ulm university universität ulm

Ecdysone Workshop 2008
July 20th - 24th Ulm, Germany

Ecdysone Workshop 2008
July 20th - 24th Ulm, Germany

The following institutions and companies supported the Ecdysone Workshop.
We gratefully acknowledge their contributions.

ulm university universität ulm

Promega
www•promega•com

Peter Karlson-Stiftung

CANDOR Bioscience GmbH

Ulmer Universitäts-Gesellschaft
UG
Ulmer Universitätsgesellschaft

VWR
Supplier Partnerships for Customer Solutions

City of Ulm

Impress

Layout: M. Burret, Ulm University, Germany
Print: SZ-Offsetdruck, Neu-Ulm, Germany

Ecdysone-Workshop 2008
July 20th - 24th Ulm, Germany

Index

Sunday 20th of July

16:00-20:00 **Registration and get together and Poster Session**

Monday 21st of July

08:00-08:30 **Registration**

08:30-09:00 **Welcome**

I. Synthesis and Metabolism

Chair: Rees H., Dauphin-Villemant C.

09:00-09:40 **Guittard E., Bijakowski C., Maria A., Lafont R., Blais C. and Dauphin-Villemant C.**
"Ecdysteroid catabolism in insects: is 26-hydroxylation catalyzed by CYP18A1?"

09:40-10:10 **Yoshiyama T., Dauphin-Villemant C., Kataoka H. and Niwa R.**
"Neverland, a conserved Rieske-type family of proteins essential for ecdysone synthesis and cholesterol metabolism in the prothoracic gland"

10:10-10:40 **Laufer H., Shin H.,Demir N. and Bagshaw J.**
"Multifunction of crustacean recombinant CHHs, including new functions of regulation of ecdysone production by ovarian cells"

10:40-11:10 ***Coffee break***

11:10-11:40 **Orgogozo V., Murat S. and Dauphin-Villemant C.**
"Loss of enzymatic activities involved in sterol metabolism during evolution: the interesting case of a cactophillic *Drosophila* species"

11:40-12:10 **Ho R., Raharivelomanana P., Cousteau P.-Y., Bianchini J.-P., Girault J.-P. and Lafont R.**
"Isolation of a new class of ecdysteroid conjugates (Glucosyl-Ferulates)"

12:10-13:40 ***Lunch***

Monday 21st of July

II. Physiology

Chair : Hiruma K., Henrich V. (dedicated to L.I. Gilbert)

13:40-14:20 **Mané-Padrós D., Nieva C., Maestro J.L., Vilaplana L., Maestro O., Bellés X. and Martín D.**

„The nuclear receptor BgFTZ-F1 is essential for the onset of the adult developmental program in the hemimetabolous insect *Blattella germanica*“

14:20-14:50 **Gruntenko N. E., Chentsova N. A., Bogomolova E. V., Adonieva N. V., Karpova E. K. and Rauschenbach I. Y.**

„The mechanisms of 20-hydrohyecdysone and dopamine interaction in *Drosophila* under normal and stress conditions“

14:50-15:20 **Manaboon, M., Iga, M., Iwami, M. and Sakurai, S.**

„Involvement of intracellular Ca^{2+} in the nongenomic action of 20-hydroxyecdysone induced programmed cell death“

15:20-15:40 ***Coffee break***

15:40-16:10 **Farkaš R., Mentelová L., Kuchárová-Mahmood S., Raška I. and Mechler B. M.**

„The interaction between $p127^{l(2)gl}$ and armadillo leads to a decreased formation of Arm/Tcf, activation of *hid* expression, and execution of cell death program in *Drosophila* salivary glands“

16:10-16:40 **Shao H.-L., Zheng W.-W., Liu P.-C., Wang Q., Wang J.-X., Zhao X.-F.**

„Establishment of a new cell line from lepidopteran epidermis and the 20-hydroxyecdysone regulation on gene expression“

16:40-18:00 ***Poster session***

Tuesday 22nd of July

II. Physiology

Chair: Hiruma K., Belles X. (dedicated to L.I. Gilbert)

08:30-09:00 Gullipalli D., Arif A. and Dutta-Gupta A.
„Novel 23kDa fat body secretory protein affects steroid levels during insect development“

09:00-09:30 Takaki K., Konopova B. and Jindra M.
„EcR is required for oocyte development in the telotrophic ovary of *Tribolium castaneum*“

09:30-10:00 Futahashi R. and Fujiwara H.
„Hormonal regulation on stage-specific larval mimicry markings of the swallowtail butterfly, *Papilio xuthus*“

10:00-10:30 *Coffee break*

10:30-11:00 Hiruma K. and Muramatsu D.
„Hormonal control of pupal commitment of the epidermis of the silkworm, *Bombyx mori*: comparison with that of *Manduca sexta*“

11:00-11:30 Shiotsuki T.
„Comparison of precocious metamorphosis induced by different conditions in the silkworm“

11:30-12:00 Li S.
„Juvenile hormone modulates PKC activity and ecdysteroids signaling to regulate fat body remodeling and pupal metamorphosis in *Drosophila*“

12:00-13:30 *Lunch*

Tuesday 22nd of July

III. JH interactions

Chair: Jindra M., Belles X.

13:30-14:10 Konopova B. and Jindra M.,
„Met and BR-C control beetle metamorphosis in response to juvenile hormone“

14:10-14:40 Singtripop T., Sereewattanachai P. and Sakurai S.
„Juvenile hormone analog and 20-hydroxyecdysone induction of the wing disc development and *EcR* mRNA expression in the larval diapause of bamboo borer, *Omphisa fuscidentalis*“

14:40-15:10 Henrich, V.C., Plotkin J., Costantino B., Andres A. J., Clifton K. C. and Callender J. A.
„Developmental effects of a dominant negative form of Ultraspiracle (USP) in *Drosophila melanogaster*“

15:10-15:40 *Coffee break*

15:40-16:10 Hopkins P., Durica D., and Washington T.
„The putative partner of the crustacean ecdysteroid receptor, UpRXR, and its isoforms from the fiddler crab, *Uca pugilator*“

15:10-16:40 Greb-Markiewicz B., Orłowski M., Gwóźdź T., Dutko-Gwóźdź J., Dobrucki J., Ożyhar A.
„Identification of NLS and NES signals for *Drosophila* methoprene-tolerant protein“

16:40-17:10 Dubrovsky E.
„RNase Z, the Drosophila homolog of the human ELAC2, is required during early larval development“

17:10-19:00 *Poster session*

Wednesday 23rd of July

IV. Nuclear receptors

Chair: Palli S. R., Horman R. E.

08:30-09:10 Lapenna S., Dinan L., Palli S. R., Barlow A., Friz J., Hopfinger A. J., Liu J., Hormann R. E.

Introductory remarks and

„Ecdysteroids as gene-switch actuators: Enhancement of physicochemical properties and trends toward orthogonality“

09:10-09:40 Graham L. D., Hannan G. N., Pawlak-Skrzecz A., Noyce L., Donya Tohidi-Esfahani D., Johnson W. M., Howell L., Lovrecz G., Lu L., Pilling P. A., Eaton R. E., Bliese M., and Ronald J. Hill R. J.

„Recombinant ligand-binding domains from insect ecdysone receptors“

09:40-10:10 Palli S. R., Parthasarathy R. and Anjiang Tan

„Molecular analysis of ecdysone action in the red flour beetle, *Tribolium castaneum*“

10:10-10:40 *Coffee break*

10:40-11:10 Tremmel Ch.

„Regulation of transcriptional activity of EcR isoforms of *Drosophila melanogaster*“

11:10-11:40 Azoitei A.

„Ligand binding of the ecdysteroid receptor is modulated by interaction with DNA“

11:40-12:10 Hönl C.

“Interaction of EcR with NF*K*B“

12:10-12:40 Vafopoulou X.

“EcR of Rhodnius prolixus (Hemiptera) co-localizes with mitochondria and microtubules“

13:00-18:00 *Excursion (Lunch in the Bus)*
Hauff Museum of the Prehistoric World, Holzmaden

20:00 *Banquet*

Thursday 24th of July

08:30-09:30 **Karlson lecture**

Dinan L.: „Phytoecdysteroids: what use are they?“

Introductory remarks by Lafont R.

V. Phytoecdysteroids and Applications

Chair: Lafont R., Dinan L.

09:30-10:10 **Panguluri S. K., Lib B., Hormann R. E. and Palli S. R.**
Introductory remarks and
„Effect of ecdysone receptor gene switch ligands on gene expression in 293 cells“

10:10-10:40 ***Coffee break***

10:40-11:10 **Beckage N. E.**
„Ecdysone agonists and mosquito molting: from basic research to applications“

11:10-11:40 **Meybeck A.**
“Ecdysteroids from plants prevent UV induced premature senescence of human skin fibroblasts“

11:40-12:10 **Tóth N., Báthori M., Ramirez G. G., Szabó A., Kacsala P., Héger J., Zádor E.**
„Effect of 20E on different rat muscle models“

12:10-13:00 ***Concluding remarks***

Ecdysteroid catabolism in insects: is 26-hydroxylation catalyzed by CYP18A1?

Guittard E., Bijakowski C., Maria A., Lafont R., Blais C. and Dauphin-Villemant C.

email: chantal.dauphin-villemant@snv.jussieu.fr

Equipe Biogenèse des Stéroïdes, Laboratoire Protéines Biochimie Structurale et Fonctionnelle, Université Pierre et Marie Curie, Paris, France

An important aspect of insect development is that regulatory processes must be tightly timed to ensure appropriate cell growth and differentiation for each defined life stage of the animals. Ecdysteroids coordinate the major developmental transitions of insects, like molting and metamorphosis. Not only the increases in the circulating levels of active hormones, but also their falls are important and both biosynthesis and inactivation processes of ecdysteroids are of physiological relevance. A widespread and prominent route of ecdysteroids inactivation is the conversion into 26-hydroxylated metabolites, and ultimately to the corresponding ecdysonoic acids. Biochemical evidences of these reactions have been obtained in several insect species and suggest that a cytochrome P450 enzyme (CYP) is involved. At the molecular level, it has been proposed on several occasions that *cyp18a1* might be a good candidate gene for coding the 26-hydroxylase, but this was never demonstrated. We will present our results concerning ecdysteroid inactivation by 26-hydroxylation in drosophila as well as experimental evidences about DmCYP18 catalytic activity. Current knowledge about CYP18 in various insects will be reviewed.

Neverland, a conserved Rieske-type family of proteins essential for ecdysone synthesis and cholesterol metabolism in the prothoracic gland

Yoshiyama T.[1], Dauphin-Villemant C.[2], Kataoka H.[1] and Niwa R.[1, 3]

email: kk57513@mail.ecc.u-tokyo.ac.jp

[1]Graduate School of Frontier Sciences, The University of Tokyo, Japan
[2]FRE2852 Protéines Biochimie Structurale et Fonctionnelle, CNRS-Université P. et M. Curie, Paris, France
[3]Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan

Conversion of cholesterol to 7-dehydrocholesterol (7-DHC) is the first step of the ecdysone biosynthesis pathway in the prothoracic gland (PG). This step is hypothesized to be catalyzed by a microsomal Cytochrome P450 oxygenase. Previously we have reported that a conserved Rieske-type oxygenase Neverland (Nvd) is essential for 7-DHC formation in the PG and insect development. During embryonic and larval development, nvd is expressed specifically in the PG. Loss of nvd function in the PG causes arrest of both molting and growth. This phenotype is rescued by application of 20-hydroxyecdysone or 7-DHC but not of cholesterol. Therefore, we have postulated that Nvd functions in the conversion of C to 7-DHC in the ecdysteroidogenic pathway. The molecular function of Nvd, however, has not been characterized completely. Here, we report the subcellular location, biochemical functions, and effects of site-directed mutagenesis of Nvd. Our results suggest that Nvd family proteins have a potential role in steroid hormone synthesis and cholesterol metabolism in eukaryotes.

Multifunction of crustacean recombinant CHHs, including new functions of regulation of ecdysone production by ovarian cells

Laufer H.[1,2], Shin H.[1], Demir N.[1] and Bagshaw J.[3]

email: hans.laufer@uconn.edu

[1]Department of Molecular and Cell Biology, University of Connecticut, USA
[2]The Marine Biological Laboratory, Woods Hole, USA
[3]Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, USA

The sinus gland X-organ complex of the crustacean eyestalk produces a family of regulatory neuropeptides, generally 72-76 ammio acids in length, called Crustacean Hyperglycemic Hormones (CHHs). We are using recombinant (r) CHHa and CHHb from the American lobster, *Homarus americanus*, produced in the yeast (*Pichia pastoris*), *in vivo* and *in vitro* bioassays, to determine the biological activities of these CHHs. Molt inhibiting hormone (MIH) activity was tested on Y-organ cells, followed by radioimmunoassay for ecdysone. Mandibular Organ Inhibitory Hormone (MOIH) activity was tested by C^{14}-methly incorporation into methyl farnesoate (MF), a juvenile hormone of crustacean, by mandibular organ cells maintained in culture, while Vitellogen Inhibiting Hormone (VIH) was assayed by immunoprecipitation of vitellogenin labeled with a mixture of S^{35}-Methionine and S^{35}-Cysteine following Vg-Ab precipitation, tested on hepatopancreas cells in culture. Both $rCHH_a$ and $rCHH_b$ were active in increasing glucose in the blood, following injection into crayfish, and thus showed CHH activity. The rCHHs possessed molt inhibiting (MIH) activity, possessed MOIH activity by reducing MF synthesis by mandibular organ cells. Recombinant CHHb inhibited vitellogenin synthesis by hepatopancreas cells and so exhibited VIH (or Gonad Inhibiting Hormone (GIH)) activity. Thus CHHs are a family of multifunctional regulatory neuropeptides.

Earlier, we found that terminally molted, mature reproductive spider crabs, which do not possess Y-organs, produced ecdysones in their gonads. Here, we investigated the regulation of ecdysone production by the maturing crustacean ovary using rCHHs. Recombinant CHH_a and CHH_b (0.5 µg) inhibited ecdysone production by ovarian cells by 60%, which was equivalent to 0.4 sinus gland extract. In conclusion, reproduction in crustacea appears to be controlled by CHHs, both by controlling MF production via MOIHs, and ecdysone production by the gonads.

Supported in part by the CT and RI Sea Grant College Programs, NOAA, and the CT Department of Environmental Protection's Long Island Sound Research Fund

Loss of enzymatic activities involved in sterol metabolism during evolution: the interesting case of a cactophilic *Drosophila* species

Orgogozo V., Murat S. and Dauphin-Villemant C.

email: virginie.orgogozo@snv.jussieu.fr

Equipe Biogenèse des Stéroïdes, Laboratoire Protéines Biochimie Structurale et Fonctionnelle, Université Pierre et Marie Curie, Paris, France

Host specialization in insects that feed on plants provides an excellent opportunity to study the genetic basis of ecological adaptation. The specialization of *Drosophila pachea* towards its single host plant, a cactus species named *Lophocereus schottii*, is remarkable in that it involves both ecotoxicological and hormonal interactions between plant and insect. While *D. pachea* is one of the few fly species that is resistant to toxic alkaloid compounds of *Lophocereus schottii*, it requires 7-dehydrogenated sterols produced by this cactus to survive, because it has lost the capacity to 7,8-dehydrogenate cholesterol (first enzymatic step of the ecdysone biosynthesis pathway).

In insects, this enzymatic reaction appears to be catalyzed by a Rieske-domain enzyme encoded by the *neverland* gene (Yoshiyama et al, 2006). To determine the genetic basis of loss of 7,8-dehydrogenation in *D. pachea* during evolution, we analyzed the evolution of neverland in *D. pachea* and other *Drosophila* species. We found that several amino acids that are otherwise conserved across insects have changed in *D. pachea* Neverland protein. Our study suggests that neverland may have acquired a new function in *D. pachea*.

Yoshiyama T, Namiki T, Mita K, Kataoka H, Niwa R. Neverland is an evolutionally conserved Rieske-domain protein that is essential for ecdysone synthesis and insect growth. Development. 2006 Jul;133(13):2565-74

Isolation of a new class of ecdysteroid conjugates (Glucosyl-Ferulates)

Ho R.[1], Raharivelomanana P.[1], Cousteau P.-Y.[1,2], Bianchini J.-P.[1], Girault J.-P.[3] and Lafont R.[2]

email: phila.raharivelomanana@upf.pf

[1]Laboratoire de Chimie des Substances Naturelles,
Université de la Polynésie Française, Tahiti, Polynésie Française
[2]Laboratoire de Biochimie Structurale et Fonctionnelle des protéines,
Université Pierre et Marie Curie, Paris, France
[3]Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques,
Université Paris Descartes, Paris, France

Microsorum membranifolium which belongs to the Polypodiaceae family is one the most popular fern species used in Polynesian traditional medicine. This plant contains ecdysteroids as major bioactive components including ecdysone, 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone and 2-deoxyecdysone. It also contains unusual ecdysteroids which have been unambiguously identified by MS and NMR.

A new class of ecdysteroid conjugates (3-glucosyl-ferulates of 2-deoxyecdysone and 2-deoxy-20-hydroxyecdysone) has been isolated, together with a new glycoside (2-deoxyecdysone 25-rhamnoside). The simultaneous presence of a sugar and an aromatic moiety results in a very particular chromatographic behaviour of these conjugates. They behave like flavonoids and polyphenols when using the classical purification on polyamide aimed at removing the latter from crude plant extracts and would therefore be lost. They elute as non-polar ecdysteroids on RP-HPLC, whereas their behaviour on NP-HPLC is strongly depending of the mobile phase composition. Our data highlight the importance of selectivity in the choice of HPLC methods used for ecdysteroid separations.

The nuclear receptor BgFTZ-F1 is essential for the onset of the adult developmental program in the hemimetabolous insect *Blattella germanica*

Mané-Padrós D., Nieva C., Maestro J. L., Vilaplana L., Maestro O., Bellés X. and Martín D.

email: cnbagr@ibmb.csic.es

Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CID, CSIC), Barcelona, Spain

Metamorphosis is one of the most fascinating as well as critical periods of insect development, by which an immature larva transforms into a mature adult insect, concomitant with a cessation of further molting. In holometabolous insects, the changes are so dramatic that an intermediate stage, the pupa, is required to accommodate the replacement of larval structures by adult ones. Conversely, in hemimetabolous insects, the adult body plan is similar to that of the immature nymph, although several stage-specific processes must occur during the nymphal-adult transition, such as the degeneration of the prothoracic gland and a remarkable activation of juvenile hormone production in the corpora allata. All these processes require a coordinated set of changes in different tissues and organs mediated by two main hormones, 20-hydroxyecdysone and juvenile hormone. A great deal of research has been devoted to uncover the molecular mechanisms regulating metamorphosis in holometabolous insects. However, little understanding has been achieved regarding the mechanisms controlling the transition to the adult stage in hemimetabolous species. We are currently using the German cockroach, *Blattella germanica*, as model to characterize the genetic network controlling the onset of the adult developmental program. The present work describes a detailed analysis of the role of several nuclear hormone receptors, and particularly BgFTZ-F1, on this process, based on systemic RNAi approaches.

The mechanisms of 20-hydrohyecdysone and dopamine interaction in *Drosophila* under normal and stress conditions

Gruntenko N. E., Chentsova N. A., Bogomolova E. V., Adonieva N. V., Karpova E. K. and Rauschenbach I. Y.
email: nataly@bionet.nsc.ru
Institute of Cytology and Genetics SD RAS, Novosibirsk 630090, Russia

Earlier we have found that in *Drosophila* upon a change in the level of one of the gonadotropins, 20-hydroxyecdysone (20E) or juvenile hormone (JH), as a result of a mutation, effect of a stressor or pharmacological agent, the hormonal balance is restored due to the relative change in the titre of the other gonadotropin. The mediator in the JH and 20E interrelationship is dopamine (DA): a rise in 20E level increases DA content in young females, thus leading to a rise in JH level; a rise in DA content increases JH level in young females, thus leading to a rise in the 20E titre. To reveal mechanisms of 20E and DA interaction we studied (i) changes in the metabolic system of DA in 20E-treated young females of *D. virilis* and *D. melanogaster* under normal and stress conditions, (ii) changes in the metabolic system of 20E in DA- and JH-treated young females of *D. virilis*, and (iii) changes in 20E metabolic system in *D. virilis* strains differed in the dynamics of 20E titre fluctuation under heat stress.

(i) DA content at any moment is determined by the ratio of its synthesis to its degradation. The rate of synthesis depends on the pool of precursors and the activity of synthesizing enzymes. The activities of alkaline phosphatase (ALP), determining at least in part the level of DA precursor, tyrosine, and tyrosine hydroxylase (TH), the first enzyme in DA synthetic pathway, were measured in 20E-treated young females of *D. virilis* and *D. melanogaster* under normal and stress conditions. 20E level was raised by feeding the flies with exogenous 20E. It was shown that an increase in the 20E titre lead to a rise in the activity of both enzymes under normal conditions and an intensification of stress response (a rise in stress-reactivity of both enzymes) under high (38oC) temperature in females of both species. The activity of DA-degrading enzyme, arylalkylamine N-acetyltransferase (AANAT), on the contrary, was found to decrease in the 20E-treated young females of both species. Thus, we conclude that 20E controls the DA level by the way of regulation of both synthesis and degradation of the amine.

(ii) 20E content is determined by the intensity of ecdysone (E) synthesis and its conversion to 20E and by its catabolism. Conversion of E to 20E is catalysed by ecdysone 20-monooxygenase (E20MO). The activity of E20MO was measured in the young wild type females of *D. virilis* treated with exogenous DA or JH. DA content was increased by feeding flies with DA precursor, L-DOPA. The effect of a higher JH titre was studied upon application of exogenous JH. It was revealed that the activity of E20MO grew in the L-DOPA- and JH-treated females.

(iii) The activity of E20MO was measured following 1h, 2h and 3h of heat stress (38oC) in the wild type and hs-mutant *D. virilis* females. The 20E titre in the wild type females started to rise after 1h of heat exposure, whereas in the hs-mutants it started to rise only after 2h of stress. It was shown that dynamics of the increase in 20E titre under stress coincided with dynamics of the increase of E20MO activity in females of both strains.

This study was supported by RFBR grants ## 06-04-48357, 07-04-00194

Involvement of intracellular Ca^{2+} in the nongenomic action of 20-hydroxyecdysone induced programmed cell death

Manaboon M., Iga M., Iwami M. and Sakurai S.
email: manaboon@stu.kanazawa-u.ac.jp
Graduate School of Natural Science&Technology, Kanazawa University, Japan

20-Hydroxyecdysone (20E) triggers programmed cell death (PCD) of anterior silk glands (ASGs) of the silkworm, *Bombyx mori*. Previous studies indicated an involvement of nongenomic action of 20E in addition to genomic action in the 20E-induced cell death. In the nongenomic action, Ca^{2+} acts as the second messenger that activates protein kinase C and caspase 3-like protease, but the early pathway up to the intracellular Ca^{2+} elevation remained to be seen. In order to investigate such an unknown pathway, we employed pharmacological tools to examine individual components in the pathway. ASGs were cultured with 1 **µ**M 20E with various concentrations of one of the following inhibitors: G protein-coupled receptor (GPCR) inhibitor, phospholipasce C (PLC) inhibitor, inositol 1,4,5-trisphosphate receptor (IP_3R) antagonist, and L- and T- type Ca^{2+} channel blockers. Although T-type Ca^{2+} channels blocker inhibited 20E-induced nuclear fragmentation and DNA fragmentation, PCD was induced by 20E in Ca^{2+} free medium, indicating that the source of Ca^{2+} is the intracellular Ca^{2+} reservoir. In fact, IP_3R antagonist inhibited nuclear and DNA fragmentation, showing that Ca^{2+} source may be the endoplasmic reticulum. In addition, nuclear fragmentation and DNA fragmentation was effectively inhibited by GPCR inhibitor and PLC inhibitor. Taken together, the intracellular Ca^{2+} increase may be resulted in response to 20E through GPCR/PLC/IP pathway, which activates the downstream pathway including protein kinase C and caspase 3-like protease.

The Interaction between p127$^{l(2)gl}$ and Armadillo leads to a decreased formation of Arm/Tcf, activation of hid expression, and execution of cell death program in *Drosophila* salivary glands

Farkaš R.[1], Mentelová L.[1, 2], Kuchárová-Mahmood S.[1], Raška I.[3] and Mechler B. M.[4]

email: ueenfark@savba.sk

[1]Laboratory of Genetics, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia,
[2]Department of Genetics, Comenius University, 84215 Bratislava, Slovakia,
[3]Laboratory of Cell Biology, 1st Faculty of Medicine, Charles University and the Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic
[4]Department of Developmental Genetics, DKFZ-ZMBH Allianz, Heidelberg, Germany

During *Drosophila* metamorphosis larval tissues, such as the salivary glands, are histolyzed whereas imaginal tissues differentiate into adult structures forming at eclosion a fly-shaped adult. The disintegration of the larval salivary glands is triggered by the steroid hormone ecdysone and takes place 12-13h after pupariation (APF), although vacuolization of the cytoplasm is already noticed 4-5h APF, much before the activation of the death genes (10-12h APF). Previously we showed that disintegration of the salivary glands requires the presence of the p127 cytoskeletal protein encoded by the *l(2)gl* tumour suppressor gene and that the timing of histolysis displays a *l(2)gl*-dose response. We found that in *l(2)gl* salivary glands the Armadillo (Arm) protein, which is normally associated with the plasma membrane, is released in the cytoplasm where it is diffusely distributed. Immuno-precipitation and the use of the yeast-two hybrid system revealed that p127 and Arm physically interacts together. While ectopic expression of *wg*, which is known to stabilize intracellular Arm, significantly delays the process of cell death, *sgg* expression, which encodes the Shaggy/Zeste-white 3 kinase, was found to accelerate this process. RT-PCR analysis showed no expression of *hid* mRNA in both *l(2)g* salivary glands or wt glands ectopically expressing *arm* or *wg*. However, we found that *sgg* can induce a level of hid expression similar to that in wild type at ~10 hr APF. The occurrence of Arm/Tcf binding sites within the *hid* gene indicates that the *wg* signalling pathway can be involved in the control of cell death gene expression and our data provide the first evidence for a crosstalk between the pathways regulated by ecdysone and *wingless*.

Establishment of a New Cell Line from Lepidopteran Epidermis and the 20-hydroxyecdysone regulation on the genes expression

Shao H.-L.[1,2], Zheng W.-W.[1], Liu P.-C.[1], Wang Q.[1], Wang J.-X.[1], Zhao X.-F.[1]
email: xfzhao@sdu.edu.cn
[1]School of Life Sciences, Shandong University, Jinan, China
[2]School of medicine, Shandong University, Jinan, China

Insect molts, a process of old cuticle on the outside of the integument is shed by apolysis and a new cuticle is formed under the old one, is completed by the epidermal cells which are controlled by 20E and juvenile hormone. To understand the molecular mechanisms of integument remolding and hormonal regulation on the gene expression, an epidermal cell line from the 5th instar larval integument of *Helicoverpa armigera* was established and named HaEpi. The cell line was cultured and sub-cultured for 52 passages beginning on June 30, 2005 until now. Cell doubling time was 64 h. The chromosomes were granular and numbered from 35 to 560. Collagenase I was used to detach the cells from the flask bottom. Non-self pathogen AcMNPV induced the cells to apoptosis. The cell line was proved to be an epidermal cell line based on its unique gene expression pattern. It responded to 20-hydroxyecdysone (20E) and the non-steroidal ecdysone agonist RH-2485. Its gene expression could be knocked down using RNA interference. Various genes in the cell line were investigated based on their response to 20E. This new cell line represents a platform for investigating the 20E signaling transduction pathway, the immune response mechanism in lepidopteran epidermis and interactions of the genes.

This work was supported by grants from the Natural Science Foundation of China (No: 30710103901; 30670265), the Special Fund for the Major State Basic Research Project (2006CB102001)

Novel 23 kDa fat body secretory protein affects steroid levels during insect development

Gullipalli D., Arif A. and Dutta-Gupta A.
email: apdgsl@uohyd.ernet.in
Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India

The postembryonic development in insect involves growth, molting and metamorphosis. It is now well established that metamorphosis, which is viewed externally as abrupt morphological transition, is in reality a smooth continuation of precisely regulated events. Molting as well as metamorphosis are hormonally mainly regulated, by an interplay of juvenile hormones and ecdysteroids. Through immunoscreening of fat body cDNA library we obtained a fat body specific clone of 639 bp. This cDNA clone was over expressed in bacterial system using pGEX4T-1 vector and protein obtained was of 23 kDa. The recombinant protein was purified for the generation of polyclonal antibody, basically to study the physiological functions of the protein during the postembryonic development of the insect. *In vivo* neutralization of this 23 kDa protein in larval forms causes a precocious rise in ecdysteroid titter, which was associated with either larval mortality or formation of larval-pupal intermediates. This along with the other data will be presented and discussed during presentation.

EcR is required for oocyte development in the telotrophic ovary of *Tribolium castaneum*

Takaki K., Konopova B. and Jindra M.
email: jindra@entu.cas.cz
Biology Center ASCR, Institute of Entomology, Ceske Budejovice, Czech Republic

The importance of ecdysone signaling for oogenesis is well studied in *Drosophila* whose polytrophic meroistic ovary consists of ovarioles, where each oocyte forms a cluster with its sibling nurse cells, enveloped with somatic follicle cells. The ecdysone receptor (EcR) and some of its early-response genes are required in the germline cells for progression of the oocyte beyond mid-oogenesis. To understand the role of the ecdysone signaling pathway in other types of insect ovaries, we utilize the red flour beetle, *Tribolium castaneum*. *Tribolium* is highly sensitive to systemic RNAi and possesses a telotrophic meroistic ovary, in which the oocytes are ensheathed with follicle cells but are not accompanied by nurse cells. All nurse cells reside in an anterior region, the tropharium, and connect to each oocyte via a nutritive cord. The germ cell proliferation is complete by the end of the pupal stage. In the adult female, oocytes proceed from the tropharium to the vitellarium and begin to grow rapidly. Normally, one ovariole contains only few early oocytes and growing follicles. To address the role of the ecdysone signaling in *Tribolium* oogenesis, we suppressed the function of EcR and its partner Usp by using dsRNA-mediated RNAi. Injection of *EcR* or *usp* dsRNA during larval and pupal stages invariantly prevented the next ecdysis. However, in adult mated females, silencing of either *EcR* or *usp* blocked egg production within a few days. Ovaries of the affected females contained extranumerary oocytes that grew only to some extent but failed to mature. The follicle cells were abnormally shaped and showed excessive proliferation along the ovariole. These phenotypes differed from anomalies caused by *EcR* mutations in *Drosophila*, where vitellogenic egg chambers were missing, and also from the effect of *EcR* RNAi in the panoistic ovary of the cockroach *Blattella*, where follicle cells proliferated less than normally and formed a defective chorion. In addition, we found that mRNA levels of two vitellogenin genes were reduced upon *EcR* RNAi in beetle females. This reduction was consistent with the fact that vitellogenin expression is controlled by ecdysone in some insects (e.g. mosquitoes), but alone could not explain the observed oogenesis defects. Thus, although ecdysone signaling appears crucial in multiple types of insect ovaries, detailed studies of its function in the *Tribolium* telotrophic ovary should bring interesting functional comparisons.

Supported by project LC07032 from the Czech Ministry of Education

Hormonal regulation on stage-specific larval mimicry markings of the swallowtail butterfly, *Papilio xuthus*

Futahashi R.[1,2] and Fujiwara H.[1]
email: ryo284@k.u-tokyo.ac.jp
[1]Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Bioscience Bldg. 501, Kashiwa, Japan
[2]National Institute of Agrobiological Sciences, 1-2 Owashi, Tsukuba, Japan

Changes in larval coloration among the larval stages are well-known in Lepidoptera. The swallowtail butterfly, *Papilio xuthus*, is a spectacular example in that young caterpillars are mimics of bird droppings whereas the final larval instar can be camouflaged in leaves of the host plant. We first compared the mRNA expression of epidermis between the third and fourth molts of *P. xuthus* using cDNA subtraction method and whole-mount *in situ* hybridization. After analyzing 2,072 clones from two subtractive libraries, we found that two genes were expressed at the presumptive green region only during the fourth molt, whereas several cuticular protein genes expressing specifically in tubercle structures only in juvenile instars. Moreover, expression of melanin synthesis genes was associated with the black markings of subsequent instar. Cuticular pigmentation occurs with precise timing just before ecdysis. We analyzed the effects of 20-hydroxyecdysone (20E) on the pigmentation and expression of the melanin synthesis genes. Topical application of 20E to molting specimens not only inhibited cuticular pigmentation but also suppressed the expression of several pigmentation genes. We next investigated the effect of juvenile hormone (JH) on stage-specific markings. We found that the level of juvenile hormone (JH) decreases during the fourth larval instar, and treatment with JH analog prevents the larval pattern switch. JH regulates not only overall color pattern but also exoskeletal structures and pigment distribution at specific markings, suggesting that JH regulates the progressive larval pattern switch.

Hormonal control of pupal commitment of the epidermis of the silkworm, *Bombyx mori*: comparison with that of *Manduca sexta*

Hiruma K. and Muramatsu D.
email: hiruma@cc.hirosaki-u.ac.jp
Faculty of Agriculture and Life Sciences, Hirosaki University, Japan

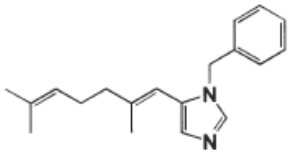
During the pupal metamorphosis in insects, cellular commitment for pupal differentiation must precede before its differentiation. The pupal commitment of *Bombyx mori* epidermis occurred from day 3 to day 6 last (5th) instar larvae in response to the gradual increase in ecdysteroid titer in the presence of a small amount of juvenile hormone (JH). Yet the concealed preparatory process of the commitment had begun in the newly synthesized 5th instar larval epidermis (~6 hr before the ecdysis) as a competence phase, in which pupal commitment *in vitro* was induced by 20-hydroxyecdysone (20E) but inhibited by JH. This competence phase continued until day 2 5th instar, and the decrease and increase in cellular sensitivity to JH and 20E respectively occurred gradually during this period. In early day 3, autonomous pupal commitment began *in vitro* and 20E stimulated the commitment, but JH could only partially prevent the commitment in both cases. This apparent reversible to irreversible transition ended in early day 6 by the completion of pupal commitment, when the cells completely lost their sensitivity to JH and no longer expressed the larval cuticle protein gene 30. The expression of the transcription factor, broad, closely followed the commitment, so that we could use this gene expression as a molecular marker for pupal commitment. These results indicate that exposure to 20E and loss of the sensitivity of the epidermal cells to JH are required for the completion of pupal commitment, and suggest that the unusually long process over 3 days could be due to the presence of the detectable JH during the commitment.

Supported by the PROBRAIN

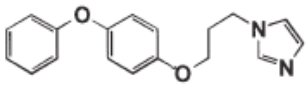
Comparison of precocious metamorphosis induced by different conditions in the silkworm

Shiotsuki T.
email: shiotsuk@affrc.go.jp
Gene Function Research Unit, Division of Insect Science,
National Institute of Agrobiological Sciences, Japan

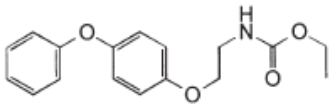
Precocious metamorphosis in the silkworm is able to induced by topical application with some substituted imidazole, such as KK-42 [1-benzyl-5-(2,6-dimethyl-1,5-heptadienyl)imidazole (**1**)]. It was reported that the KK-42 inhibited the synthesis of ecdysteroids in both *in vitro* and *in vivo*. In addition, it was found the KK-42 induced enzymatic activity of juvenile hormone esterase (JHE) in hemolymph. It has been known well that an allatectomy can also induce the precocious metamorphosis at 3rd or 4th stadium of the silkworm. The duration of the larval stadium before the precocious metamorphosis was prolonged and normal cocoons were formed for the KK-42 treated or allatectomized larvae. The precocious metamorphosis was also occurred in the larvae of transgenic silkworm with over-expression of JHE at 3rd stadium. In contrast to KK-42 treated or allatectomized larvae, the JHE over-expressed larvae metamorphosed precociously with no extension of the duration of 3rd stadium, and made no cocoons. On the other hand, 1-[3-(4-phenoxyphenoxy)-propyl]imidazole (KS-175, **2**) is a hybrid compound with two characteristic moieties of insect growth regulators, that is, the phenoxyphenoxyalkyl group in the juvenile hormone analogs (JHAs, such as fenoxycarb, **3**) and substituted imidazole. When 4th instar larvae were treated topically with KS-175, the larvae did not molt for more than 20 days without any toxic phenomenon. Low ecdysteroid levels in the hemolymph and irreversible damage of prothoracic gland were observed.



KK-42 (**1**)



KS-175 (**2**)



fenoxycarb (**3**)

This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN)

Juvenile hormone modulates PKC activity and ecdysteroids signaling to regulate fat body remodeling and pupal metamorphosis in *Drosophila*

Liu Y., Sheng Z., An S., Sun Y., Wen D., Shao W., Liu H., Wang S., Jiang R.-J., Huang J., Bendena W. G., Wang J., Song Q., Li S.[1]

email: shengli@sippe.ac.cn

[1]Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

In larvae of Lepidoptera, juvenile hormone (JH) prevents ecdysteroids (Ecd) from initiating metamorphosis, so that after molt another larval stage follows. For this reason, JH is referred as a “status quo” hormone of insects. Previous studies show JH plays little role in regulating *Drosophila* postembryonic development except its adult reproduction. In this report, we provide genetic evidences that in contrast with Lepidoptera, JH is required for pupal metamorphosis in *Drosophila* at the presence of Ecd. JH regulates *Drosophila* metamorphosis partially by controlling fat body remodeling. In JH-deficiency flies, fat body undergoes precocious remodeling with dramatic changes of protein and mRNA levels during larval-pupal transition, as consequentially results in pupal lethality. JH controls fat body remodeling by blocking the Ecd-triggered transcriptional cascade, which causes autophagy and apoptosis in this tissue. JH blocks Ecd signaling in the fat body via modulating PKC activity, phosphorylation and nuclear localization of the Ecd receptor complex EcR/USP. The *Drosophila* fat body might be a useful system to understand the long-standing puzzle of JH signal transduction.

Met and BR-C control beetle metamorphosis in response to juvenile hormone

Konopova B. and Jindra M.
email: jindra@entu.cas.cz
Biology Center ASCR, Institute of Entomology, Ceske Budejovice, Czech Republic

Insect metamorphosis is a prime example of how hormones regulate development. Growth, differentiation, tissue remodeling and death are all orchestrated by the morphogenesis-promoting ecdysteroids and the morphostatic juvenile hormone (JH), whose presence precludes metamorphosis. How tissues interpret the combinatorial effect of these two hormonal cues is poorly understood, mainly because JH signal transducers remain unknown. Genetic studies on JH regulation of metamorphosis are hindered by the lack of a robust effect of JH on *Drosophila*: unlike in beetles or moths, JH cannot prevent larval-pupal transition or induce extra larval instars in the fly. We therefore chose to study metamorphosis in the flour beetle, *Tribolium castaneum*, which is both amenable to systemic RNAi and sensitive to the typical anti-metamorphic JH effect. Here, we show that JH controls the entry of beetle larvae to metamorphosis through the ortholog of the *Drosophila* PAS domain protein Methoprene-tolerant (Met). Loss of *Met* confers resistance to JH but alone causes no developmental defects in *Drosophila* [1]. In contrast, *Tribolium* larvae deficient in *Met* function enter the pupal program prematurely, before reaching their final instar. Met RNAi also makes *Tribolium* pupae resistant to JH [2]. These findings define Met as an essential transducer of the anti-metamorphic JH signal and encourage the idea that Met could be the missing JH receptor. Next, we show that in response to JH, Met regulates expression of the *Tribolium Broad-Complex* gene (*BR-C*), which is required for pupal development and for the temporal coordination of metamorphosis in the beetle. *BR-C* is upregulated in precocious beetle prepupae that result from Met knockdown, and ectopic JH induces *BR-C* in normal pupae but not in pupae deficient for Met [3]. Thus, Met and its downstream target *BR-C* play a critical role in the JH-dependent regulation of insect metamorphosis.

Supported by project 204/07/1032 from the Czech Science Foundation

1. Wilson, T.G. and Ashok, M. (1998) Insecticide resistance resulting from an absence of target-site gene product. Proc. Natl. Acad. Sci. USA 95, 14040-
2. Konopova, B. and Jindra, M. (2007) Juvenile hormone resistance gene Methoprene-tolerant controls entry into metamorphosis in the beetle Tribolium castaneum. Proc. Natl. Acad. Sci. USA 104, 10488-
3. Konopova, B. and Jindra, M. (2008) Broad-Complex acts downstream of Met in juvenile hormone signaling to coordinate primitive holometabolan metamorphosis. Development 135, 559-

Juvenile hormone analog and 20-hydroxyecdysone induction of the wing disc development and EcR mRNA expression in the larval diapause of bamboo borer, *Omphisa fuscidentalis*

Singtripop T.[1], Sereewattanachai P.[1] and Sakurai S.[2]
email: scboi020@chiangmai.ac.th
[1]Department of Biology Faculty of Science, Chiangmai University, Thailand
[2]Division of Life Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Japan

The bamboo borer *Omphisa fuscidentalis* (Lepidoptera, Pyralidae) is a univoltine insect with a larval diapause period lasting up to 9 months. Mature larvae enter diapause in September and remain in diapause for 9 months without feeding. Application of methoprene, a JH analog (JHA), terminates larval diapause by increasing ecdysteroids in the hemolymph (Singtripop et al., 2002). To study the growth and development of the wing discs during the larval diapause, protein contents in the fore and hind wing discs were measured in the diapause larvae from September to March and after JHA application or 20-hydroxyecdysone (20E) injection. Results showed that the protein contents in the fore and hind wing discs increased greatly in February and March. In the larvae treated with 0.1, 0.5 and 1 µg JHA or injected with 0.1, 0.5 and 1µg 20E, the protein contents increased significantly ($P<0.01$) when they entered to G0 stage. An in vitro culture of wing discs in 20E showed that the protein contents in the wing discs increased by 24 hr of culture and significantly increased after 72 hr of culture in 0.5 and 1 µg/ml 20E ($P<0.01$). Immunohistochemical observations of S and M phase cells in the cultured wing discs showed that 0.1 µg 20E induced wing disc growth by increasing the number of S and M phase cells in a wing disc($P<0.01$). RT- PCR analysis of *O. fuscidentalis* ecdysone receptor (*OfEcR*) mRNA expression in the wing disc showed that 1 µg JHA application induced *OfEcR-A* mRNA expression that peaked at G1. *OfEcR-B1* mRNA expression displayed two peaks at day 8 and G1. When the bamboo borer larvae were injected with 1 µg 20E, they entered G0 in 2 days and *OfEcR-A* mRNA expression peaked at G0 and *OfEcR-B1* mRNA peaked at Day2 and G1. Cultured wing discs in 0.1 µg/ml 20E exhibited an increase in *OfEcR-A* and *OfEcR-B1* mRNA expression after 24 hr of culture, and *OfEcR-B1* mRNA expression level increased 2 folds of *OfEcR-A* mRNA. The present results indicate that JHA elicite stimulatory effects on the growth of wing imaginal discs, resulted from the increase in ecdysteroid concentration in the hemolymph followed by increase in expression levels of *OfEcR-A* and *EcR-B1* mRNAs.

Developmental effects of a dominant negative form of Ultraspiracle (USP) in *Drosophila melanogaster*

Henrich, V. C.[1], Plotkin J.[1], Costantino B.[2], Andres A. J.[2], Clifton K. C.[1] and Callender J. A.[1]
email: vincent_henrich@uncg.edu
[1]Center for Biotechnology, Genomics, and Health Research;
University of North Carolina- Greensboro, Greensboro, NC, USA
[2]School of Life Sciences, University of Nevada-Las Vegas, Las Vegas, NV, USA

The predominant receptor for ecdysteroid signals in insects has been shown to be a heterodimer of EcR and Ultraspiracle (USP) proteins. Previous studies using mutants of *Drosophila melanogaster* indicate that deletions involving the DNA-binding domain of USP cause both repressive and inductive changes in the pattern of target gene expression. Cell culture studies further suggest that the EcR/USP regulation of ecdysteroid-inducible transcriptional activity is isoform-specific. To investigate these effects further, a modified form of *D. melanogaster* USP under the control of a UAS promoter has been tested with a variety of GAL4 lines (UAS-ΔDBD-*Dm*USP). When expressed broadly during larval development, ΔDBD-*Dm*USP causes an arrest in the late third instar that resembles the lethal phenotype seen in the absence of USP activity. Further, when tested in cell types that predominantly express the EcR-A isoform, the phenotypic effects of the ΔDBD-*Dm*USP resemble those caused by mutations of the EcR-A isoform, and developmental arrest occurs during pupal-adult development. Similar morphological defects are evoked by a UAS-USP+, though the wild-type form does not cause lethality. These developmental studies indicate that the ΔDBD-*Dm*USP exerts a variety of dominant negative effects that vary by cell type, thus providing a basis for investigating the role of USP in specific cellular processes. The effects of the ΔDBD-*Dm*USP on transcriptional activity in the larval salivary gland and other tissues are currently being investigated.

The putative partner of the crustacean ecdysteroid receptor, UpRXR, and its isoforms from the fiddler crab, *Uca pugilator*

Hopkins P., Durica D., and Washington T.
email: phopkins@ou.edu
Department of Zoology, University of Oklahoma, Norman, OK, USA

In the fiddler crab, *Uca pugilator*, there are at least two isomers of the UpRXR in the regenerating limb that differ from one another by a 33 amino acid insert in the ligand binding domain - $UpRXR_{(larger)}$ and $UpRXR_{(smaller)}$. mRNAs for both RXR isomers are expressed in limb bud tissues of the crab in specific patterns. Injection of ds mRNA for UpRXR into the compartment in which the limb blastema develops significantly lowers the relative amounts of *UpRXR* but has no effect on the levels of *UpEcR* (nor control gene, *GAPDH*). Both isoforms of UpRXR heterodimerize with UpEcR in the absence of ligand. The *in vitro* binding of UpEcR to ecdysteroids is, however, affected by the UpRXR isoform with which it is paired. Equilibrium K_d's of UpEcR binding to ^{3}H-ponasterone A (PA) vary depending on which UpRXR isoform is present. When partnered with the smaller isoform, UpEcR binds PA with greater affinity than when paired with the larger isoform (K_d's = 1.0 nM versus 1.9 nM). UpEcR (with either of the isoforms) binds to PA with greater affinity than to 20-hydroxyecdysone (20E) or ecdysone (E), PA has no effect, however, on dimerization. UpEcR dimerization to the larger isoform is significantly increased in the presence of high levels of 20E. Putative endogenous ligands for the UpRXRs are present in the regenerating limb bud blastema. Retinoids, retinoid metabolites, and retinoid metabolizing enzymes have been isolated from blastemas and identified using HPLC, UV absorption, and mass spectrometry. The apparent K_d for the putative UpRXR ligands is very high (0.2- 0.4 mM) Putative UpRXR ligands at high concentrations have little or no effect on *in vitro* homodimerization. These variations in dimer function will be discussed in light of variations in circulating steroids in crab hemolymph during the regeneration/molting cycle and the role of UpRXR is limb blastema regeneration.

Identification of NLS and NES signals for *Drosophila* methoprene-tolerant protein

Greb-Markiewicz B.[1], Orłowski M.[1], Gwóźdź T.[1], Dutko-Gwóźdź J.[1], Dobrucki J.[2], Ożyhar A.[1]

email: greb_markiewicz@yahoo.com

[1]Department of Biochemistry, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland

[2]Division of Cell Biophysics, Faulty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Introduction:
Although juvenile hormone (JH) is an important regulator of insect development and reproductive maturation, neither the molecular mechanism of JH action nor JH receptor is known. Met is one of proposed and very probable candidate for this function. It has been shown that Met belongs to the family of bHLH-PAS transcription factors. Their functional activity often is correlated with shuttling between nucleus and cytoplasm according to the masking and unmasking, by interacting partners, signals directing this proteins to nuclear or cytoplasmic compartment of the cell. The first report concerning *Drosophila melanogaster* Met has informed about localization of this protein in cytoplasm. Later on it has been shown as nuclear protein by *in vivo* immunostaining of *Drosophila* tissues and by using chimeric proteins with GFP in S2 cells. Till now, no NLS and NES motifs has never been elucidated for Met.

Methods:
In order to determine the sequences of nuclear localization signals (NLSs) and nuclear export signals (NESs), series of deletion and point mutants tagged by yellow fluorescence protein (YFP) were prepared by cloning into EYFP-C1 vector and expressed in mammalian cells.

Results:
Our results show the presence of dominant NLS in area linking bHLH and PAS A domains and recessive NLS in area of PAS B repeat. Additionally, we have detected presence of active NES motifs in PAS A, PAS B repeats and the C-terminal part of Met.

Conclusion:
Met is cytoplasm-nucleus shuttling protein with very complicated control system of localization of this transcription factor in the cell compartments.

RNase Z, the *Drosophila* homolog of human ELAC2, is required during early larval development

Dubrovsky E. B.
email: dubrovsky@fordham.edu
Department of Biology, Fordham University, New York, USA

Juvenile hormone (JH) is an important component of the insect endocrine system. In adults of many insect species, JH regulates reproductive maturation, affects sexual behavior and controls reproductive diapause.

Previously we found that in *Drosophila* cultured cells and adult females, JH rapidly and specifically up-regulates the expression of the *dRNaseZ* gene. *dRNaseZ* is a member of the ELAC1/2 gene family, which is conserved from bacteria to man. In human, *ELAC2* was identified as a Prostate Cancer susceptibility gene. ELAC2 protein possesses the tRNA endonuclease activity. There is also data indicating that ELAC2 possibly plays a role in growth control and cell division. The hypothesis is that perturbation of ELAC2 might promote tumorogenesis through irregular cell division. However, the *in vivo* biological function of the ELAC2 protein has yet to be demonstrated.

Maturation of a functional tRNA is a multi-step process, and dRNaseZ, the fly ortholog of ELAC2, catalyses one of them - the removal of the 3‘ trailer sequence from the primary tRNA transcript. We performed the RNAi study in *Drosophila* S2 cells, and found that dsRNA-mediated silencing of *dRNaseZ* disrupted tRNA maturation and triggered accumulation of pre-tRNA molecules with 3’ extensions. In our next study, we used the imprecise excision mutagenesis to create a series of *dRNaseZ* knockout mutations. The preliminary analysis showed that: (i) *dRNaseZ* is an essential gene; (ii) the dRNaseZ protein is a part of the tRNA maturation pathway; (iii) dRNaseZ has an additional role besides tRNA processing, perhaps involving the cell cycle regulation.

Ecdysteroids as Gene-Switch Actuators: Enhancement of Physicochemical Properties and Trends Toward Orthogonality

Lapenna S.[1], Dinan L.[1], Palli S. R.[2], Barlow A.[1], Friz J.[3], Hopfinger A. J.[4,5], Liu J.[5], Hormann R. E.[1]

email: rhormann@intrexon.com

[1]Department of Biological Sciences, University of Exeter, Devon, UK
[2]Department of Entomology, College of Agriculture, University of Kentucky, Lexington, KY, USA
[3]Intrexon Corp., Norristown, PA , USA
[4]The Chem21 Group, Inc., Lake Forest, IL, USA
[5]College of Pharmacy, MSC09 5360, University of New Mexico, Albuquerque, NM, USA

The ligand-inducible, ecdysteroid receptor (EcR) gene-expression system can add critical control features to protein expression in cell- and gene therapy. However, potent natural ecdysteroidal ligands possess absorption, distribution, metabolism and excretion (ADME) properties that have not been optimized for use as gene-switch actuators *in vivo*. On the notion that alkylation might improve the potency or bioavailability of these ligands, the representative ecdysteroids 20-hydroxyecdysone (20E) and ponasterone A (PoA) were singly- and multiply-methylated at the 2-, 3-, 14-, 22- and 25-positions, or singly-alkylated at the 22 position. The semi-synthetic steroids were assayed in engineered gene-switch systems in mammalian cells using EcRs from several species. Gene-switch potency is maintained, or even enhanced, for certain steroid/EcR combinations. Calculated ADME properties using the membrane-interaction (MI)-QSAR methodology indicate more balanced, and generally desirable, trends toward lower solubility, higher permeability and higher blood-brain barrier penetration without excessive modulation of logP or plasma protein binding.

In separate experiments, several dozen natural and semi-synthetic ecdysteroids were assayed in a gene switch format in murine 3T3 cells across ten different ecdysteroid receptor (EcR) ligand binding domains derived from nine arthropod species. Several duplex ligand/EcR combinations exhibit an inversion of relative potency and therefore lend themselves to construction of orthogonal duplex gene switches. Ecdysteroid SAR and orthogonality is described in the context of LBD amino acid sequences and docking.

Recombinant ligand-binding domains from insect ecdysone receptors

Graham L. D.[1], Hannan G. N.[1], Pawlak-Skrzecz A.[1], Noyce L.[1], Tohidi-Esfahani D.[1], Johnson W. M.[2], Howell L.[2], Lovrecz G.[3], Lu L.[3], Pilling P. A.[3], Eaton R. E.[1], Bliese M.[2], and Hill R. J.[1]
email: lloyd.graham@csiro.au

[1]CSIRO Molecular & Health Technologies, North Ryde, NSW, Australia
[2]CSIRO Molecular & Health Technologies, Clayton, VIC, Australia
[3]CSIRO Molecular & Health Technologies, Parkville, VIC, Australia

Cloned EcR and USP cDNAs encoding the ecdysone receptors of four insect pests (*Lucilia cuprina, Myzus persicae, Bemisia tabaci, Helicoverpa armigera*) were manipulated to allow the co-expression of their ligand binding domains (LBDs) in insect cells using a baculovirus vector. Cognate EcR and USP LBDs associated spontaneously with high affinity to form heterodimers that avidly bound ecdysteroids. Recombinant heterodimers could readily be affinity-purified via a His6 tag on the EcR LBD.

The K_d values for [^{3}H]-ponasterone A binding and K_i values for ecdysteroid and dibenzoylhydrazine competitors will be discussed. The dissociation constants for the only E/F heterodimer studied were several times higher than those for its DE/F counterpart. Kinetic rate constants were estimated for a representative ecdysteroid-LBD heterodimer interaction.

A fluorescein-inokosterone A conjugate was used to develop a novel *in vitro* binding assay that relies upon fluorescence polarization (FP). An FP-based competitive binding assay ranked the affinity of competitor ecdysteroids in the same order as the [^{3}H]-ponasterone A binding assay. The FP assay is well suited to high-throughput screening, and has been automated to screen chemical libraries for new ecdysone receptor ligands.

The binding data obtained *in vitro* using recombinant LBD heterodimers reflects the ability of agonists to induce transgene expression in recombinant mammalian cells, and can also reflect their efficacy as insecticides. A survey of published data for tebufenozide confirms that its *in vitro* binding affinity for the ecdysone receptor from a particular insect correlates positively with its biological efficacy in that species and negatively with the phylogenetic distance of the EcR LBD from a lepidopteran reference sequence.

Molecular analysis of ecdysone action in the red flour beetle, *Tribolium castaneum*

Palli S. R., Parthasarathy R. and Tan A.
email: rpalli@email.uky.edu
Department of Entomology, University of Kentucky, Lexington, KY, USA

We used quantitative real-time reverse-transcriptase PCR (qRT-PCR) and RNA interference (RNAi) analyses to study action of ecdysteroids in the red flour beetle, *Tribolium castaneum*. Nineteen canonical and two Knirps family NRs were identified in the genome of *T. castaneum*. RNAi analysis showed that 10 out of the 19 canonical NRs, TcE75, TcHR3, TcHR4, TcEcR, TcUSP, TcFTZ-F1, TcHR51, SVP, TcHR38, TcHR39 are important for metamorphosis. Knocking down the expression of five NRs, TcTll, TcDsf, TcHNF4 and TcHR78 caused defects in production of offspring. TcHNF4, TcHR78, TCHR51 and TcDsf affected egg production and TcTll affected embryonic development. Knocking down the expression of non-canonical NR Knirps-like affected adults and caused reduction in egg production. The other Knirps family member, Eagle, and five canonical NRs, TcE78, TcHR83, TcHR96, TcPNR-like and TcERR did not show much effect on metamorphosis or production of offspring. Two isoforms each of EcR and USP have been identified. qRT-PCR analysis showed isoform-specific developmental expression of both EcR and USP in the epidermis and the midgut dissected from the final instar larvae and pupae. Injection of double-stranded RNA (dsRNA) prepared using the common or isoform-specific regions of EcR or USP as a template caused derailment of development. EcR common region or EcRA dsRNA caused more severe effects, and most of the treated larvae died prior to pupation. EcRB dsRNA caused less severe effects and most of the treated larvae became pupae but showed developmental defects. Only dsRNA prepared against USP common region but not against USPA or USPB isoform-specific regions caused developmental defects during larval-pupal metamorphosis. Determination of mRNA levels of EcR isoforms and 20-hydroxyecdysone-response (20E) genes (broad, E75, E74, HR3 and FTZ-F1) by qRT-PCR in the larvae injected with EcRA, EcRB or EcR common region dsRNA showed that EcRA initiates ecdysteroid action by regulation the expression of EcRB and 20E-response genes. These data suggest that the EcR but not USP isoforms play distinct roles during the larval-pupal metamorphosis and EcRA plays a dominant role in transduction of ecdysteroid response in *T. castaneum*. RNAi studies also revealed important roles for EcR, USP, Broad and Met in 20E induced programmed cell death of polyploid larval cells and proliferation of intestinal stem cells in the midgut remodeling during larval pupal metamorphosis.

Regulation of transcriptional activity of *Drosophila melanogaster* ecdysteroid receptor isoforms

Tremmel Ch., Ruff H., Braun S., Azoitei A., Spindler-Barth M.
email: christian.tremmel@uni-ulm.de
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

The Drosophila melanogaster ecdysteroid receptor consists of the three isoforms EcR-A, EcR-B1 and EcR-B2, which differ in length and sequence of their AB domain. One of their main heterodimerization partners is Ultraspiracle (Usp).

Our examination on the regulation of transcriptional activity of the EcR/Usp complex include reporter gene assays, studies on receptor stability (quantification of specific Western Blot signals), DNA binding (EMSA), intramolecular interaction of the EcR isoforms (FRET technique) and interaction with comodulators in wildtype and mutated receptors.

Constitutive transactivation potency is different for EcR isoforms and is enhanced after dimerization with Ultraspiracle and in the presence of hormone. Modulation of transactivation potency is not only a result of changes in receptor activity but also of altered receptor stability. EcR proteins are stabilized by Usp and hormone in an isoform-specific manner. Our experiments with different Usp variants show, that the AB domain of Usp encompasses an inhibitory function in the N-terminus and an activation function located in the hexapeptide adjacent to the C-domain. The hexapeptide also influences DNA binding and is active only in the absence of hormone. Mutation of the Usp amino acid leu259, which is important for the antagonistic conformation of Usp helix 12, impairs transactivation potency of EcR/Usp only, if the hexapeptide is present and hormone is absent. These results confirm the active role of Ultraspiracle in the modulation of ecdysteroid receptor activity.

Using mutated receptor isoforms we show that lys497 in helix 4 of EcR ligand binding domain is part of a corepressor binding site. The hexapeptide in the AB domain of Usp interacts with this corepressor binding site, further supporting our hypothesis of an activation function located in the hexapeptide.

In addition, transcriptional activity is regulated by intramolecular interaction of the EcR isoforms. FRET analysis (fluorescence resonance energy transfer) revealed that transactivation functions AF1 (AB domain) and AF2 (ligand binding domain) of EcR interact in an isoform-specific manner independent of the absence or presence of Usp. This interaction is modified in some, but not all isoforms by hormone.

DNA Affects Ligand Binding of the Ecdysone Receptor of *Drosophila melanogaster*

Azoitei A., Spindler-Barth M.
email: anca.azoitei@uni-ulm.de
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

In the absence of a heterodimerization partner hormone binding to ecdysone receptor (EcR) is stimulated by the presence of ecdysone response elements (EcREs) to different degrees depending on the isoform. If Ultraspiracle (Usp), the invertebrate orthologue of RXR, is used as dimerization partner, all EcR isoforms of *Drosophila* bind the ligand ponasterone A with the same affinity. In the presence of EcREs ligand binding of the heterodimer (EcR/Usp) is also increased depending on the type of EcRE, the EcR isoform and the Usp variant. Ligand binding to heterodimers with wild type Usp is enhanced about 5 fold with hsp27, Pal-1 and DR-1. The same results are obtained with monomeric and pentameric EcRE´s. In the absence of DNA hormone binding is not affected, if the AB domain of wild type Usp was replaced by the AB domain of VP16. In contrast substantial differences were observed with Vp16-Usp fusion proteins in the presence of DNA. Hsp 27 monomers have no effect, but ligand binding to EcR/ Vp16-Usp is enhanced with 5xhsp27 for all EcR isoforms. In the presence of Pal-1 and DR-1 an increase in ligand affinity is already observed with EcRE monomers, which is further enhanced with 5x Pal-1 and 5x DR-1. Ligand affinity is especially improved (about 10 fold) in the presence of all EcREs, if Usp III ($Vp16_{AB}$- Usp_{DE}), which lacks the C-domain, is used as heterodimerization partner. RXR confers ligand binding to the receptor complex only in the presence of an EcRE.

Interaction between the Ecdysteroidreceptor (EcR) and the NF*K*B- homologue Dorsal in *Drosophila melanogaster* cells

Hönl C., Spindler-Barth M.
email: celine.hoenl@uni-ulm.de
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

The NFKB-pathway in vertebrates has a central role in signal transduction in inflammatory and immunological processes and is also important for cell differentiation. It is highly conserved, having a homologous pathway system also in *Drosophila melanogaster*: the Toll/Dorsal pathway, also involved in innate immunity and cell differentiation.

Like NFKB, the Ecdysteroidreceptor (EcR) belongs to the family of nuclear receptors and is known to be a ligand-dependent transcription factor. In insect cells Ecdysteroids can bind to EcR and thus influence various developmental processes like metamorphosis, embryogenesis or reproduction. Many studies already revealed that steroid hormone receptors are able to interact with the NFKB-pathway by suppressing the activation of NFKB-dependent target genes. This effect can also be shown in the *Drosophila melanogaster* Schneider cell line S2, where the presence of EcR suppresses the activity of an NFKB-homologue. The interaction between EcR and NFKB can be shown in insect cells as well as in a mammalian cell system (CHO cells) via Western Blot expression studies.

EcR of *Rhodnius prolixus* (Hemiptera) co-localizes with mitochondria and microtubules

Vafopoulou X.
email: xanthev@yorku.ca
Biology Department, York University, Toronto, Ontario, Canada

We have shown previously that EcR in larval *Rhodnius* undergoes daily cycling in abundance in the cytoplasm (Cell Tissue Research 323, 443-455, 2006). Here, we report the subcellular distribution and potential significance of cytoplasmic EcR. EcR was localized immunohistochemically using several antibodies to EcR of *Manduca* and *Drosophila* in a confocal laser scanning microscope. Double labels were made to visualize EcR and a) mitochondria (using a fluorescent MitoTracker probe), b) microtubules (MTs) (using an antibody to tyrosylated α-tubulin) after stabilization of MTs with taxol (paclitaxel) and c) acidic organelles such as lysosomes (using a fluorescent LysoTracker probe). EcR co-localized with both mitochondria and tubulin in MTs but not with lysosomes. All different EcR antibodies produced similar co-localization patterns. EcR was seen in the perinuclear aggregation of mitochondria and was distributed throughout the MT network. EcR co-localized with tubulin for some distance down nerve axons in the brain. Mitochondria were themselves closely associated with MTs. Depolymerization of MTs with cochicine abolished the co-localization of EcR with MTS, but not with mitochondria. EcR co-localization with mitochondria and MTs has not been seen before in insects, despite several reports of similar co-localization of steroid hormone receptors in vertebrates. We infer that mitochondria are targets of ecdysteroids. The co-localization of EcR with MTs suggests that MTs may be responsible for intracellular transport of EcR or they may play a role in EcR signal transduction in the extranuclear compartment (i.e. non-genomic actions of EcR; see Mol. Cell. Endocrinol. 247, 64-72).

Karlson lecture: Phytoecdysteroids: what use are they?

Dinan L.
email: lauriedinan@fsmail.net
Université Pierre et Marie Curie, Labo. Protéines: Biochimie Structurale et Fonctionnelle, Paris, France

During the course of this lecture I intend to answer the rhetorical question of the title by clearly demonstrating that phytoecdysteroids have many uses in a surprisingly wide variety of areas and considerable potential to contribute to future agricultural and medical developments, as well as in fundamental research, and that they even have commercial potential. Since their initial discovery in the mid-60s, the number of known phytoecdysteroids has steadily increased to a current level of ca. 300 analogues. The ecdysteroids are unique amongst plant steroids in how they have been catalogued and in how extensive and systematic information is available concerning their chromatography and identification. The general availability of much of this information through Ecdybase is a considerable boon to Ecdysonists. Equally, the kindness of Nature in providing all these analogues (and some of them in large and readily available amounts) should not be underestimated. Up to now this has not been fully exploited, but the time is ripe for their use in structure-activity studies of nuclear, membrane and taste receptors, enzyme specificity, invertebrate deterrence studies, pharmaceutical studies on humans etc. There are many intriguing reports of the largely beneficial effects of ecdysteroids on vertebrates, but these have not been yet thoroughly documented or substantiated by proper clinical trials. The purported anabolic and adaptogenic effects of ecdysteroids have already led to a large (and unregulated) commercial market for preparations containing these compounds for humans, their pets and for farm animals. This brings a number of concerns; are these preparations safe, are they effective and should their use be controlled, at least under certain circumstances?

I shall first consider the chemical, biochemical and biological characteristics of phytoecdysteroids. I shall then go on to examine their use to the plant which produces them, before considering what applications mankind has found for them in agricultural and medical spheres. I shall finish by exploring the potential of this class of molecules and indicating the directions in which I believe future research should go.

Effect of ecdysone receptor gene switch ligands on gene expression in 293 cells

Panguluri S. K.[1], Lib B.[2], Hormann R. E.[2] and Palli S. R.[3]
email: email: rpalli@email.uky.edu
[1]Department of Entomology, College of Agriculture, University of Kentucky, Lexington, KY, USA
[2]Intrexon Corp., Norristown, PA, USA.

Structural differences between insect ecdysteroids and mammalian steroids make them one of the attractive ligands for regulation of transgene expression *in vivo*. Ecdysteroids also appear to have a benign pharmacology without adverse interference with mammalian signaling systems. Consequently, the ecdysone receptor (EcR) gene switches are being developed for applications in medicine. The effect of inducers of EcR gene switches on the expression of endogenous genes in HEK 293 cells was determined. Four ligand chemotypes, represented by a tetrahydroquinoline (RG-120499), one amidoketone (RG-121150), two ecdysteroids (20-hydroxyecdysone, 20E, and ponasterone A, Pon A), and four diacylhydrazines (RG-102240, RG-102277, RG-102398 and RG-100864), were tested in HEK 293 cells. The cells were exposed to ligands at concentrations of 1 µM (RG-120499) or 10 µM (all others) for 72 h and the total RNA was isolated and analyzed using microarrays. Microarray data showed that the THQ ligand, RG-120499 caused cell death at concentrations ≥10 µM. At 1 µM, this ligand caused changes in the expression of genes such as TNF, MAF, Rab and Reprimo. At 10 µM, the amidoketone, RG-121150, induced changes in the expression of genes such as v-jun, FBJ and EGR, but was otherwise non-interfering. Of the two steroids tested, 20E did not affect gene expression, but Pon A caused some changes in the expression of endogenous genes. At lower concentrations pharmacologically relevant for gene therapy, intrinsic gene expression effects of ecdysteroids and amidoketones may actually be insignificant. *A fortiori*, even at 10 µM, the four diacylhydrazine ligands did not cause significant changes in expression of endogenous genes in 293 cells and therefore should have minimum pleiotropic effects when used as ligands for the EcR gene switch.

Ecdysone agonists and mosquito molting: From basic research to applications in human disease vector control

Beckage N. E.
email: nancy.beckage@ucr.edu
Departments of Entomology & Cell Biology and Neuroscience,
University of California-Riverside, Riverside, CA, USA

Ecdysone agonists are extremely useful experimental tools for studying ligand-receptor interactions and the molecular basis of insect hormone action. Several of these non-steroidal bisacylhydrazine molecules bind to the ecdysone receptor-ultraspiracle protein complex (EcR-Usp) with high affinities, activating gene transcription and the initiation of a premature lethal molt in immature insects. Disruption of embryogenesis, alterations in diapause programming, and deleterious effects on vitellogenesis and spermatogenesis in adult insects are also induced by treatment with ecdysone agonists aside from their disruptive action on larval molting. As these non-steroidal ecdysone agonists target several insect life stages and induce a wide range of developmental anomalies that are lethal, several agonists have been commercially formulated as biopesticide products to kill insect pests in agriculture, turf management, forestry, and ornamental crops. Our laboratory is focusing on assessing their effects on human disease vectors including the larval stages of mosquito species that transmit West Nile Virus, dengue, malaria, encephalitis, and yellow fever. Treatment of all instars of *Culex quinquefasciatus* and *Aedes aegypti* larvae with methoxyfenozide (RH-2485), as either the technical grade compound or its formulation Intrepid® diluted in water, induces dose-dependent developmental disruption in the larval or pupal stage, with a range of molting-related abnormalities being seen. Sublethal effects detectable in adult mosquitoes following treatment of larvae also occur. A model to explain the physiological basis of ecdysone agonist-induced developmental disruption in mosquitoes will be presented. We conclude that future development of new ecdysone agonist–based biopesticides specifically targeted to mosquitoes represents a valuable option to achieve implementation of biologically-based strategies for control of human disease vector populations which in many geographic areas of the world have rapidly evolved high levels of resistance to conventional chemical pesticides.

Ecdysteroids from plants prevent UV induced premature senescence of human skin fibroblasts

Meybeck A.[1], Yang C.-R.[2], Zhang Y.-J.[2], Salmon M.[3], Belot N.[3], Toussaint O.[4], Wurtz J.-M.[5], Moras D.[5], Ho R.[6] and Raharivelomanana P.[6]

email: ameybeck@club-internet.fr

[1]AM Phyto-Conseil, Courbevoie, France
[2]State Key Laboratory of Phytochemistry and Western Plant Resources,Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Yunnan, China
[3]StatiCELL SA, Crealys Science Park, Gembloux, Belgium
[4]Research Unit of Cell Biology, Laboratory of Biochemistry and Cellular Biology, University of Namur, Namur, Belgium
[5]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France
[6]Laboratoire de Chimie des Substances Naturelles, Université de la Polynésie Française, Faa'a, Tahiti

The present work was undertaken in order to determine whether 20-Hydroxyecdysone from the Chinese plant *Cyanotis arachnoidea* (*C.a.*) and an ecdysteroid fraction from the Polynesian plant *Microsorum scolopendria* (*M.s.*) (2) are able to prevent stress induced premature senescence (SIPS) (3) of human dermal fibroblasts (HDF) which has effects similar to replicative senescence , including irreversible growth arrest , and which is characterized by a high proportion of cells positive for senescence-associated β-galactosidase or SA β-gal (1)

In the first SIPS experiment, the repeated UV treatment of BJ dermal fibroblasts increased the % of senescent cells expressing SA β-gal from 31 to 38.6 . But for the cultures containing 20-E from *C.a.* before and after the irradiations, the proportion of senescent cells was even lower as for the control,at 25.3,25.5 and 26.5 respectively for 50,125 and 250 µg/ml. And for the cultures containing fraction F of *M.s.* before and after the irradiations, the proportion of senescent cells was also lower than for the control at 26, 25.6 and 26 respectively for 50, 125 and 250 µg/ml, showing total protection from senescence even at the lowest level tried . In the second SIPS experiment, the control culture had 41.2, and the repeatedly UV irradiated cultures had 49.2 % of senescent cells. In this trial the antioxidant Trolox completely protected the cultures and the proportion of SA β-gal positive cells remained at 41.5 % while 20-E from C.a.brought down the proportion of senescent cells lower than for the control respectively at 33.6, 34.0 and 34.5 for 50, 125 and 250 µg/ml. And the fraction F of *M. s.* extract brought down this proportion of senescent cells also further down as the non-irradiated control, with a dose effect, to 36.4, 36.0 and 30.4 respectively for concentrations of 50, 125 and 250 µg/ml.

20-E increased the p53 content of nuclear proteins by 49% . (By comparison, the treatement by UVB induced a 404% increase)

The best GOLD score (5) found for the in silico docking of 20-E in the alternative pocket of the Vitamin D receptor (VDR-A) described by Mizwicki et al (4), was 38,6 which is lower than the 50 limit considered as satisfactory. However, in its enol configuration, the molecule gave a GOLD score of 50.6, while 1**α**,25$(OH)_2$-vitamin D3 (1,25D) gave 54.9 in this alternative active site, and the positive standard 1α, 25 dihydroxy-lumisterol (JN) gave 82.1.

The present work shows that 20-Hydroxyecdysone from *Cyanotis arachnoidea* and the ecdysteroid fraction of *Microsorum scolopendria* (rich in ecdysone and 20-E) completely protects BJ dermal fibroblasts from stress induced premature senescence, and could therefore be used in skin care products (6). This anti-senescence effect might be due to activation of the "guardian of the genome" p53. This activation of p53 might be due to the binding of 20-E to the alternative pocket of the Vitamine D receptor thought to be responsible for rapid non-genomic effects. Indeed it has been found (7) that JN entirely mimics the protective effects against UV irradiation of 1,25(OH)(2)D(3) which is known to activate p53 (8).

References

1- Dimri G et al. J.Proc.Natl.Acad.Sci.USA 92 : 9363-67, 1995
2- Ho R.Ph D Thesis , Université de la Polynésie Française,Faa'a,Tahiti.2007
3- de Magalhaes JP et al.FEBS Letters 523:157-162,2002
4- Mizwicki M et al. J.Proc.Natl.Acad.Sci.USA 101 : 12876-81 , 2004
5- Verdonck ML et al. Proteins. 52 : 609-23 , 2003
6- Meybeck A and Yang CR. PCT Patent WO/2007/060302.
7- Dixon KM et al. J Steroid Biochem Mol Biol.103:451-6, 2007
8- Gupta R et al. J Invest Dermatol. 127:707-15, 2007

Effect of 20E on different rat muscle models

Tóth N.[1], Báthori M.[1], Ramirez G. G.[2], Szabó A.[3], Kacsala P.[4], Héger J.[3], Zádor E.[3]
email: hunyadi.a@pharm.u-szeged.hu
[1]Department of Pharmacognosy, University of Szeged, Hungary
[2]Department of Pathophysiology, Lab of Respiratory Muscles, Katholieke Universiteit Leuven, Belgium
[3]Institute of Biochemistry, University of Szeged, Hungary
[4]Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

It is well known that 20-hydroxyecdysone (20E), the main ecdysteroid in plants, has an anabolic effect in vertebrates, i. e. increasing muscle size without an androgen influence. These data were mostly based on measuring the muscle weight and the incorporation of amino acids into proteins, while the effect of 20E on the different muscle fibers of distinct muscles has not been reported yet. Here we present that the 20E affects the size of muscle fibers (cross-sectional area, CSA) in a muscle specific-manner in rat, which is different in case of a predominantly slow-twitch (m. soleus) than in a fast-twitch muscle (m. extensor digitorum longus). The effect was influenced by the site of treatment and the presence of a regenerating muscle in the other leg of the animal. The 20E also increased the myonuclear number in the muscle fibers suggesting the activation of satellite cells. We found that 20E also promotes the regeneration of muscle fibers after notexin induced necrosis. We also examined the efficacy of 20E in muscle atrophy models and found that it may be beneficial to protect the muscles from the deleterious effect of corticosteroids, extensively used in therapeutics. Based on these data we propose that 20E may provide an alternative of anabolic-androgenic steroids in the medical treatment against muscle atrophy.

Smt3 is required for ecdysone synthesis in *Drosophila melanogaster*

Talamillo A.[1]*, Sánchez J.[1]*, Cantera R.[2,3], Pérez C.[1], Martín D.[4] Lopitz F.[1], Rodriguez M.[1] and Barrio R.[1]

email: rbarrio@cicbiogune.es

[1]CIC Biogune, Derio, Bizkaia, Spain
[2]Stockholm University, Stockholm, Sweden
[3]IIBCE, Montevideo, Uruguay
[4]Institut de Biologia Molecular de Barcelona-CSIC, Barcelona, Spain
* Equal contribution of these authors to the work

To study *in vivo* the role of the ubiquitin-like protein Smt3 (Sumo) during *Drosophila* development, we generated transgenic flies carrying the transgene *UAS-smt3i* to reduce *smt3* mRNA levels in specific groups of cells. Low *smt3* in the prothoracic gland prevents metamorphosis. RNAi knockdown larvae stop their development in their last larval stage and remain alive for up to a month, during which they continue to eat and gain weight. Their prothoracic glands have fewer, but larger cells than normal. They also have lower ecdysteroid titre than WT. After dietary administration of exogenous ecdysone these larvae form pupal cases, but do not proceed further in development and die. We observed that, in larvae with lower levels of *smt3*, the subcellular localization and expression levels of factors involved in the regulation of ecdysteroids synthesis, are altered. These results are in concordance to larvae mutant in *lesswright*, the homologue of the Sumo conjugating enzyme gene *Ubc9*. Interestingly, prothoracic gland cells present reduced intracellular channels and a reduced content in lipid droplets and cholesterol, which could contribute to the deficit in steroidogenesis.

Effect of insulin and other peptides in the testis ecdysteroid biosynthesis of *Spodoptera littoralis*

Iga M., Vleugels T. and Smagghe G.

email: masatoshi.iga@ugent.be

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

Ecdysteroids are key hormones in insect growth and development. Prothoracic glands are considered as the source of ecdysteroids during postembryonic development. Ecdysteroids are found in adult insects, although the glands degenerate around the time of the imaginal ecdysis. Over the last decade there have been some reports on ecdysteroids production in testis and a testis ecdysiotropin (LTE) from gypsy moth, *Lymantria dispar*, and blood-sucking bug, *Rhodnius prolixus*. In this project, we tested the potency of the testes from the sixth (last) larval instar of the cotton leafworm, *Spodoptera littoralis*, to produce ecdysteroids at different levels. First we measured the *in vivo* ecdysteroid titers in the hemolymph and the testes at different moments of the last instar larvae by using enzyme immunoassay (EIA). The ecdysteroids titers in hemolymph and testes are significantly increased and they are peaked at 104 h after ecdysis (about 40 h before pupation). Then testes were cultured under *in vitro* conditions and were tested the effect of insulin and other peptides reported as inducers of ecdysteroid biosynthesis. The ecdysteroids amounts in the cultured testes and the culture medium were followed. In addition, we cloned the Halloween genes in *S. littoralis* and they were used as a criterion of *de novo* ecdysteroid biosynthesis. Finally the results are discussed in relation to potential different resources of ecdysteroids and developmental stages.

Q-RT-PCR profiling of Cyp-coding genes in the biosynthetic pathways of 20-hydroxy-ecdysone and juvenile hormone in the desert locust, *Schistocerca gregaria*

Marchal E., Badisco L., Verlinden H., van Wielendaele P., van Soest S., Vanden Broeck J.
email: elisabeth.marchal@bio.kuleuven.be
Department of Animal Physiology and Neurobiology, Zoological Institute K.U.Leuven, Leuven, Belgium

In insects, body growth is linked to ecdysis, the regular replacement of the outer part of the cuticle. This process is highly dependent on two developmental hormones: 20OH-ecdysone (20E), the molting hormone, and juvenile hormone (JH), which controls the nature of the molt. Extensive research recently led to the identification of several genes coding for cytochrome P450 (Cyp) enzymes involved in the biosynthesis of both hormones. For 20E, they are called the 'Halloween genes'. This significant progress allows us to characterize and measure the expression of Cyp-coding genes in the biosynthetic pathways of both classic insect hormones in our model organism, the desert locust *Schistocerca gregaria*. For centuries, this locust has threatened the agricultural production in large parts of Africa, the Middle-East and Asia. Affecting the livelihood of about one tenth of the world population, it is probably the most dangerous of all locust pests. It has the ability to undergo phase (solitary-gregarious) transition and to form huge swarms. Our research will hopefully result in a better understanding of the physiology of this remarkable insect and generate new leads in the development of selective pest control strategies.

Molecular cloning of phantom from the Y-organ of the kuruma prawn, *Marsupenaeus japonicus*

Asazuma H., Nagata S., Nagasawa H.

email: asa-ch@msc.biglobe.ne.jp

Laboratory of Bioorganic Chemistry, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo, Tokyo, Japan

The molting in crustaceans is controlled by ecdysteroids as in insects. Recently, the enzymes catalyzing the ecdysteroid biosynthesis were identified and characterized as CYP450s in *Drosophila melanogaster* and *Bombyx mori*. In the kuruma prawn, Marsupenaeus japonicus, the Y-organ, a molting organ in crustaceans, mainly produces 3-dehydroecdysone, and probably involves ecdysteroidogenic enzymes encoded by the Halloween genes. PCR was performed with cDNA prepared from the Y-organ using degenerate primers based on the conserved sequences among insect Halloween genes. The resulting cDNA fragment was similar to the insect phantom (Cyp306a1, 25-hydroxylase) and was termed *Mj-Phm*. The complete cDNA sequence of *Mj-Phm* was obtained by 5'- and 3'-RACE. The deduced amino acid sequence of Mj-Phm showed ~40% identity to the insect Phantom. Some motif sequences were conserved and the ER-targeted sequence was also found in Mj-Phm. Expression analyses revealed that *Mj-Phm* mRNA was specifically expressed in the Y-organ and the expression level of *Mj-Phm* was increased at the pre-molt stage and decreased after ecdysis. However, the expression of *Mj-Phm* was not completely supressed during the inter-molt stage. When the Y-organ was incubated with molt-inhibiting hormone (MIH), the expression of *Mj-Phm* was significantly decreased. These results indicated that the ecdysteroidogenic pathway is conserved between insects and crustaceans. In addition, one of the molt-inhibiting mechanism by MIH involves at least the transcriptional regulation of *Mj-Phm*.

Targeted and untargeted metabolomics of calves urine samples upon 20E administration: application to the control of its potential misuse in cattle

Destrez B.[1], Pinel G.[1], Antignac J.-P.[1], Lafont R.[2] and Le Bizec B.[1]

email: destrez@vet-nantes.fr

[1]LABoratoire d'Etude des Résidus et Contaminants dans les Aliments,
Ecole Nationale Vétérinaire de Nantes, Route de Gachet, Nantes, France
[2]Université Pierre et Marie Curie-CNRS FRE 2852,
Laboratoire Protéines: Biochimie Structurale et Fonctionnelle, Paris, France

Several studies underlined 20-hydroxyecdysone (20E) potential to enhance the protein synthesis rate in mammalian tissue. In consequence, this compound could be potentially used as anabolic agent in food producing animals. The aim of this study was to develop an efficient analytical method based on LC-MS^n-HRMS measurements and dedicated to ecdysteroids and their metabolites analysis in biological matrices at trace level. Extraction and purification procedure was set up on urine.

Two complementary approaches were conducted in order to assure efficient control of ecdysteroids misuse in meat producing animals:
A targeted approach combining on-line accurate mass LC-HRMS and MS^n measurements allowed unambiguous elemental composition determination and structural elucidation of several 20E urine metabolites (at least 14-deoxy-20-hydroxyecdysone, 20,26-dihydroxyecdysone, 14-deoxy-20,26-dihydroxyecdysone). This strategy allowed the detection of 20E and its metabolites over 4 days after 20E administration to calves.

An untargeted approach based on comparison of multiple urine full scan HRMS acquisition and dedicated statistical retreatment allowed to establish urine fingerprints as a tool to discriminate treated animals from controlled one.

HPLC-EIA and nano LC-MS/MS analysis of ecdysteroid diversity in *Drosophila*

Blais C.[1], Blasco T.[2], Maria A.[1], Dauphin-Villemant C.[1], Lafont R.[1]
email: catherine.blais@snv.jussieu.fr
[1]Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines,
Université Pierre et Marie Curie, Paris, France
[2]Plateforme Spectrométrie de masse et protéomique - IFR 83, Université Pierre et Marie Curie, Paris, France

Even if 20-hydroxyecdysone (20E) is classically considered as the major steroid hormone in *Drosophila melanogaster*, the reality is more complex. Drosophila contains both 27C and 28C ecdysteroids, which results from both its inability to dealkylate phytosterols and the use of several sterols to produce its steroid hormones. The relative proportions of these molecules are dependent on the sterol composition of the diet of larvae (Redfern, 1986, Feldlaufer et al., 1995). Early studies demonstrated that drosophila ring glands produce both ecdysone and 20-deoxymakisterone A (20dMaA), but a third less polar unidentified ecdysteroid was also observed (Redfern, 1986; Pak and Gilbert, 1987). Drosophila larvae feed partly on yeasts, which are known to produce peculiar sterols, differing from those of plants by the stereochemistry of their 24-alkyl substituent. When used by insects to produce ecdysteroids, such fungal sterols do not give rise to makisterone A (MaA), but instead to its 24-epimer (Maurer et al., 1993). The presence of 24-epimakisterone A (24epi-MaA) in drosophila was therefore an attractive hypothesis.

The availability of reference phytoecdysteroids, MaA, 24epi-MaA and more recently 20dMaA (Snogan et al., 2007) prompted us to reinvestigate the nature of drosophila ecdysteroids. Three different sets of samples were analyzed: (1) larval extracts, (2) the secretory products of ring glands and (3) the secretory products of ring glands co-cultured with fat body. Sensitive analytical techniques, namely HPLC-EIA and nanoLC-MS-MS were used, and the latter allowed to get MS-MS spectra in the low femtomolar range. 20E, MaA and 24epi-MaA were identified from both larval extracts and ring glands + fat body. On the other hand, culture media of ring glands alone showed the presence of ecdysone, 20dMaA and a third slightly less polar compound with a mass spectrum very similar to that of 20dMaA, and which is therefore assumed to be its 24-epimer.

Drosophila EcR binds both 20E and MaA with a high affinity and 24epi-MaA with a lower affinity (Dinan and Horman, 2005), and we may wonder whether the amounts present allow it to play an active hormonal function in this species.

References :

Dinan, L. & Hormann, R.E. (2005). In Comprehensive Molecular Insect Science. (L.I. Gilbert, K. Iatrou & S.S. Gill, Eds.) Oxford, Elsevier. Vol. 3, pp. 197-242.

Feldlaufer, M.F., Weirich, G.F., Imberski, R.B. & Svoboda, J.A. (1995). Insect Biochem. Molec. Biol. 25: 709-712.

Maurer P., Girault J.-P., Larcheveque, M. & Lafont, R. (1993). Arch. Insect Biochem. Physiol. 23: 29-35.

Pak, M.D. & Gilbert, L.I. (1987). J. Liq. Chromatogr. 10: 2591-2611.

Redfern, C.P.F. (1986). Experientia 42: 307-309.

Snogan, E. et al. (2007). Phytochem. Anal. 18(5): 441-450.

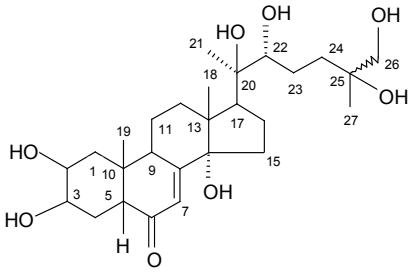
Polar Ecdysteroids and Biological Activity of the total Ecdysteroids from the Plant *Silene viridiflora*

Mamadalieva N. Z.[1], Egamberdiyeva D.[2], Lafont R.[3], Syrov V. N.[1], Girault J.-P.[4]
email: mnilufar76@yahoo.com
[1]S.Yu.Yunusov Institute of the Chemistry of Plant Substances AS RUz, Tashkent, Uzbekistan
[2]Department of Biotechnology and Microbiology National University of Uzbekistan, Tashkent, Uzbekistan
[3]Université Pierre et Marie Curie, Institut de Biologie Intégrative,
Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Paris, France
[4]Université René Descartes, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques,
Paris, France

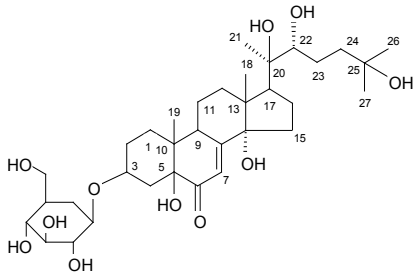
Nowadays according to the available data, ecdysteroids have been found in more than 120 species of Silene (*Caryophyllaceae*).
We previously reported that the plant *Silene viridiflora* (cultivated in the botanical garden of the Institute of Chemistry of Plant Substances AS RUz) is ecdysteroid-rich (the general yield of the total ecdysteroids from air-dried raw material is up to 1.5 % of dry weight). From the aerial parts of the plant have been isolated 2-deoxy-20-hydroxyecdysone, polypodine B, 20-hydroxyecdysone, 26-hydroxypolypodine B, integristerone A, 2,22-diacetate- and 3,22-diacetate- of 20,26-dihydroxyecdysone, sileneoside A and sileneoside D [1, 2].

Further investigation of buthanol extract from *S. viridiflora* allowed the isolation of the more polar ecdysteroids (*1*) and (*2*). Compounds 1 and 2 were identified using CI/D-mass spectrometry and NMR spectroscopy as 20,26-dihydroxyecdysone (podecdysone C) (1) and 2-deoxypolypodine B-3-β-D-glucoside (2). These ecdysteroids have been isolated for the first time from the plant *S. viridiflora.*

1



2



We investigated the biological activity of the ecdysteroid mixture isolated from aerial parts of *S. viridiflora*. When given orally to experimental (mice, rats) at doses 5-10 mg/kg body weight, they increased the duration of the animals dynamical work, increasing the endurance in hard conditions (hypoxia, hypothermia) and decreased the rate of tiredness.

1. N.Z. Mamadalieva, L.N. Zibareva, Z. Saatov, R. Lafont. Chem. Nat. Comp., 39 (2), 199 (2003)
2. N.Z. Mamadalieva, L.N. Zibareva, N. Evrard-Todeschi, J.P. Girault, A. Maria, N.Sh. Ramazanov, Z. Saatov, R. Lafont. Coll. Czech. Chem. Comm., 69, 1675 (2004)

The mutual influence of 20-hydroxyecdysone and octopamine on the one another metabolism in *Drosophila* under normal and stress conditions

Bogomolova E. V., Adonieva N. V., Chentsova N. A., Gruntenko N. E. and Rauschenbach I. Y.
email: iraushen@bionet.nsc.ru
Institute of Cytology and Genetics SD RAS, Novosibirsk 630090, Russia

The influence of 20-hydroxyecdysone (20HE) on activities of the rate-limiting enzyme of octopamine (OA) synthesis, tyrosine decarboxylase (TDC), and enzyme of OA catabolism, arylalkylamine N-acetyltransferase (AANAT) in *Drosophila virilis* and *D. melanogaster* wild type females under normal and heat stress (38oC) conditions was studied. Increase of 20E titre (feeding with enzyme) was found to lead to a rise of TDC activity and decrease of TDC response intensity (stress reactivity) on heat stress in both species females. At the same time AANAT activity doesn't alter under 20E treatment of females of both species. Thus 20E regulates OA metabolism at synthesis level.

Influence of OA titre increase (feeding with OA) on ecdysone-20-monooxygenase (E20MO), converting ecdysone to 20E and determining to a considerable degree 20E titre, in *D. virilis* wild type females was studied. OA treatment was found to provoke the increase of E20MO activity and 20E titre in females. The influence of a decrease in OA level on 20E metabolism was estimated after measurements of E20MO activity and 20E titre in the octopamineless females of the strain $T\beta h^{nM18}$, in females of the strain P845 (precursor of $T\beta h^{nM18}$ strain) and in wild type females, *Canton S*, of *D. melanogaster*. It was established that the absence of octopamine leads to a considerable decrease in the enzyme activity and in 20E titre. Thus E20MO occupies a key position in the regulation of 20E titre under the conditions that lead to changes in OA levels.

The work was supported by RFBR grants ## 07-04-00194, 06-04-48357.

Ecdysteroid Signaling in the Parasitic Copepode *Lepeophtheirus salmonis*

Skern-Mauritzen R., Eichner Ch., Dalvin S. T.
email: rasmus.skern@imr.no
Havforskningsinstituttet, Bergen, Norway

Salmon lice (*Lepeophtheirus salmonis*) is an ectoparasite which cause major economical losses in aquaculture of farmed salmon. In addition, the reproductive output from the large number of lice present in fish farms, cause a threat to the offspring of wild living salmon. The lifecycle of the salmon louse comprise three planktonic stages, where the third stage attaches to the skin of the host. After attachment the lice undergo seven moltings before adulthood. Mating and subsequent egg production takes place while attached the salmonid host. Limited information regarding sexual maturation of the salmon louse reproductive system is available. After the final molt, the salmon louse is not fully developed but undergoes a process that includes a large increase of the genital segment and abdomen. Once fully developed, the adult female begins to produce batches of eggs approximately every 10 days. From an EST library we have isolated an Ultraspiracle (USP) homolog and several vitellogenins. A microarray experiment to characterize female salmon louse transcriptomes during post molting growth and egg production has also been done. Several vitellogenins were upregulated in adult females. We hypothesize that vitellogenesis and possibly also ecdysis is controlled by ecdysone acting through an USP/EcR heterodimer. We are presently using western blotting, Q-PCR, RNAi and microarrays to study the roles of USP and vitellogenins in maturation and egg production.

Ecdysone modulates agonistic behavior in crayfishes

Delbecque J. P., Minni A. and Cattaert D.
email: jp.delbecque@cnic.u-bordeaux1.fr
Université de Bordeaux, Centre de Neurosciences Intégratives et Cognitives, Talence, France

The agonistic behavior of *Procambarus clarkii*, a very aggressive crayfish species, has been the subject of numerous studies. Till now, however, most investigations have been done on intermolt animals, because the molting process increases the vulnerability of animals and strongly interferes with their behavior. Our observations show that crayfish aggressiveness significantly changes during the successive molt stages, decreasing in late premolt, then progressively returning to normal during postmolt. Crayfishes, behaving as dominants during the intermolt stage, progressively become subordinates during the molting process, but generally recover their aggressiveness and their social status at the end of postmolt. Injections of ecdysone into such intermolt animals, leading them to undertake the whole molting process including postmolt, also induced the complete sequence of progressive decrease and increase of aggressiveness. Ecdysone is thus involved in the cyclic modulation of agonistic behavior in crayfishes: it probably triggers a complex neurohormonal cascade, in order to regulate the whole behavioral sequence.

Identification of genes involved in choriogenesis of *Blattella germanica* by subtractive hybridization approaches and correlation with ecdysteroid patterns

Irles P., Castresana J., Bellés X. and Piulachs M. D.
email: mdpagr@cid.csic.es
Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona, Barcelona, Spain

Blattella germanica is a basal insect with panoistic ovaries whose development is governed by juvenile hormone III (JHIII). JHIII regulates vitellogenin expression in the fat body, the patency in the follicular epithelium and the uptake of vitellogenin by growing oocytes. However, the presence of 20-hydroxyecdysone (20-E) in the ovaries plays an important role in choriogenesis, among other reproductive functions, as previously demostrated in a number of insects. The monolayer of follicle cells surrounding the oocyte secretes the components of the three chorion layers. Due to the relevance in embryo development, we are interested in elucidating the molecular mechanisms and the role of 20-E underlying the regulation of genes involved in choriogenesis.

With this aim, we isolated those genes that are differentially expressed in the ovary at the end of the first gonadotrophic cycle, corresponding with the highest levels of 20-E, using suppressive subtractive hybridization techniques (SSH).

The *tester* library was constructed with 1.0 µg of mRNA from chorionated 6 to 7 day-old females, and the driver library was obtained from ovaries from 3 to 4 day-old females. The subtracted PCR amplification products were cloned and sequenced. The sequences were compared against non redundant nucleotide and protein databases of NCBI using BLAST. From 293 useful sequences, only 10% showed significant similarity with other proteins in the databank. Therefore, most of the found sequences appear to be undescribed. Among the labeled cDNAs, and according to the Gene Ontology classification, we have found genes related with different functions, like endopeptidase activity, peroxidase activity, structural activity. A number of clones, representative of these processes, were chosen and its expression was studied by real-time PCR, during the first gonadotrophic cycle. The expression patterns obtained were compared with 20-E levels in the hemolymph and in the ovary in order to infer cause-effect hypotheses.

Dual role of BgFTZ-F1 during nymphal development in the hemimetabolous insect *Blattella germanica*

Nieva C., Cruz, J., Mané-Padrós D., Martín D. and Bellés X.
email: cnbagr@cid.csic.es
Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona, Barcelona, Spain

In insects, the ecdysteroidal hormone 20-hydroxyecdysone (20E) controls key developmental processes during embryogenesis, molting, metamorphosis and reproduction, as it has been extensively studied in holometabolous insects, especially in *Drosophila melanogaster*. The situation is very different in hemimetabolous insects, which do not develop through complete metamorphosis, their juvenile forms being morphologically similar to the adult. Indeed, in hemimetabolous insects the wealth of information concerning the molecular basis of the 20E-triggered genetic hierarchy is practically non-existent. However, if we aim to understand the molecular basis of the evolution towards complete metamorphosis in insects, then characterization of the 20E-induced genetic hierarchy in primitive species becomes of paramount importance. In the cockroach *Blattella germanica*, we have previously characterized the two components of the heterodimeric ecdysone receptor, as well as several early and early-late genes. In the present work we extend the knowledge of the 20E genetic hierarchy in *B. germanica* by identifying a FTZ-F1 homologue in this cockroach, by studying its regulation by ecdysteroids and by determining its functions in nymphal development with RNAi approaches. RNAi experiments reveal that FTZ-F1 is required to molt and to control the timing of ecdysteroid production during the last nymphal stage.

Prothoracicotropic hormone induces tyrosine phosphorylation in prothoracic glands of the silkworm, *Bombyx mori*

Lin J.-L., Gu S.-H.
email: jlsky@yahoo.com
Department of Zoology, National Museum of Natural Science, Taichung, Taiwan, ROC

Protein tyrosine kinases mediate the transduction and processing of many extra- and intracellular signals. They are critical in regulating cell growth and differentiation. This study aims to investigate the tyrosine phosphorylations of the silkworm prothoracic glands upon prothoacicotropic hormone (PTTH) stimulation and their relation with ecdysteroidogenesis. Western blot analysis using phosphotyrosine-specific antibodie showed that PTTH stimulates a rapid increase in tyrosine phosphorylation of at least 2 proteins in prothoracic glands, one of which was identified as extracellular signal-regulated kinase (ERK). The phosphorylation of another 120 kDa protein showed dose- and time-dependent to stimulation by PTTH in vitro. In vitro activation of tyrosine phosphorylation was also verified by in vivo experiments: injection of the PTTH into day 6 last instar larvae greatly increased the tyrosine phosphorylation. The treatment of the prothoracic glands with the protein tyrosine phosphatase inhibitor, sodium orthovanadate, also results in tyrosine phosphorylation of several proteins and increased ecdysteroidogenesis. Genistein, a broad-spectrum tyrosine kinase inhibitor, markedly attenuated the ability of PTTH to tyrosine phosphorylation and ecdysteroidogenesis. PP2, a more selective inhibitor of the Src-family tyrosine kinases, inhibited PTTH-stimulated tyrosine phosphorylation, but not ecdysteroidogenesis. The result suggests that the tyrosine kinase, other than the Src-family tyrosine kinases, is involved in both PTTH-stimulated tyrosine phosphorylation and ecdysteroidogenesis.

Neuropeptides involved in the physiological regulation of the reproduction in the desert locust *Schistocerca gregaria*

van Wielendaele P.
email: pieter.vanwielendaele@bio.kuleuven.be
Department of Animal Physiology and Neurobiology, Zoological Institute K.U.Leuven, Leuven, Belgium

For thousands of years, desert locust (*Schistocerca gregaria*) outbreaks have been threatening the agricultural production in large parts of Africa, the Middle East and South-West Asia. The swarms that make up these outbreaks can migrate over large distances and cause crop damage, which often leads to famine and death. Given certain favourable environmental conditions, locusts are able to reproduce massively. Due to this, the population densities increase vigorously as a result of which these insects undergo phase-transition and start to aggregate. Prevention of locust outbreaks, by controlling their reproduction, could be a suitable solution for this problem. Therefore, a better understanding of the complex regulation of the reproductive physiology seems to be desirable. Neuropeptides play a crucial role in this regulation (e.g. by regulation of ecdysteroidogenesis and juvenile hormone biosynthesis). In the desert locust, some neurohormones involved in this regulation have already been identified, e.g. sNPF and OMP's (respectively short neuropeptide F and ovary maturating parsins). It has been shown that sNPF and OMP's play a role in the regulation of the vitellogenesis and ovarian maturation while the OMP's also regulate the ovarian ecdysteroidogenesis. The precise role and working mechanism of these peptides are however not yet known. Also about the functional link between these signal molecules and the regulation of their synthesis and release, we're still left in the dark. By means of techniques like quantitative real-time-RT-PCR, SDS-PAGE and enzym-immuno-assay for ecdysteroids, we'll try to find out more about all this.

Hormonal Regulation of *E75* Gene Expression in *Drosophila*

Bernardo T., Dubrovskaya V., Dubrovsky E. B.
email: dubrovsky@fordham.edu
Department of Biology, Fordham University, New York, USA

Drosophila development is regulated by two hormones, 20-hydroxyecdysone (ecdysone) and juvenile hormone (JH). Ecdysone binds to the EcR/Usp heterodimer, which then binds to an ecdysone response element and increases gene expression. While ecdysone action is well characterized, both the receptor and the mode of action of JH are currently unknown. We previously found that expression of the *E75* gene is induced by both ecdysone and JH. The *E75* gene occupies 100 kb of genomic DNA; it has 4 alternative promoters, producing isoforms E75A, B, C, and D, as well as two polyadenylation sites. Our current efforts are focused on identifying DNA elements involved in the hormonal regulation of the E75A and E75D isoforms. To that end, we are employing several experimental approaches. First, since conserved non-coding elements may be of functional importance, we are identifying sequences that are conserved among distantly related drosophila species and among functionally distinct genes, which share a common hormonal inducibility. Second, we are using chromatin immunoprecipitation (ChIP) followed by real-time PCR to identify non-coding regions with elevated levels of two known markers for enhancer regions: monomethylated histone H3 at lysine 4 and nucleosomal depletion. Finally, we are testing the functionality of putative regulatory elements identified by both the sequence conservation and ChIP analysis using transient transfection assays. We have performed ChIP on *Drosophila* Schneider Line 2 (S2) cells using antibodies to a region common to all forms of H3, as well as antibodies specific to H3 monomethylated at lysine 4. We have found several non-coding regions upstream of the E75A and E75D isoforms that display peaks of H3K4 monomethylation concomitant with nucleosomal depletion. The results suggest the presence of enhancers at some of these regions, which may be involved in the transcriptional regulation of *E75* possibly by ecdysone or JH.

Effect of juvenile hormone mimics and insect growth regulators on ecdysteroid receptor activity in a heterologous cell culture assay

Beatty J. M. and Henrich V. C.
email: jmbeatty@hotmail.com
Center for Biotechnology, Genomics, and Health Research, University of North Carolina at Greensboro, Greensboro, NC, USA

A mammalian cell culture system has been employed to investigate the effects of several compounds for their effect on ecdysone receptor/Ultraspiracle dimer on transcriptional activity. The aim of this work is to develop an *in vitro* assay to screen plant extracts as insecticidal candidates based on their ability to affect transcriptional activity in cell cultures. Two types of activity have been studied: agonist-induced activity evoked by both natural and nonsteroidal agonists, and potentiation, that is, a reduction of the agonist dosage necessary to obtain a maximal response by as much as tenfold observed in the presence of juvenile hormone and several juvenile hormone analogues. When compared directly, the EcR/USP heterodimer from *Drosophila melanogaster* and *Leptinotarsa decemlineata* displayed different levels of inducibility to various agonists. Additionally, potentiation by juvenile hormone III and some JH precursors is considerably stronger in *D. melanogaster* than in *L. decemlineata*. Several plant-synthesized JH mimics, synthetic JH analogues, and insect growth regulators were also tested since these include several isoprenoids and terpenoids that serve as a defense against insect predation. These compounds evoked different levels of potentiation from the two species' receptors. The receptors of neither species were potentiated when diacylhydrazines are used as an agonist, indicating that the shape of the ligand-bound EcR is a crucial determinant for potentiation, which also rules out the possibility that cellular metabolism of the potentiators is responsible for the effects observed.

Identification of NLS and NES signals for *Drosophila* methoprene-tolerant protein

Greb-Markiewicz B.[1], Orłowski M.[1], Gwóźdź T.[1], Dutko-Gwóźdź J.[1], Dobrucki J.[2], Ożyhar A.[1]

email: greb_markiewicz@yahoo.com

[1]Department of Biochemistry, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland

[2]Division of Cell Biophysics, Faulty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Introduction:
Although juvenile hormone (JH) is an important regulator of insect development and reproductive maturation, neither the molecular mechanism of JH action nor JH receptor is known. Met is one of proposed and very probable candidate for this function. It has been shown that Met belongs to the family of bHLH-PAS transcription factors. Their functional activity often is correlated with shuttling between nucleus and cytoplasm according to the masking and unmasking, by interacting partners, signals directing this proteins to nuclear or cytoplasmic compartment of the cell. The first report concerning *Drosophila melanogaster* Met has informed about localization of this protein in cytoplasm. Later on it has been shown as nuclear protein by *in vivo* immunostaining of *Drosophila* tissues and by using chimeric proteins with GFP in S2 cells. Till now, no NLS and NES motifs has never been elucidated for Met.

Methods:
In order to determine the sequences of nuclear localization signals (NLSs) and nuclear export signals (NESs), series of deletion and point mutants tagged by yellow fluorescence protein (YFP) were prepared by cloning into EYFP-C1 vector and expressed in mammalian cells.

Results:
Our results show the presence of dominant NLS in area linking bHLH and PAS A domains and recessive NLS in area of PAS B repeat. Additionally, we have detected presence of active NES motifs in PAS A, PAS B repeats and the C-terminal part of Met.

Conclusion:
Met is cytoplasm-nucleus shuttling protein with very complicated control system of localization of this transcription factor in the cell compartments.

Novel functions of Ecdysone receptor B1 isoform during *Drosophila* oogenesis

Romani P.[1], Bernardi F.[1], Duchi S.[1], Hackney J.[2], Dobens L.[2], Gargiulo G.[1], Cavaliere V.[1]
email: patrizia.romani@unibo.it
[1]Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, Bologna, Italy
[2]Division of Molecular Biology and Biochemistry School of Biological Sciences
University of Missouri-Kansas City, Kansas City, MO, USA

In *Drosophila*, ecdysone signaling is mediated by a heteromeric receptor composed of the Ecdysone Receptor protein (EcR) and the RXR homolog transcription factor Ultraspiracle (USP). There are three different EcR isoforms: EcR-A, EcR-B1 and EcR-B2 that differ in their N termini and have different spatial–temporal functions. Here we analized the effects of EcR-B1 or USP loss of function in the follicular epithelium during oogenesis.

We find that targeting RNAi of EcR-B1 by using the ubiquitous Tubulin-Gal4 driver causes severe alteration in egg chamber development. Silencing of EcR-B1 isoform in flp-out clones causes apoptotic follicle cell death and affects follicular epithelium monolayer structure. Multilayered follicle cells are also detected knocking down EcR-B1 isoform by using enhancer trap lines that drive Gal4 expression at mid-oogenesis. We show that multilayered follicle cells lack proper cell polarity with altered distribution of apical and baso-lateral cell polarity markers (atypical-PKC, Armadillo, Discs-large and Scribble). Furthermore, these delaminating follicle cells show accumulation of adherens junctions (DE-Cadherin) and their F-actin cytoskeleton is strongly affected.

Interestingly, we found that mosaic follicle cells homozigous for the usp^3 mutation that interferes with USP repressor activity die by apoptosis at early stages of oogenesis and show only slightly increased Discs-large expression levels but not alteration in DE-cadherin expression levels.

These data indicate follicular epithelium morphogenesis and follicle cell survival require EcR-B1 and USP activities.

Structural studies and biophysical characterization of the binding of various ecdysteroids to the ligand-binding domains of EcR/USP

Browning C., Chavant V., Moras D. and Billas I. M. L.
email: browning@igbmc.fr
Département de Génomique et de Biologie Structurales, Université Louis Pasteur, Illkirch, France

Having solved the structures of EcR/USP in complex with 20E and ponA, we recently reported on the determination of the difference in binding affinities for the two ligands towards EcR. Using theoretical calculations based on docking and free energy methods, the results suggest that the free energy gained from the extra H-bond formed when 20E is bound is less than the cost of desolvating 20E compared to ponA[1].

Following up from these observations, we are interested in the determination of the EcR/USP LBD bound to numerous ecdysteroids which have additional or fewer OH groups on the alkyl chains compared to 20E. With the aid of structural and biophysical methods, it allows us to further understand how solvation of the ligand affects the binding affinity of these ecdysteroids. Furthermore, it also gives us an insight into how these ecdysteroids interact with residues lining the binding pocket of EcR.

Browning,C., Martin,E., Loch,C., Wurtz,J.M., Moras,D., Stote,R.H., Dejaegere,A.P., and Billas,I.M.L. (2007). Critical role of desolvation in the binding of 20-hydroxyecdysone to the ecdysone receptor. Journal of Biological Chemistry 282, 32924-32934.

The AB-Domain of Ultraspiracle (Usp) Interacts with a Corepressor Binding site of the Ligand Binding Domain of the Ecdysone Receptor (EcR)

Ruff H.[1], Tremmel Ch.[1], Azoitei A.[1], Braun S.[1], Henrich V. C.[2], Spindler-Barth M.[1]
email: heike.ruff@uni-ulm.de
[1]Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany
[2]Center for Biotechnology, Genomics, and Health Research, University of North Carolina at Greensboro, Greensboro, NC, USA

Constitutive, but not hormone induced transcriptional activity of the ecdysone receptor (EcR) is enhanced, if lysine 497 in helix 4 of the ligand binding domain, which is involved in hormone induced formation of a salt bridge between helix 4 and helix 12, is mutated. In contrast, constitutive transcriptional activity is severely impaired in heterodimers of mutated EcR and wild type Usp, but not with $VP16_{AD}$-Usp_{CDE}. As already shown by Ruff et al. (see poster), the inhibitory action of the AB-domain of Usp is mainly mediated by the N-terminus, whereas an activating function is present in the hexapeptide of wild type Usp situated adjacent to the N-terminus of the C-domain of Usp. Using a mutated EcR we show in this study, that the Usp_{AB} interacts with the ligand binding domain of EcR wildtype and is inhibited by the mutation in the LBD of EcR. Comparison of reporter activity obtained with heterodimers of mutated EcR and $VP16_{AD}$-Usp_{CDE} with and without this hexapeptide supports the hypothesis, that mutation of lys497 to glutaminic acid interrupts the interaction of the hexapeptide of Usp with the E-domain of EcR. This interruption only takes place in the absence of hormone, when the salt bridge is not formed and affords the presence of the C-domain of Usp. No effect of the mutation is seen in the presence of hormone, which is in accordance with the hypothesis that interaction of corepressor or hexapeptide with K497 in the E-domain of EcR is possible only in the absence of hormone, when the salt bridge is not formed. We conclude that K497 is part of a comodulator binding site, which is occupied by a corepressor in the absence of Usp and interacts in the presence of Usp with the hexapeptide of its AB-domain, which harbors an activating function.

Transcriptional activity of ecdysone receptor (EcR) isoforms is regulated by modulation of receptor stability and interaction with AB- and C-domains of the heterodimerization partner Ultraspiracle (Usp)

Ruff H., Tremmel Ch., Spindler-Barth M.
email: heike.ruff@uni-ulm.de
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Besides the modulatory effect of Usp and hormone on transactivation potency of the receptor complex of EcR isoforms, transcriptional activity is enhanced to a considerable extent by stabilization of the receptor protein. Depending on the combination of EcR isoforms, heterodimerization partner and ligand, the change in transcriptional activity is composed of altered stability and interaction of various Usp domains and the AB-domain of EcR to different degrees and demonstrates the complex interplay of the partners involved. Basal transcriptional activity of the ecdysteroid receptor (EcR) expressed in CHO-K1 cells is determined by its AB-domain (B1>B2>A). The AB-domain of Usp also modifies the transcriptional activity of the ecdysone receptor complex. Comparison of different Usp variants revealed that the hexapeptide adjacent to the C-domain of Usp has a stimulatory influence on transcriptional activity of the heterodimer. According to Western Blots enhanced transcriptional activity of the receptor complex in case of EcR-B1 and EcR-A heterodimers is responsible for the increased reporter activity. This is in contrast to EcR-B2, which seems to be stabilized in the presence of Usp. Since the influence of the hexapeptide is restricted to receptor complexes in the absence of ligand, we assume that interaction of the hexapeptide in the AB-domain of Usp with the LBD of EcR takes place only in the absence of hormone. Our results show that the AB-domains of EcR and Usp act synergistically and not only additiv, the N-termini of both dimerization partners regulate transcriptional activity of the receptor complex. This means that a crosstalk between both N-terminal domains takes place in the absence of hormone. Deletion of the C-domain of Usp increases hormone induced transcriptional activity of the receptor complex EcR/Usp with EcR-B1 and EcR-A, although DNA binding is considerably reduced indicating an inhibitory influence of the DNA binding domain. Since this effect depends on the EcR isoform, we conclude that the ligand induced change in the 3 D architecture, which leads to an altered position of helix 12 allows interaction of the AB-domain of EcR with the AF2 domain, either directly or indirectly. This mechanism is already known from vertebrate nuclear receptors like the androgen receptor, RAR and RXR.

Modulation of transcriptional activity of ecdysone dependent genes by different hormone response elements

Schauer S., Braun S., Spindler-Barth M.
email: sebastian.schauer@uni-ulm.de
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Molting hormone action is mediated mainly by the heterodimer of ecdysteroid receptor (EcR) and Ultraspiracle (Usp), which act as ligand-dependent transcription factors. Therefore the recognition of their cognate hormone response element (HRE) is essentially. EcR/Usp binds to direct repeats (like DR1), to perfect inverted repeat sequences (like PAL1) and to imperfect palindromes (like hsp27).

We studied the impact of various hormone response elements on constitutive and hormone induced transcriptional activity in CHO-1 cells, which were transiently transfected with EcR and Usp, to eliminate the influence of endogenous ecdysone receptor activity. Since receptor stability varies depending on receptor isoform and presence or absence of hormone, we normalized reporter (luciferase activity) not on transfection efficiency, but on receptor concentration. We therefore quantified specific receptor signals of Western blots, since comparison with Scatchard analysis revealed that this quantification method is reliable.

The activity of the reporter luciferase is dependent on the kind of hormone response element, the EcR isoform and the Usp variant.

Cell cycle as a regulator of EcR distribution and expression in vertebrate cell culture system

Betanska K., Spindler-Barth M., Spindler K.-D.
email: katarzyna.betanska@uni-ulm.de
Institute of General Zoology and Endocrinology, University of Ulm, Ulm, Germany

The *Drosophila melanogaster* ecdysteroid receptor (dmEcR) is distributed differently within the CHO-K1 cells and is observed exclusively in the nucleus, nucleus and cytoplasm and as well exclusively in the cytoplasm (Nieva et al., 2005). This heterogeneous localization was a starting point for the investigation considering cell cycle as a regulator of EcR distribution and expression. CHO-K1 cells were reversibly arrested in G2 phase using nocodazole. They were released from the G2 state after 12 hours. The ability of the cell population to re-enter the cell cycle and to maintain the synchronization was controlled by FACS analysis after different time intervals. The distribution and expression of EcR were studied using fluorescent microscopy and Western blotting. The highest EcR concentration was observed in G1 and S phases, but a progressive decrease in its expression during late S and G2/M. We presume that the changes in EcR level are not due to its degradation because of low stability or different efficiency of transfection. Firstly, the same result was obtained in the presence of Ultraspiracle that stabilizes EcR. Secondly, prolonged expression of EcR to 20 hours showed that CHO-K1 cells were transfected efficiently. Similarly, EcR distribution varies according to the cell cycle. By the decrease of the percentage of cells in S phase, the number of cells with cytoplasmic distribution of EcR decreases, whereas the level of cells with nuclear localization is increased. We are currently investigating the interplay between ecdysteroids receptor and cell cycle regulatory protein cyclin D1.

Intramolecular interaction of the *Drosophila melanogaster* ecdysteroid receptor isoforms analysed by FRET technique

Tremmel Ch.[1], Schäfer M.[2], Spindler-Barth M.[1], Spindler K.-D.[1]

email: christian.tremmel@uni-ulm.de

[1]Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany
[2]Institute for Pharmacology, Charité, Berlin, Germany

The isoforms EcR-A, EcR-B1 and EcR-B2 of the *Drosophila melanogaster* ecdysteroid receptor differ only in length and sequence of their AB domain. Constitutive transactivation potency is different for the EcR isoforms and is enhanced after dimerization with Ultraspiracle (Usp) and in presence of hormone.

FRET (fluorescence resonance energy transfer) is a method to detect (intra)molecular interaction between proteins or protein domains tagged with fluorescent proteins. Aim of our study is to investigate, whether intramolecular interactions between transactivation functions AF1 and AF2 of the EcR isoforms are responsible for the differences in their transactivation potency as is reported from vertebrate nuclear receptors, e.g. the androgen receptor (Schaufele et al. 2005, PNAS 102, 9802-9807).

Performing FRET analysis we could show that AF1 (AB domain) and AF2 (ligand binding domain) of EcR interact in an isoform-specific manner independent of the absence or presence of Usp. With EcR-A interaction between AF1 and AF2 is increased significantly after addition of hormone (1 µM Muristerone A). In contrast EcR-B1 shows no hormone-induced increase of the AF1–AF2 interaction. The type of Usp variant used has no influence on the AF1–AF2 interaction of EcR.

Wild-type ecdysteroid receptor signaling in ecdysteroid-resistant cell lines from the polyphagous noctuid pest *Spodoptera exigua*

Swevers L.[1], Soin T.[2], Mosallanejad H.[2], Iatrou K.[1] and Smagghe G.[2]

email: swevers@bio.demokritos.gr

[1]Insect Molecular Genetics and Biotechnology, Institute of Biology, National Centre for Scientific Research "Demokritos", Athens, Greece

[2]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

In this project we describe an analysis of ecdysteroid receptor signaling in cell lines derived from the polyphagous noctuid pest *Spodoptera exigua* (Se4 cells), that were selected for continuous growth in the presence of high concentrations of 20-hydroxyecdysone (20E) or methoxyfenozide. In contrast to other ecdysteroid-resistant cell lines described to date, normal functioning of the ecdysteroid receptor complex was demonstrated in the resistant Se4 cell lines by two criteria: (1) activation of an ecdysteroid-responsive luciferase cassette; and (2) induction of expression of the early gene *HR3*. Sequencing of PCR fragments also revealed the presence of *SeEcR* mRNA with a wild-type ligand-binding domain in resistant cells.

It was also observed that the gene *FTZ-F1*, whose expression correlates with the absence of circulating ecdysteroids during insect development, is constitutively expressed in Se4 cells and that its expression is not regulated by the addition of ecdysteroid. It is therefore proposed that the resistance mechanism in Se4 cells resides at the coupling between the conserved hierarchical cascade of early and early-late gene expression and the differentiation program in the Se4 cell line.

Molecular cloning of the ecdysone receptor (*EcR*) and the retinoid X receptor (*RXR*) and expression analysis of *EcR, RXR* and the early gene *E75* from the mysid shrimp *Neomysis integer*

Soin T.[1,2], Janssen C.[2], Smagghe G.[1]
email: thomas.soin@ugent.be
[1]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[2]Laboratory of Environmental Toxicology and Aquatic Ecology, Department Applied Ecology and Environmental Biology, Ghent, Belgium

While research efforts into effects of potential endocrine disrupting chemicals (EDCs) have mainly focused on vertebrates, significantly less attention has been paid to potential endocrine disruption in invertebrates. Given that invertebrates account for at least 95% of all known animal species and are indispensable for ecosystem functioning, it remains essential to tackle this discrepancy in knowledge and research. Of the crustaceans, mysid shrimps have been proposed as most suitable organisms for the regulatory testing of potential EDCs in the USA, Europe and Japan (deFur et al. 1999).

Our goal is to develop an *in vitro* binding assay with the functional ecdysteroid receptor complex of *Neomysis integer* in order to test which potential EDCs can influence the activity of this receptor complex. To achieve this we first cloned the ecdysteroid receptor (*EcR*) and the retinoid X receptor (*RXR*) through degenerate PCR and RACE. The similarity with the sequences of other crustacean and insect EcRs and RXRs is described. Also the *in vivo* expression patterns of *EcR*, *RXR* and the early gene *E75* are presented.

This study will contribute to the current knowledge about crustacean endocrinology, which is necessary to gain more insight into the mode of action of potential EDCs.

deFur P.L., Crane M., Ingershold C., Tattersfield L. (1999). Endocrine disruption in invertebrates: ecotoxicology, testing and assessment. Society of Environmental Toxicology and Chemistry, Pensacola, FL, USA

Tissue and ligand specificities of DmUSP, TcUSP and HsRXR LBDs in hormonal responses in *Drosophila*

Beck Y.[1], Delaporte C.[1], Moras D.[1], Richards G.[2] and Billas I. M. L.[1]

email: beck@igbmc.fr

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Département de Biologie et de Génomique Structurales, Illkirch, France

[2]Human Frontier Science Program, Strasbourg, France

In insects, 20-hydroxyecdysone acts by binding the Ecdysone receptor (EcR). EcR functions as a heterodimer with the Ultraspiracle protein (USP), the homolog of the Retinoid X Receptor. In contrast to RXR, no ligand has clearly been identified for USP. In the beetle *Tribolium castaneum*, the USP LBD protein sequence shows a higher sequence homology with mammalian RXR LBD than with *Drosophila* USP LBD. However, structural and functional studies demonstrated that TcUSP is in a stable apo conformation and acts as a constitutively silent partner of EcR. In order to gain more insight into the functional role of USP, we used transgenic flies expressing fusion proteins coding for Gal4 DBD fused with DmUSP, TcUSP or the human RXR LBDs. Expression of these fusion proteins in the presence of an UAS-GFP reporter gene allows to follow their activity *in vivo* during the late third larval ecdysone response and in selected tissues incubated with different known or putative ligands. Here, we show that DmUSP, TcUSP or HsRXR LBDs exhibit tissue- and ligand-dependant activities. In addition, we observe that juvenile hormone has no direct effect on our fusion proteins activity. However, it seems to trigger the expression of a factor repressing their activity in presence of ecdysteroids or RXR ligands.

Molecular characterization and functional analysis of two isoforms of the Ets transcription factor E74 in the hemimetabolous insect *Blattella germanica*

Borràs F., Bellés X. and Martín D.
email: fbcagr@cid.csic.es
Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CID, CSIC), Barcelona, Spain

In insects, major developmental transitions occurring throughout their life cycles are finely regulated by changes in the titer of the ecdysteroidal hormone, 20-hydroxyecdysone (20E). 20E regulates these transitions upon binding to its heterodimeric receptor formed by two members of the nuclear receptor superfamily, EcR and the RXR homolog USP. In addition, the activated receptor elicits cascades of gene expression that mediate and amplify the ecdysteroidal signal. Molecular characterization of most of the cascade genes revealed that they encode transcription factors, namely E75, E74 and Broad complex. Here, we report the cloning and hormonal regulation of two isoforms of E74 from the hemimetabolous direct-developing insect *Blattella germanica*, namely BgE74A and BgE74B. Within the DNA-binding ETS domain of the protein, BgE74 is 95% similar to that of *D. melanogaster* homologue, whereas the 5'-isoform-specific regions of BgE74A and BgE74B show significantly lower similarity. The two isoforms present characteristic expression patterns during embryo and nymphal development, and experiments *in vitro* with fat body tissue have shown that the two isoforms display specific 20E responsiveness. Finally, we characterized the functions of BgE74 during nymphal and adult development by using RNAi experiments *in vivo*.

Dual role of BgFTZ-F1 during nymphal development in the hemimetabolous insect *Blattella germanica*

Nieva C., Cruz J., Mané-Padrós D., Martín D. and Bellés X.
email: fbcagr@cid.csic.es
Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CID, CSIC), Barcelona, Spain

In insects, the ecdysteroidal hormone 20-hydroxyecdysone (20E) controls key developmental processes during embryogenesis, molting, metamorphosis and reproduction, as it has been extensively studied in holometabolous insects, especially in *Drosophila melanogaster*. The situation is very different in hemimetabolous insects, which do not develop through complete metamorphosis, their juvenile forms being morphologically similar to the adult. Indeed, in hemimetabolous insects the wealth of information concerning the molecular basis of the 20E-triggered genetic hierarchy is practically non-existent. However, if we aim to understand the molecular basis of the evolution towards complete metamorphosis in insects, then characterization of the 20E-induced genetic hierarchy in primitive species becomes of paramount importance. In the cockroach *Blattella germanica*, we have previously characterized the two components of the heterodimeric ecdysone receptor, as well as several early and early-late genes. In the present work we extend the knowledge of the 20E genetic hierarchy in *B. germanica* by identifying a FTZ-F1 homologue in this cockroach, by studying its regulation by ecdysteroids and by determining its functions in nymphal development with RNAi approaches. RNAi experiments reveal that FTZ-F1 is required to molt and to control the timing of ecdysteroid production during the last nymphal stage.

Comparative analysis for ecdysone receptor functionality in selected lepidopteran and dipteran methoxyfenozide-resistant insect cell lines

Mosallanejad H.[1], Soin T.[1], Swevers L.[2], Iatrou K.[2] and Smagghe G.[1]

hadi.mosallanejad@ugent.be

[1]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[2]Institute of Biology, National Centre of Scientific Research 'Demokritos', Athens, Greece

Using an ecdysteroid-resistant cell line derived from an important polyphagous noctuid, the beet armyworm *Spodoptera exigua* (Se4), we recently reported that the resistance mechanism in the cells likely was not due to differential metabolism or uptake of the ecdysone (agonist) compared to sensitive cells (Mosallanejad et al. 2008). In continuation of this research we have demonstrated the presence of an active ecdysone receptor (EcR) complex in these resistant Se4 cells. To gain more insight into resistance mechanisms against the ecdysone agonist methoxyfenozide, we have selected two other lepidopteran cell lines (CF203, a midgut line of *Choristoneura fumiferana* and Bm5, an ovarian line of *Bombyx mori*) and one dipteran cell line (S2, an embryonic line of *Drosophila melanogaster*) towards methoxyfenozide.
In this work, a comparative analysis for EcR functionality in these resistant cells was carried out using an ecdysone-responsive reporter gene. Our data indicate that the EcR complex in the lepidopteran methoxyfenozide-resistant cell lines is functional whereas in the methoxyfenozide-resistant dipteran S2 cells the ecdysone-responsive reporter gene is almost not active during continuous presence of ecdysone agonist. However, induction of the reporter by methoxyfenozide is observed when selected cells are cultured first in agonist-free medium for several passages. In the latter case the cells were still resistant to growth inhibition by methoxyfenozide.
In conclusion, for lepidopteran cell lines, it is conceivable that the receptor is not the cause of the resistance and most likely resistance will be associated to factor(s) located downstream from the receptor. For dipteran S2 cells, however, the resistance mechanism may reside at the level of the EcR complex. For instance, long exposure to methoxyfenozide may have caused a decrease in expression levels of EcR and/or USP or a decrease in their activity through posttranslational modification mechanisms. As a general conclusion, analysis of resistance mechanisms in selected lepidopteran and dipteran cell lines may provide new insights in the function of the ecdysteroid signaling pathway.

Mosallanejad H., Soin T., Smagghe G. (2008). Selection for resistance to methoxyfenozide and 20-hydroxyecdysone in cells of the beet armyworm Spodoptera exigua. Arch. Insect Biochem. Physiol. 67, 36-49.

Ecdysteroids from *Cyanotis longifolia* (Commelinaceae) grown in Paris

Crouzet S.[1], Girault J.-P.[2], Maria A.[1], Dinan L.[1] and Lafont R.[1]

email: rene.lafont@snv.jussieu.fr

[1]Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines,
Université Pierre et Marie Curie, Paris, France.
[2]Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques,
Université Paris Descartes, Paris, France

The *Cyanotis* genus comprises at least two ecdysteroid-rich species (*C. arachnoidea and C. vaga*) which grow in Asia and are used on a large scale to prepare ecdysteroids, which are proposed on the market as 25 kg drums claimed to contain 97% pure 20-hydroxyecdysone (20E)! *Cyanotis* extracts are used for improving silk production by silkworms and also by bodybuilders for increasing their muscle mass. Beside 20E, *C. arachnoidea* has previously been shown to contain a wide array of different ecdysteroids. As we had the opportunity to access the closely related species *C. longifolia* from the “Serres d’Auteuil” in Paris, we decided to undertake a phytochemical analysis of this species.

Aerial parts and roots were collected separately, extracted and purified using a combination of NP- and RP-HPLC, and purified ecdysteroids were then characterized by MS and NMR. Much higher concentrations of ecdysteroids were found in roots as compared with aerial parts, but there appeared also significant qualitative differences concerning ecdysteroids present in both plant parts. Aerial parts contained essentially 20E, 20E 3-acetate and ajugasterone C, together with minor amounts of polypodine B. In addition to the above compounds, roots contained several side-chain cleavage products not detected is the aerial parts, among which isovitexirone, poststerone, and several new ecdysteroids, among which 5β-hydroxy-poststerone and poststerone 2-acetate.

As a conclusion, *C. longifolia* accumulates large amounts of ecdysteroids, and its ecdysteroid pattern is close, albeit somewhat different from that of *C. arachnoidea*. It appears that the *Cyanotis* genus is of much interest, and additional studies are under way to check other available species.

Spinach ecdysteroids: biosynthesis and regulation

Takvorian N.[1], Bakrim A.[1,2], Maria A.[1] and Lafont R.[1]

email: najat.takvorian@snv.jussieu.fr

[1]UPMC, Univ. Paris 06, Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Paris, France
[2]CEEM, Laboratoire de Biologie Appliquée & Sciences de l'Environnement,
Faculté des Sciences et Techniques, Tangier, Morocco

Many plant species produce phytoecdysteroids (PEs) as a chemical defence against non-adapted insects and nematodes. PEs are good candidates for the development of an environmentally safe approach to crop protection, but most crop species do not accumulate PEs. Many arguments, however, support the idea that most (if not all) plant species have the genetic ability to produce PEs. Thus a better understanding of the PE biosynthetic pathway and its regulation appears as a prerequisite for allowing cultivated species to accumulate PEs.

Spinach is one of the very few crop plants which produce large amounts of PEs, of which 20-hydroxyecdysone (20E) is the major component, thus it represents an interesting model system for a detailed analysis of the regulation of PE biosynthesis and transport. We focussed on aerial parts and performed radiolabelling experiments of excised leaves with various precursors (mevalonic acid and putative biosynthetic intermediates) and 20E itself.
PEs are stable molecules produced in old leaves (source) and continuously redistributed in the aerial parts, so that they always reach their highest concentration in the apical tips (sink). Although unable to perform the whole biosynthetic pathway, young leaves nevertheless possess several active enzymes involved in late biosynthetic steps. 20E directly regulates PE synthesis by a direct negative feed-back, thus the sustained synthesis by older leaves requires that those can export the PEs they produce.

Evidence for differences between the biosynthetic pathways of spinach and insects has been obtained for the sub-cellular localization and the substrate-specificity of two "late" enzymes (the 2- and 20-hydroxylases), and the preferred sequence of hydroxylation in spinach (2dE -> 2d20E -> 20E) markedly differs from that of insects.

The Chemotaxonomic Usefulness of Phytoecdysteroid profiles in the Genus *Silene* (Caryophyllaceae)

Zibareva L.[1], Yeriomina V. I.[1], Munkhjargal N.[1], Girault J.-P.[2], Dinan L.[3] and Lafont R.[3]

email: rene.lafont@snv.jussieu.fr

[1]Tomsk State University, Siberian Botanical Garden, Tomsk, Russia
[2]Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris, Paris, France
[3]Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Université Pierre et Marie Curie, Paris, France

Phytoecdysteroids have the potential to act as chemosystematic markers for the identification of plant species by relating ecdysteroid content and profiles to taxonomic position, but the validity of this needs to be demonstrated. The genus *Silene* is ideal material for testing this concept, because i) there are many species in the genus, ii) a substantial proportion of them contain phytoecdysteroids and iii) a wide variety of ecdysteroid analogues are found in this genus. Extending our previous work in this area, we have now selected a dozen key ecdysteroid-containing species from different Sections of the genus. The phytoecdysteroid profiles of extracts of whole plants of these 12 species (*S. fridvalskyana* Hampe, *S. gigantea* L., *S. graminifolia* Otth, *S. jenissensis* Willd, *S. mellifera* Boiss. & Reuter, *S. melzheimeri* Greuter, *S. oligantha* Boiss. & Heidr. in Boiss., *S. repens* Patr., *S. roemeri* Friv., *S. schmuckeri* Wettst., *S. sendtneri* Boiss. and *S. viscosa* (L.) Pers.) have been examined and identified by HPLC and, in the case of new compounds, also by mass spectrometry and NMR. New minor ecdysteroids have been purified (by column chromatography on SiO2 and HPLC) and identified: 26-hydroxyintegristerone A from *S. frivaldskyana* and 2-deoxy-20-hydroxyecdysone 25-glucoside from *S. gigantea*. There is considerable variability with regard to ecdysteroid profiles within the genus Silene, since the extracts differ not only in levels of ecdysteroids, but also in terms of the complexity of the 'cocktails' and the identities of the analogues present. The chemotaxonomic value of ecdysteroid profiles within the genus *Silene* will be discussed.

Phytoecdysteroids from *Ajuga macrosperma var. breviflora* roots

Castro A.[1], Coll J.[1], Tandrón Y. A.[1], Pant A. K.[2] and Mathela C. S.[3]

email: jctqob@iiqab.csic.es

[1]Departament de Química Orgànica Biològica, Institut d'Investigacions Químiques i Ambientals de Barcelona "Josep Pascual Vila", Consejo Superior de Investigaciones Científicas. Barcelona, Spain
[2]Chemistry Department, G. B. Pant University of Agriculture and Technology, Pantnagar. India
[3]Chemistry Department, College of Basic Sciences and Humanities, Kumaun University, Nainital, India

Three new phytoecdysteroids out of eight isolated compounds have been obtained from *Ajuga macrosperma* Wall. *var. breviflora* and the structures established on the basis of extensive NMR spectral studies. Five of them were common ecdysteroids, namely 20-hydroxyecdysone, cyasterone, makisterone A, 20-hydroxyecdysone 2-acetate and 20-hydroxyecdysone 3-acetate. New compounds side chain presented some particular functions as a lactone bridge across C(26),C(23) showed by breviflorasterone; acetal oxygen bridges between C-26 and C-20/C-22 (showing the unprecedented dioxabicyclo[3.2.1]octene acetal function), or C-26/C-23 named ajugacetalsterone C and D, respectively.

Linking Endocrine disrupting chemicals with the decline in the North Sea brown shrimp (*Crangon crangon*) stock: a key role for the ecdysteroid receptor?

Verhaegen Y.[1,2,3], Parmentier K.[1], De Coen W.[2] and Smagghe G.[3]

email: yves.verhaegen@ugent.be

[1]Department of Fisheries, Institute for Agricultural and Fisheries Research, Ostend, Belgium
[2]Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Antwerp, Belgium
[3]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

The North Sea is of vital economical importance for North-Western Europe. On the one hand it serves as a major transport route, as a natural source of gas, oil, gravel and sand, and as the sink for land-based pollution. On the other hand it was formerly considered as an inexhaustible source of fish and shellfish. Of the economical important shellfish the brown shrimp (*Crangon crangon*) exhibits a gradual decrease in abundance since the late 1970's, which has now become critical for the sustainability of the North-Western brown shrimp fisheries, especially the Flemish. Next to overfishing as the plausible major cause, pollution has been proposed as an important contributory factor, but the magnitude of its impact at the population level is currently unknown. Like other crustaceans, *C. crangon*, and more specifically the ecdysteroid signalling pathway, is believed to be very sensitive to pesticides and other potential endocrine disrupting chemicals (EDC's) that are observed in alarmingly high concentrations in the North Sea and its biota. In order to finally clarify the contribution of these field-levels of EDC's to the population decline, we initially need to clarify the effects of the relevant EDC's at the molecular level.

In this study we cloned and sequenced the EcR and RXR nuclear receptors and their isoforms out of adult *C. crangon* ovaria through degenerated PCR primers and RACE, in order to further analyse the EDC-receptor interaction in brown shrimp and to elucidate the relationship between the brown shrimp population decrease and the concentrations of potential ecdysteroid signalling disrupting chemicals observed in the North Sea. The sequence similarities of CcEcR and CcRXR with other Arthropod ecdysteroid receptors will also be discussed.

Effects of an ecdysteroid agonist (halofenozide) on reproduction events in the German cockroach *Blattella germanica* (Dictyoptera, Blattellidae)

Aribi N.[1], Kilani-Morakchi S.[1], Farine J. P.[2], Smagghe G.[3] and Soltani N.[1]

email: nadaribi@yahoo.fr

[1]Laboratoire de Biologie Animale Appliquée, Département de Biologie Animale, Université de Annaba, Annaba, Algeria
[2]CNRS-UMR 5548, Université de Bourgogne, Dijon, France
[3]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering. Ghent University, Ghent, Belgium.

Due to the secondary effects of conventional insecticides on the environment, a new class of selective insect growth regulators (IGRs) which mimic the steroid insect moulting hormone 20-hydroxyecdysone (20E), have been developed. Halofenozide, a benzoylhydrazine derivative belonging to ecdysteroïd agonist class, was investigated on reproductive events of the most prevalent and resistant cockroaches, *Blattella germanica*. The compound was applied topically (10 µg) on newly emerged adults. Behavioral tests, conducted on 6 days old-adult, revealed that treated females caused a significant decrease in males wings raising. The characterization of the cuticular hydrocarbons was made on 1, 3 and 6 days old virgin adults of both sexes using gas chromatography. Thirteen major compounds were investigated. No qualitative difference was observed with regard to sex and treatment. However, but our results revealed quantitative hydrocarbon variations. In control males and females, significant differences between both sexes were clearly observed depending to the treatment. Halofenozide significantly reduced all cuticular components in both sexes at 1, 3 and 6 days during treatment. In addition, enzyme immunoassay measurements showed a decrease in the whole body ecdysteroïd titers, in treated series of both gender at 6 days. Finally, the coumpound reduced the number of oocytes produced by females during the first 6 days of adults life.

Comparison of the activity of ecdysone agonists in *Bombyx mori* and *Spodoptera littoralis* by *in vitro* reporter assays and *in vivo* toxicity assays

Soin T.[1], Mosallanejad H.[1], Martín D.[2], Iatrou K.[3], Ozaki S.[4], Kitsuda S.[4], Harada T.[4], Nakagawa Y.[4], Smagghe G.[1] and Swevers L.[3]

email: swevers@bio.demokritos.gr

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

[2]Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CSIC), Barcelona, Spain

[3]Insect Molecular Genetics and Biotechnology, Institute of Biology, National Centre for Scientific Research "Demokritos", Aghia Paraskevi Attikis, Athens, Greece

[4]Division of Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Ecdysone agonists such as dibenzoylhydrazines have been successfully used in biological control programs of lepidopteran and coleopteran pests. However, little information exists with respect of the specificity of the action of such compounds towards different species of Lepidoptera or Coleoptera. In this study, we compare the activity of ecdysone agonists belonging to three different chemical classes (dibenzoylhydrazines, acylaminoketones and tetrahydroquinolines) between the lepidopteran pest *Spodoptera littoralis* and a beneficial lepidopteran species, the silkworm *Bombyx mori*. The activity of ecdysteroid agonists is measured both *in vitro*, using an ecdysone-responsive reporter assay based on transfected or transformed *Spodoptera*- or *Bombyx*-derived cell lines (Sl2 and Bm5 cells, respectively), and in vivo, by toxicity tests on *Spodoptera* and *Bombyx* larvae. The study aims at the elucidation of the species-specific factors that determine the activity of ecdysone agonists against Lepidoptera.

Isolation of new phytoecdysteroids from *Silene viridiflora* and investigation of the natural origin of ecdysteroid acetonides

Hunyadi A.[1], Tóth N.[1], Simon A.[2], Tóth G.[2], Báthori M.[1]
email: hunyadi.a@pharm.u-szeged.hu
[1]Department of Pharmacognosy, University of Szeged, Szeged, Hungary
[2]Institute for General and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Hungary

The distribution of phytoecdysteroids in *Silene* genus is wide; the *Silene* species are characterized by a high accumulation of ecdysteroids even with unusual structures. More than 70 ecdysteroids (about 25% of the known phytoecdysteroids) were detected in plants belonging to the *Silene* genus.

Here we report the isolation of 9 ecdysteroids from the *S. viridiflora*: integristerone A (1), 5,20,26-trihydroxyecdysone (26 hydroxypolypodine B) (2), 20,26-dihydroxyecdysone (3), 2-deoxy-20-hydroxyecdysone (4), 2 deoxyintegristerone A (5), 2-deoxy-polypodine B 25-**α**/**β**-D-glucopyranoside (6) and 5**α**- (7) and 5**β**- (8) isomer pair of 2 deoxy 5,20 dihydroxyecdysone 20,22-acetonide and the 2,3;20,22-diacetonide derivative of makisterone C (9). Compound 6, 7 and 9 are new natural compounds while 3, 4 and 5 are reported for the first time from the *Silene viridiflora*.

In view of both our previous and present results, the species was found to be particularly rich in various ecdysteroid acetonides. To exclude the occurrent artefact-formation caused by the use of acetone during certain purification steps, the presence of these compounds in the extract was also investigated and confirmed by using NP and RP-HPLC.

Algeria

Nadja Aribi **nadaribi@yahoo.fr**
Laboratoire de Biologie Animale Appliquée, Département de Biologie Animale, Université de Annaba, Annaba, Algeria

Australia

Lloyd Graham **lloyd.graham@csiro.au**
CSIRO Molecular & Health Technologies, North Ryde, NSW, Australia

Belgium

Masatoshi Iga **masatoshi.iga@ugent.be**
Laboratory of Agrozoology, Department of Crop Protection,
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Elisabeth Marchal **elisabeth.marchal@bio.kuleuven.be**
Department of Animal Physiology and Neurobiology,
Zoological Institute K.U. Leuven, Leuven, Belgium

Hadi Mosallanejad **hadi.mosallanejad@ugent.be**
Laboratory of Agrozoology, Department of Crop Protection,
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Guy Smagghe **guy.smagghe@ugent.be**
Laboratory of Agrozoology, Department of Crop Protection,
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Thomas Soin **thomas.soin@ugent.be**
Laboratory of Agrozoology, Department of Crop Protection,
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Yves Verhaegen **yves.verhaegen@ilvo.vlaanderen.be**
Department of Fisheries, Institute for Agricultural and Fisheries Research,
Ostend, Belgium

Pieter van Wielendaele **pieter.vanwielendaele@bio.kuleuven.be**
Department of Animal Physiology and Neurobiology,
Zoological Institute K.U.Leuven, Leuven, Belgium

Canada

Xanthe Vafopoulou **xanthev@yorku.ca**
Biology Department, York University, Toronto, Ontario, Canada

Czech Republic

Marek Jindra **jindra@entu.cas.cz**
Biology Center ASCR, Institute of Entomology, Ceske Budejovice, Czech Republic

Keiko Takaki **keiko@entu.cas.cz**
Biology Center ASCR, Institute of Entomology, Ceske Budejovice, Czech Republic

China

Sheng Li **shengli@sippe.ac.cn**
Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Xiao-Fan Zhao **xfzhao@sdu.edu.cn**
School of Life Sciences, Shandong University, Jinan, China

France

Yannick Beck **beck@igbmc.u-strasbg.fr**
Institut de Génétique et de Biologie Moléculaire et Cellulaire, Département de Biologie et de Génomique Structurales, Illkirch, France

Catherine Blais **catherine.blais@snv.jussieu.fr**
Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Université Pierre et Marie Curie, Paris, France

Christopher Browning **browning@igbmc.fr**
Département de Génomique et de Biologie Structurales, Université Louis Pasteur, Illkirch, France

Chantal Dauphin-Villemant **chantal.dauphin-villemant@snv.jussieu.fr**
Equipe Biogenèse des Stéroïdes, Laboratoire Protéines Biochimie Structurale et Fonctionnelle, Université Pierre et Marie Curie, Paris, France

Jean-Paul Delbecque **jp.delbecque@cnic.u-bordeaux1.fr**
Université de Bordeaux, Centre de Neurosciences Intégratives et Cognitives, Talence, France

Blandine Destrez **destrez@vet-nantes.fr**
LABoratoire d'Etude des Résidus et Contaminants dans les Aliments,
Ecole Nationale Vétérinaire de Nantes, Route de Gachet, Nantes, France

Laurie Dinan **lauriedinan@fsmail.net**
Université Pierre et Marie Curie, Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Paris, France

Rene Lafont **rene.lafont@snv.jussieu.fr**
Université Pierre et Marie Curie, Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Paris, France

Alain Meybeck **ameybeck@club-internet.fr**
AM Phyto-Conseil, Courbevoie, France

Virginie Orgogozo **virginie.orgogozo@snv.jussieu.fr**
Université Pierre et Marie Curie, Equipe Biogenèse des Stéroïdes, Laboratoire Protéines Biochimie Structurale et Fonctionnelle, Paris, France

Najat Takvorian **najat.takvorian@snv.jussieu.fr**
Université Pierre et Marie Curie, Laboratoire de Biochimie Structurale et Fonctionnelle des Protéines, Paris, France

French Polynesia

Phila Raharivelomanana **phila.raharivelomanana@upf.pf**
Laboratoire de Chimie des Substances Naturelles,
Université de la Polynésie Française, Tahiti, Polynésie Française

Raimana Ho **raimanah@yahoo.fr**
Laboratoire de Chimie des Substances Naturelles,
Université de la Polynésie Française, Tahiti, Polynésie Française

Germany

Anca Azoitei **anca.azoitei@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Katarzyna Betanska **katarzyna.betanska@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Simone Braun **simone.braun@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Michael Burret **michael.burret@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Susan Czogalla **susan.czogalla@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Celine Hönl **celine.hoenl@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Umesh Gangishetti **umesh.gangishetti@tuebingen.mpg.de**
Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Gemany

Hartmut Kayser **hartmut.kayser@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Bernard M. Mechler **dev.genetics@dkfz-heidelberg.de**
Department of Developmental Genetics, DKFZ-ZMBH Allianz, Heidelberg, Germany

Bernard Moussian **bernard.moussian@tuebingen.mpg.de**
Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Gemany

Marie Mühl **marie.muehl@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Heike Ruff **heike.ruff@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Sebastian Schauer **sebastian.schauer@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Klaus-Dieter Spindler **klaus-dieter.spindler@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Margarethe Spindler-Barth **margarethe.spindler-barth@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Christian Tremmel **christian.tremmel@uni-ulm.de**
Institute of General Zoology and Endocrinology, Ulm University, Ulm, Germany

Greece

Luc Swevers **swevers@bio.demokritos.gr**
Insect Molecular Genetics and Biotechnology, Institute of Biology,
National Centre for Scientific Research Demokritos", Athens, Greece

Hungary

Attila Hunyadi **hunyadi.a@pharm.u-szeged.hu**
Department of Pharmacognosy, University of Szeged, Szeged, Hungary

Noemi Toth **hunyadi.a@pharm.u-szeged.hu**
Department of Pharmacognosy, University of Szeged, Szeged, Hungary

India

R. K. Chaitanya **chaitanyark@gmail.com**
Department of Animal Sciences, School of Life Sciences,
University of Hyderabad, Hyderabad, India

Aparna Dutta-Gupta **aparnaduttagupta@gmail.com**
Department of Animal Sciences, School of Life Sciences,
University of Hyderabad, Hyderabad, India

Italy

Patrizia Romani **patrizia.romani@unibo.it**
Dipartimento di Biologia Evoluzionistica Sperimentale,
Università di Bologna, Bologna, Italy

Japan

Hideaki Asazuma **asa-ch@msc.biglobe.ne.jp**
Laboratory of Bioorganic Chemistry, Department of Applied Biological Chemistry,
Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi,
Bunkyo, Tokyo, Japan

Kiyoshi Hiruma **hiruma@cc.hirosaki-u.ac.jp**
Faculty of Agriculture and Life Sciences, Hirosaki University, Japan

Ryo Futahashi **ryo284@k.u-tokyo.ac.jp**
Department of Integrated Biosciences, Graduate School of Frontier Sciences,
The University of Tokyo, Kashiwa, Japan

Manaboon Manaporna **manaboon@stu.kanazawa-u.ac.jp**
Graduate School of Natural Science&Technology, Kanazawa University, Japan

Takahiro Shiotsuki **shiotsuk@affrc.go.jp**
Gene Function Research Unit, Division of Insect Science,
National Institute of Agrobiological Sciences, Ibaraki, Japan

Takukji Yoshiyama **kk57513@mail.ecc.u-tokyo.ac.jp**
Graduate School of Frontier Sciences, The University of Tokyo, Japan

Norway

Sussie Dalvin **sussie.dalvin@imr.no**
Havforskningsinstituttet, Bergen, Norway

Christiane Eichner **christiane.eichner@imr.no**
Havforskningsinstituttet, Bergen, Norway

Rasmus Skern-Mauritzen **rasmus@imr.no**
Havforskningsinstituttet, Bergen, Norway

Poland

Beata Greb-Markiewicz **greb_markiewicz@yahoo.com**
Department of Biochemistry, Faculty of Chemistry,
Wrocław University of Technology, Wrocław, Poland

Russia

Elena Bogomolova **iraushen@bionet.nsc.ru**
Institute of Cytology and Genetics SD RAS, Novosibirsk 630090, Russia

Nataly Gruntenko **nataly@bionet.nsc.ru**
Institute of Cytology and Genetics SD RAS, Novosibirsk 630090, Russia

Larissa Zibareva **zibareval@inbox.ru**
Tomsk State University, Siberian Botanical Garden, Tomsk, Russia

Slovakia

Robert Farkas **ueenfark@savba.sk**
Laboratory of Genetics, Institute of Experimental Endocrinology,
Slovak Academy of Sciences, Bratislava, Slovakia

Spain

Rosa Barrio **rbarrio@cicbiogune.es**
CIC Biogune, Derio, Bizkaia, Spain

Xavier Belles **xbragr@cid.csic.es**
Institut de Biologia Molecular de Barcelona
Department of Physiology and Molecular Biodiversity
Barcelona, Spain

Ferràn Borrás **fbcagr@cid.csic.es**
Department of Physiology and Molecular Biodiversity,
Institut de Biologia Molecular de Barcelona (CID, CSIC), Barcelona, Spain

Josep Coll **jctqob@iiqab.csic.es**
Departament de Química Orgànica Biològica, Institut d'Investigacions Químiques i Ambientals de Barcelona "Josep Pascual Vila",
Consejo Superior de Investigaciones Científicas, Barcelona, Spain

Paula Irles **piiagr@cid.csic.es**
Institut de Biologia Molecular de Barcelona
Department of Physiology and Molecular Biodiversity
Barcelona, Spain

David Martin **dmcagr@cid.csic.es**
Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CID, CSIC), Barcelona, Spain

Claudia Nieva **cnbagr@ibmb.csic.es**
Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona, Barcelona, Spain

Dolors Piulachs **mdpagr@cid.csic.es**
Institut de Biologia Molecular de Barcelona
Department of Physiology and Molecular Biodiversity
Barcelona, Spain

Amaya Castro Ruiz **jctqob@iiqab.csic.es**
Departament de Química Orgànica Biològica, Institut d'Investigacions Químiques i Ambientals de Barcelona "Josep Pascual Vila",
Consejo Superior de Investigaciones Científicas, Barcelona, Spain

Jonatan Sanchez **rbarrio@cicbiogune.es**
CIC Biogune, Derio, Bizkaia, Spain

Ana Talamillo **atalamillo@cicbiogune.es**
CIC Biogune, Derio, Bizkaia, Spain

Taiwan

Ju-Ling Lin **jlsky@yahoo.com**
Department of Zoology, National Museum of Natural Science, Taichung, Taiwan

Thailand

Tippawan Singtripop **scboi020@chiangmai.ac.th**
Department of Biology Faculty of Science, Chiangmai University, Thailand

United Kingdom

Huw H. Rees **reeshh@liv.ac.uk**
School of Biological Sciences, University of Liverpool, Liverpool, UK

Robert Hormann **rhormann@intrexon.com**
Department of Biological Sciences, University of Exeter, Devon, UK

USA

Joshua M. Beatty **jmbeatty@hotmail.com**
Center for Biotechnology, Genomics, and Health Research,
University of North Carolina at Greensboro, Greensboro, NC, USA

Nancy E. Beckage **nancy.beckage@ucr.edu**
Departments of Entomology & Cell Biology and Neuroscience,
University of California-Riverside, Riverside, CA, USA

Edward B. Dubrovsky **dubrovsky@fordham.edu**
Department of Biology, Fordham University, New York, USA

Veronica Dubrovskaya **dubrovsky@fordham.edu**
Department of Biology, Fordham University, New York, USA

Lawrence Gilbert **lgilbert@bio.unc.edu**
University of North Carolina at Chapel Hill, Department of Biology, Insect Endocrinology, Biochemistry and Molecular Biology, Chapel Hill, North Carolina, USA

Vincent Henrich **vincent_henrich@uncg.edu**
Center for Biotechnology, Genomics, and Health Research
University of North Carolina- Greensboro, Greensboro, NC, USA

Penny Hopkins **phopkins@ou.edu**
Department of Zoology, University of Oklahoma, Norman, OK 73019, USA

Hans Laufer **hans.laufer@uconn.edu**
Department of Molecular and Cell Biology, University of Connecticut, USA

Subba R. Palli **rpalli@uky.edu**
Department of Entomology, College of Agriculture,
University of Kentucky, Lexington, KY, USA

Uzbekistan

Mamadalievaa Nilufar Z. **mnilufar76@yahoo.com**
S.Yu.Yunusov Institute of the Chemistry of Plant Substances AS RUz,
Tashkent, Uzbekistan

Authors Index

Cloning and characterization of L-chain and P-chain fibroin from an insect silk gland: Synchronous developmental and hormones induced changes in transcription

Chaitanya R. K. and Dutta-Gupta A.
email: apdgsl@uohyd.ernet.in
Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India

A choreographic precision of titer of mainly the ecdysteroids and juvenile hormones and their interaction is required for almost all the processes that an insect undergoes during its life cycle. Silk production of lepidopteran insects is one such process. Silk fibroin in insects generally constitutes of a complex of three proteins namely, H-chain fibroin, L-chain fibroin and P-chain fibroin with few exceptions. In our present work, we cloned and characterized L-chain fibroin and P-chain fibroin from *Corcyra cephalonica* commonly known as rice moth that belongs to the order lepidoptera. The effect(s) of physiological concentrations of 20-hydroxyecdysone (20-HE) and juvenile hormone (JH) at the molecular level of these genes are not known. Hence, we exogenously administered basal levels of 20-HE and JH and showed the effect of transcription of these genes in the late last instar larvae. We found that 20-HE decreases the transcription and JH does not effect the transcription of these genes. We also showed the developmental regulation and tissue specific expression of these genes.

Ecdysone-Workshop 2008
July 20th - 24th Ulm, Germany

THE ECDYSONE TEAM

Ecdysone-Workshop 2008
July 20th - 24th Ulm, Germany

Dear ecdysonists,

The 17th ecdysone workshop is approaching rapidly. We would like to give you some additional information:

1. **Our team will wear T-shirts in orange throughout the workshop in order to be easily recognized (see figure).**
2. **Two of the team will be at Ulm main station on Sunday (07/20/2008) from 12 to 6.pm near the information desk. They will give some advice, how to reach your hotel and the university (see also point 3).**
3. **All participants will get bus tickets, which are valid from Sunday to Thursday within Ulm. You can take the bus as often as you wish. Those, who contact our team already at the main station, will receive their bus tickets there. All others get the tickets at the registration desk at the university. The price for the bus ticket is already included in the registration fee.**
4. **We need your quick response (till 07/07/2008) to the following questions:**

   vegetarian **non-vegetarian**

   if you do not reply to „info@ecdysone2008.de“, you will be treated as a non vegetarian

5. **During the Visit of the Hauff Museum there is an option to collect your own fossils in the field nearby. You could spend an additional hour for fossil collection. In order to organize the trip we need your answer (till 07/07/2008). Are you interested to collect your own fossils**

   yes or no

   (If you do not respond, you will be treated as a person, who does not like to collect fossils).
   Depending on the number of persons in the two categories (and primarily on the weather!), the participants will be split in two groups, one which departs immediately after visit of the museum and the other after fossil hunting.

   Urweltmuseum Hauff: http://www.urweltmuseum.de
6. **Please see our homepage for the final program: http://www.ecdysone2008.de**
7. **The Power-Point presentations of the oral presentations will be collected on a CD-ROM which will be distributed on Thursday. Only these presentations will be included which will be deposited at the registration desk on Monday (07/21/2008).**
8. **In order to avoid problems with the presentation, it would be advisable to store your PowerPoint presentation under MS-Office 2003 or as a “Portable Executable“ file.**