 between midday and midnight hours. Cell survival assay (MTT-test) showed that oxidative stress reduced the percentage of viable cells, which could be counteracted by PACAP only during the dark phase. In summary, PACAP modulated the phosphorylation of p38 MAPK and the appearance of 14-3-3 protein, and promoted survival in chicken pineal cells, but these effects were dose-dependent and also depended on the time of day.
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P06-03 Expression of the circadian clock gene clock in human melanoma skin biopsy

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Emerging findings suggest that circadian timing mechanisms are involved in cell cycle regulation. In skin, possible relation was suggested between circadian clock genes and cell proliferation by recent studies on the upregulation of CLOCK/BMAL1 target genes in telogen/early anagen phase of hair follicle cycle in mice. At the same time, we have found no data on the role of clock genes in human skin tumorigenesis. Therefore, we aimed to detect mRNA expression of clock gene in excised melanoma and adjacent normal skin biopsies taken from melanoma patients.
A 3 mm dermatolological punch biopsy was obtained from the primary melanoma at 10:00 a.m. The paired non-cancerous tissue was collected from the adjacent healthy skin. Tissue RNA extracts were processed with quantitative real-time RT-PCR methodology.
Clock mRNA expression was detected in both tumorous and healthy skin samples. However, clock mRNA levels in melanoma were significantly lower than in the non-cancerous biopsy.
Our data provide evidence for the first time that clock mRNA is present in human melanoma with altered intensity of gene expression if compared to adjacent healthy skin. Based on these and recently published data on other tumor types (e.g. breast, prostate, colon) we plan to compare circadian expression patterns of clock genes between melanoma and healthy skin biopsies.
This work was supported by the Hungarian Medical Research Council (ETT50072-1133-99).

P07-01 A comparative analysis of collecting ducts morphometry and Aquaporine 2 expression in nectar feeding birds from contrasting habitats

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Nectar feeding birds ingest large amounts of water while feeding, so eliminating water excess becomes a problem for these species. On the contrary, in arid zones, these birds must avoid huge water loss by means of efficient morphological and physiological mechanisms. In this work we aim to investigate some molecular and morphological parameters on the avian kidney collecting ducts (CDs) implicated in water loss regulation. Aquaporin (AQP) is a membrane protein involved in the transfer of water and small solutes across cellular membranes, and it plays a critical role in water reabsorption. Avian kidney expresses Aquaporin 1, 2, and 4. We performed a semi-quantitative image analysis of immunoreactive AQP2 homologs with integrated optical densities (IOD) in the CDs of 3 nectar-feeding bird species: the hummingbirds Amazilia tobaci (At) inhabiting montane rainforest, and Leucippus fallax (Lf) from arid zones, and the passerine Coereba flaveola (Cf), common to both environments. We also determined the following morphometric variables for CDs: density, total diameter, and luminal diameter. At presented the highest IOD, followed by Lf, and Cf. CD density was higher in Lf, followed by At, and Cf. Regarding the CD diameter, At presented the largest CDs, however the luminal diameters were lower for Lf, and At with respect to Cf. Both IOD and luminal diameter of CDs were correlated in Lf and Cf except for At. Lf, which is a bird of arid zones, facing water-availability restrictions presented the highest CD density and AQP2 expression. Cf, which lives in both habitats, shows positive correlation between the lumen size and IOD with a low CD density. While in Lf, with the smaller lumen, this correlation was negative. At apparently has a different form of water loss regulation, modulating CDs density and the quantity of AQD2 expressed. Although At presented more AQP2 than Lf , the CDs density were lower and it cannot be compensated with by large CDs diameters. However, At, inhabiting wet zones, does not have the same handicap than a bird from arid zones. The differences in CD density and size, and AQP2 expression, reveal the existence of complex water loss regulatory mechanisms which are not species-specific but adapted by similar species, such as the nectarivores, occupying the same habitats. Key words: Nectar feeding bird, collecting ducts, aquaporins

P07-02 Seasonal changes in courtship behavior, testicular development and in hypothalamic aromatase immunoreactivity in male free-living European starlings (Sturnus vulgaris)

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Male European starlings (Sturnus vulgaris) sing prior to copulation and together with wing-waving these behaviors play essential role in mate atraction. In birds the activity of testis - as a primary source of androgens production-gradually increases during the breeding season (spring). The aromatization of testosterone - the most important androgen - in brain plays an important role in regulating the behavior. Our goal was to determine wheather the seasonal changes in male courtship behavior (measured by song bout length and wing-waving) are related to seasonal changes in androgen activity (measured by testis volume and aromatase (ARO) activity in the preoptic area (POA)) of free-living male starlings. Song bout length was recorded with a presence of a female and a nesthole. The number of ARO cells - together with the testis volume - increased during the courtship and egg laying period but outside the breeding season (August) they were on minimum. Song bout length showed simmilar pattern, namely the peak was reached during the courtship and egg laying period and after that males stoped their singing, when chicks started to hatch. A second elevation of song bout length was observed during fledging of youngs. The short and fast wing-flicking behavior was observed in males which were singing in the near of the nestholes but the typical wing-waving display occured almost exclusively after introduction of a female. This behavior did not show seasonal changes. Summarizing, we found that song bout length of free-living male starlings went parallel with testicular growth and ARO activity in the brain, except at the hatching time, which suggest that although androgens are essential for inducing male sexual arousal, but they are not exclusive regulators of courtship behavior.

P09-01 Structural identification and possible functional determination of the pea aphid, Acyrthosiphon pisum adipokinetic hormone

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A methanolic extract of whole bodies from virginoparous females of A. pisum was purified by RP HPLC and subjected to LC/MS and positive ESI tandem mass spectrometry analyses. The procedure resulted in the identification of a decapeptide with the mass of MH+ = 1159.6 Da and the primary sequence of pGlu-Val-Asn-Phe-Thr-Pro-Thr-Trp-Gly-Gln-NH2. The sequence was identical to that recently published for the adipokinetic hormone derived from the genome sequence study of A. pisum (Acypi-AKH) (Huybrechts et al., 2010). To evaluate a metabolic effect of the Acypi-AKH on the virginoparous females, we applied commercially synthesized Acypi-AKH into the insects by two methods - by injection and by application via the artificial diet. First results indicated that Acypi-AKH applied per os does not enter into the aphid body. Injection of Acypi-AKH revealed modulation of activities of unspecific glycosidases and proteinases in the body of A. pisum. Possible consequences of the results to the aphid biology are discussed.
Huybrechts J., Bonhomme J., Minoli S., Prunier-Leterme N., Dombrovsky A., Abdel-Latief M., Robichon A., Veenstra J.A. and Tagu D. (2010) Neuropeptide and neurohormone precursors in the pea aphid, Acyrthosiphon pisum. Insect Molecular Biology, 19 87-95.
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P09-02 The foraging gene of the desert locust

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Our knowledge of how genes act on the nervous system in response to the environment to generate behavioral plasticity is limited. A number of recent advancements in this area concern feeding and locomotion-related behaviors, and a specific gene family: foraging (for), which encodes a cGMP-dependent protein kinase (PKG). Locusts are notorious for their destructive feeding and long term migratory behavior. Locust phase polyphenism is an extreme example of environmentally-induced behavioral plasticity. In response to changes in population density locusts dramatically alter their behavior, from solitary and relatively sedentary behavior to active aggregation and swarming. Little is known about the molecular and genetic basis of this striking behavioral phenomenon. In this work we focused on the locust for gene. We identified and cloned the gene of the desert locust (Schistocerca gregaria). We comparatively studied its expression in the brain of gregarious and solitary-reared locusts. Last, we determined the phylogenetic relationship between the locust PKG and other known PKG proteins in insects. FOR expression was found to be confined to neurons of the anterior midline of the brain - the pars intercerebralis. The PKG activity of gregarious locusts was found to be significantly higher than that of solitary ones. Differences in PKG activity are sex specific, with higher PKG activity found in males than in females, in both solitary and gregarious locusts. Our findings are correlated to well-established phase-related behavioral differences and thus lay the ground work for functional studies of the locust for gene and its possible relations to locust phase polyphenism.

P09-03 Transfer function analysis reveals that trout pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) decrease baroreflex sensitivity in trout

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) belong to the secretinglucagon superfamily of peptides. PACAP and VIP share sequence similarities and these peptides have been remarkably well conserved from fish to mammals. Our recent studies have shown that in trout, the intracerebroventricular (ICV) injection of picomolar doses of trout PACAP but not VIP produced a slight but significant increase in mean dorsal aortic blood pressure with no significant change in heart rate. The lack of heart rate response to elevation of blood pressure suggests that the baroreflex sensitivity (BRS) may be depressed following ICV PACAP. Therefore, the aim of this study was to examine in our experimental model, the unanesthetized rainbow trout Oncorhynchus mykiss, whether PACAP and VIP are involved centrally in the regulation of the BRS. Cross spectral analysis techniques using a fast Fourier transform algorithm were employed to calculate the coherence, phase and transfer functions between spontaneous fluctuations of systolic arterial blood pressure (SAP) and R-R intervals (R-Ri) of the electrocardiogram. The transfer function provides a measure of the degree to which the input signal (SAP), at a given frequency, appears in the output (R-Ri) energy. The BRS (msec/kPa) was estimated as the mean of the gain of the transfer function between SAP and R-Ri when the coherence was high. The power spectral density of SAP and R-Ri, reflecting the SAP and R-Ri variabilities were also determined. Compared to the vehicle-injected group of trout (n= 16), ICV administration of a threshold dose of 50 pmol PACAP (n=8) and VIP (n=8) did not significantly change the overall variability of R-Ri but attenuated the high frequency component located in the 0.1-0.2 Hz frequency band. In contrast, the spontaneous variability of SAP was significantly increased (vehicle: 3971 ± 595 kPa²/Hz versus PACAP: 13981 ± 5442 kPa²/Hz or versus VIP: 10247 ± 5228 kPa²/Hz, P< 0.05). The coherence value between SAP and R-Ri was decreased but remained significant, the gain of the transfer function was also reduced whereas the transfer phase remained unchanged indicating that the BRS was significantly reduced (vehicle: 2914 ± 311 msec/kPa versus PACAP: 1150 ± 369 msec/kPa or versus VIP: 1693 ± 250 msec/kPa, P < 0.05). To our knowledge, our results demonstrate for the first time in any vertebrate class that exogenously administered PACAP and VIP act on the brain to reduce the BRS and suggest that these endogenous peptides might be implicated in the central control of baroreflex functions.

P09-04 Cardiovascular and ventilatory actions of trout neuropeptide Y and peptide YY in trout

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Neuropeptide Y (NPY) and peptide YY (PYY) are two members of the pancreatic polypeptide family of regulatory peptides. In mammals, these two peptides and their receptors are widely distributed in the central nervous system and peripheral tissues, and NPY in particular is known to elicit cardiorespiratory effects. The amino acid sequences of NPY and PYY have been well conserved during evolution but little is known about their cardiovascular and ventilatory actions in non-mammalian vertebrates. The present study was undertaken to explore the peripheral and central effects of graded doses (25, 100 and 500 pmol) of trout NPY and trout PYY on cardiovascular and ventilatory variables in the unanesthetized rainbow trout. Compared to the vehicle-injected group of trout, intra-arterial (IA) administration of NPY and PYY elicited a slight, non dose-dependent and non-significant elevation of the mean dorsal aortic blood pressure (PDA), but significantly decreased heart rate (¦H) and elevated the pulse pressure. Interestingly, although the maximal increase in PDA was similar between the two peptides at a dose of 100 pmol (NPY: + 0.35 ± 0.06 kPa; PYY: + 0.35 ± 0.13 kPa), the bradycardic response was significantly greater after IA PYY injection than after IA NPY injection (-7.19 ± 1.38 versus - 4.47 ± 0.78 beats min-1, P< 0.001). In contrast, IA injection of NPY and PYY was without action on the ventilation rate or ventilation amplitude. Preliminary results obtained following intracerebroventricular injection of the two peptides at a dose of 100 pmol did not demonstrate any significant changes in cardiovascular or ventilatory variables. In conclusion, native NPY and PYY elicited negative chronotropic actions only after peripheral injection. It remains to be determined whether the potent PYY- induced bradycardia is due to potentiation of the cardiac baroreflex or is caused by a direct negative chronotropic action on the heart.

P09-05 Brain extirpation stimulate PACAP expression in the central nervous system of the earthworm

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Recently, we have shown the distribution of PACAP- and PAC1-receptor like immunoreactivity (IR) in the nervous system of the earthworm, E. fetida. It is now well-established that PACAP is a neurotrophic factor, playing important roles in the development of the nervous system and regeneration of neural processes in vertebrate animals. Based on the apparent evolutionary conservation of PACAP and on the several common mechanisms of vertebrate and invertebrate nervous regeneration, the question was raised whether PACAP has any role in the regeneration of the earthworm nervous system. As a first step, we studied the distribution, concentration, and time-course of PACAP-like IR in the central nervous system of control and brain extirpated animals by RIA. A strong upregulation of PACAP-like immunoreactivity was observed in the subesophageal ganglion after brain extirpation. A decreasing gradient of PACAP-like molecules from the subesophageal ganglion to circumpharyngeal connectives was found in regenerating earthworms if the connectives were cut. If circumpharyngeal connectives were cauterized, PACAP-like compounds accumulated in the connectives, suggesting that neural processes could transport these compounds to the site of regeneration. Our present results show that the injury of the central nervous system stimulates the synthesis of PACAP/PACAP-like peptides in neural structures of earthworms, suggesting trophic functions in earthworm neural structures similarly to vertebrate species.
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P09-06 Effects of crustacean hyperglycemic hormone (CHH) on carbohydrate metabolism-related enzymes in the kuruma prawn, Marsupenaeus japonicus

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In crustaceans, eyestalk ablation causes various physiological events, including decrease of the hemolymph glucose level by lacking of a neuropeptide, crustacean hyperglycemic hormone (CHH). Although the hyperglycemic activity of CHH is well documented, little has been investigated on its functional regulation at a molecular level. In this study, to clarify the mechanism of the regulation, we analyzed the effects of CHH on glycogen metabolism and gluconeogenesis.
We first cloned cDNAs encoding four carbohydrate metabolism-related enzymes; glycogen phosphorylase (MjGP), glycogen synthase (MjGS), fructose 1,6-bisphosphatase (MjFBPase), and phosphoenolpyruvate carboxykinase (MjPEPCK) from Marsupenaeus japonicus by degenerate PCR, and then analyzed their expression levels by RT-PCR. The results showed that eyestalk ablation two days before the experiment decreased the mRNA level of MjGP, while this treatment increased that of MjGS in a main tissue for glycogen storage, hepatopancreas as well as in other tissues, indicating that the eyestalk-ablated prawns declined to the metabolic condition for glycogen accumulation. On the other hand, little changes in the levels of MjFBPase and MjPEPCK were observed, indicating that hypoglycemic condition caused by eyestalk ablation might not be due to hormonal regulation via gluconeogenesis. We next analyzed the mRNA levels of the four enzymes in the hepatopancreas and muscle by ex vivo stimulation of CHH. Quantative RT-PCR analyses showed that the mRNA levels of these enzymes in the examined tissues did not change after 1 hour exposure to 100 nM CHH, indicating that carbohydrate metabolism may not be transcriptionally regulated by CHH at least in a short period.

P09-07 LC/MS analysis of peptide hormones and their processing in the larval and adult Drosophila melanogaster midgut

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Peptide hormones play important roles in the regulation of many physiological processes. In mammals, peptide signals originating from the nervous system, the digestive tract and adipose tissue influence satiety and energy balance. Also for insects, it has been shown that metabolism and food intake are regulated by neuropeptide hormones, some of which are both structurally and physiologically homologous to vertebrate peptides. In contrast, little is known about the functions and structures of regulatory peptides from endocrine cells of the insect gut.
In the last years, we characterized the peptide hormone complement of the neurohemal organs of Drosophila by direct mass spectrometric profiling. We have now extended this work and report here on the first chemical characterization of the peptidome of midgut endocrine cells in Drosophila by capillary RP-HPLC and off-line MALDI-TOF/TOF mass spectrometry.
Our results corroborate existing immunofluorescent data and show that midgut endocrine cells produce members of at least 7 families of regulatory brain-gut peptides. The amino acid sequences of the processed molecules are in accordance with peptides found in the nervous system. Thus, there seem to be identical processing pathways in both neuroendocrine and gut endocrine tissue. The amon gene of Drosophila codes for a homolog of the vertebrate prohormone convertase 2. Recent data suggest that AMON plays a key role in the processing of neuropeptide hormones and is expressed also the midgut. By immunofluorescent double stainings, we found amon-Gal4 driven GFP expression to be colocalized with different peptide hormones in midgut endocrine cells of 3rd instar larvae. Furthermore, restrained AMON availability strongly affected peptide hormone signals in LC/MS analysis of midgut tissue.
In conclusion, we chemically identified the peptidome of the Drosophila midgut and provide evidence for a functional role of AMON in the proteolytical processing of peptide hormones from midgut endocrine cells.
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P09-08 Examination of the role of crustacean hyperglycemic hormone in stress response using the whiteleg shrimp, Litopenaeus vannamei

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Appropriate response to stress is important for animals to adapt to environmental change. Stress response is often mediated by hormones, which have the effect of increasing energy supply. While there is an abundant knowledge of stress hormones in vertebrates, much less is known concerning those in invertebrates. Thus, in this study, we aimed to clarify stress hormones in a crustacean species. To this end, we focused on peptides classified in the crustacean hyperglycemic hormone (CHH) family, some of which possess hyperglycemic activity. We chose Litopenaeus vannamei as the model animal, and emersion stress as the experimental stress factor. Firstly, we analyzed CHH contents in the sinus glands by HPLC to clarify which CHHs show response to stress. It was seen that one of the CHHs in L. vannamei, SGP-G, decreased significantly when animals were exposed to stress. In Western-blotting analysis of hemolymph, SGP-G was detected from only animals exposed to stress. These results suggest that SGP-G is excreted from the sinus glands to the hemolymph when animals are placed under stressful conditions. Next, we examined the effects of SGP-G on hemolymph glucose levels in animals injected with SGP-G. Glucose levels in the hemolymph increased in animals placed under stressful conditions as well as in those injected with SGP-G. Thus, this study indicated that some CHHs mediate energy supply as a stress response, and may serve as stress hormones in crustaceans.

P09-09 Antioxidant effect of adipokinetic neuropeptides in Spodoptera littoralis

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Degradation of plant allelochemicals in digestive tract of herbivorous insect is often associated with a production of oxidative radicals which could trigger extensive oxidative stress (OS) and subsequent damage of vital tissues. Considerable OS was elicited in gut of the Egyptian moth larvae Spodoptera littoralis after feeding on the diet supplemented with tannin (natural plant allelochemical). Besides an indisputable role in energetic metabolism, some of the insect adipokinetic hormones (AKHs) have been recently proven to enhance antioxidant response in two insect species (the firebug Pyrrhocoris apterus and the potato beetle Leptinotarsa decemlineata) after exposure to OS induced by a herbicide paraquat. Here, an antioxidative effect of two neuropeptides from AKHs family (Manse-AKH and Helze-HrTH) in S.
littoralis 6th instar larvae gut after feeding on 5% tannin diet has been examined. Individual as well as simultaneous effect of both hormones has been studied. Extent of the OS after the hormonal treatment has been determined by measuring of protein carbonylation and activity of three enzymes: catalase, ascorbate peroxidase and glutathione-S-transferase with peroxidase activity.
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P09-10 Intracellular calcium signaling is essential for coelomocyte activation in earthworm

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Calcium signaling is basically important in the activation of innate and adaptive immunity; however, the evolutionary aspects are not clarified yet. Currently limited data are available about intracellular calcium levels of coelomocytes, cellular mediators of earthworm immunity. We aimed to observe basal and induced Ca2+ levels of coelomocyte subgroups after various stimulations in Eisenia fetida and Allolobophora caliginosa using the Fluo3-AM Ca2+-sensitive dye. E. fetida chloragocytes had the highest basal Ca2+ levels among subpopulations; however there was no detectable Ca2+ influx after any stimuli, while coelomocytes showed strong Ca2+ increase after ionomycin treatment, which could be attenuated using the PMA phorbol ester. A. caliginosa coelomocytes showed a weak response to ionophore, while chloragocytes, similar to those in E. fetida, exhibited no changes after this stimulation. Intracellular calcium is mainly stored in the endoplasmic reticulum of coelomocytes as proved by thapsigargin treatments. Among several mitogens only phytohemagglutinin caused increased Ca2+ level in E. fetida coelomocytes, but not in A. caliginosa coelomocytes. Moreover, the bacterial chemoattractant fMLP revealed calcium influx of Eisenia coelomocytes. For the first time we observed various basal Ca2+ levels and sensibility to Ca2+ influx inducers (including mitogens and chemoattractant) of coelomocyte subgroups using flow cytometry. These observations suggest that Ca2+ influx and signal transduction may play crucial roles in the innate immunity of the earthworm.

P10-01 Characterization of growth hormone in green iguana (Iguana iguana)

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Growth hormone (GH) is a protein studied in many vertebrates, however few studies exist on reptiles. The hypophysis of green iguana is constituted by pars distalis and pars nervosa, divided by pars intermedia more developed in this species. The present study describes GH in green iguana (giGH) purified from hypophysis by immunoaffinity chromatography using anti-chicken GH (cGH) antiserum. The giGH has a weight of 22 kDa and a molecular variant mass of 44 kDa as determinated by Western blot. Furthermore, the giGH has at least four charge variants between 6.2-7.4 isoelectric point.The giGH cDNA consisted of 1016 bp that encoded a prehormone of 218 aa. The mature protein has 191 aa and a signal peptide of 27 aa. The phylogenetic analysis of giGH shows crocodiles and turtles are closer to birds, but the iguana (squamata) goes in other branch. Although in situ hibridization and immunohistochemistry was found the somatotropes distribution in the caudal region of the pars distalis. These cells have a diameter of 6.5 -10 *f*Êm with granules of 250-300 nm where the giGH is stored.
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P10-02 Insulin and IGF-I effects on the proliferation of an osteoblast primary culture from seabream (Sparus aurata)

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Bone deformities in several fish species, like seabream (Sparus aurata), are currently a main problem in aquaculture. In order to gain knowledge on fish bone development and skeletal growth, a primary cell culture model has been established from vertebra of seabream. The initial fibroblastic phenotype of the cells changes to a more polygonal shape throughout the culture, and the addition of an osteogenic medium containing ascorbic acid, β-glycerophosphate and calcium chloride, promotes the deposition of minerals in the extracellular matrix (ECM). The capacity of the cells to differentiate into osteoblasts and to mineralize the ECM has been evaluated using von Kossa and Alizarin red staining after three weeks of growing on osteogenic medium. Also, the cells have been induced to differentiate into adipocytes, by the addition of an adipogenic medium consisting on insulin, dexamethasone, IBMX and lipid mixture, and the accumulation of lipids into the cells has been detected with Oil Red O staining, demonstrating the pluripotentiality of these cells.
The viability and proliferation of the osteogenic cells has also been analyzed using the MTT assay based on the reduction of the tetrazolium salts under normal and mineralizing conditions at different days along the culture (0, 5, 10, 15 and 20 days). At all times, the cells showed an increased rate of proliferation in the presence of osteogenic medium. Subsequently, the effects of insulin (1, 10 and 100 nM) and IGF-I (0.1, 1 and 10 nM) on cell proliferation have been evaluated at day 3 of the culture in the presence of 2% fetal bovine serum. IGF-I significantly stimulated the proliferation of the cells in a dose response manner, while insulin had only slight effects. Next, the hormonal regulation of osteoblast differentiation will also be analyzed.
In summary, a primary culture of seabream osteoblasts has been characterized. This cellular system can be a good model to identify the molecules involved in the process of osteoblastogenesis in fish and its endocrine regulation; and to study bone development to contribute to improve the quality of the product in aquaculture.
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P10-03 The knockdown of the maternal estrogen receptor-beta2 mRNA affects embryo transcript contents and larval development in zebrafish

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In zebrafish, vitellogenic oocytes can incorporate significant amounts of 17beta-estradiol released from nearby granulosa cells according to a first-order kinetics, since the steroid low polarity ensures high permeability and affinity for yolk lipids. Estrogen bioactivity is likely, because the maternal mRNA for the estrogen receptor-beta2 (erbeta2) is highly expressed in ovulated oocytes. This transcript is available for translation in the embryo until its sharp decline from 4 to 8 hours post-fertilization (hpf), being replaced by low levels of zygotic erbeta2 mRNA from 24 hpf to hatching at 48 hpf, as determined by qRT-PCR. Estrogen receptors-alpha and -beta1 are only expressed zygotically at low levels from 24 hpf onwards. To test the functional role of maternal erbeta2 mRNA, 1-or 2-cell embryos were injected with 10.3 ng each of morpholino to knockdown translation (ATGMO) of both maternal and zygotic erbeta2 transcripts, missplicing morpholino (splicMO) to block post-transcriptionally the zygotic transcript alone, and a nonspecific morpholino (stdMO) as a control. Treatment with ATGMO caused severe malformations in 63% of 1-5 dpf larvae, as compared to 10-11% in those treated with splicMO and stdMO. Defects included body growth delay and curved shape, abnormal brain and splanchnocranium development, enlarged and hemorrhagic pericardial cavity, uninflated swim bladder and rudimentary caudal fin with aberrant circular motion. Affected larvae could survive for only 12-14 days. Co-injection of an anti-p53 MO failed to rescue the ATGMO-phenotypes, eliminating the possibility of off-target effects. Pangenomic microarray analysis revealed that 240 and 219 significantly expressed transcripts were up- and down-regulated, respectively, by maternal ERbeta2 protein deficiency in 8-hpf ATGMO-embryos. Also at 48 hpf, 162 and 120 presumably zygotic transcripts were up- and down-regulated, respectively, but only 18 were in common with each of the 8-hpf sets. Whole-mount in situ hybridization revealed an intensified expression of the genes six3.1 and emx1 in ATGMO-embryos at 24-48 hpf, as compared to controls. These findings suggest the involvement of maternal erbeta2 mRNA in the epigenetic programming of zebrafish development.

P10-04 The deubiquitinating enzyme mUBPy in the brain and sensory organs of mouse during embryonic development

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Mouse UBPy (mUBPy) belongs to the family of ubiquitin-specific processing proteases (UBPs). In this study we have investigated the expression of mUBPy in the brain and sensory organs of mouse at different embryonic stages (E9, E11, E13, E15, E17, E19) and during the postnatal stages P0, P1, P2, P4 and P5 using Western blot and immunohistochemistry.
mUBPy-immunoreactive cell bodies first appeared at stage E11 in several brain regions, particularly in the walls surrounding the vesicles and the ventricles. Subsequently, at stage E13, new mUBPy-positive cells appeared in the corpus striatum, the caudate nucleus, the thalamus, the epithalamus, the hypothalamus and the pons. At E15 the mUBPy pattern was very similar to that observed at E13, whereas at stage E17 mUBPy-immunoreactivity significantly decreased and a high number of mUBPy-immunoreactive cells was found only to line the third ventricle and within the mantle layer of the fourth ventricle. At E19 and P0, no mUBPy-immunoreactive element was found in the brain. At the postnatal stages P2 and P5, mUBPy-positive cells were detected in all subdivisions of the brain, with high concentrations in several cortex regions. Double labelling with the mUBPy antiserum and antisera against specific cells markers showed that the enzyme is expressed both in neurons and astrocytes.
Outside the brain, mUBPy was detected, from stage E11, in the eye, within the lens and the cornea, the inner ear, at level of the cochlear and vestibular systems and in the olfactory epithelium. The spatio-temporal expression of mUBPy suggests that the enzyme may be involved in neuroregulatory processes during embryogenesis.

P10-05 Identification of PINK1 in the brain, eye and ear of mouse during embryonic development

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PINK1 is a 581 amino acid protein with a serine/threonine kinase domain and an N-terminal mitochondrial targeting motif. The enzyme is expressed in the brain as well as in several tissues such as heart, skeletal muscle, liver, kidney, pancreas and testis. In the present study, we have investigated by Western blot analysis and immunohistochemistry the presence and distribution of PINK1 in the brain, eye and inner ear of mouse during embryonic development. In the brain we detected a PINK1 molecular form of 66 kDa. Immunoreactive perikarya first appeared at stage E15 in the diencephalon within the thalamus, the hypothalamus, the periventricular layers of the third ventricle and in the rhombencephalon at level of the pons. Subsequently, new PINK1-positive neurons were found in the midbrain within the floor and the periventricular layers of the ventral wall of the mesencephalic vesicle (stage E17) as well as in the neopallial cortex, the tegmentum of the midbrain and the periventricular region of the caudal part of the rhombencephalon (stage E19). At P0, PINK1immunoreactive cells appeared in the striatum, the mantle layer and caudal part of the medulla oblongata and the cerebellum. The spatio-temporal expression of PINK1 and its heterogeneous distribution suggest that the enzyme might be involved in neuroregulatory processes during embryogenesis.
In the eye, PINK1-immunoreactivity was found in lens and in the cornea, whereas in the inner ear the enzyme was expressed in the ependymal and subependymal cells of the saccule and in the semicircular canals indicating that PINK1 plays a role in the development of these sensory organs.

P10-06 Expression of PINK1 in the zebrafish D. rerio during development

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PINK1 is a highly conserved 581 amino acid serine/threonine kinase that possesses an N-terminal mitochondrial targeting sequence. In spite of its predicted ubiquitous distribution, several evidence showed that the enzyme is present in discrete cell populations.
In this study, we have investigated by Western blot analysis and immunohistochemistry the expression of PINK1 in the brain and eye of zebrafish during development. We detected two PINK1-positive bands in the whole zebrafish lysates: a smaller one, of 33 kDa, and a larger one, of 66 kDa, as early in zebrafish larvae at 48 hpf and throughout development up to 5-day larva.
According with Western Blot analysis, PINK1-immunoreactivity appeared in the brain at 48 hpf. Immunoreactive cells were confined to some regions of the medulla oblongata and the hypothalamus. The expression was also maintained at later stage of development in 5-day larva in the same regions. At this stage a new expression domain appeared in the lens and in the retina of the eye and in the neuromasts of the lateral line. Outside the nervous system PINK1 was also expressed in the muscle and in the gut.
In the developing nervous system a similar expression of PINK1 has been reported for comparable stages of mouse development. Finally, our data showed a restricted expression pattern of PINK1 suggesting an involvement of this enzyme in developmental pathway linked to neuroregulatory mechanism.

P10-07 Molecular cloning and characterization of Dmc1, a meiosis-specific gene, from the whiteleg shrimp, Litopenaeus vannamei

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The molecular events of meiosis are highly conserved among eukaryotes. In meiosis, the recombination of maternal and paternal genomic information occurs via homologous recombination. In mammals, the Dmc1 gene is known to be expressed specifically in meiosis-stage germ cells, and the DMC1 protein is critical for the proper pairing and synapsis of homologous chromosomes leading to homologous recombination. However, in Crustacea, meiotic events are at present poorly understood. In this study, in order to obtain a better understanding of meiosis in Crustacea, the Dmc1 homologue gene was partially cloned from the whiteleg shrimp, Litopenaeus vannamei, using a homology cloning approach. The cDNA sequence obtained in this study showed a high level of identity to black tiger shrimp Dmc1 homologue (95%), deer tick Dmc1 homologue (72%), and zebrafish Dmc1 homologue (69%). Phylogenetic analysis revealed that the sequence belonged to the Dmc1 family, but not the RecA nor Rad51 family which are Dmc1 homologues but do not show meiosis-specific expression. It was confirmed by RT-PCR analysis that the transcripts are specifically expressed in the gonads. These results indicated that the sequence obtained in this study was a partial sequence of the L. vannamei Dmc1 (LvDmc1) homologue gene. Moreover, RT-PCR results suggest that LvDmc1 is expressed specifically during meiosis, and may be involved in homologous recombination in Crustacea.

P10-09 The brown shrimp (Crangon crangon L.) ecdysone receptor complex: cloning, structural modeling of the ligand-binding domain and functional expression in an EcR-deficient Drosophila cell line

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The cDNAs encoding ecdysone receptor (EcR) and retinoid X receptor (RXR) were cloned and sequenced from brown shrimp Crangon crangon (Crustacea: Decapoda), a common faunal species and commercially important in the North-West European coastal waters. A 3D model of the ligand binding domain (LBD) of EcR was created and docking of ponasterone A (PonA) was simulated in silico. Finally, we report the transfection of expression plasmids for these receptors in the mutant Drosophila L57-3-11 cell line. Through an ecdysone responsive reporter assay we clearly prove the functionality of shrimp ecdysone receptor in the transfected cell line. Our results indicate that the Drosophila cell line and in silico LBD modeling can be used to study the function of crustacean ecdysone receptors and be applied to assess endocrine disrupting effects on non-target crustacean species.

P10-10 The RXR receptor: a target of endocrine disruption in the brown shrimp (Crangon crangon L.)?

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The arthropod retinoid-X-receptor (RXR) influences the nuclear signalling of many other nuclear receptors, such as the ecdysteroid receptor (Wu et al., 2004), through heterodimerization. It is likely that RXR isoform expression is tissue dependent and as such the influence of RXR on nuclear signalling is tissue specific. Here we report the cloning of four RXR isoforms of the North Sea brown shrimp Crangon crangon, an economical and ecological important species. We compared the expression of these isoforms in tissues of brown shrimp through semi-quantitative RT-PCR.
Furthermore, we modelled the ligand binding domain of CrcRXR and docked tributyltin (TBT) in silico. TBT is an important marine contaminant and acts as a potent nanomolar activator of RXR, as observed in Daphnia magna (Wang and LeBlanc, 2009). Finally we exposed brown shrimps to TBT and investigated the effect on RXR isoform expression.
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P10-11 Potential of de novo ecdysteroid biosynthesis in the testis of Spodoptera littoralis

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Ecdysteroids are the key hormones in insect growth and development, and the prothoracic glands are known as the major source of ecdysteroid biosynthesis during postembryonic development. To date, six important genes, a Rieske-domain protein neverland and five cytochrome P450 hydroxylases named Halloween genes (CYP307A1, spook; CYP306A1, phantom; CYP302A1, disembodied; CYP315A1, shadow; CYP314A1, shade) involved in the ecdysteroids biosynthesis, were identified and studied thoroughly. On the other hand, over the last few decades there have been several reports on ecdysteroid production in testis and the triggering factor, Lymantria testis ecdysiotropin (LTE), while the molecular aspects are not fully understood. Therefore, we used the gene expression of these six essential genes as a criterion of de novo ecdysteroids biosynthesis, and focused on the sixth (last) instar period in the cotton leafworm, Spodoptera littoralis, which is an important lepidopteran insect causing high damage in agriculture. In testis, the titer of ecdysteroids showed two peaks: a small peak at day 2 and a large peak at day 4 in the last instar, being similar to the hemolymph ecdysteroid titer. All the genes were expressed in testis, but the expression pattern did not concert with the ecdysteroid titer. Since there are some reports that the ecdysteroids are synthesized in testis sheath part, we compared the expression of these genes in whole testis and in testis sheath only. The expression pattern of neverland and shade showed no difference between whole testis and testis sheath, while that of the other genes was significantly different. Furthermore, we measured the ecdysteroid composition in hemolymph and testis at day 2 and day 4 in the last instar. Finally the results are discussed in relation to potency of ecdysteroid biosynthesis in testis.

P10-12 High-throughput screening of ecdysone agonists using a dipteran-specific EcR reporter assay

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Diacylhydrazine (DAH) analogs were developed as a new group of insect growth regulators, called ecdysone agonists or molting accelerating compounds. These DAHs manifest their toxicity via interaction with the ecdysone receptor (EcR) in susceptible insects as does the natural molting hormone 20-hydroxyecdysone (20E). A notable feature is their high activity and specificity against lepidopteran insects, raising the question whether non-lepidopteran-specific analogs can be isolated.
We have developed a dipteran specific reporter-based screening system with transfected S2 cells of Drosophila melanogaster to discover and evaluate compounds with ecdysone agonistic or antagonistic activity. A library of non-steroidal ecdysone agonists with 150 DAH, 6 acylaminoketon (AAK) and 7 tetrahydroquinoline (THQ) analogues was tested. None of the compounds was as active as 20E. Of note was the identification of two THQ compounds with activity in S2 but not in lepidopteran cells, confirming the relative specificity of this group of compounds towards dipteran insects. Although marked differences in activity exist with respect to the activation of EcR between dipterans and lepidopterans, there exists a positive correlation between the median effective dose values in S2 and lepidopteran cells.
In parallel, in silico modeling of the ligand-binding domain of Drosophila EcR (DmEcR) and comparison with the structure of its lepidopteran counterpart reveals the absence of a second lobe at the bottom of the ecdysone-binding pocket for DmEcR, which prevents the anchoring of the B-ring of DAH. The structural models therefore offer an explanation for the differences in binding activity and activation potential between dipteran and lepidopteran receptors.

P10-13 Relationship between larval-pupal metamorphosis and gene expression of insulin-like peptide and insulin receptor in Spodoptera littoralis

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Insulin-like peptides (ILPs) affect a wide variety of biological events, such as metabolism, lifespan, growth and reproduction. Two ILPs (Sl-ILP1 and Sl-ILP2) were identified in the cotton leafworm, Spodoptera littoralis, while the functions and developmental characters are not fully understood. In the present study, we identified the partial sequence of the S. littoralis insulin receptor (Sl-InR) in addition to Sl-ILPs, and followed the expression profile of these genes during larval-pupal development. Sl-ILP1 and Sl-ILP2 were specifically expressed in brain, and their gene expressions were gradually decreased in concert with larval-pupal development. On the other hand, Sl-InR was expressed in all the selected tissues (brain, testis, fat body, Malpighian tubules, prothoracic glands, midgut), though the gene expression pattern was different among the tissues. Interestingly, the gene expression pattern of Sl-InR in fat body seemed to relate with larval-pupal development. In a parallel experiment, the juvenile hormone mimetic methoprene was able to prolong the larval period, and the gene expression of Sl-ILPs and Sl-InR was affected. These results clearly showed the relationship between gene expression of Sl-ILPs and larval-pupal development, and suggested the effect of Sl-ILPs may be controlled by not only Sl-ILPs expression but also Sl-InR expression.

P10_14 Teneurin C-terminal Associated Peptides (TCAPs): a conserved peptide system in metazoans that regulate cell growth and metabolism

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Oxygen is one of the central components driving life processes. As such, oxygen metabolism must be tightly regulated in order to maintain the delicate balance between oxygen deprivation and oxygen toxicity. Therefore, elaborate and highly regulated mechanisms must exist to defend against the accumulation of reactive oxygen species (ROS) and the effects of inadequate oxygen that threaten cellular integrity and survival.
TCAP is found in both deuterostome and protostome metazoans and may be key in helping organisms adapt to different oxygen levels through its neuroprotective actions. Compared to unicellular animals and bacteria, multi-cellular metazoans require more sophisticated systems to coordinate responses to change in oxygen tensions. Perhaps for these reasons, there are four TCAP paralogs in vertebrates and only one in invertebrates.
TCAP-1 reduces superoxide radical formation in immortalized brain cells by upregulating the superoxide dismutasecatalase pathway. This present study shows that TCAP-1 treatment increases proliferation and modulates neurite number in immortalized mouse hypothalamic cells under hypoxia. A promising target of TCAP-1 actions is brain-derived neurotrophic factor (BDNF) due to its survival-promoting effects against the adverse effects of oxidative stress and hypoxic injury. First, we establish these cells as a suitable model for studying the interactions between TCAP-1, BDNF and oxygen-related stress. We then observe that TCAP-1 treatment modulates BDNF protein and leads to differential regulation of BDNF splice variants (I-XA). We hypothesize that TCAP-1 may confer neuroprotection during hypoxic stress and modulates axonal and neurite outgrowth by regulating BDNF which can then activate a series of cell survival cascades.

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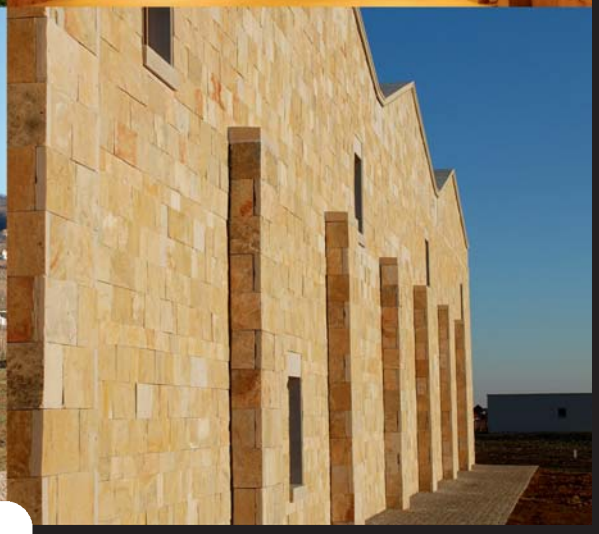

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Rosé 2009 (taste or buy it at the EXCURSION TO ORFŰ) *Character*: Due to the vintage, this is a deep colored rose with a nose of wild strawberries, raspberry, and elderberry with mild floral notes. In the mouth, it combines its gentle acidity with lush fruit, resulting in a bold vibrant wine. *Acid:* 5.5 g/l, *Alcohol:* 13.8%.
Sauska Siller Cuvée (taste or buy it at the CECE BANQUET dinner) *Character:* A rich dark rose the colour of fresh cherry juice. On the nose, raspberries, sweet cherries, and strawberry jam burst from the glass. Big, fresh, and juicy in the mouth flavours of candied fruits linger with the fresh bright finish. *Alcohol:* 15%
Sauska Cuvée 13 2008 (taste or buy it at the CECE BANQUET dinner) *Character:* Bright red/purple coloured. This Syrah based cuvee has a distinct mix of spice and fruit. A very open and giving nose of black currants, plums, and white and black pepper. Great volume in the mouth, with ripe fruit and spice, a pleasant finish and bold young tannin. *Alcohol:* 14%
Casino Cuvée 2001 (taste ot buy it at the CECE BANQUET dinner) *Character:* Dark gold colour with a hint of amber. A typical vibrant taste and scent, in which sugar and acidity are in a perfect harmony. Among this wine's remarkably fruity aroma (apricot, fig, walnut, black tea, propolis, honey, spices and crust), its creamyness is also unforgettable. A suitable wine for desserts, or goose liver. *Acid:* 7,4 g/l, *Alcohol:* 14,0 %
Cuvée 7 2006 (taste or buy it at the CECE BANQUET – wine tasting) *Character:* Deep red purple colour. Aromas of black fruits and cherry mingle with anise and graphite. Flavors of sweet black berries, currant and plum exude from a dense core just starting to reveal its full depth. A bold and ripe wine, the sweet tannins persist, creating a long pleasant finish. *Acid:* 5.9 g/l, *Alcohol:* 15%.
Chardonnay Makár 2007 (taste or buy it at the CECE BANQUET – wine tasting) *Character:* Yellow-gold with a hint of green. Tropical fruit and floral nose, with hints of citrus and brioche, well-integrated wood, clean and focused in the mouth with ample acid, and a long finish with great balance. *Acid:* 5 g/l, *Alcohol:* 14.5%.
Sauska Pinot Noir 2008 (taste or buy it at the CECE BANQUET – wine tasting) *Character:* Warm purple colour. Earthy - forest floor, and fresh cherry nose, combine with the spice of the new oak barrels. In the mouth, the taste is intense and full with gentle tannins and a long pleasant finish. *Alcohol:* 14.3%, *Acid:* 5.3 g/l.
Sauska Merlot 2006 (limited item, taste or buy it at the CECE BANQUET – wine tasting) *Character:* Black and ruby coloured wine. An attractive earthy nose of fresh raspberry and wild strawberry with hints of tobacco and vanilla. Its thick, dense palate opens slowly to reveal forest fruits and sour cherry. Medium-bodied wine with a long, pleasant finish. *Alcohol:* 14,7% *Acid:* 5.4 g/l

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