8th Intercongress Symposium of the
AOSCE 2018
Asia and Oceania Society for Comparative Endocrinology

Veterinary Science Conference Centre
The University of Sydney
July 8-12 2018

Sponsored by
ELSEVIER General and Comparative Endocrinology
Canadian Science Publishing FACETS Journal
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Bronwyn McAllan
Melissa Cameron
The University of Sydney

Samantha Richardson
Janine Danks
RMIT University

John Donald
Deakin University
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<td>List of Delegates</td>
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Welcome Message

It is our great honour to welcome you to the 8th Intercongress Symposium of the Asia and Oceania Society for Comparative Endocrinology (AOSCE), held at Veterinary Science Conference Centre at The University of Sydney, Sydney, NSW Australia from Sunday 8 July 2018 to Thursday 12 July 2018.

The program will cover a wide range of topics in invertebrate and vertebrate endocrinology, a tradition of the society that it is our privilege to host in 2018. There are delegates from 14 countries from across the region and beyond, and the large number of student presentations signals a healthy future for the society.

Comparative Endocrinology has embraced many of the major changes in scientific endeavor, from the use of comparative genomics to identifying new model organisms for specific problems. The presentations in this meeting reflect these changes. Advances in chronobiology, neuroendocrinology, reproductive biology, evolutionary biology, responses of animals to their environment and medical endocrinology are just some of the topics that will be covered in the Intercongress. The endocrinology of diverse organisms such as corals, sea stars, marsupials, birds and fishes will also be presented.

We would also like to welcome you to Sydney, the largest city in Australia. The city is one of the most multicultural in the world and The University of Sydney is close to two restaurant areas with over 130 restaurants serving food from around the world. Nearby are also vibrant shopping precincts, as well as Sydney’s famous harbour, which you can explore on the free day on Wednesday; day trip recommendations have been provided.

We appreciate all the support of the AOSCE council members and all colleagues who provided symposia suggestions, and we are pleased that we could accommodate all those who planned to present their research. We thank the journal, General and Comparative Endocrinology, for providing the student prizes, and FACETS for supporting the student/postdoc dinner.

We look forward to meeting you all in Sydney and wish you a successful and enjoyable intercongress.

Samantha Richardson
Janine Danks
RMIT University

Bronwyn McAllan
Melissa Cameron
The University of Sydney

John Donald
Deakin University
# Schedule at a glance

## 8 July 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Veterinary Science Conference Centre Webster Lecture Theatre</th>
<th>Veterinary Science Conference Centre Pfizer Foyer Level 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1400 - 1600</td>
<td></td>
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</tbody>
</table>
| 1600 - 1730| **Welcome**: Professor Katherine Belov, PVCR for Global Exchange, The University of Sydney  
Plenary Lecture: Professor Ching-Fong Chang  
The reproduction and related hormones in fish and coral | Registration |
| 1730 - 2100| Welcome BBQ Pfizer foyer level 1                            |                                                          |

## 9 July 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Veterinary Science Conference Centre Webster Lecture Theatre</th>
<th>Symposia Veterinary Science Conference Centre Seminar Room 115</th>
</tr>
</thead>
</table>
| 0900 - 1000| **Plenary Lecture**: Professor Vance L Trudeau  
The granin-derived peptide secretoneurin as a new vertebrate reproductive hormone |                                                          |
| 1000 - 1100| Morning Tea Pfizer Foyer Level 1                            |                                                          |
| 1100 - 1300| Symposium 1: Reproductive endocrinology of fishes: Session 1 | Symposium 2: Environmental light and color modulating endocrine functions: Session 1 |
| 1300 - 1400| Lunch Pfizer Foyer Level 1                                  |                                                          |
| 1400 - 1600| Symposium 3: Genomic and nongenomic signaling and functions of steroid receptors | Symposium 2: Environmental light and color modulating endocrine functions: Session 2 |
| 1600 - 1630| Afternoon Tea Pfizer Foyer Level 1                          |                                                          |
| 1630 - 1730| Symposium 1: Reproductive endocrinology of fishes: Session 2 |                                                          |
| 1730 - 1900|                                                            | AOSCE Council meeting |

## 10 July 2018

<table>
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</table>
| 0900 - 1000| **Plenary Lecture**: Professor Ishwar Parhar  
Social stress and the serotonergic system |                                                          |
| 1000 - 1100| Morning Tea Pfizer Foyer Level 1                            |                                                          |
| 1100 - 1230| Symposium 4: Neurobiology of social behaviour                | Symposium 5: Marine invertebrates: Session 1               |
| 1230 - 1430| Lunch Pfizer foyer level 1 AND                               |                                                          |
| 1330 - 1430| **Poster Session 1**: Jurox Foyer Level 2                    |                                                          |
| 1430 - 1600| Symposium 8: Neuroendocrinology and reproduction              | Symposium 7: Reproductive endocrinology                   |
| 1600 - 1630| Afternoon Tea Pfizer Foyer Level 1                          |                                                          |
| 1630 - 1800| Symposium 6: Bone and mineral endocrinology                  | Symposium 9: Environmental endocrinology: responses to the environment |
| 1800 - 2100| Explore Newtown (Student/Postdoc only dinner) “Atom Thai” 130 King St Newtown  
Sponsored by FACET |                                                          |
### 11 July 2018

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>1800 - 2100</td>
<td>Veterinary Science Conference Centre Seminar Room 115</td>
<td><strong>FREE DAY TO EXPLORE SYDNEY</strong>&lt;br&gt;<strong>SEE CONFERENCE BAG FOR SUGGESTED ACTIVITIES</strong></td>
</tr>
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</table>

### 12 July 2018

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>0930 - 1030</td>
<td>Veterinary Science Conference Centre Webster Lecture Theatre</td>
<td><strong>Plenary Lecture:</strong> Professor Marilyn B. Renfree&lt;br&gt;<em>The endocrinology of marsupial sexual differentiation</em></td>
</tr>
<tr>
<td>1030 - 1100</td>
<td>Webster Lecture Theatre</td>
<td>Morning tea Pfizer Foyer Level 1</td>
</tr>
<tr>
<td>1100 - 1230</td>
<td>Webster Lecture Theatre</td>
<td><strong>Symposium 5:</strong> <em>Marine invertebrates</em>: Session 2&lt;br&gt;<strong>Symposium 10:</strong> Neuroendocrinology</td>
</tr>
<tr>
<td>1230 - 1430</td>
<td>Webster Lecture Theatre</td>
<td>Lunch Pfizer foyer level 1 AND&lt;br&gt;<strong>Poster Session 2:</strong> Jurox Foyer Level 2</td>
</tr>
<tr>
<td>1300 - 1430</td>
<td>Webster Lecture Theatre</td>
<td><strong>Symposium 11:</strong> Stress endocrinology&lt;br&gt;<strong>Symposium 12:</strong> Fish endocrinology</td>
</tr>
<tr>
<td>1600 - 1630</td>
<td>Webster Lecture Theatre</td>
<td>Afternoon tea Pfizer Foyer Level 1</td>
</tr>
<tr>
<td>1630 - 1700</td>
<td>Webster Lecture Theatre</td>
<td><strong>Farewell Comments (Professor Ching-Fong Chang)</strong>&lt;br&gt;<strong>Presentation of student Prizes (Professor Mark Sheridan and Professor Deborah Power Editors in Chief General and Comparative Endocrinology)</strong></td>
</tr>
</tbody>
</table>
# Schedule

## Sunday 8 July 2018

<table>
<thead>
<tr>
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<td>1400 - 1600</td>
<td>Welcome: Professor Katherine Belov, PVCR for Global Exchange, The University of Sydney Plenary Lecture: Professor Ching-Fong Chang <em>The reproduction and related hormones in fish and coral</em></td>
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</tr>
<tr>
<td>1730 - 2100</td>
<td>Welcome BBQ Pfizer foyer level 1</td>
<td></td>
</tr>
</tbody>
</table>

## Monday 9 July 2018

* Indicates student presentation, eligible for the General and Comparative Endocrinology Student Prizes

<table>
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<tr>
<td>1000 - 1100</td>
<td>Morning Tea Pfizer Foyer Level 1</td>
<td></td>
</tr>
<tr>
<td>1100 - 1200</td>
<td>Symposium 1: Reproductive Endocrinology of Fishes: Session 1 Wei Ge <em>Folliculogenesis and Its Control in the Zebrafish – A Genetic Approach</em></td>
<td>Symposium 2: Environmental light and color modulating endocrine functions: Session 1 1100 - 1130 Akiyoshi Takahashi, Daisuke Shimizu, Satoshi Kasagi, Kanta Mizusawa and Tadashi Andoh <em>Fish growth under chromatic light</em></td>
</tr>
<tr>
<td>1130 - 1200</td>
<td>Kanta Mizusawa, Satoshi Kasagi and Akiyoshi Takahashi (Kitasato University, Sagamihara Japan) <em>Endocrinological regulation of light wavelength-specific skin color change in larval zebrafish</em></td>
<td></td>
</tr>
<tr>
<td>1200 - 1230</td>
<td>Kouhei Matsuda, Kazuki Minami, Akiyoshi Hamaguchi, Morio Azuma, Makito Kobayashi, Tomoya Nakamachi and Norifumi Konno <em>Effect of recombinant somatolactin-apha and -beta on melanophore migration in goldfish melanocytes</em></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Speaker(s)</td>
</tr>
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<td>---------</td>
<td>------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1220 - 1240</td>
<td>Hirofumi Ohga, Kojiro Matsumori, Ryuto Kimura, Hajime Kitano, Keishi Sakaguchi, Kohei Ohta, Michiya Matsuyama</td>
<td>Leptin potentially leads to puberty via follicle-stimulating hormone regulation in chub mackerel, a scombroid teleost</td>
</tr>
<tr>
<td>1240 - 1300</td>
<td>Takafumi Amagai*, Daisuke Izumida and Kiyoshi Soyano</td>
<td>The effects of male-released pheromone on the pituitary-gonadal axis and oocyte maturational competence during final oocyte maturation in the honeycomb grouper Epinephelus merra</td>
</tr>
<tr>
<td>1300 - 1400</td>
<td>Lunch Pfizer Foyer Level 1</td>
<td>Symposium 3: Genomic and Nongenomic Signaling and Functions of Steroid Receptors</td>
</tr>
<tr>
<td>1400 - 1600</td>
<td>Symposium 2: Environmental light and color modulating endocrine functions: Session 2</td>
<td>1400 - 1430 Takashi Yoshimura Seasonal changes in color perception and behavior in medaka</td>
</tr>
<tr>
<td>1430 - 1500</td>
<td>Hironori Ando, Md. Shahjahan, Takashi Kitahashi and Atsuhiko Hattori</td>
<td>Melatonin, the hormone of gloom: Implications in the semilunar-synchronized spawning of the grass puffer</td>
</tr>
<tr>
<td>1500 - 1530</td>
<td>Kodai Fukunaga, Fumika Yamashina, Yuki Takeuchi, Hiroki Takekata, and Akihiro Takemura</td>
<td>Interplay between endocrine and clock systems in the lunar-synchronized spawning of tropical groupers</td>
</tr>
<tr>
<td>1530 - 1550</td>
<td>Fadi A. Issa, Katie N. Clements, Matt Chilton, Julia Brown, Yong Zhu</td>
<td>Opposing actions of two steroid receptors and dopaminergic regulation of social aggression and spinal motor circuits in zebrafish (Danio rerio)</td>
</tr>
<tr>
<td>1530 - 1600</td>
<td>Kiyoshi Soyano, Yuji Mushirobira, Mitsuru Niida, Daisuke Izumida, Takarou Hotta and Yuichiro Fujinami</td>
<td>The role of photoperiod and water temperature in the regulation of gonadal development and maturation in the yellowtail</td>
</tr>
</tbody>
</table>

**Note:** The table represents a schedule of presentations, sessions, and symposiums related to various topics in endocrinology and developmental biology, including mechanisms related to body color changes and puberty.
Afternoon Tea Pfizer Foyer Level 1

1600 - 1630

**Symposium 1: Reproductive Endocrinology of Fishes: Session 2**

1630-1650

**Linyan Zhou** and Deshou Wang

Endocrine regulation of fish sex differentiation

1650-1710

**Kun Wu*** and Wei Ge

Disruption of dmrt1 rescues the all-male phenotype of cyp19a1a mutant in the Zebrafish – Evidence for interaction between Dmrt1 and aromatase in directing gonadal differentiation

1710-1730

Xianbo Zhang, **Deshou Wang**

Mutation of foxl2 or cyp19a1a results in female to male sex reversal in XX Nile tilapia

1730 - 1900

AOSCE Council meeting

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**Tuesday 10 July 2018**

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<td></td>
<td>Social stress and the serotonergic system</td>
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<tr>
<td>1000 - 1100</td>
<td>Morning Tea Pfizer Foyer Level 1</td>
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</tbody>
</table>

1100 - 1230

**Symposium 4: Neurobiology of Social Behaviour**

1100 - 1130

**Terence Y Pang**, Annabel K Short, Katie A Fennell, Shlomo Yeshurun, Arina Rawat, Vicky Batchelor, Anthony J Hannan

Transgenerational impacts of paternal stress on offspring behavioural phenotypes and stress-response

1130 - 1150

Sachuriga, Daisuke Yoshida, Naoto Inuma, Tomoya Nakamachi, Norifumi Konno and **Kouhei Matsuda**

Distribution of cholecystokinin (CCK)-like immunoreactivities in the goldfish brain, and effect of intracerebroventricular administration of sulfated CCK octapeptide on psychomotor activity in goldfish

**Symposium 5: Marine Invertebrates: Session 1**

1100 - 1130

**Meaghan K. Smith***, Utpal Bose, Abigail Elizur, Cherie A. Motti, Scott F. Cummins

Brainless Matters. Neural investigations in the crown-of-thorns seastar, Acanthaster planci

1130 - 1150

**Masatoshi Mita**, Keitaro Nakamura, and Hidekazu Katayama

Effect of chimera relaxin-like gonad-stimulating peptides on spawning in ovaries of starfish *Patiria pectinifera*
<table>
<thead>
<tr>
<th>Time</th>
<th>Session 1</th>
<th>Session 2</th>
</tr>
</thead>
</table>
*The involvement of BDNF in maintaining HPG-axis function – relevance to schizophrenia* | 1150 - 1210  
Yan Zhang, Yu-Ying Ho, Yi-Ling Chiu, Jack I-Chen Yao, Shinya Shikina, Ching-Fong Chang  
*Identification and characterization of an Antho-RFamide neuropeptide that induces tentacle contraction in a stony coral Euphyllia ancora* |
| 1210 - 1230| Tomoko Soga, and Ishwar S. Parhar  
*Social isolation and the GnIH neurons in the brain* | 1210 - 1230  
*Production of a sea cucumber recombinant Relaxin-like Gonad-stimulating Peptide (RGP) that induces spawning* |
| 1230 - 1430| Lunch Pfizer foyer level 1 AND  
Poster Session 1: Jurox Foyer Level 2 | Symposium 6: Neuroendocrinology and Reproduction  
1430 - 1500  
Jeremy T. Smith  
*Kisspeptin neurons are central regulators of fertility and metabolism*  
Symposium 7: Reproductive Endocrinology  
1430 - 1450  
Andriyanto, Wisnu Hendra, Nur H. Safitri, Firda Agustin, Natalia F. Lyla, and Wasmen Manalu  
*Administration of Water Extracts of Ocimum sanctum and Curcuma longa prior to laying period to improve serum estrogen and vitellogenin concentration, growth performances and liver functions in poultry by using mojosari duck as a model* |
| 1430 - 1610|  
1500 - 1520  
Takayoshi Ubuka and Ishwar Parhar  
*Transmembrane protease serine 2 and forkhead box A1 as potential bisphenol A responsive genes in the neonatal male rat brain*  
1520 - 1540  
Haimei Tang, Yu Chen, Le Wang, Xiaochun Liu, Haoran Lin  
*New insights into the role of estrogens in sex differentiation and male fertility based on findings in aromatase-deficient zebrafish*  
1540 - 1600  
Satoshi Ogawa, Felix S.K. Thomas, Tomoko Soga and Ishwar Parhar  
*GnIH function as a novel stress regulator*  
1510 - 1530  
Wasmen Manalu, Andriyanto, Firda Agustin, Diki Yulianzah, Kharisma Mardatillah, and Natalia F. Lyla  
*Administration of Jamu ATOKE prior to mating in maternal rats improves serum estradiol and progesterone concentrations during pregnancy and the quality of the born offspring*  
1530 - 1550  
Sangita and Suresh Yenugu  
*The epididymis specific gene, sperm associated antigen 11 (Spag11) as a possible marker for cancer* |
### 1600 - 1630
**Afternoon Tea Pfizer Foyer Level 1**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>1600 - 1630</td>
<td><strong>Symposium 8:</strong> Bone and mineral endocrinology</td>
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<tr>
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<td><strong>1630 - 1700</strong></td>
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<tr>
<td></td>
<td><strong>Deborah M Power.</strong> Ana Patricia Mateus, Rita Costa and Patricia IS Pinto**</td>
</tr>
<tr>
<td></td>
<td><em>Regulation of skin and scale regeneration in marine teleost fish</em></td>
</tr>
<tr>
<td>1630 - 1800</td>
<td><strong>Symposium 9:</strong> Environmental endocrinology: responses to the environment</td>
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<td><strong>1630 - 1700</strong></td>
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<tr>
<td></td>
<td><strong>John Cockrem</strong></td>
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<tr>
<td></td>
<td><em>The hypothalamo-pituitary-adrenal (HPA) axis and endocrine responses of animals to changes in their environment</em></td>
</tr>
<tr>
<td></td>
<td><strong>1700 - 1720</strong></td>
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<tr>
<td></td>
<td><em><em>Awf A. Al-Khan</em>. Judith S. Nimmo, Mourad Tayebi, Stewart D. Ryan, James Simcock, Raboola Tarzi, Charles Kuntz, Eman S. Saad, Michael J. Day, Samantha J. Richardson and Janine A. Danks</em>*</td>
</tr>
<tr>
<td></td>
<td><em>Parathyroid hormone receptor 1 (PTHR1) is a prognostic indicator in osteosarcoma</em></td>
</tr>
<tr>
<td></td>
<td><strong>1720 - 1740</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Janine Danks</strong></td>
</tr>
<tr>
<td></td>
<td><em>Calcium regulation was required for the evolution of the skeleton</em></td>
</tr>
<tr>
<td>1800 - 2100</td>
<td>Explore Newtown (Student/Postdoc only dinner) &quot;Atom Thai&quot; 130 King St Newtown</td>
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<td>Sponsored by <strong>FACET</strong></td>
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### Wednesday 11 July 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1800 - 2100</td>
<td>Conference Dinner “The Refectory”</td>
</tr>
</tbody>
</table>

**FREE DAY TO EXPLORE SYDNEY**

SEE CONFERENCE BAG FOR SUGGESTED ACTIVITIES
**Thursday 12 July 2018**

* Indicates student presentation, eligible for the *General and Comparative Endocrinology* Student Prizes

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<td>Plenary Lecture: Professor Marilyn B. Renfree&lt;br&gt;&lt;br&gt;<em>Indicates student presentation, eligible for the General and Comparative Endocrinology Student Prizes</em>&lt;br&gt;&lt;br&gt;The endocrinology of marsupial sexual differentiation</td>
<td></td>
</tr>
</tbody>
</table>

| 1030 - 1100| Morning tea Pfizer Foyer Level 1 |  |

| 1100 - 1230| Symposium 5: Marine Invertebrates: Session 2<br>Chieh-Jhen Chen*, Shinya Shikina, Ching-Fong Chang<br>*A novel female-specific and sexual reproduction-associated dmrt gene discovered in the stony coral, Euphyllia ancora* | Symposium 10: Neuroendocrinology<br>Seong-sik Yun, Yoo-Na Lee, Arfaxad Reyes-Alcaraz, Jong-Ik Hwang, Jae Young Seong<br>*Neuropeptide spexin and its receptor GALR2: A link between depression and appetite* |
| 1130 - 1150| Shinya Shikina, Yi-Ling Chiu, Ching-Fong Chang<br>*De novo* transcriptome analysis of the gonads in a stony coral Euphyllia ancora (Anthozoa, Cnidaria) to explore the intrinsic mechanisms underlying sexual reproduction of corals | 1130 - 1150<br>Ryo Sakanoue*, Hirofumi Ohga, Fumiko Akase, Hajime Kitano, Keishi Sakaguchi, Kohei Ohta, Michiya Matsuyama<br>*Molecular identification and ligand activity of Kiss1 and Kiss2 core peptides in interspecies of sixteen Scombridae fish* |
| 1150 - 1210| Kyeong Seop Kim, Mi Ae Kim and Young Chang Sohn<br>*Phylogenetic and functional analysis of 5-Hydroxytryptamine receptors in Pacific abalone, Haliotis discus hannai* | 1150 - 1210<br>Hitomi Seike, Yi Jun Zhou, and Shinji Nagata<br>*Effects of RF-amide peptides on the feeding behavior in the two-spotted cricket, Gryllus bimaculatus, a new model insect* |
| 1210 - 1230| Chi Chen*, Yao-Tse Chung, Guan-Chung Wu, Ching-Fong Chang<br>*The physiological adaptation to high sulfide extreme environment in hydrothermal vent crab, Xenograpsus testudinatus* | 1210 - 1230<br>Kazuyoshi Ukena, Kenshiro Shikano, Masaki Kato, Megumi Furumitsu, Eiko Iwakoshi-Ukena<br>*Identification and function of two novel small secretory proteins, neurosecretory protein GL and neurosecretory protein GM, in the chicken hypothalamus* |

| 1230 - 1430| Lunch Pfizer foyer level 1 AND Poster Session 2: Jurox Foyer Level 2 |
| 1300 - 1430|  |  |

<p>| 1430 - 1600| Symposium 11: Stress Endocrinology&lt;br&gt;Edward J. Narayan&lt;br&gt;<em>Assessing chronic stress in rescued wild koalas using non-invasive stress hormone monitoring</em> | Symposium 12: Fish Endocrinology&lt;br&gt;1430 - 1500&lt;br&gt;Guokun Yang, Chaobin Qin, Caiyun Sun, WenSheng Li&lt;br&gt;<em>C1q/TNF-Related protein 9 in the orange-spotted grouper: molecular cloning, identification and its function</em> |
| 1430 - 1500| Hera Maheshwari, and Koekoeh Santoso&lt;br&gt;<em>Corticosterone response of Japanese quails exposed to prolonged heat stress</em> | 1430 - 1500&lt;br&gt; Shotaro Irachi*, Katsuhisa Uchida and Stephen D. McCormick&lt;br&gt;<em>Na+/K+-ATPase response to ion-deficient environment in the gill ionocytes of</em> |</p>
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<td>1520 - 1540</td>
<td>Takashi Bungo, Kouichi Yoshidome and Yoshimitsu Ouchi</td>
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<td>Probing the effects of bisphenol a (BPA) on gonadal differentiation and development using a novel genetic zebrafish model</td>
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<td>Yada Takashi, Miyamoto Kouta and Michihisa Abe</td>
<td>Pedro F S Palma, Bruno Louro, Patrícia I S Pinto, Deborah M Power, Pedro M Guerreiro and <em>Adelino V M Canário</em>.</td>
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Editors in Chief General and Comparative Endocrinology
Plenary Lectures

Sunday 8 July 1600 - 1730

Welcome: Professor Katherine Belov, PVCR for Global Exchange, The University of Sydney

Presidential Plenary Lecture
Professor Ching-Fong Chang
The reproduction and related hormones in fish and coral

Monday 9 July 0900 - 1000

Professor Vance L Trudeau
The granin-derived peptide secretoneurin as a new vertebrate reproductive hormone

Tuesday 10 July 0900 - 1000

Professor Ishwar Parhar
Social stress and the serotonergic system

Thursday 12 July 0930 - 1030

Professor Marilyn B. Renfree
The endocrinology of marsupial sexual differentiation
Presidential Plenary Lecture

Sunday 8 July 1600 - 1730

The Reproduction and Related Hormones in Fish and Coral

Guan-Chung Wu, Shinya Shikina, Ching-Fong Chang
Department of Aquaculture, National Taiwan Ocean University; The Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 20224, Taiwan. b0044@email.ntou.edu.tw.

Abstract
We had long-term comparative investigation on the reproduction and hormones in fish (protandrous black porgy, Acanthopagrus schlegelii) and scleractinian coral (Euphyllia ancora). In fish, the hypothalamic-pituitary-gonadal axis releases GnRH, LH/FSH and sex steroids (estradiol, testosterone, 11-ketotestosterone, 17,20β-dihydroxy-pregn-3-one, 17,20β,21-trihydroxy-4-pregnen-3-one) for the regulation of reproduction, and gamete growth and maturation. We hypothesize that the endocrine system or hormones play an important role as an endogenous factor both in the synchronous mass spawning of corals and fish seasonal reproduction. We comparatively identified the vitellogenesis in coral and fish. Immunoreactive testosterone and estradiol in the free and glucuronidated forms were identified and consistently detected in the coral polys throughout the year. Peak levels of free estradiol and glucuronidated estradiol were obtained in the coral tissue just prior to spawning. The presence of specific aromatase activity was demonstrated in coral tissue according to the tritiated-androstenedione as a substrate and tritiated H2O formation, with a significantly high aromatase activity detected during the spawning period. Higher concentrations of free estradiol than glucuronidated estradiol were detected in the coral tissue throughout the year. In contrast, higher levels of glucuronidated estradiol than free estradiol and glucuronidated testosterone were found in seawater during coral mass spawning. Furthermore, immunoreactivity and biological activity of immunoreactive gonadotropin-releasing hormone (irGnRH) were detected and peak levels of irGnRH were observed in coral tissue during spawning. Coral extracts and mammalian (m)GnRH agonist had a similar dose dependent effect on the release of LH in the black porgy fish pituitary cells; while mGnRH receptor antagonist blocked the stimulatory effects of coral extracts. Our recent studies demonstrated that a steroidogenic enzyme 17β-hydroxysteroid dehydrogenase type 14 transcript and protein were detected in the coral tissues. Some hormones are possibly conserved in the regulation of seasonable reproduction of fish and coral.
Plenary Lecture

Monday 9 July 0900 - 1000

The granin-derived peptide secretoneurin as a new vertebrate reproductive hormone

Vance L. Trudeau, Kim M. Mitchell, Wo Su Zhang, Brendan Kelly, Paige Benson, Bin Bin Tao, Chunyu Lu, Wei Hu

a Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5. trudeauv@uottawa.ca
b State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China.

Abstract
At the hypothalamo-pituitary interface, multiple neurotransmitters and neuropeptides interact to control luteinizing hormone (LH) and follicle stimulating hormone (FSH) release from gonadotrophs. The LH surge is essential for fertility as it triggers ovulation in females and sperm release in males. While it is well-established that gonadotropin-releasing hormone (GnRH) and/or kisspeptin are required for pulsatile and surge release of LH in mammalian species, their essentiality is challenged by studies showing knockouts (KO) in zebrafish and medaka do not block reproduction. In goldfish, we have discovered that secretoneurin a (SNa), a neuropeptide derived from secretogranin-IIa (SgIIa) processing, stimulates LH release in vivo and from dispersed pituitary cells in vitro. SN does not bind to the human GnRH receptor and can enhance LH release when applied directly to mouse LbetaT2 cells. KO of SgIIa and SgIIb genes using TALENs in zebrafish indicates that these genes are required for optimal reproduction. Rates of oviposition for double SgII-KO females are 6% and 11% when crossed with double SgII-KO and wild-type (WT) males, compared to 62% in virgin WT pairings. Comprehensive video analysis demonstrates that SgII-KO reduces all stereotypical courtship behaviours. Severe reductions in the expression of GnRH3 in the hypothalamus and LH in the pituitary suggest that SgII-derived peptides drive the GnRH-LH control system. Spawning success was partially rescued in double SgII-KOs following one injection (i.p.) of synthetic SNa in which it increased from 11% to 30% although embryo survival rate was lower than saline-injected WT pairings. Injection of human chorionic gonadotropin increased double SgII-KO spawning success to 41%, thus comparing favourably to 47% in saline-injected WT controls. These data support our proposal that SNa is a key reproductive hormone. The high conservation of SN from lamprey to human suggests a broader importance of this emerging peptide family. Supported by NSERC, uOttawa-IHB collaborative grants.
Plenary Lecture

Tuesday 10 July 0900 - 1000

Social Stress and the Serotonergic System

Ishwar Parhar
Brain Research Institute, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia,

Abstract
Social stress caused by social defeat leads to psychological disorders such as depression as well as other health problems including sexual dysfunction, which is closely associated with altered serotonergic (5-HT) system in the brain. However, the aetiology of 5-HT related disorders remains unknown. We have been studying the molecular mechanisms of 5-HT regulation by social stress using tilapia fish as a social defeat model. In socially defeated male tilapia, the ratio of 5-HT metabolite to 5-HT was significantly increased, while monoamine oxidase A (MAO-A), an enzyme that degrades 5-HT was significantly decreased. Transcription factors regulating MAO-A and 5-HT1A receptor including transcription factors RING finger protein 38 (RNF38) and DEAF 1 respectively were significantly low in socially stress animals. These studies suggest that the 5-HT system is affected by social stress. In this talk, I will summarize the neuro-molecular pathways which include Habenula kisspeptin, 5-HT1A receptors and transcription factors involved in the control of 5-HT system under socially stressed conditions.
Plenary Lecture

Thursday 12 July 0930 - 1030

The endocrinology of marsupial sexual differentiation.

Marilyn B Renfree
School of BioSciences, The University of Melbourne, Melbourne, Victoria, Australia
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Abstract
Marsupials are only 6% of living mammals but have many characteristics that can inform us about the endocrine control of reproduction and development. A striking characteristic is that marsupials give birth to highly altricial young after a relatively short gestation period supported by the chorio-vitelline placenta. They complete much of their development within the pouch, dependent on a long and highly sophisticated lactation during which the composition of the milk changes dynamically to coordinate the specific growth requirement of the developing young. We have focussed on the control of sexual differentiation, which all takes place post-natally, and discovered some unexpected findings that have caused re-evaluation of accepted dogma. We have described a new alternate androgen synthesis pathway in the developing young that is responsible for virilisation of the prostate and phallus at specific windows of sensitivity. We also overturned the powerful Jost paradigm that sexual differentiation simply depended on hormones secreted by the testis when we demonstrated a number of hormone-independent sexual dimorphisms before the testicular differentiation. We now know that there are many other hormone-independent sexual dimorphisms, not only in mammals but also in birds, and that the female pathway is not the “DEFAULT” pathway. In addition, because of the post-natal gonadal development, we have been able to achieve testicular sex reversal after treatment with oestrogen in vivo and in vitro, and furthermore can induce sex reversal and hypospadias of the penis with oestrogen treatment, and sex reversal of the clitoris with androgen treatment in vivo. Exogenous steroids induce changes in the phallus, not only morphological ones but also in the expression of the controlling coding and non-coding genes. Marsupials may only be 6% of living mammals, but their unique post-natal sexual differentiation provides novel opportunities for further understanding the evolution and control of sexual differentiation of the internal and external genitalia.

Acknowledgements: Supported by the Australian Research Council and the Australian National Health and Medical Research Council.
Monday 9 July 2018

Symposium 1: Reproductive Endocrinology of Fishes

Chairs: Vance L Trudeau and Wei Ge

Session 1 1100 - 1300
1100 - 1140
Wei Ge
Folliculogenesis and Its Control in the Zebrafish – A Genetic Approach
State of the Art lecture 40 minutes

1140 - 1200
Shinji Kanda, Daichi Kayo, Chika Fujimori, Akiko Takahashi, Yoshitaka Oka
Role of GnRH in the gonadotropin release: changes during vertebrate evolution
15 minutes + 5 minutes questions

1200 - 1220
Xingyong Liu*, Hesheng Xiao, Xianbo Zhang, Minghui Li and Deshou Wang
Loss of Amh signaling causes the dysregulation of folliculogenesis in XX Nile tilapia.
15 minutes + 5 minutes questions

1220 - 1240
Hirofumi Ohga, Kojiro Matsumori, Ryuto Kimura, Hajime Kitano, Keishi Sakaguchi, Kohei Ohta, Michiya Matsuyama
Leptin potentially leads to puberty via follicle-stimulating hormone regulation in chub mackerel, a scombroid teleost
15 minutes + 5 minutes questions

1240 - 1300
Takafumi Amagai*, Daisuke Izumida and Kiyoshi Soyano
The effects of male-released pheromone on the pituitary-gonadal axis and oocyte maturational competence during final oocyte maturation in the honeycomb grouper Epinephelus merra.
15 minutes + 5 minutes questions

Asterisk (*) indicate student presentation
State of the Art Lecture

Folliculogenesis and Its Control in the Zebrafish – A Genetic Approach

Wei GE

Centre of Reproduction, Development and Aging (CRDA), Faculty of Health Sciences, University of Macau, Taipa, Macau, China. weige@umac.mo or gezebrafish@gmail.com

Abstract

Ovarian folliculogenesis is one of the most dynamic physiological and developmental processes in vertebrates. Despite numerous studies on this issue in mammals, the molecular mechanisms that control follicle development, especially the early phase of folliculogenesis, still remain poorly understood. Gonadotropins are primary endocrine hormones that control ovarian development and function. In addition, various local paracrine and autocrine factors in the ovary also play important roles in this process. Although the endocrine and paracrine controls of folliculogenesis have been studied in fish, there has been a lack of genetic data on their functional importance, mostly due to unavailability of the gene knockout approach in fish models.

Using zebrafish as the model and the emerging genome editing technologies, TALEN and CRISPR/Cas9, we have generated a series of zebrafish knockout mutants for both endocrine hormones and local ovarian paracrine factors and analyzed their impacts on folliculogenesis. We first studied the importance of FSH and LH in follicle development by disrupting fshb, lhb, fshr and lhcgr genes. FSH-deficient zebrafish (fshb−/-) were surprisingly fertile in females; however, the puberty onset or start of vitellogenic growth was significantly delayed. In contrast, LH-deficient zebrafish (lhb−/-) showed normal gonadal growth, but the females failed to spawn and were therefore infertile. In contrast to fshb deficiency, the FSH receptor (fshr)-deficient females showed a complete failure of follicle activation; however, the deletion of lhcgr gene caused no obvious phenotypes. In addition to gonadotropins, we have also investigated potential involvement of local paracrine factors in controlling folliculogenesis. Our data showed that follicle development in the zebrafish involves both external endocrine hormones and internal paracrine factors in the ovary.

ACKNOWLEDGEMENT

This study was supported by grants from the University of Macau (MYRG2014-00062-FHS, MYRG2015-00227-FHS, and CPG2014-00014-FHS) and The Macau Fund for Development of Science and Technology (FDCT114/2013/A3 and FDCT/089/2014/A2).
Role of GnRH in the gonadotropin release: changes during vertebrate evolution

Shinji Kanda, Daichi Kayo, Chika Fujimori, Akiko Takahashi, Yoshitaka Oka
Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo 113-0033
shinji@bs.s.u-tokyo.ac.jp

Abstract
Since the discovery of GnRH as the regulator of gonadotropin release in mammals, quite a few number of studies in various vertebrate species have indicated that exogenous GnRH potently induces the release of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). However, not a few unanswered questions still remain as for the role of GnRH in folliculogenesis or ovulation in non-mammalian species.

We have been studying the mechanisms of central regulation of reproduction from the evolutionary view points by using a teleost, medaka. Interestingly, teleosts possess a unique morphological characteristic that their LH and FSH are secreted from distinct cells, which is experimentally proven to have arisen in the early actinopterygian lineage. Taking advantage of this characteristic, a previous Ca$^{2+}$ imaging study have given evidence that GnRH induces the release of both FSH and LH (Karigo et al., 2014). However, more recently, GnRH knockout (KO) medaka showed a phenotype similar to LH-KO, which shows a failure of ovulation, but different from FSH-KO, which shows a failure in folliculogenesis (Takahashi et al., 2016). On the other hand, there is a consensus that GnRH in mammals plays an essential role in the generation of LH pulse, which facilitates folliculogenesis. Given that kisspeptin plays an important role in reproduction of mammals but not of teleosts and birds, it can be hypothesized that mammals uniquely acquired the pulsatile mode of LH release, which is dependent on the GnRH pulse generator including kisspeptin neurons. Here, we found the importance of FSH, which may have been somehow neglected in studies of mammalian reproduction, and started to analyze the mechanisms of estrogenic regulation of FSH release. From the analysis of KO medaka lines of nuclear estrogen receptors, we found evidence to suggest that the negative feedback action of estrogen on FSH expression is mediated by esr2a.
Loss of Amh signaling causes the dysregulation of folliculogenesis in XX Nile tilapia

Xingyong Liu, Hesheng Xiao, Xianbo Zhang, Minghui Li* and Deshou Wang*

Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education), School of Life Sciences, Southwest University, 400715, Chongqing, P.R. China. liuxy2017@email.swu.edu.cn; wdeshou@swu.edu.cn

Abstract
Anti-Müllerian hormone (AMH) and its type II receptor AMHRII are reported to be expressed in the granulosa cells and plays important roles in folliculogenesis in mammals. However, in non-mammalian species, expression and mutation of amh were only reported in zebrafish, and mutation of amhrII was only reported in medaka. Here we showed Amh expression in granulosa cells of the previtellogenic follicles but not in the vitellogenic follicles in the ovary of Nile tilapia by immunohistochemistry. Amh expression was also detected in the neuron cells of the midbrain and the neurohypophysis. AmhrII expression was detected in leydig cells, oogonia and phase I oocytes in the ovary, and its expression was also detected in glial cells of the midbrain and lactotropes of the adenohypophysis. amh and amhrII knockout lines were established in XX females by CRISPR/Cas9. Both amh and amhrII homozygous mutants were infertile. Most of the follicles were arrested at the primary growth stage, with a few follicles at vitellogenic stage, and developed mild hypertrophic ovaries, in the amh mutants. In contrast, all follicles were completely arrested at the primary growth stage in the amhrII mutants. The amhrII mutants developed severe hypertrophic ovaries due to over proliferation of follicles. Gene expression analysis revealed that fsh and lh expressions in the pituitary and fshr and lhr expressions in the gonads were significantly decreased in both amh and amhrII mutants. Serum E2 concentration was down-regulated but 11-KT not affected in amh mutants; while both serum E2 and 11-KT concentrations were significantly down-regulated in amhrII mutants. In all, our results demonstrated that both amh and amhrII play essential roles in fish folliculogenesis. Loss of amhrII resulted in more serious defects compared with loss of amh in females, indicating that amhrII might have other ligands besides amh in tilapia.
Leptin potentially leads to puberty via follicle-stimulating hormone regulation in chub mackerel, a scombroid teleost

Hirofumi Ohga\textsuperscript{a}, Kojiro Matsumori\textsuperscript{b}, Ryuto Kimura\textsuperscript{b}, Hajime Kitano\textsuperscript{a}, Keishi Sakaguchi\textsuperscript{a}, Kohei Ohta\textsuperscript{b}, Michiya Matsuyama\textsuperscript{b}

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Abstract

Nutritional status and reproduction are closely related. In mammals, leptin is an adipocyte-derived hormone and is secreted in proportion to fat stores, and acts within the brain and pituitary to signal adequate energy reserves and satiety required for puberty onset. This suggest that leptin signal is important and essential for successful of pubertal onset. We already identified two leptin genes (lepa and lepb) and a single leptin receptor (lepr) in chub mackerel (cm); showed that lepr mRNA especially strongly expressed in the pituitary grand and hepatic lepa expression gradually increased according to pubertal process. In this study, we produced recombinant leptin in chub mackerel and investigated the effect on gonadotropin secretion in pubertal male fish. Recombinant chub mackerel leptin-a (cm-rLepA) was produced by Escherichia Cori (E.cori) expression system. The cm-rLepA was biologically active in promoting STAT3-Luc activation in CHO cells transfected with the cmLepR. Primary pituitary cells were collected from pre-puberty and overt-puberty male fish, respectively. After 4 days of culture, cells were exposed for 1, 2, and 4 h to 1 mM of cm-rLepA or synthetic cm gonadotropin-releasing hormone 1 (Gnrh1). Cm-rLepA significantly stimulated follicle-stimulating hormone (Fsh) secretion in 1 and 2 h culture in pre-pubertal pituitary cells. Gnrh1 showed no effect in Fsh secretion. In contrast, only Gnrh1 significantly stimulated the secretion of luteinizing-hormone (Lh). Both leptin and Gnrh1 showed no effects in gonadotropin secretion in overt-puberty pituitary cells. These results indicate that leptin stimulates Fsh secretion in the juvenile period just before puberty. We speculate that leptin may be the factor that brings to gate of puberty in not only mammals but also teleost fish species.
The effects of male-released pheromone on the pituitary-gonadal axis and oocyte maturational competence during final oocyte maturation in the honeycomb grouper Epinephelus merra

Takafumi Amagai\textsuperscript{a}, Daisuke Izumida\textsuperscript{b} and Kiyoshi Soyano\textsuperscript{a}

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\textsuperscript{b}Akkeshi Station, Hokkaido National Fisheries Research Institute, Japan Fisheries Research and Education Agency, 2-1 Chikushiko, Akkeshi 088-1188, Hokkaido, Japan. izumida@affrc.go.jp

Abstract
In the honeycomb grouper that is a lunar-related spawner, the final oocyte maturation (FOM) is regulated by male pheromonal cue. In order to understand the physiological roles of male pheromone on the pituitary-gonadal axis during FOM, we investigated the changes of the gonadotropins (GtHs) gene expression in pituitary and plasma sex steroids (E2 and T) levels. In addition, the response to hormones in oocyte was investigated by in vitro culture using male-pheromone-exposed oocyte for understanding the mechanisms of oocyte maturational competence (OMC) after pheromonal stimuli.

Females and males were separately reared until start of the following experiments. Exp.1) At two days after full moon, females containing fully-grown oocytes (not yet FOM phase) were divided into three experimental groups as follows. 1) HCG (100 IU/kg BW) injection group. 2) male rearing water (MW) exposure group. 3) control group (females alone without treatment). Sampling of experimental groups was conducted every 24 h after start of experiment. Exp.2) Ovaries of MW and control groups were cultured in L-15 medium with FOM inducers (HCG and maturation inducing steroid, DHP) and/or cycloheximide (protein synthesis inhibitor). The developmental stage of cultured oocyte was observed after incubation.

Exp.1) FOM was observed in HCG and MW groups at 48 and 72 h, respectively. The male-released pheromone promoted the pituitary GtHs expression and E2 and T synthesis. Moreover, both steroids levels were increased by HCG injection. Exp.2) The cultured oocyte of control group did not complete FOM under all culture conditions. In contrast, the oocyte of MW group completed FOM by HCG and DHP treatment. However, FOM was not induced in the oocyte of MW groupe with cycloheximide. These results suggest that the pheromone contained in male rearing water plays a role on accelerating the hormones synthesis in pituitary-gonadal axis, and acquiring the OMC via acquisition of DHP sensitivity.
Monday 9 July 2018

Symposium 2: Environmental light and color modulating endocrine functions

Chairs: Akiyoshi Takahashi and Arimune Munakata

Session 1: 1100 - 1300

1100 - 1130
Akiyoshi Takahashi, Daisuke Shimizu, Satoshi Kasagi, Kanta Mizusawa and Tadashi Andoh
*Fish growth under chromatic light*
25 minutes + 5 minutes questions

1130 - 1200
Kanta Mizusawa, Satoshi Kasagi and Akiyoshi Takahashi
*Endocrinological regulation of light wavelength-specific skin color change in larval zebrafish*
25 minutes + 5 minutes questions

1200 - 1230
Kouhei Matsuda, Kazuki Minami, Akiyoshi Hamaguchi, Morio Azuma, Makito Kobayashi, Tomoya Nakamachi and Norifumi Konno
*Effect of recombinant somatolactin-pha and -beta on melanophore migration in goldfish melanocytes.*
25 minutes + 5 minutes questions

1230 - 1300
Arimune Munakata
*Mechanisms and roles of body color changes during smoltification in Pacific salmon.*
25 minutes + 5 minutes questions
Fish growth under chromatic light

Akiyoshi Takahashi\textsuperscript{a}, Daisuke Shimizu\textsuperscript{b}, Satoshi Kasagi\textsuperscript{c}, Kanta Mizusawa\textsuperscript{d} and Tadashi Andoh\textsuperscript{e}

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\textsuperscript{b}Miyako Laboratory, Tohoku National Fisheries Research Institute, Japan Fisheries and Education Agency, Miyako, Iwate 027-0097, Japan. dshimizu@affrc.go.jp
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Abstract
The growth and body colors of fishes are affected by their photic environments. Melanin-concentrating hormone (MCH) produced in the hypothalamus plays various roles in both the brain and the peripheral tissues. The major biological functions of MCH are the stimulation of appetite (central function) and aggregation of melanosomes in melanophores (peripheral function). The production and secretion of hormones fluctuate in response to changes in background color; thus, we hypothesized that fish under a white background with high MCH levels would grow rapidly. A flatfish species barfin flounder \textit{Verasper moseri} under a white background grew faster than those under a black background. Moreover, when we examined the effects of chromatic light, growth was stimulated by blue, green, and red LED light. Among them, green light elicited the greatest effects, followed by blue light. Growth facilitation under LED lights was also observed in spotted halibut \textit{V. variegatus} and Japanese flounder \textit{Paralichthys olivaceus}, while red and white light had no effect. Based on the results obtained in three flounders, we examined the effects of green light on the growth and endocrine systems at various water temperatures in \textit{V. variegatus}. The growth of the fish under green light was facilitated at four different water temperatures examined, 12, 15, 18, and 21°C, over approximately 4 weeks of rearing. Of the genes encoding several hypothalamic hormones, expression levels of \textit{mch1} were enhanced by green light under the four water temperatures. Increased expression was also observed for \textit{mch2}, \textit{neuropeptide Y}, \textit{pituitary adenylate cycle-activating polypeptide}, and \textit{proopiomelanocortin-c} under certain temperature conditions. No difference was observed in the expression of pituitary hormone genes, including those of growth hormone, and plasma levels of insulin family peptides. The results suggest that MCH is a key hormone, whose production is stimulated by green light; in turn, MCH augments food intake.
Endocrinological regulation of light wavelength-specific skin color change in larval zebrafish

Kanta Mizusawa\textsuperscript{a}, Satoshi Kasagi\textsuperscript{b} and Akiyoshi Takahashi\textsuperscript{c}

\textsuperscript{a,b,c}School of Marine Biosciences, Kitasato University, Sagamihara, Kanagawa 252-0373, Japan.
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Abstract

Teleosts can change their color in response to the light environment. This color change is regulated by the endocrine and sympathetic nervous systems. In adult fish, melanin-concentrating hormone (MCH) and noradrenaline induce paleness, whereas melanocyte-stimulating hormone (α-MSH), derived from proopiomelanocortin (POMC), induces a dark skin color. In this study, we reveal that zebrafish larvae exhibit light wavelength-specific skin color changes. We also investigated the endocrinological mechanisms regulating these color changes. After irradiation of LED light from 1 to 4 days post fertilization (dpf), melanosomes in the skin melanophores were more dispersed under short wavelength light (λ\textsubscript{max} \leq 400 nm) than under fluorescent light (FL). Conversely, melanosomes were more aggregated under mid–long wavelength light (λ\textsubscript{max} \geq 476 nm) than under FL. We also found that the expression of a gene for MCH was down-regulated by 400 nm light but up-regulated by 530 nm light. Meanwhile, a gene for POMC was up-regulated under 400 nm light. Melanosomes in 4 dpf larvae exposed to a black background under FL aggregated when immersing the larvae in MCH solution. However, melanosomes did not disperse in larvae exposed to a white background under FL when immersing the larvae in α-MSH solution. An α\textsubscript{2}-adrenergic receptor antagonist, yohimbine, did not induce melanosome dispersion in larvae exposed to a white background. These results suggest that MCH plays a key role in the light wavelength-dependent response of melanophores, flexibly mediating the transmission of light wavelength information between photoreceptors and melanophores. Short wavelength light-dependent suppression of MCH expression, inducing melanosome dispersion, may contribute to protecting the internal organs of organisms against ultraviolet light. On the contrary, mid–long wavelength light-dependent stimulation of MCH expression, inducing melanosome aggregation, may contribute to the camouflage when changing the skin color according to the intensity of visible light.
Involvement of somatolactin (SL)-α and SL-β in the regulation of body pigmentation in goldfish

Kouhei Matsuda\textsuperscript{a,b}, Kazuki Minami\textsuperscript{a}, Akiyoshi Hamaguchi\textsuperscript{a}, Morio Azuma\textsuperscript{c}, Makito Kobayashi\textsuperscript{d}, Tomoya Nakamachi\textsuperscript{a} and Norifumi Konno\textsuperscript{a}

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Abstract
Somatolactin (SL) is a pituitary hormone that belongs to the growth hormone/prolactin family of adenohypophysial hormones. SL is present only in bony fish, and two cDNAs encoding distinct SL subtypes, SL-α and SL-β, have been characterized in the pars intermedia of bony fish. Functional studies have suggested that SL is involved in the regulation of steroidogenesis, water-mineral balance and lipid metabolism. However, there is little information about the effect of SL-α and SL-β on body pigmentation of fish. Therefore, we examined the effect of background color on SL-α and SL-β release and expression of their mRNAs in dispersed and cultured pituitary cells from goldfish. Goldfish were kept for 1 month in a tank with black or white background color. They were then transferred in a tank with opposite background color, and kept for 1 month again. SL-α mRNA expression levels increased in cultured pituitary cells prepared from goldfish transferred from the white to the black background, and SL-α-like immunoreactivity of cultured medium also increased in cultured pituitary cells prepared from goldfish under same condition. On the other hand, SL-β mRNA expression levels increased in cultured pituitary cells prepared from goldfish transferred from the black to the white background, and SL-β-like immunoreactivity of cultured medium also increased in cultured pituitary cells prepared from goldfish under same condition. We prepared recombinant goldfish SL-α and SL-β, and we also investigated the effect of recombinant SL-α and SL-β on concentration and dispersion of melanin granules in melanophores of goldfish scales. Melanin granules dispersed in treatment with recombinant SL-α, and melanin granules concentrated in treatment with recombinant SL-β. These data strongly suggested that SL is involved in the regulation of body pigmentation in goldfish.
Mechanisms and roles of body color changes during smoltification in Pacific salmon

Arimune Munakata

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Abstract
Masu salmon (*Oncorhynchus masou*), a Pacific salmon (*genus Oncorhynchus*) drastically change their body color especially during parr to smolt transformation (smoltification). Before this period, masu juveniles regularly show dark brown skin with black parr marks and spots. Such body color is seemed to be so called “camouflage color” against potential predators such as mammalian and avian species while the juveniles live in upper and mid river reaches where the rocks and fallen trees are located on the bottom. Our study demonstrated that masu juveniles changed body color into brighter one within 30 minutes when they were transferred into an aquarium where white stones are placed, suggesting that they can modulate their body color depending on background color to minimize predation risks. Injection of Melanin-concentrating hormone (MCH) alone can induce such body color change, further suggesting that body color changes occur via endocrine control systems. During the river life phase, masu juveniles frequently compete for suitable feeding area and then they regularly differentiate into dominants (resident parr) that will live continuously in rivers and subordinates (smolts) that will undergo smoltification and downstream migratory behavior. During this period, majority of smolts show silvery skin with black dorsal fin tip. Such body color is seemed to be camouflage color against predators while juveniles live in the sea. Injection of thyroxine (T4), one of thyroid hormones can induce such body color change through stimulating accumulation of guanine on the skin. Thus, masu and other Pacific salmon juveniles change and modulate their body color in relation to their life stages and surrounding environments using some endocrine factors. In this talk, I would like to introduce detailed mechanisms and roles of body colors in masu and other Pacific salmons.
Monday 9 July 2018

Symposium 3: Genomic and Nongenomic Signaling and Functions of Steroid Receptors

Chairs: Yong Zhu and Yun-Bo Shi

1400 - 1600

1400 - 1430
Yun-Bo Shi
Epigenetic modifications in the regulation of intestinal stem cell development by thyroid hormone receptor.
State of the Art talk 40 minutes

1430 - 1450
Chengdong Wang, Weili Shi and Hui Zhao
Bridging the ER stress and nuclear receptor: Heat shock 70kDa protein 5 (Hspa5) regulates retinoic acid signaling and is essential for pronephros formation.
15 minutes + 5 minutes questions

1450 – 1510
Samantha J. Richardson, Vydanathan Ravi and Byrappa Venkatesh
Duplication of the transthyretin-like protein gene to the transthyretin gene could have been part of a whole genome duplication.
15 minutes + 5 minutes questions

1510 - 1530
Fadi A. Issa, Katie N. Clements, Matt Chilton, Julia Brown, Yong Zhu
Opposing actions of two steroid receptors and dopaminergic regulation of social aggression and spinal motor circuits in zebrafish (Danio rerio).
15 minutes + 5 minutes questions

1530 - 1550
Xin-Jun Wu and Yong Zhu
Molecular mechanisms of progestin receptor in the regulation of metalloproteinases and ovulation.
15 minutes + 5 minutes questions
State of the Art Lecture

Epigenetic modifications in the regulation of intestinal stem cell development by thyroid hormone receptor
Yun-Bo Shi

Section on Molecular Morphogenesis, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bldg. 18T, Rm. 106, Bethesda, MD 20892, USA. shi@helix.nih.gov

Abstract
Adult organ-specific stem cells are essential for organ homeostasis and tissue repair and regeneration, but the underlying mechanism for their development is unclear. Intestinal remodeling during frog metamorphosis offers a unique opportunity to study the formation of such stem cells during vertebrate development. During the transition from an herbivorous tadpole to a carnivorous frog, the intestine is completely remodeled with the larval epithelial cells undergo apoptosis and are replaced by adult epithelial cells formed de novo. The entire metamorphic process is under the control of thyroid hormone (T3). We have shown that adult epithelial stem cells are induced by T3 through dedifferentiation of some larval epithelial cells. T3 exerts its metamorphic effects through T3 receptors (TRs). TRs recruit, in a T3-dependent manner, cofactor complexes for chromatin remodeling/histone modifications. We have demonstrated that the expression of two histone methyltransferases, Dot1L and PRMT1, are activated by T3 during intestinal remodeling. Our studies further suggest that both are recruited by TR during metamorphosis to function as TR coactivators to promote gene regulation and intestinal stem cell formation and/or proliferation through histone methylation.
**Bridging the ER stress and nuclear receptor: Heat shock 70kDa protein 5 (Hspa5) regulates retinoic acid signaling and is essential for pronephros formation**

Chengdong Wang, Weili Shi and Hui Zhao*

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**Abstract**

Endoplasmic reticulum (ER) is the organelle where membrane-bound and secreted proteins are synthesized. Overload of unfolded or misfolded proteins in the ER will lead to a stress condition called ER stress resulting in disruption of ER functions. In response to ER stress, cells activate the unfolded protein response (UPR) to maintain homeostasis of the ER by attenuating protein translation, promoting protein folding and secretion and protein degradation, in part, through activation of the transcription factor XBP1, which further activate the heat shock 70kDa protein 5 (Hspa5). The Hspa5 also known as the binding immunoglobulin protein (Bip) or glucose regulated protein 78 (Grp78), belongs to the heat shock protein 70kDa family. As a multifunctional protein, it participates in protein folding, calcium homeostasis and serves as an essential regulator of the ER stress.

In this study, we used morpholino antisense oligonucleotides (MO) to knockdown Hspa5 activity in *Xenopus* embryos. In Hspa5 morphants, pronephros formation was strongly inhibited with the reduction of pronephric marker genes *lhx1*, *pax2* and *atp1b1*. Pronephros tissue is induced in vitro by treating animal caps with all-trans retinoic acid (atRA) and activin. Depletion of Hspa5 in animal caps, however, blocked the induction of pronephros as well as reduced the expression of RA-responsive genes, suggesting that knockdown of Hspa5 attenuated RA signaling. Knockdown of Hspa5 in animal caps resulted in decreased expression of *lhx1*, a transcription factor directly regulated by RA signaling and essential for pronephros specification. These results suggest that the RA-*lhx1* signaling cascade is involved in Hspa5MO induced pronephros malformation. We also found that the RA receptor, RARα, a nuclear receptor, was reduced in HSPA-depleted cells. The underlying mechanism is still under investigation. Taken together, our study indicates that Hspa5, a key regulator of the unfolded protein response, plays an essential role in pronephros formation, which is mediated in part through RA signaling during early embryonic development.
Duplication of the transthyretin-like protein gene to the transthyretin gene could have been part of a whole genome duplication.

Samantha J. Richardson¹, Vyedianathan Ravi² and Byrappa Venkatesh²
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Abstract
Transthyretin (TTR) is a homotetrameric protein involved in the binding and distribution of thyroid hormones in the blood of all vertebrates and in the cerebrospinal fluid of reptiles, birds and mammals. TTR also binds up to two molecules of retinol/retinol-binding protein and is involved in the distribution of retinol. The TTR gene is only found in vertebrates and is believed to have arisen as a duplication of the TTR-like protein (TLP) gene. TLP is an enzyme involved in uric acid oxidation, and does not bind thyroid hormones or retinol-binding protein. We investigated if the gene duplication of TLP to TTR was an isolated gene duplication event or if it was part of a whole genome duplication event. Our synteny analyses suggested that that the gene duplication of TLP to TTR was part of a whole genome duplication.
Steroidal and dopaminergic regulation of social behavior and spinal motor circuits in zebrafish (*Danio rerio*)

Fadi A. Issa, Katie N. Clements, Matt Chilton, Julia Brown, Yong Zhu
East Carolina University, Greenville, NC, USA. Issaf14@ecu.edu

Abstract
Adaptive social and motor behavior is under tight hormonal and neuronal control. However, our understanding of the interplay between hormonal and neuromodulatory pathways and their effects on social activity is not fully understood. Here, we investigated the effects of nuclear progestin (Pgr) and nuclear androgen receptor (Ar) knockouts on the social behavior, dopaminergic signaling and regulation of spinal motor circuits of adult male zebrafish. We show that the two receptors play opposing roles in regulating aggressive activity, territorial displays and stability of social dominance. Brain gene expression analysis of Pgr KO fish show significant increase of key proteins that control dopaminergic signaling. We probed the effects of social behavior on dopaminergic regulation of motor activity focusing specifically on the activation patterns of the escape and swim motor circuits in socially dominant and subordinate wild type fish. Our results show social-status dependent shift in the activation pattern of the two competing neural circuits. Subordinates favor escape over swim while dominants favor swimming over escape. We investigated the role of dopamine in regulating these differences by augmenting dopamine levels. Consequently, differences in escape behavior between the two social phenotypes were diminished, suggesting a social status-dependent regulation of dopamine. Further analysis of gene expression in brains reveal significant upregulation of the dopamine transporter (dat) in dominants and downregulation of the dopamine receptor 1b (drd1b) in subordinates. Pharmacological manipulation of drd1 and drd3 receptors can shift the activation pattern of the motor circuits in a social status-dependent manner mediated indirectly via activation GABAergic and glycineric inputs. Taken together, these results implicate Pgr and Ar in regulating social behavior and that social status can shift the activation of competing neural circuits mediated, in part, through the balance of dopamine supply regulated by dat and interpretation of dopamine by differences in drd1 and drd3 expression.
**Pgrmc1 Knockout Impairs Oocyte Maturation via Reduced Expression of mPRα in Female Zebrafish**

Xin-Jun Wu\textsuperscript{a} and Yong Zhu\textsuperscript{a}
Department of Biology, East Carolina University, Greenville, NC, USA. zhuy@ecu.edu

**Abstract**

Recent investigations suggest progestin receptor membrane component 1 (Pgrmc1) transports and associates with a wide range of molecules such as heme, cytochromes P450, progestin, membrane progestin receptor, G-protein coupled receptor, and insulin receptor. It is difficult to discriminate true functions of Pgrmc1 from functions of its associated molecules using biochemical and pharmacological approaches. To determine physiologic function(s) of Pgrmc1, we generated global knockouts for \textit{pgrmc1} (\textit{pgrmc1}\textsuperscript{−/−}) in zebrafish. We found a reduction in both spawning frequency and the number of embryos in female mutants. We also observed reduced sensitivity of fully-grown immature oocytes to progestin and reduced number of oocytes undergoing maturation both \textit{in vivo} and \textit{in vitro} in \textit{pgrmc1}\textsuperscript{−/−}. This reduced sensitivity to progestin corresponds well with significant reduced expression of membrane progestin receptor α (mPRα), a receptor mainly responsible for oocyte maturation and meiosis resumption in fish. We have provided \textit{in vivo} and \textit{in vitro} evidence, and a plausible molecular mechanism for the physiological functions of Pgrmc1 in oocyte maturation and fertility, likely via regulation of mPRα, which in turn directly regulate oocyte maturation and affect fertility in zebrafish.
Monday 9 July 2018

Symposium 2: Environmental light and color modulating endocrine functions

Chairs: Hironori Ando and Takashi Yoshimura

Session 2 1400 - 1600

1400 - 1430
Takashi Yoshimura
*Seasonal changes in color perception and behavior in medaka.*
25 minutes + 5 minutes questions

1430 - 1500
Hironori Ando, Md. Shahjahan, Takashi Kitahashi and Atsuhiko Hattori
*Melatonin, the hormone of gloom: Implications in the semilunar-synchronized spawning of the grass puffer.*
25 minutes + 5 minutes questions

1500 - 1530
Kodai Fukunaga, Fumika Yamashina, Yuki Takeuchi, Hiroki Takekata, and Akihiro Takemura
*Interplay between endocrine and clock systems in the lunar-synchronized spawning of tropical groupers.*
25 minutes + 5 minutes questions

1530 - 1600
Kiyoshi Soyano, Yuji Mushirobira, Mitsuru Niida, Daisuke Izumida, Takurou Hotta and Yuichiro Fujinami
*The role of photoperiod and water temperature in the regulation of gonadal development and maturation in the yellowtail.*
25 minutes + 5 minutes questions
Seasonal changes in color perception and behavior in medaka

Takashi Yoshimura\textsuperscript{a-c}

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\textsuperscript{c}Division of Seasonal Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan

Abstract

The appropriate timing of various seasonal processes, such as reproduction, migration and hibernation, is crucial to the survival of animals living in temperate regions. Although this phenomenon attracts great interest, its underlying mechanisms are not well understood. By using non-model organisms that have highly sophisticated seasonal responses, we have uncovered the universality and diversity in the signal transduction pathway regulating seasonal rhythms in vertebrates. Although humans are not typically considered seasonal animals, some evidence suggests that seasonal variation in physiology and behavior also exists in humans. For example, the wavelength settings for the unique yellow hue are shifted to shorter wavelengths in summer compared with those in winter. Seasonal affective disorder patients, experiencing recurrent winter episodes of depressed mood, overeating and hypersomnia, show electroretinogram changes in winter, with lower sensitivity compared with healthy subjects. These observations highlight the potential importance of the retina in seasonality, but the molecular basis of these seasonal changes remains unknown. We have recently discovered dynamic plasticity in phototransduction regulates seasonal changes in color perception in Japanese medaka fish \textit{(Oryzias latipes)}, an excellent model for studying seasonal adaptation. I will discuss the seasonal adaptation strategy of medaka.
Melatonin, the hormone of gloom: Implications in the semilunar-synchronized spawning of the grass puffer

Hironori Ando, Md. Shahjahan, Takashi Kitahashi and Atsuhiro Hattori

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Abstract
Grass puffer (\textit{Takifugu alboplumbeus}), a semilunar spawner, provides a unique and excellent animal model for studying the neuroendocrine mechanisms underlying circadian, lunar and seasonal control of reproduction because spawning occurs several hours before high tide at dusk during spring tide in early summer. Observations of spawning behavior in spawning grounds and in aquarium revealed that the spawning rhythm is tightly connected to the tidal changes and is also endogenously maintained possibly under the control of lunar- or tidal-related clock. In the hypothalamus, the expression of genes for gonadotropin-releasing hormone, kisspeptin and gonadotropin-inhibitory hormone show specific daily, circadian and lunar age-dependent oscillations. These changes are consistent with the changes in expression of four subtypes of melatonin receptor (MelR) genes. The plasma levels of melatonin increased during dark phase, and were also dependent on the lunar age. The treatment with melatonin in vivo significantly stimulated the expression of these neurohormone genes. These results suggest that melatonin may transmit the photoperiodic information (dark phase and lunar age) to the hypothalamus. Furthermore, in the pineal gland, the expression of MelR genes showed ultradian oscillations with a period of 15 hours under constant dark conditions. Transcriptome analyses of the diencephalon showed that more than 200 genes from 2425 genes were potential ultradian expression genes. These results suggest that, in addition to the circadian clock, there is a tidal-related clock in the pineal gland. Although the link between the melatonin signal that transmits photic information and these two biological clocks is missing at present, they may play important roles in the precisely-timed daily and semilunar spawning.
Interplay between endocrine and clock systems in the lunar-synchronized spawning of tropical groupers

Kodai Fukunaga\textsuperscript{a,b}, Fumika Yamashina\textsuperscript{a}, Yuki Takeuchi\textsuperscript{a,c}, Hiroki Takekata\textsuperscript{a,b}, and Akihiro Takemura\textsuperscript{a}

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Abstract

Some fish inhabiting coral reefs undergo lunar-related reproduction: their gametogenesis and gamete release are observed periodically in accordance with the lunar cycle. In particular, groupers are commercially high value fish, which show lunar-related reproductive activity. To date, however, little is known about how the lunar-related reproduction is regulated endocrinologically. The present study aimed to explain the interplay between the endocrine and clock system in the honeycomb grouper \textit{Epinephelus merra}, which is a small and common grouper in Okinawa, Japan. When females were collected every week from May to June, vitellogenic oocytes in an ovary developed toward the full moon (FM) and disappeared afterwards. Ovulatory follicles could be observed in the ovary around the last quarter moon (LQM), suggesting that spawning occurred between these lunar phases. Real-time quantitative polymerase chain reaction (qPCR) analyses revealed that b-subunit of gonadotropins (\textit{fsh}β and \textit{lh}β) in the pituitary increased toward FM, while \textit{gnrhl} and \textit{gnrh2} in the diencephalon peaked around LQM and the first quarter moon (FQM), respectively. When a \textit{cryptochrome2} (\textit{cry}2) expression in the subdivided brain regions was measured at midnight, it increased from LQM to the new moon (NM) in the diencephalon and around NM in the pituitary. Implantation of an osmotic pump containing melatonin in the body cavity of matured females lowered \textit{fsh}β and \textit{lh}β in the pituitary, suggesting that melatonin has a negative impact on oocyte development through alternation of gonadotropin levels. Such treatment did not alter \textit{cry}2 in the diencephalon, although its abundance was acutely influenced by manipulation of “brightness at night” in certain groupers. It is concluded that “brightness at night” is related to fluctuation of hormones at the HPG axis through melatonin, and that clock gene(s) act as a lunar oscillator or a driver to generate lunar periodicity.
The role of photoperiod and water temperature in the regulation of gonadal development and maturation in the yellowtail.

Kiyoshi Soyano\(^a\), Yuji Mushirobira\(^a\), Mitsuru Niida\(^{a,b}\), Daisuke Izumida\(^{b,c}\), Takouro Hotta\(^d\) and Yuichiro Fujinami\(^d\)

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Abstract
In the seed production of the yellowtail *Seriola quinqueradiata*, which is one of the important species for aquaculture in Japan, the artificial manipulation of environmental factors is an effective technique for controlling the gonadal development and maturation. In particular, water temperature and photoperiod play an important role of regulatory factors of reproductive phenomena. Although it is known that they are important factors for maturational control in other teleost, the role in the endocrine mechanism of reproduction has not been clarified. Therefore, we investigated the seasonal changes in gonadal development, plasma steroid levels (T and E\(_2\)), and gene expression of gonadotropins (GtHs) in the female yellowtail in order to know the relationship between reproductive endocrine activity and changes in water temperature and photoperiod. In this study, the yolk vesicle stage which is the first step of ovarian development was observed and GtHs slightly increased in January after the photoperiod turned to long from short at the winter solstice. Initiation of vitellogenesis and increase of plasma T and E\(_2\) levels were observed in early March, while water temperature began to rise. These results indicate that GtHs synthesis are started by changes in photoperiod from short to long, and yolk accumulation via steroids synthesis is initiated by increase in water temperature. Based on the results of these studies, the promoting and inhibiting effects of water temperature and photoperiod on gonadal development were investigated by artificial manipulation of both factors using 2-3 years-old cultured yellowtail. The yellowtail were kept under short and long photoperiod and three different temperature (18, 22, and 26°C). The long photoperiod and 18 and 22°C induced the ovarian development, although the continuous short photoperiod or 26°C inhibited the development. These results show that the initiation and progression of ovarian development is dependent on the changes in photoperiod and water temperature.
Monday 9 July 2018

Symposium 1: Reproductive Endocrinology of Fishes

Chairs: Vance L Trudeau and Wei Ge

Session 2: 1630 - 1730

1630 - 1650
Linyan Zhou and Deshou Wang
Endocrine regulation of fish sex differentiation.
15 minutes + 5 minutes questions

1650 - 1710
Kun Wu* and Wei Ge
Disruption of dmrt1 rescues the all-male phenotype of cyp19a1a mutant in the Zebrafish – Evidence for interaction between Dmrt1 and aromatase in directing gonadal differentiation 15 minutes + 5 minutes questions

1710 - 1730
Xianbo Zhang, Deshou Wang
Mutation of foxl2 or cyp19a1a results in female to male sex reversal in XX Nile tilapia.
15 minutes + 5 minutes questions

Asterisk (*) indicate student presentation
Endocrine regulation of fish sex differentiation

Linyan Zhou and Deshou Wang

Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education); School of Life Sciences, Southwest University (400715), Chongqing, China. (E-mail: yanlinzhou916@126.com; wdeshou@swu.edu.cn)

Abstract

It is well accepted that Estradiol-17β (E2) and 11-Ketotesterone (11-KT) play critical role in both ovarian and testicular differentiation in teleosts. Intriguingly, several studies showed that DHP also play an essential role in the early stages of oogenesis and spermatogenesis. We aimed to investigate the collaboration between E2, 11-KT and DHP during sex differentiation in a teleost, Nile tilapia (Oreochromis niloticus). We find that Simultaneous treatment with estrogen and androgen before sexual differentiation resulted in all females in both XX and XY fish. Blockage of androgen synthesis and simultaneous administration of estrogen in XY fish can even induce the transdifferentiation of differentiated testis into functional ovary. Blockage of DHP signaling pathway resulted in the disruption of spermatogenesis, whereas 11-KT was insufficient to rescue the delay of entry of spermatogenesis. On the other hand, deficiency of DHP led to the masculinization of XX fish, while E2 treatment partially restore the oogenesis. Taken together, our data indicated that the interaction and synergism of E2, 11-KT and 11-KT are required for sexual differentiation in tilapia.
Disruption of dmrt1 Rescues the All-male Phenotype of cyp19a1a Mutant in the Zebrafish – Evidence for Interaction between Dmrt1 and Aromatase in Directing Gonadal Differentiation

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Abstract
Sex determination and differentiation are complex developmental processes controlled by many different factors. The regulatory relationships between different factors are still poorly understood. The plasticity of zebrafish gonadal development, with many different factors involved, provides an excellent model to investigate the interaction among them. Ovarian aromatase (cyp19a1a) and dmrt1 are considered to be key factors in directing ovarian and testis differentiation respectively and their functions are conserved among diverse species. Recent knockout studies on cyp19a1a and dmrt1 in zebrafish further confirmed the importance of these molecules in gonadal differentiation. Knockout of zebrafish cyp19a1a led to all-male offspring whereas loss of dmrt1 resulted in dysgenesis of the testis. In the present study, we established a dmrt1 and cyp19a1a double mutant zebrafish, and discovered that introduction of dmrt1 mutation into cyp19a1a mutant could rescue the all-male phenotype of the latter, resulting in females in the cyp19a1a mutant. Although dmrt1 and cyp19a1a double mutant zebrafish could develop ovaries, the folliculogenesis was arrested at previtellogenic stage or stage II. This result suggests that the ovarian aromatase may play a role in determining ovarian differentiation by suppressing dmrt1 expression; however, this enzyme is not essential for early follicle development afterwards until previtellogenic stage. The arrest of folliculogenesis in the double mutant is likely due to the failure of estrogen-dependent vitellogenin synthesis in the liver. Our results provide a solid piece of evidence for interactions between cyp19a1a and dmrt1 in directing gonadal differentiation in zebrafish.

ACKNOWLEDGEMENT This study was supported by grants from the University of Macau (MYRG2014-00062-FHS, MYRG2015-00227-FHS, and CPG2014-00014-FHS) and The Macau Fund for Development of Science and Technology (FDCT114/2013/A3 and FDCT/089/2014/A2)
Mutation of *foxl2* or *cyp19a1a* results in female to male sex reversal in XX Nile tilapia

Xianbo Zhang, Deshou Wang

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Abstract

It is well accepted that Forkhead box protein L2 (Foxl2) and aromatase (Cyp19a1; the enzyme responsible for estrogen synthesis) are critical for ovarian development in vertebrates. Knockouts of Foxl2 and Cyp19a1 in goat, mouse, and zebrafish have revealed similar but not identical functions across species. Functional analyses of these two genes in other animals are needed to elucidate their conserved roles in vertebrate sexual development. In this study, we established *foxl2* and *cyp19a1a* mutant lines in Nile tilapia. Both *foxl2*−/− and *cyp19a1a*−/− XX fish displayed female-to-male sex reversal. Sf1, Dmrt1, and Gsdf were upregulated in the *foxl2*−/− and the *cyp19a1a*−/− XX gonads. Downregulation of Cyp19a1a and serum estradiol-17β level, and upregulation of Cyp11b2 and serum 11-ketotestosterone level were observed in *foxl2*−/− XX fish. The mutant phenotype of *foxl2*−/− XX individuals could be rescued by 17β-estradiol treatment from 5 to 30 days after hatching. Upregulation of Star1, the enzyme involved in androgen production in tilapia, was also observed in the *foxl2*−/− XX gonad at 30 and 90 dah. In vitro promoter analyses consistently demonstrated that Foxl2 could suppress the transcription of *star1* in a dose-dependent manner. In addition, compared with the control XX gonad, fewer germ cells were detected in the *foxl2*−/− XX, *cyp19a1a*−/− XX, and control XY gonads at 10 dah. These results demonstrate that Foxl2 promotes ovarian development by upregulating Cyp19a1a expression and repressing male pathway gene expression. These results extend the study of Foxl2 and Cyp19a1a loss of function to a commercially important fish species.
Symposium 4: Neurobiology of Social Behaviour

Chairs: Ishwar S. Parhar and Tomoko Soga

1100 - 1230

1100 - 1130
Terence Y Pang, Annabel K Short, Katie A Fennell, Shlomo Yeshurun, Arina Rawat, Vicky Batchelor, Anthony J Hannan
Transgenerational impacts of paternal stress on offspring behavioural phenotypes and stress-response
State of the Art Lecture 25 minutes + 5 minutes

1130 - 1150
Sachuriga, Daisuke Yoshida, Naoto Inuma, Tomoya Nakamachi, Norifumi Konno and Kouhei Matsuda
Distribution of cholecystokinin (CCK)-like immunoreactivites in the goldfish brain, and effect of intracerebroventricular administration of sulfated CCK octapeptide on psychomotor activity in goldfish
15 minutes + 5 minutes questions

1150 - 1210
The involvement of BDNF in maintaining HPG-axis function – relevance to schizophrenia
15 minutes + 5 minutes questions

1210 - 1230
Tomoko Soga, and Ishwar S. Parhar
Social isolation and the GnIH neurons in the brain.
15 minutes + 5 minutes questions
State of the Art Lecture

Transgenerational impacts of paternal stress on offspring behavioural phenotypes and stress-response

Terence Y Pang\textsuperscript{a,b}, Annabel K Short\textsuperscript{a,c}, Katie A Fennell\textsuperscript{a}, Shlomo Yeshurun\textsuperscript{a}, Arina Rawat\textsuperscript{a}, Vicky Batchelor\textsuperscript{a}, Anthony J Hannan\textsuperscript{a,b}
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Abstract

Exposures of the parental generation to non-genetic environmental challenges such as stress, malnutrition, and drugs of abuse can result in a range of ill-consequences for offspring health and their risk for disease. There is a growing body of evidence for this phenomenon of ‘transgenerational inheritance’ from retrospective human studies and a range of multiple animal models. While the maternal influence and the modes of transmission are relatively well-studied, the extent of the paternal influence remains ill-defined. In this presentation, I will discuss paternally transmitted transgenerational influences of preconceptional stress on offspring behaviour and health. I will highlight the evidence from studies of different mouse models of early life and adult psychosocial stress, as well as the mouse model of physical stress via glucocorticoid supplementation developed by my lab. Our studies revealed that paternal corticosterone treatment resulted in juvenile female F1 offspring with impaired fear extinction and adult male F1 offspring displaying increased anxiety-related behaviours. We have also reported that the F1 offspring also have a differential behavioural response to the selective serotonin reuptake inhibitor sertraline. Consistent with the ‘mis-match hypothesis’, F2 offspring displayed reduced anxiety behaviours along with a contradictory emergence of depression-related behaviours. RNA sequencing analysis of sperm isolated from male breeders was conducted to investigate the molecular origins of paternal transgenerational inheritance and this revealed significant changes in the levels of sperm-borne microRNAs. Bioinformatics analyses predicted the paternally-imprinted gene igf2 to be differentially resulted and this was confirmed in the hippocampus of F1 and F2 offspring despite being unaltered in F0 breeders. Finally, I will discuss the most recent studies within the field seeking to elucidate the mechanisms underlying stress-induced alterations to sperm epigenetic profile.
Distribution of cholecystokinin (CCK)-like immunoreactivities in the goldfish brain, and effect of intracerebroventricular administration of sulfated CCK octapeptide on psychomotor activity in goldfish

Sachuriga\textsuperscript{a}, Daisuke Yoshida\textsuperscript{a}, Naoto Inuma\textsuperscript{a}, Tomoya Nakamachi\textsuperscript{a}, Norifumi Konno\textsuperscript{a} and Kouhei Matsuda\textsuperscript{a,b}

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Abstract
Sulfated cholecystokinin octapeptide (CCK-8s) is involved in the appetite and satiety regulation as an anorexigenic factor in vertebrates. In rodents, intracerebroventricular (ICV) administration of CCK-8s has been shown to affect not only food intake, but also psychomotor activity. However, as there is still no information regarding the psychophysiological effects of CCK-8s in goldfish, we then observed CCK-like immunoreactivities in the goldfish brain, and we also investigated the effect of synthetic goldfish (gf) CCK-8s on psychomotor activity in this species. CCK-like immunoreactivities were observed widely in the telencephalon, diencephalon, mesencephalon, cerebellum and medulla oblongata. ICV administration of gfCCK-8s at 0.5, 1 and 2.5 pmol/g body weight (BW) did not affect locomotor activity. Since intact goldfish prefer a black to a white background area, or the lower to the upper area of a tank, we used two types of preference test (black/white and upper/lower tests) for measuring anxiety-like behavior in goldfish. ICV administration of gfCCK-8s at 2.5 pmol/g BW shortened the time spent in the white background area, and in the upper area. These actions of gfCCK-8s mimicked that of the central-type benzodiazepine receptor inverse agonist, FG-7142 (an anxiogenic agent), at 5 and 10 pmol/g BW. The anxiogenic-like effect of gfCCK-8s was abolished by treatment with the CCK receptor antagonist, proglumide, at 100 pmol/g BW. These data indicate that gfCCK-8s potently affects psychomotor activity, and that gfCCK-8s exerts an anxiogenic-like effect via the CCK receptor-signaling pathway in goldfish.
The involvement of BDNF in maintaining HPG-axis function – relevance to schizophrenia

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Abstract
Females are less likely to develop schizophrenia than males and female patients have later onset of symptoms. This difference is attributed to the female sex hormone estradiol. Recent clinical evidence suggests that a large proportion of women with schizophrenia exhibit abnormal menstrual cycle lengths and reduced circulating estradiol levels, which may contribute to symptoms such as cognitive deficits. While this phenomenon is largely ascribed to antipsychotic-induced hyperprolactinaemia, innate pathophysiological changes related to the disease may also contribute in some cases. One potential mechanism is brain-derived neurotrophic factor (BDNF), which is a regulator of the hypothalamic-pituitary-gonadal (HPG) axis and which is reduced early in schizophrenia. Hence we propose that a reduction of BDNF may be sufficient in inducing pathological changes in the HPG-axis, thereby leading to alterations of menstrual cycling and reductions in estradiol production. To examine this hypothesis, we examined the estrous cycling of both female wild-type and BDNF heterozygous mice, the latter expressing ~50% of wild-type BDNF level. Daily monitoring from 7-9 weeks of age revealed an abnormal elongation of estrous cycle in the BDNF heterozygous mice, similar to what is reported in young female schizophrenia patients, who displayed elongated menstrual cycles. Furthermore, molecular analyses show alterations of HPG-axis regulators in the BDNF heterozygous mice, such as reductions of the protein expression of gonadotropin-releasing hormone receptor in the hypothalamus and hippocampus, reduction in hypothalamic oxytocin/neurophysin 1 and an increase in hypothalamic kisspeptin. Our data show that reduction in BDNF is a sufficient pathoetiological factor to cause an abnormal shift in estrous cycling in female mice. These findings provide a mechanism of HPG-axis disruption that may apply to schizophrenia patients and which may modulate symptoms such as cognitive deficits. They further open up the possibility of treatments that utilise the estrogen signalling pathway for these hitherto untreatable symptoms.
Social Isolation and the GnIH Neurons in the Brain

Tomoko Soga\textsuperscript{a}, Ishwar S. Parhar\textsuperscript{b}

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Abstract
Social stress activates the hypothalamic–pituitary–adrenal axis and increases the risk of developing mental disorders. In fact, social stress such as social isolation or social defeat stress deregulates the neuroendocrine system and the reproductive pathways. In recent years, animal models have been developed that use social isolation or social defeat stress to induce neuronal dysfunctions and behaviour changes that might be similar to those occurring in the course of the development of depression. I will discuss the work we have done to develop a rat social stress model by post-weaning social isolation. These animals were exposed to social stress to understand the brain serotonergic systems and the regulation of its target neurons. In particular, I will focus on gonadotropin-inhibitory hormone (GnIH) neurons in the dorsomedial hypothalamic nucleus, which have an inhibitory effect on central reproductive regulation. The serotonergic regulations of GnIH neurons elucidates the neuronal mechanisms of reproductive dysfunction and social stress induced depression and provides pathways for the discovery of better target therapies.
Tuesday 10 July 2018

Symposium 5: Marine Invertebrates

Chair: Ching-Fong Chang

Session 1 1100 - 1230

1100 - 1130
Meaghan K. Smith*, Utpal Bose1, Abigail Elizur, Cherie A. Motti, Scott F. Cummins
*Brainless Matters. Neural investigations in the crown-of-thorns seastar, Acanthaster planci
State of the Art Lecture 25 minutes + 5 minutes

1130 - 1150
Masatoshi Mita, Keitaro Nakamura, and Hidekazu Katayama
Effect of chimera relaxin-like gonad-stimulating peptides on spawning in ovaries of starfish Patiria pectinifera
15 minutes + 5 minutes questions

1150 - 1210
Yan Zhang, Yu-Ying Ho, Yi-Ling Chiu, Jack I-Chen Yao, Shinya Shikina, Ching-Fong Chang
Identification and characterization of an Antho-RFamide neuropeptide that induces tentacle contraction in a stony coral Euphyllia ancora
15 minutes + 5 minutes questions

1210 - 1230
Production of a sea cucumber recombinant Relaxin-like Gonad-stimulating Peptide (RGP) that induces spawning
15 minutes + 5 minutes questions

*Asterisk (*) indicate student presentation
State of the Art Lecture


Meaghan K. Smith¹, Bronwyn Rotgans², Utpal Bose¹, Abigail Elizur¹, Cherie A. Motti², Michael Hall², Scott F. Cummins¹,*

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The Crown-of-Thorns Seastar (COTS), *Acanthaster planci* cf. *solaris* causes unprecedented damage to coral on the Great Barrier Reef, due to explosive population outbreaks. Current control methods are laborious and expensive with extrapolated estimates predicting a decline to less than 10% coral cover remaining on the reef in coming decades, unless intervened. With cyclic outbreaks of COTS a constant threat to the reef, there is a necessity to better understand COTS biological cycles and factors driving the population explosions and subsequent collapse. Lack of knowledge into molecular regulation of COTS biological processes (e.g. growth and reproduction) is one area where effective control methods are currently hindered. Exploration of the neural system and neurotransmitters in COTS is one area in critical need of research. Through this study, we present the most inclusive investigation of COTS neural system to date. The ultrastructure of COTS radial nerve cord reveals unique bulbous projections from the ectoneural surface which appear only in *A. planci* and not in the close relative, *Acanthaster brevispinus*, nor any other seastar. Employment of -omic technologies allowed for a thorough investigation of the neuropeptide and small molecule neurotransmitters present in the radial nerve cord, specifically the bulbous structures. Neuropeptide investigations revealed a total of 48 neuropeptide precursors, including 10 novel neuropeptides, some of which elicit contractile bioactivity in COTS tube-feet. The seastar neuropeptide responsible for final oocyte maturation, Relaxin-Gonad-Stimulating Peptide (RGP) was produced in a recombinant system and successfully induced oocyte maturation and spawning, with fertilization success and larval development. This peptide is of critical relevance to COTS reproductive biology and is a strong candidate for novel biocontrol strategies. A comparative mass spectral analysis looking at small molecule neurotransmitter abundance between adult male and female COTS, and when food-deprived (~90 days) revealed (i) females contain higher levels of GABA, (ii) histamine and epinephrine increase following food deprivation, and (iii) serotonin and melatonin decrease following food deprivation. Most enzymatic biosynthesis pathway genes were identified in the COTS genome. These findings are consistent with food deprivation causing reproductive and regenerative downregulation in COTS, indicating a possible cause of the population collapse following mass outbreaks.
Effect of chimera relaxin-like gonad-stimulating peptides on spawning in ovaries of starfish *Patiria pectinifera*

**Masatoshi Mita**, Keitaro Nakamura, and Hidekazu Katayama

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**Abstract**

Relaxin-like gonad-stimulating peptide (RGP) of starfish is the first identified invertebrate gonadotropin to trigger final gamete maturation. RGP stimulates ovarian follicle cells to produce maturation-inducing hormone, 1-methyladenine (1-MeAde). In this sense, RGP is functionally analogous to the vertebrate luteinizing hormone. RGP is a heterodimeric peptide comprising an A- and B-chains with disulfide cross-linkages of one intra-chain and two inter-chain disulfide bonds. Recently, three orthologous of RGP molecules were found in the class Asteroida; PpeRGP of *Patiria pectinifera*, AamRGP of *Asterias amurensis*, and AjaRGP of *Aphelasterias japonica*. The chemical structure of AamRGP is close to that of AjaRGP, although the amino acid sequence of PpeRGP is quite different from that of AamRGP or AjaRGP. Cross-experiments among *P. pectinifera*, *A. amurensis*, and *A. japonica* showed that PpeRGP could induce oocyte maturation and ovulation in ovarian fragments of *A. amurensis*, and *A. japonica*. In contrast, neither AamRGP nor AjaRGP induced spawning in the ovary of *P. pectinifera*. This is possibly due to species specificity of an RGP receptor. In this study, chimera RGPs in which each A- and B-chains had been exchanged were used to examine the interaction of RGP with its receptor. PpeRGP derivatives (Aam A-chain/Ppe B-chain and Aja A-chain/Ppe B-chain) replaced with the A-chain of either AamRGP or AjaRGP were failed to induce spawning in the *P. pectinifera* ovaries. On the contrary, the B-chain replaced PpeRGP derivatives (Ppe A-chain/Aam B-chain and Ppe A-chain/Aja B-chain) could induce oocyte maturation and ovulation in *P. pectinifera* ovaries but their spawning activities were relatively lower than that of PpeRGP. These results suggest that A-chains of RGP are important for spawning activity. It may be possible that the A-chain of RGP interacts with its receptor protein to produce 1-MeAde in follicle cells.
Identification and characterization of an Antho-RFamide neuropeptide that induces tentacle contraction in a stony coral *Euphyllia ancora*

Yan Zhang\(^a\), Yu-Ying Ho\(^a\), Yi-Ling Chiu\(^{b,c}\), Jack I-Chen Yao\(^a\), Shinya Shikina\(^{d,e}\), Ching-Fong Chang\(^{a,d}\)

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\(^e\)Institute of Marine Environment and Ecology, National Taiwan Ocean University, Keelung, Taiwan. shikina@mail.ntou.edu.tw (S. S.)

**Abstract**

FMRFamide-like peptides (FLPs) are low-molecular-weight neuropeptides exerting various physiological functions in invertebrates. In cnidarians, studies of the distribution, structures and functions of FLPs have been widely conducted in sea anemones, hydras and jellyfish. However, little is known about the presence and function of FLPs in stony corals. To gain a better understanding of FLPs in stony corals, we established a transcriptome database of the stony coral *Euphyllia ancora*, and searched for genes possibly encoding FLP precursor proteins by local blast analysis against the database. We identified a sequence exhibiting a similarity to a FLPs precursor protein that was firstly identified in the sea anemone *Anthopleura elegantissima* (named Antho-RFamide). Deduced amino acid sequence of the precursor protein in *E. ancora* contained 17 copies of Glu-Gly-Arg-Phe (QGRF) motifs. Quantitative RT-PCR analysis showed that the transcripts were expressed higher in the tentacle compared with other coral tissues such as gonads (ovary and testis) and mesenterial filament in the coral polyp. Immunohistochemical analysis with a polyclonal anti-FMRFamide antibody showed that the cells exhibiting immunoreactive signals, probably the ganglion neurons, were mainly distributed at the base of the epithelium of the tentacle. Furthermore, treatment of the adult *E. ancora* polyps with a synthetic tetrapeptide QGRFamide could induce the contraction of the tentacles. These results strongly suggested that QGRFamide is involved in the control of tentacle contraction in stony corals. To the best of our knowledge, this study provides the first evidence for the presence and possible function of FLPs in stony corals.
Production of a sea cucumber recombinant Relaxin-like Gonad-stimulating Peptide (RGP) that induces spawning

Chieu Hoang Dinh1,2, Luke Turner3,4, Meaghan K. Smith1, Tianfang Wang1, Josephine Nocillado1, Peter Palma1,5, Saowaros Suwansa-ard1, Abigail Elizur1, and Scott F. Cummins1

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2Research Institute for Marine Fisheries (RIMF), 224 LeLai Street, HaiPhong City, Vietnam
3Tasmanian Seafoods Pty. Ltd., Tasmania, Australia
4Darwin Aquaculture Centre, Darwin, Australia
5SEAFDEC Aquaculture Department in Philippines

Abstract
Wild sea cucumbers have been exhausted rapidly and need to be restocked by proactive actions to produce seed through artificial breeding. The neurohormone relaxin-like gonad-stimulating peptide (RGP) has been identified as the active gonad-stimulating peptide in starfish, which could also be effective in other echinoderms. However, RGP’s quaternary structure is complex and difficult to synthesise. In this study, a recombinant Holothuria scabra RGP was produced in the yeast Pichia pastoris, then prepared to four levels of purification: crude RGP supernatant (CS), crude concentrated RGP (CR), Sep-pak-purified RGP (SR), and His-tag with Amicon filter-purified RGP (HR). The most pure form, HR, induced 98.56 ± 1.19% germinal vesicle breakdown (GVBD) in H. scabra and 89.57 ± 1.19% GVBD in Holothuria leucospilota. Both CS and HR induced spawning in H. scabra females; CS induced spawning at 80 min after second injection (2nd injection at 60 min after first injection), while HR induced spawning at 90 min after first injection. All mature oocytes from this spawning bioassay were fertilised successfully, developed into juveniles and settled for growth at the Darwin Aquaculture Centre for conservation programs. Our results provide a key finding for the development of a break-through new artificial breeding approach in sea cucumber aquaculture.

Keywords: Juvenile, oocyte maturation, recombinant RGP, sea cucumber, spawning.
Tuesday 10 July 2018

Poster session 1

1300 - 1430

Poster Number:

1. **Daichi Kayo***, Shinji Kanda, Yoshitaka Oka
   *The role of estrogen receptor β1 (Esr2a) in the negative feedback regulation of follicle stimulating hormone by 17β-Estradiol in a teleost medaka.*

2. **Yuji Mushirobira**, Mitsuru Niida, Takurou Hotta, Yuichiro Fujinami and Kiyoshi Soyano
   *Hepatic expression profiles of three subtypes of vitellogenin and estrogen receptor during vitellogenesis in cultured female yellowtail*

3. **Ken Ueno***, Hiroshi Miyanishi, Hirohiko Kagawa, Katsuhisa Uchida
   *Endocrine aspects of landlocked masu salmon in freshwater and seawater aquaculture conditions: their smoltification, seawater adaptation, growth and reproduction*

4. **Mageswary Sivalingam***, Priveena Nair Ramadasan, Satoshi Ogawa, Ishwar Parhar
   *Potential interaction between kisspeptin and tachykinin in aversive response*

5. Mohammad Lutfar Rahman, Md. Shahjahan and Hironori Ando
   *Immunolocalization of kisspeptin and kisspeptin receptor in the brain of grass puffer*

6. **Nurul M. Abdal Satar***, Satoshi Ogawa and Ishwar Parhar
   *Evidences of kisspeptin-1 indirectly control dopamine system in the zebrafish brain*

7. **Daisuke Moue***, Tatae Kitani, Kazue Nagasawa, Motoichi Kato, Toshihito Naiki, Makoto Osada
   *Differentiation and development of monoamines and GnRH neuron during larval development of bivalves*

8. Katsuhisa Uchida, Yuma Yokoyama, Kazuaki Yamaguchi, Shigehiro Kuraku, Hiroshi Miyanishi and Hirohiko Kagawa
   *Molecular and cellular evidences for shark GnRHs and their receptors in the brain and pituitary of cloudy catshark, Scyliorhinus torazame*

9. **Anu Shahapal***, Hyo Jeong Yong, Sunam Mander, Huong Thi Nguyen, Jong-Ik Hwang, Jae Young Seong
   *Expression pattern of neuroglia development factor 5 (NGDF5) in the central nervous system*

10. **Hyo Jeong Yong***, Anu Shahapal, Sunam Mander, Huong Thi Nguyen, Jong-Ik Hwang, Jae Young Seong
    *Expression pattern of a novel chemokine like peptide, BDNF 4 during mouse brain development*
11. Zulviker Syambani Ulhaq, and Mitsuyo Kishida
Ritanserin, a 5-HTR2 antagonist, impairs the expression of cyp19a1b in the eye and the eye development in zebrafish

12. Md. Shahjahan, Muhammad Lutfar Rahman, Takashi Kitahashi and Hironori Ando
Thermoregulatory expression of GnIH, GnIH receptor, GH and PRL genes in the grass puffer during the spawning season

13. Eiko Iwakoshi-Ukena, Masaki Kato, Tetsuya Tachibana, Kazuyoshi Ukena
Expression and localization of growth hormone-releasing hormone in the chicken hypothalamus

14. Sun CY, Huang JF, Kuang ZL, Liang ZW, Yuan X, Cai RJ, Li WS
Regulation of recombinant protein of activin IIB receptor extracellular domain on the growth of tilapia (Oreochromis niloticus)

Intracerebroventricular administration of sulfated cholecystokinin (CCK) octapeptide induces anxiety-like behavior in zebrafish

16. Chuin Hau Teo*, Tomoko Soga, Ishwar S. Parhar
The role of β-catenin in gonadotropin-inhibitory hormone neurons during social isolation

Asterisk (*) indicate student presentation
Poster 1

The role of estrogen receptor β1 (Esr2a) in the negative feedback regulation of follicle stimulating hormone by 17β-Estradiol in a teleost medaka

Daichi Kayo, Shinji Kanda, Yoshitaka Oka
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Abstract
17β-Estradiol (E2) is one of the major sex steroid hormones in female vertebrates and plays crucial roles in reproduction. As the ovarian follicles mature, E2 suppresses the secretion of follicle stimulating hormone (FSH) from the pituitary, which is required for normal folliculogenesis. Recent studies have indicated that FSH but not luteinizing hormone (LH) is required for folliculogenesis in teleosts. However, the mechanism of down-regulation of FSH by E2 has not been well understood. In a teleost medaka, we previously reported that females in which one of the three subtypes of estrogen receptor (ER), Esr2a, has been knocked out (KO) show higher expression levels of fshb mRNA. Thus, it has been suggested that Esr2a mediates the down-regulation of FSH induced by E2 in medaka (Kayo et al., ICZ2016 meeting abstract).
In the present study, we further analyzed the contribution of Esr2a to the FSH negative feedback. For the elucidation of the feedback mechanism, we first optimized the experimental design for the artificial control of blood E2. To optimize the protocol for E2 application, we analyzed the blood E2 level of ovariectomized (OVX) and E2-administrated medaka by ELISA at multiple time points. The blood E2 levels of OVX female medaka were decreased, comparable to the that of the intact males three days after ovariectomy. On the other hand, the E2 level of males, which is usually low, showed an increase to reach the level comparable to that of intact females four hours after administration of E2-containing food.
To analyze the possible involvement of Esr2a in the down-regulation of FSH by E2, we examined E2 effect on FSH expression by using OVX and OVX+E2 model in esr2a+/+ and esr2a−/−. OVX+E2 medaka of esr2a+/+, but not those of esr2a−/−, showed significant difference in the expression of FSH compared to that of OVX medaka.
Poster 2

Hepatic expression profiles of three subtypes of vitellogenin and estrogen receptor during vitellogenesis in cultured female yellowtail.

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Abstract
Vitellogenin (Vtg) is a major yolk protein precursor, and produced by estradiol-17β (E2) in hepatocyte. Generally, E2 promotes transcription of Vtg gene via estrogen receptor (ER) belonging to nuclear receptors. In perciforms, it is known to exist three subtypes of Vtg (VtgAa, VtgAb, and VtgC) and ER (ERα, ERβ1, and ERβ2). However, knowledge of transcriptional regulation for three vtg subtypes is quite limited, and difference of roles for ER subtypes during vitellogenesis is also unclear. This study aimed to elucidate transcriptional regulation of Vtg subtypes via ER subtypes in our model perciforms, yellowtail (<i>Seriola quinqueradiata</i>).

Two-year-old female yellowtail used as experimental fish were reared under natural condition in sea cage. Liver and blood samples were collected by every 4 ~ 14 days from vitellogenesis to end of spawning season. Hepatic expressions of vtg and er subtypes were measured by quantitative RT-PCR. Serum E2 levels were quantified with ELISA. Hepatic expression profiles of three vtg subtypes were very similar, but expression levels were vtgAb > vtgAa > vtgC. In the hepatic expression of er subtypes, its profile in era was slightly different from those of erβ1 and erβ2 which are similar pattern. The expression profiles of era strongly correlated with those of three vtg subtypes. In serum E2 levels, weakly positive correlations were observed against hepatic expressions of three vtg subtypes. When vtg subtypes were significantly expressed in the liver, hepatic expressions of er subtypes also increased regardless of similar serum E2 levels. Particularly, expression levels of era were higher than those of erβ subtypes then.

This study revealed that transcription of three vtg genes are synchronously regulated during vitellogenesis in yellowtail. Moreover, these results suggest that high expressions of vtg subtypes are regulated via not blood concentration of E2 but hepatic abundance of ER subtypes, especially ERα.
Poster 3

Endocrine aspects of landlocked masu salmon in freshwater and seawater aquaculture conditions: their smoltification, seawater adaptation, growth and reproduction

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Abstract
Generally, masu salmon (Oncorhynchus masou masou) do not migrate to the sea in southern Kyushu Island due to high water temperature through spring to autumn, showing they are geographically landlocked and important for FW aquaculture resources. In this study, we have demonstrated that FW-aquaculture fish transformed into moderately-smoltified fish in late autumn. We established SW aquaculture system of the smolts starting from December to March, when the SW temperature was suitable for salmon aquaculture. After SW cultivation, the body weight of the fish was ten-fold greater than that of FW-reared fish. We analysed physiological and endocrine factors related with their smoltification, SW tolerance and somatic growth both in FW- and SW-reared fish. Coincident with increased pituitary TSH mRNA levels, the smolts showed cellular activation of thyroid gland in autumn. The mRNA levels of gill Na, K-ATPase α1b, which is mainly expressed in branchial salt-secreting pump, were rapidly increased after SW transfer in smolts, indicating their SW adaptability. The mRNA levels of pituitary GH and hepatic IGF-I were higher in the smolts than those of non-smolts during SW aquaculture period. In early April, the SW-aquaculture fish were transported to the FW ponds. They gradually progressed in their gonadal development and spawn in October. Their eggs were much higher in their size and number compared with those of entirely FW-aquaculture fish, suggesting the higher productivity of seeds and edible egg products caused by SW-aquaculture process. Coincident with increased pituitary FSH mRNA and plasma estradiol-17β levels in female, gonad-somatic index (GSI) was gradually increased through June to October, whereas the GSI of FW-aquaculture fish was rapidly increased from the end of August. The earlier initiation of oocyte growth under the endocrine controls may contribute to efficient egg productivity, particularly in the egg size of the gigantic masu salmon.
Poster 4

Potential interaction between kisspeptin and tachykinin in aversive response

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Abstract

Morphine is a potent analgesic in treating acute and chronic pain. Habenula, an evolutionarily conserved epithalamic structure, has been known as one of action target of morphine analgesia. We have previously shown expression of a reproductive neuropeptide, kisspeptin (Kiss1) in the habenula of zebrafish and its role in serotonergic modulation of aversive stress. Recently, the role of kisspeptin in pain sensitivity has been demonstrated in mammals. However, its neuronal mechanism is still unknown. Antinociceptive properties of tachykinin peptides such as substance P (SP encoded by tac1 gene) have been well studies, but their possible actions on the habenula remained obscure. In the present study, we examined association between habenular Kiss1 and tachykinin signaling, and their involvement in aversive response induced by alarm substance. In addition, effect of morphine on their gene expression was examined in zebrafish. Administration of SP upregulated kiss1 gene and this phenomenon was successfully attenuated by sendide, a SP receptor (NK1 encoded by tacr1) antagonist. Sendide also blocked the effect of alarm substance on kiss1 accompanied by anxiolytic behaviour upon exposure to alarm substance. We found that Kiss1 neurons co-express tacr1 gene, suggesting that SP can modulate aversive response via habenular Kiss1 neurons. In fish administered with morphine, Kiss1 and tac1 gene expression were significantly decreased, while tacr1 gene was increased in the brain. In fish treated with morphine, npas4a, a neuronal activity marker gene was highly expressed in the periventricular preoptic area containing high densities of SP neurons. These results suggest that habenular Kiss1 could be mediated by morphine via preoptic SP neurons.
Poster 5

Immunolocalization of kisspeptin and kisspeptin receptor in the brain of grass puffer

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Abstract

Kisspeptins play a key role in the neuroendocrine regulation of reproduction through its stimulatory action on GnRH neurons in mammals, although its roles in the control of reproduction are still controversial in teleosts. Studying the detailed distribution of kisspeptin as well as its receptor, originally identified as an orphan G-protein coupled receptor, GPR54, in the brain is important for understanding their multiple action sites and potential functions. Previously we identified the expression of genes encoding kisspeptin (kiss2) and its receptor (kiss2r) in the brain of grass puffer, which is a semilunar spawner, by in situ hybridization. kiss2 mRNA-positive cells are localized in a single neuronal population in the preoptic area (POA), and kiss2r mRNA-positive cells are localized in the POA and the nucleus dorsomedialis thalami. The expression levels of both kiss2 and kiss2r show daily and circadian variations. In the present study, to examine the Kiss2 neuronal structure and potential action sites, we generated specific antibodies against Kiss2 peptide and its receptor and examined their distributions in the brain of grass puffer by immunohistochemistry. Grass puffer Kiss2-immunoreactive (-ir) cells were localized in the single cell population in the magnocellular preoptic nucleus in the POA, consistently with the ISH study, whereas Kiss2r-ir cells were distributed in three different areas including the previous mRNA-positive cell groups, and the third population of Kiss2r-ir cell bodies were scattered in the medial preglomerular nucleus. In addition, brain samples were collected in the daytime and nighttime, and the immunoreactivity of Kiss2-ir cells tended to be stronger in the nighttime than daytime, suggesting that the Kiss2 peptide content may also show daily variations in the grass puffer brain.
Poster 6

Evidences of kisspeptin-1 indirectly control dopamine system in the zebrafish brain

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Abstract
We have previously shown predominant expression of a reproductive neuropeptide, kisspeptin (Kiss1) in the habenula and its role in serotonergic modulation of aversive responses in zebrafish. However, the possible association between Kiss1 and dopaminergic system in teleost fish remains unknown. Here, we demonstrated the possible link between habenular Kiss1 and dopaminergic system in zebrafish. The effect of Kiss1 administration on dopamine-related genes was examined in different brain regions by real-time PCR and followed by measurement of dopamine levels using liquid chromatography-mass-spectrometry (LCMS). Central administration of Kiss1 peptides significantly increased expression of dopamine-related genes in the whole-brain. Dopamine quantification using LCMS showed that high levels of dopamine content in the telencephalon and the hypothalamic area of Kiss1-treated fish. Furthermore, expression of kiss1 and npas4a, a neuronal activity marker gene was significantly decreased in the habenula, indicating suppression of the habenular Kiss1 neurons in fish treated with Kiss1 peptides. These results suggest that habenular Kiss1 neurons may play inhibitory role in modulation of dopaminergic system.
Poster 7

Differentiation and development of monoamines and GnRH neuron during larval development of bivalves

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Abstract

Pacific oyster (Crassostrea gigas) and Manila clam (Ruditapes philippinarum) are commercially important species and are known to have a free swimming larval stage. The larval settlement and metamorphosis are important events to change from planktonic stages to benthic stages. Nevertheless, little is known about the mechanisms of larval settlement and metamorphosis. Coon and Bonar (1985) reported that catecholamines (e.g., norepinephrine, epinephrine) induced the larval settlement and metamorphosis in oyster. However, this observation has not been seen in any other bivalves. In addition, Kamiya et al (2014) reported gonadotropin-releasing hormone (GnRH) induced metamorphosis in protochordate, Ciona intestinalis. The objective of this study was to understand mechanisms controlling larval settlement and metamorphosis in bivalves by using quantitative analyses of monoamines (i.e., catecholamines, serotonin and their metabolites) in two species and GnRH mRNA in oyster. In this study, we performed high performance liquid chromatography (HPLC) for simultaneous determination of monoamines and real-time qPCR for GnRH mRNA expression during larval development from unfertilized eggs to post-settled larva in oyster and Manila clam. Our findings showed that all catecholamines rapidly increased after the stage of shell length 100µm in oyster as found in the previous study and after D-shaped larva in Manila clam except for norepinephrine. In particular, norepinephrine was detected at very low level and did not increase during larval development in Manila clam unlike oyster. On the other hand, serotonin showed similar increasing pattern in two species. GnRH mRNA was detected from blastula to post-settled larva and its mRNA level increased from gastrula to D-shaped larva. In conclusion, norepinephrine may not be a common amine to induce settlement and metamorphosis among bivalves. In addition, serotonin was thought to be a common amine to induce settlement and metamorphosis and GnRH may be involved with settlement and metamorphosis in oyster.
Poster 8

Molecular and cellular evidences for shark GnRHs and their receptors in the brain and pituitary of cloudy catshark, Scyliorhinus torazame

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Abstract
Cartilaginous fishes are ancient groups of jawed-vertebrates, and they have diversified reproductive patterns such as oviparity, ovoviviparity and viviparity. However, our knowledge on the endocrine controls of their reproductive strategies has been poorly understood. Recently, we have identified three glycoprotein hormones (GPβ/FSHβ/LHβ/TSHβ) from the pituitary of cloudy catshark. In our current study, to clarify the functional relationship between pituitary GTHs (FSH/LH) and brain GnRH, we initially examined effects of GnRH analog on mRNA levels of pituitary GTHs by in vitro and in vivo experiments. Intraperitoneal injection of GnRH analog caused significant increase of FSHβ mRNA levels whereas no effect on LHβ gene expression. GnRH analog also stimulated the mRNA synthesis of FSHβ in the cultured pituitary tissues in vitro, showing possible presence of functional GnRH molecules in catshark. Based on the PCR cloning and sequence analysis, we have identified three paralogous genes for GnRH (GnRH1, GnRH2 and GnRH3) and four paralogous genes for GnRH receptor (GnRHR1a, GnRHR1b, GnRHR2a and GnRHR2b) from catshark brain. Tissue distributional analysis of three GnRH transcripts in the brain has shown that GnRH1 and GnRH3 genes expressed specifically in the olfactory bulb, whereas GnRH2 expressed in the midbrain. Among four paralogous genes of GnRH receptors, GnRHR1a was expressed specifically in the ventral lobe of pituitary. Moreover, GnRHR1a gene expressing cells were partially coincident with FSHβ gene expressing cells in the ventral lobe of pituitary. These results strongly suggest that shark has functional GnRH molecules to regulate pituitary GTH functions via GnRH receptors.
Expression pattern of neuroglia development factor 5 (NGDF5) in the central nervous system

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Abstract

NGDF5 (neuroglia development factor 5) is a novel peptide of approximately 100 amino acids. In this study, the expression of NGDF5 gene was examined using NGDF5-LacZ knock-in mouse model. The mouse brains with different developmental stages including embryonic, early postnatal, juvenile, and adult stages were subjected to X-gal staining. Whole mount embryo staining showed predominant expression of NGDF5 in the central nervous system (CNS), including brain and spinal cord, with some expression in peripheral tissues like cartilages. In addition, sagittal sections of the whole embryo showed expression in heart. Overall expression pattern in brain regions were observed. Further, brain regions like olfactory bulb, piriform cortex, caudate putamen, corpus callosum, cerebral cortex, septal nuclei, ventricular zone, hippocampus (CA regions and polymorphic layer of dentate gyrus), thalamus, hypothalamus, and cerebellum were stained relatively high. Though the expression seems to be non-specific to cell types and brain region, this study shows the possible role of NGDF5 in CNS development and normal functioning of brain.
Poster 10

Expression pattern of a novel chemokine like peptide, BDNF 4 during mouse brain development

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Abstract

Brain development and its functions require a complex association of various proteins which are expressed at certain locations and time points to acquire specific brain regional properties. Understanding patterns of gene expression may provide clues to reveal brain assembly and its functional mechanisms. Brain development neurofactor 4, BDNF4, is a novel chemokine like peptide, which is highly expressed in various brain regions with relatively low expressions in other tissues. In order to study its expression pattern and functional properties, a transgenic mouse with lacZ reporter gene inserted BDNF4 gene was established. Starting from E14.5, BDNF4 was expressed in premature piriform cortex and entorhinal cortex, whereas the neocortical expression of BDNF4 started after birth. In early postnatal days, BDNF4 was initially observed in somatosensory, visual and auditory cortical areas and this expression gradually expanded to all cortical areas in a layer specific way, indicating BDNF4 may involve in cortical layer development and maturation. In the mature brain, BDNF4 was expressed in the pyramidal cells of cortical layer 2/3 and 5 which might be callosal projection neurons and subcerebral projection neurons, respectively. BDNF4 was also expressed in various limbic areas, such as amygdala and hippocampus, suggesting its possible roles in the limbic system related brain functions. Although BDNF4 was not detected in the olfactory bulb, it may be still related to the olfaction, as it was expressed in other olfactory related brain regions such as piriform cortex and anterior olfactory nucleus. In addition, BDNF4 was located along the motor tract and in some of intermediated zones, such as dorsal cochlear nucleus and optic nerve layer of the superior colliculus where connections are made between neocortical pyramidal neurons and incoming signal nerve fibers. These findings provide an overview of BDNF4 expression pattern during brain development and its possible association in diverse brain functions.
Poster 11

Ritanserin, a 5-HTR2 antagonist, impairs the expression of cyp19a1b in the eye and the eye development in zebrafish

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Abstract

Estrogen is known to have regenerative and protective effects on neuronal cells including retina. Although expression of aromatase is detected in retina, the role of aromatase and locally produced estrogen is not well understood. Previously we showed that knockdown of brain aromatase, cyp19a1b, impaired eye development and visual function of developing zebrafish. Since we recently reported that brain aromatase modulates serotonergic neuron, which is known to be involved in neurogenic activity, in this study we examined the role of serotonin (5-HT) transmission in eye development of zebrafish. Embryos exposed to 10 μM ritanserin, a 5-HTR2 antagonist, exhibited the decrease in eye/body length ratio at 48 hpf, optic nerve diameter at 48 hpf, and the increase in apoptosis in the eye at 24 hpf. Assays for visual background adaptation at 5 dpf and optomotor response at 7 dpf showed the decreased visual response in the ritanserin-exposed fish. The effects of ritanserin on all the parameters were significantly reversed by co-incubation with 50 μM 5-HT or 1 μM E2 compared to the ritanserin exposure, but not to the control level except for apoptosis. Ritanserin exposure decreased the relative expression of cyp19a1b and a retinal progenitor marker, pax6a in the eye of 6-dpf larvae by RT-PCR. Taken together, our data indicate that 5-HT transmission plays an important role in eye development, and that one of the possible pathways for 5-HT to exert effects on eye development may be through modulating cyp19a1b expression in the eye.
Poster 12

Thermoregulatory expression of GnIH, GnIH receptor, GH and PRL genes in the grass puffer during the spawning season

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Abstract
Gonadotropin-inhibitory hormone (GnIH) plays an important role in the reproduction of fish through regulation of various pituitary hormones. To know the role of temperature in the regulation of reproductive function, previously we investigated the expression of genes encoding kisspeptin (kiss2), kisspeptin receptor (kiss2r) and three GnRHs (gnrh1, gnrh2 and gnrh3) in the brain and genes encoding GTH subunits (gpa, fshb and lhb) in the pituitary of sexually mature male grass puffer exposed to a low temperature (14°C), normal temperature (21°C) and high temperature (28°C) for 7 days. In parallel to gonadosomatic index, the kiss2 and kiss2r mRNA levels were significantly decreased by the low and high temperature exposures, concomitant with the decrease in gnrh1, fshb and lhb expressions. In this study, we examined changes in expression of gnih and gnihr genes in the brain and pituitary along with growth hormone (gh) and prolactin (prl) genes in the pituitary of grass puffer exposed to the three temperature conditions. The levels of gnih and gnihr mRNA were significantly decreased in both low and high temperature conditions compared to normal temperature condition. Similarly, the gh mRNAs were significantly decreased in both low and high temperature conditions. The prl mRNAs were drastically decreased at low temperature but showed no significant changes at high temperature. As the plasma levels of cortisol were significantly increased at low temperature and remain unchanged at high temperature, the fish were considered to be under stress at the low temperature conditions. Taken together, the present results indicate that anomalous temperature has an inhibitory effect on reproductive function through the suppression of gnih/gnihr/gh/fshb and lhb expression in the grass puffer and these changes may occur in a normal physiological response (at high temperature) as well as in a malfunctional stress response (at low temperature).
Expression and localization of growth hormone-releasing hormone in the chicken hypothalamus

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Abstract
Growth hormone-releasing hormone (GHRH) plays important roles not only in GH release but also in feeding behavior in birds. However, the expression and localization of GHRH in the avian hypothalamus remain to be elucidated. In this study, we investigated the expression of the GHRH precursor mRNA in the chicken brain using quantitative RT-PCR analysis and in situ hybridization. Subsequently we also analyzed the localization and distribution of mature GHRH in the hypothalamus using the specific antibody against mature GHRH corresponding to 47 amino acids. Quantitative RT-PCR analysis indicated the highly expression of the GHRH precursor mRNA in the hypothalamic infundibulum. Furthermore, the mRNA levels in the hypothalamic infundibulum increased during post-hatching development. In addition, food deprivation decreased the mRNA expression. In situ hybridization and immunohistochemical analysis revealed that GHRH-producing cells were restricted to the infundibular nucleus within the hypothalamic infundibulum. Immunoreactive fibers of GHRH were densely observed in the outer layer of the median eminence. Our findings indicate that GHRH participates in hypophysiotropic action on GH, growth, and energy metabolism including feeding behavior in chickens.
Regulation of recombinant protein of activin IIB receptor extracellular domain on the growth of tilapia (*Oreochromis niloticus*)

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Abstract
Activin type IIB receptor (ActRIIB), a member of serine/threonine kinase super-family, is one of the receptors of Myostatin. ActRIIB can bind with Myostatin to negatively modulate skeletal muscle growth through the Myostatin-ActRIIB-Smad signaling pathway. Previous studies have demonstrated that a soluble form of recombinant ActRIIB could promote skeletal muscle growth by disturbing or blocking the Myostatin/Activin signaling. In the present study, the ORF of ActRIIB with the size of 1545bp was cloned from tilapia, and it encoded 515 a.a. Tissue distribution study showed that ActRIIB was mainly expressed in white muscle, gonad, brain and liver of tilapia. To study the effect of extracellular domain of ActRIIB (ActRIIB-ECD) on the growth of tilapia, the recombinant protein of tilapia ActRIIB-ECD was obtained by *Pichia pastoris* expression system. Functional confirmation study showed that the ActRIIB-ECD recombinant protein not only could stimulate the mRNA expression of *myogenin*, but also could reduce the inhibitory effect of Myostatin on *myogenin* gene expression in mouse myoblast cell line C2C12. Feeding tilapia with different dosages (0μg/g feed, 2.5μg/g feed, 5μg/g feed and 10μg/g feed) of ActRIIB-ECD recombinant protein showed no effect on the body weight of tilapia, but this protein (2.5μg/g feed, 5μg/g feed and 10μg/g feed) could significantly increase the diameter of muscle fibers of tilapia. Continuous i.p. injection (one injection per week) of ActRIIB-ECD recombinant protein study showed that 10μg/g BW ActRIIB-ECD recombinant protein significantly stimulated the body weight of tilapia from the third week (the time point of the end of the third time injection). These results, taken together, suggested that ActRIIB-ECD recombinant protein could upregulate muscle growth of tilapia. Acknowledgements: Supported by: China Agriculture Research System (CARS-46), the Guangdong Provincial Science and Technology Program (2012B020308001) and the Modern Agriculture Talents Support Program (2016-2020) to Dr. Li WS.
Poster 15

Intracerebroventricular administration of sulfated cholecystokinin (CCK) octapeptide induces anxiety-like behavior in zebrafish

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Abstract

Sulfated cholecystokinin octapeptide (CCK-8s) is involved in the appetite and satiety regulation as an anorexigenic factor in vertebrates. In rodents, intracerebroventricular (ICV) administration of CCK-8s has been shown to affect not only food intake, but also psychomotor activity. However, there is still no information regarding the psychophysiological effects of CCK-8s in fish model. As zebrafish has several merits as a laboratory animal model, we investigated the effect of synthetic zebrafish (zf) CCK-8s A on psychomotor activity in this species. ICV administration of zfCCK-8s A at 1, 5 and 10 pmol/g body weight (BW) did not affect locomotor activity. Since intact zebrafish prefer a black to a white background area, or the lower to the upper area of a tank, we used two types of preference test (black/white and upper/lower tests) for measuring anxiety-like behavior in zebrafish. ICV administration of zfCCK-8s A at 10 pmol/g BW shortened the time spent in the white background area, and in the upper area. These actions of zfCCK-8s A mimicked that of the central-type benzodiazepine receptor inverse agonist, FG-7142 (an anxiogenic agent). The anxiogenic-like effect of zfCCK-8s A was abolished by treatment with the CCK receptor antagonist, proglumide, at 100 pmol/g BW. These data indicate that zfCCK-8s A potently affects psychomotor activity, and that zfCCK-8s A exerts an anxiogenic-like effect via the CCK receptor-signaling pathway in zebrafish.
The Role of β-catenin in Gonadotropin-Inhibitory Hormone Neurons During Social Isolation

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Abstract

Gonadotropin-inhibitory hormone (GnIH) has an inhibitory effect on gonadotropin-releasing hormone (GnRH) neurons and the reproductive system. Decreased activity of the GnIH-GnRH system have been observed under post-weaning social isolation conditions. Depressive behavior appeared to be linked to a daily light-dark cycle, with enhanced anxiety-like behavior during the dark phase in socially isolated rats. This study further investigates the changes in GnIH neuronal activity in different phases of the day. To verify a social isolation model using transgenic Enhanced Fluorescent Green Protein (EGFP)-GnIH rats, isolated rats were tested for sucrose preference. Immunohistochemistry of CLOCK protein was performed on EGFP-GnIH neurons, revealing an expression pattern of CLOCK within GnIH neurons. The amplitude of this pattern is reduced by social isolation. In pursuit of a possible link between the disruption of circadian control and GnIH activity, we investigated β-catenin expression via immunohistochemistry. β-catenin is associated with the Wnt signalling pathway, where it first saturates the cytoplasm before translocating into the cell’s nucleus to act as a transcription factor. Isolated rats exhibited increased nuclear β-catenin expression in the dark phase, whereas cytoplasmic β-catenin was elevated during the light phase for control rats, suggesting that different time phases affect expression of beta-catenin differently. Coupled with the discovery of modified CLOCK expression patterns under social isolation, these findings suggest that β-catenin and the Wnt signalling pathway may be important in understanding social isolation-induced sexual dysfunction.
Tuesday 10 July 2018

Symposium 6: Neuroendocrinology and Reproduction

Chairs: Ishwar S. Parhar and Tomoko Soga

1430 - 1600

1430 - 1500
Jeremy T. Smith
*Kisspeptin neurons are central regulators of fertility and metabolism*
State of the Art Lecture 25 minutes + 5 minutes

1500 - 1520
Takayoshi Ubuka and Ishwar Parhar
*Transmembrane protease serine 2 and forkhead box A1 as potential bisphenol A responsive genes in the neonatal male rat brain*
15 minutes + 5 minutes questions

1520 - 1540
Haipei Tang, Yu Chen, Le Wang, Xiaochun Liu, Haoran Lin
*New insights into the role of estrogens in sex differentiation and male fertility based on findings in aromatase-deficient zebrafish*
15 minutes + 5 minutes questions

1540 - 1600
Satoshi Ogawa, Felix S.K. Thomas, Tomoko Soga and Ishwar Parhar
*GnIH function as a novel stress regulator*
15 minutes + 5 minutes questions
State of the Art Lecture

Kisspeptin neurons are central regulators of fertility and metabolism

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Abstract

Kisspeptin is a neuropeptide produced by neurons in the hypothalamus and is critical for fertility through stimulation of gonadotropin-releasing hormone (GnRH) neurons. In addition to key roles in puberty onset, kisspeptin neurons govern underlying mechanism for sex steroid positive- and negative-feedback, and it is now commonly accepted – at least in rodents – that the ARC kisspeptin neurons act as the GnRH pulse generator. Moreover, kisspeptin neurons are now recognized as a central pathway responsible for conveying key homeostatic information to GnRH neurons to modulate fertility. Thus, in states of severely altered energy balance (either negative or positive) fertility is compromised, as is kisspeptin gene (Kiss1) expression in the arcuate nucleus. Furthermore, in addition to being expressed in GnRH neurons, the kisspeptin receptor (Kiss1r) is also expressed in other areas of the brain, as well as in the periphery, suggesting kisspeptin may have additional functions outside of governing reproductive status. Evidence is building for a direct role for kisspeptin in regulating energy balance and metabolism. Interestingly, kisspeptin neurons located in the arcuate nucleus are anatomically linked to, and can directly excite, anorexigenic POMC neurons and indirectly inhibit orexigenic NPY neurons. Thus, kisspeptin may have a role in energy balance and our observations indicated that Kiss1r knockout mice displayed late onset obesity and reduced energy expenditure. Moreover, recent data suggest that this obesity may be primarily due to altered uncoupling protein-1 (UCP1) mRNA expression in brown adipose tissue. Thus, in addition to regulating reproduction, kisspeptin signaling may also be an important regulator of metabolism and body weight.
Transmembrane protease serine 2 and forkhead box A1 as potential bisphenol A responsive genes in the neonatal male rat brain

Takayoshi Ubuka and Ishwar Parhar

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Abstract
Exposure to environmental chemicals has been suggested as a contributing factor to neurodevelopmental disorders. Although perinatal exposure of Bisphenol A (BPA), an organic synthetic compound widely used to make polycarbonate plastics and epoxy resins, modifies the behavior of rodents in later life, its mechanism is not known. To understand how BPA modifies the neurodevelopmental process, we searched for genes responsive to BPA from androgen and estrogen receptor signaling target genes by polymerase chain reaction array in the neonatal male rat brain. Wistar rats carrying enhanced green fluorescent protein tagged to gonadotropin-inhibitory hormone (GnIH) promoter was used to further investigate the possible action of BPA responsive molecules on GnIH neurons. Upregulation of transmembrane protease serine 2 (tmprss2) and downregulation of forkhead box A1 (foxa1) were found in the medial amygdala of male rats that were subcutaneously administered with BPA from day 1 to 3. Tmprss2-immunoreactive (ir) cells were distributed in the olfactory bulb, cerebral cortex, hippocampus, amygdala, and hypothalamus in 3-days-old male rats. Tmprss2 immunoreactivity was observed in 26.5% of GnIH neurons distributed from the ventral region of the ventromedial hypothalamic nucleus to the dorsal region of the arcuate nucleus of 3-day-old male rat. However, tmprss2 mRNA decreased significantly in 1-month-old male rats. Foxa1 mRNA was highly expressed in the hypothalamus and foxa1-ir cells were only found in the peduncular part of lateral hypothalamus in 3-days-old male rats. Foxa1 mRNA expression in the hypothalamus also decreased significantly at 1 month. As immunoreactivities to tmprss2, a transmembrane protein that may modify the construction of neuronal architecture, and foxa1, a DNA binding protein that may regulate gene transcription for neuronal development, were not detected in 1-month-old male rats, BPA may disturb the neurodevelopmental process such as that of GnIH neurons by modifying tmprss2 and foxa1 expressions in the neonatal male rat brain.
New insights into the role of estrogens in sex differentiation and male fertility based on findings in aromatase-deficient zebrafish

Haipei Tang, Yu Chen, Le Wang, Xiaochun Liu, Haoran Lin

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Abstract

Aromatase is deeply involved in the reproduction process of vertebrates. It is preliminarily shown that aromatase is essential for sex differentiation in fish. Moreover, the functional significance of aromatase in male reproduction is defined in mammals. Herein, we generated aromatase-deficient lines (cyp19a1a mutant, cyp19a1b mutant and cyp19a1a; cyp19a1b double mutant lines) via TALENs. Our results showed that cyp19a1a mutants and cyp19a1a; cyp19a1b double mutants, but not cyp19a1b mutants, had impaired sex differentiation, and all cyp19a1a mutants and cyp19a1a; cyp19a1b double mutants were males. During sex differentiation, the ovary-like gonads were not observed and the male sex differentiation program was retarded in cyp19a1a mutant fish, and these phenotypes could be partially rescued by E2 treatment. Gene expression analysis indicated that male and female sex-differentiation-related genes were significantly decreased in cyp19a1a mutant in this period. By the age of one year after fertilization, the fertility of all aromatase-deficient zebrafish remained normal. Interestingly, greater number of spermatozoa were found in cyp19a1a and double mutant males than in the wild-type and cyp19a1b mutant males. The whole-body androgen levels, FSHb and LHb protein levels in the pituitary, and expression levels of spermatogenesis and steroidogenesis related genes in the testes were significantly higher in cyp19a1a mutant and double mutant males, suggesting the cause for increased number of spermatozoa. Taken together, functional significance of gonad aromatase (cyp19a1a) in both male and female sex differentiation was determined in this work. Moreover, our results in adult mutant lines strongly indicated that estrogens are dispensable for achievement and maintenance of fertility in male zebrafish, which challenges the traditional view that estrogens are indispensable for male fertility.
GnIH Function as a Novel Stress Regulator

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Abstract
In vertebrates, gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH), respectively, regulate reproduction in positive and negative manners. GnIH belongs to the LPXRFa family of peptides previously identified in mammalian and nonmammalian vertebrates. We have previously generated antisera against tilapia GnIH and its receptor (GnIH-R) and localized their immunoreactivities in the brain and pituitary of Nile tilapia. We found that neither tilapia GnIH-immunoreactive fibers nor GnIH-R is closely associated or coexpressed with GnRH, or kisspeptin (Kiss2) neurons. In the pituitary, GnIH fibers are closely associated and GnIH-R are expressed in adrenocorticomelanotropic cells, suggesting role of GnIH signalling in the HPA axis. In socially defeated male tilapia, GnIH mRNA levels and GnIH-immunoreactive cell numbers were significantly increased. RT-PCR combined with laser-microdissection showed expression of glucocorticoid receptor (GR1 and GR2b) types in GnIH neurons. In socially stressed male, GnIH-R but not GnIH mRNA was significantly increased in the pituitary. Taken together, these results suggest that GnIH may act as a novel hypothalamic stress regulator in teleosts.
Tuesday 10 July 2018

Symposium 7: Reproductive and Energetic Endocrinology

Chair: Kanta Mizusawa

1430 - 1610

1430 - 1450
Andriyanto, Wisnu Hendra, Nur H. Safitri, Firda Agustin, Natalia F. Lyla, and Wasmen Manalu
Administration of Water Extracts of Ocimum sanctum and Curcuma longa prior to laying period to improve serum estrogen and vitellogenin concentration, growth performances and liver functions in poultry by using mojosari duck as a model
15 minutes + 5 minutes questions

1450 - 1510
Thrissawan Traijitt*, Noppadon Kitana, Kazue Nagasawa, Makoto Osada, Jirarach Kitana
Chronology of gonadal sex differentiation and steroidogenic potential in the rice field frog Hoplobatrachus rugulosus
15 minutes + 5 minutes questions

1510 - 1530
Wasmen Manalu, Andriyanto, Firda Agustin, Diki Yulianzah, Kharisma Mardatillah, and Natalia F. Lyla
Administration of Jamu ATOKE prior to mating in maternal rats improves serum estradiol and progesterone concentrations during pregnancy and the quality of the born offspring
15 minutes + 5 minutes questions

1530 - 1550
Sangita and Suresh Yenugu
The epididymis specific gene, sperm associated antigen 11 (Spag11) as a possible marker for cancer
15 minutes + 5 minutes questions

1550 - 1610
Sumihiro Matsumoto*, Natsumaro Kutsuna, and Shinji Nagata
Enteroendocrine peptides can regulate insect feeding behavior via controlling intestinal contraction
15 minutes + 5 minutes questions

Asterisk (*) indicate student presentation
Administration of Water Extracts of *Ocimum sanctum* and *Curcuma longa* prior to Laying Period to Improve Serum Estrogen and Vitellogenin Concentration, Growth Performances and Liver functions in Poultry by using Mojosari duck as a Model

Andriyanto\(^a\), Wisnu Hendra\(^b\), Nur H. Safitri\(^a\), Firda Agustin\(^c\), Natalia F. Lyla\(^a\), and Wasmen Manalu\(^a\)

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Abstract

Eighty Mojosari ducks with the age of 30 days were used in the experiment. The ducks were assigned into 4 treatments i.e., 1) control ducks without administration of water extracts of *Ocimum sanctum* or *Curcuma longa*, 2) ducks administered water extracts of *Ocimum sanctum*, 3) ducks administered water extracts of *Curcuma longa*, and 4) ducks administered water extracts of *Ocimum sanctum* and *Curcuma longa*. Each group consisted of 20 experimental ducks. The treatment was administered through drinking water with 10 mL of water extract in 1 liter of drinking water. The treatments were administered every day for 60 days. The data were collected at the end of the experiment when the experimental ducks were at the age of 90 days. The results showed that the experimental ducks administered water extract of *Ocimum sanctum, Curcuma longa, and* combination of *Ocimum sanctum and Curcuma longa* had higher body weight gain and final weight with lower mortality compared to control group. The experimental ducks administered water extract of combination of *Ocimum sanctum and Curcuma longa* had lower serum SGPT and SGOT concentrations compared to the other groups. The experimental ducks administered water extract of combination of *Ocimum sanctum and Curcuma longa* had higher serum estrogen and vitellogenin concentrations compared to the other groups. All of the experimental ducks had the same serum ureum concentrations. The experimental ducks administered water extracts of *Curcuma longa* and combination of *Ocimum sanctum and Curcuma longa* had higher creatinine and BUN concentration compared to control ducks and those administered *Ocimum sanctum*. It was concluded that the administration of water extracts of *Ocimum sanctum, Curcuma longa, and* combination of *Ocimum sanctum and Curcuma longa* prior to laying period at a dose of 10 mL/L drinking water increase growth performance and improve the liver function of laying ducks.

Key words: *Ocimum sanctum, Curcuma longa*, duck, estrogen, liver function.
Chronology of gonadal sex differentiation and steroidogenic potential in the rice field frog *Hoplobatrachus rugulosus*

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Abstract

*Hoplobatrachus rugulosus* is a frog that widely distribute throughout wetlands from central China to peninsular Malaysia. This frog is an economically important species and has potential to be an animal model in many fields of research. In this study, the process of gonadal sex differentiation of this frog was examined. The morphology of gonad in each stage of tadpole were observed under stereomicroscope. The results showed that the gonad could be observed at Gosner stage 35 (14 days post hatch, dph), while distinct testis and ovary were evidenced during 3 – 4 weeks after complete metamorphosis (stage 46, 29 dph). Histology showed that the ovarian differentiation began at stage 42 (23 dph). The testicular differentiation seemed to begin during the first week after complete metamorphosis. During this period, some individuals had intersex gonads with developing testicular tissue and oocytes/atretic oocytes. The fully developed testis, as evidenced by spermatogenesis, was identified at 6 weeks after complete metamorphosis. The result proves that, the males developed later than the females and the testis differentiation may occur through the process of oocyte degeneration. Further investigation on localization of *CYP17* (Cytochrome P450 17-hydroxylase/C17–20 lyase) mRNA was carried out to examine steroidogenic activity of the differentiated gonad. The results showed that positive signals for *CYP17* was found in interstitial cells of the testis at 5 weeks after complete metamorphosis as well as the subsequent weeks until the frogs were mature (16 weeks), but was not found in any ovarian tissue of the female frogs at the same cohort. The results suggested that *CYP17* may play a crucial role after the onset of testicular differentiation in maintaining spermatogenesis in *H. rugulosus*. Overall results suggested that the pattern of gonadal sex differentiation in *H. rugulosus* could be a semi-differentiated type that the testis developed through the intersex gonad.
Administration of Jamu ATOKE Prior to Mating in Maternal Rats Improves Serum Estradiol and Progesterone Concentrations during Pregnancy and the Quality of the Born Offspring

Wasmen Manalu\textsuperscript{a}, Andriyanto\textsuperscript{a}, Firda Agustin\textsuperscript{b}, Diki Yulianzah\textsuperscript{a}, Kharisma Mardatillah\textsuperscript{a}, and Natalia F. Lyla\textsuperscript{a}

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Abstract
Eighty female white Sprague-Dawley rats at the age of 10 weeks and weights range of 180-250 g were assigned in a Completely Randomized Design with 3 treatments and 6 replications. The treatment was dose of Jamu ATOKE consisted of 0, 2.5, and 5%. Jamu ATOKE was added to and mixed with drinking water and administered for 30 days before the experimental rats were pregnant. Parameters measured were serum estradiol and progesterone concentrations, motoric activity, and blood profile of maternal rats on day 16 postmating. The weights of the offsprings were measured on days 5 and 30 postpartum. Motoric activities and blood profiles of the offsprings were measured on day 30 postpartum. The results showed that the qualities of reproductive performance and reproductive health of rats administered with Jamu ATOKE were significantly improved compared to control rats without Jamu ATOKE administration. The rats administered with Jamu ATOKE at doses of 2.5 and 5% increased serum estradiol concentrations (by 30.62 and 39.19%, respectively) and progesterone concentrations (by 121.6 and 175.17%, respectively). The offspring rats born to maternal rats administered with Jamu ATOKE for 30 days before pregnancy had higher body weights and weight gain by 28.46% and 32.87% compared to control. The offspring born to rats administered with Jamu ATOKE at a dose of 2.5% showed the better motoric activity compared to those born to control group without Jamu ATOKE administration. This better intelligence was shown by the shorter duration of time required by the offspring born to maternal rats administered with Jamu ATOKE to find light and food (82.23 and 77.36%, respectively) compared to those born to controls maternal rats without Jamu ATOKE administration. It was concluded that administration of Jamu ATOKE was able to improve the quality of offspring by improving the quality of fertilities of the maternal rats.

Key words: Jamu ATOKE, estradiol, progesterone, health condition, motoric activity
The epididymis specific gene, Sperm associated antigen 11 (Spag11) as a possible marker for cancer

Sangita and Suresh Yenugu

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Abstract
The incidence of cancer is very rare in the epididymis. The molecular mechanisms underlying this fact are not clear and it is speculated that epididymal specific genes may govern such protection. We observed that the epididymis specific Sperm associated antigen 11e (Spag11e) gene expression was not detectable in rat epididymal and testicular cancer cells, though its expression is abundant in primary cells isolated from the tissues, indicating that Spag11e gene suppression may a critical event for cancer initiation and progression. To determine the role of Spag11e gene in contributing to the cancer free state of epididymis, we standardized isolation of epididymal primary epithelial cells and analyzed Spag11e gene expression. siRNA mediated suppression of Spag11e gene expression in isolated primary epididymal cells increased cell proliferation and influenced the expression of genes and proteins involved in cell proliferation and cancer progression. On the other hand, ectopic over expression of Spag11e gene in epididymal cancer cells decreased cell proliferation rate and also altered the expression of genes and proteins involved in cell proliferation and cancer progression. Results of our study indicate a possible role for Spag11e gene in protecting the epididymis from cancer.
Enteroendocrine peptides can regulate insect feeding behavior via controlling intestinal contraction

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Abstract
Here, we report a significant enteroendocrine regulatory mechanism in insect feeding behavior by controlling intestinal contraction. As the crude extract of the midgut of the silkworm B. mori larvae inhibited effectively on feeding motivation, we have purified and identified allatotropin (AT) and RYamide from the crude extract as the major feeding regulatory principles. Hypodermic injection of synthetic AT and RYamide into the transparent larvae fed ad libitum decreased their spontaneous esophagus contraction. Exposure of these peptides also inhibited the spontaneous contraction of the larval pharynx and ileum. Interestingly, RYamide did not inhibit the pharynx contraction in the starved larvae, indicating the possibility of different sensitivity against RYamide according to feeding states. Semi-quantitative analyses of RYamide in the larval midgut using MALDI-TOF MS spectrometry revealed that increased RYamide level was observed in the midgut of the starved larvae, while the RYamide level was significantly decreased in the midgut of the refed larvae compared with that of the starved larvae. This fluctuation of RYamide at the peptide level rather than the transcriptional level indicates that secretion of RYamide can be regulated by feeding condition. These findings proposed a novel enteroendocrine system, by which AT and RYamide modulate intestinal contraction, accompanied by transition of their feeding state, eventually influencing their feeding termination.
Tuesday 10 July 2018

Symposium 8: Bone and Mineral Endocrinology

Chair: Janine Danks

1630 - 1740

1630 - 1700
Deborah M Power\textsuperscript{1,2}, Ana Patricia Mateus\textsuperscript{1}, Rita Costa\textsuperscript{1} and Patricia IS Pinto\textsuperscript{1}

\textit{Regulation of skin and scale regeneration in marine teleost fish}
State of the Art Lecture 25 minutes + 5 minutes

1700 - 1720
Awf A. Al-Khan\textsuperscript{*}, Judith S. Nimmo, Mourad Tayebi, Stewart D. Ryan, James Simcock, Raboola Tarzi, Charles Kuntz, Eman S. Saad, Michael J. Day, Samantha J. Richardson and Janine A. Danks

\textit{Parathyroid hormone receptor 1 (PTHR1) is a prognostic indicator in osteosarcoma}
15 minutes + 5 minutes questions

1720 - 1740
Janine Danks

\textit{Calcium regulation was required for the evolution of the skeleton}
15 minutes + 5 minutes questions

\textbf{Asterisk (*) indicate student presentation}
State of the Art Lecture

Regulation of Skin and Scale Regeneration in Marine Teleost Fish

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Abstract
In vertebrates skin has a relatively well conserved organisation and is the largest neuro-immuno-endocrine organ and a key interface between the endocrine, nervous and immune systems. Fish skin resembles that of other vertebrates but it has become specialized for life in an aquatic environment and has an outer non-keratinized epithelia that covers calcified scales imbricated in the dermis. The endocrine regulation of skin in fish and the involvement of endocrine factors in damage repair when scales are lost is not very well explored. Microarray technology and next-generation sequencing have revealed hormones and receptors from different systems (including steroid or thyroid hormones and calcemic hormones) are expressed in the skin and are modified during damage repair. Comparative analysis of genes and their protein products that are present in the teleost fish skin transcriptome and that have a deleterious effect in humans and provoke ectopic/modified calcification may give insight into factors that regulate scale regeneration. Two candidate genes were analyzed to determine if they are involved in scale regeneration after damage, the ATP-binding cassette transporter 6 (ABCC6) gene and osteoglycin (OGN) both of which induce ectopic calcification in humans. The results indicated regulation of abcc6, the related gene abcc1 and the ogn duplicate genes during scale regeneration, revealing the strength of comparative approaches as a way to uncover structural and regulatory factors important in scale regeneration.

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Parathyroid Hormone Receptor 1 (PTHR1) is a Prognostic Indicator in Osteosarcoma

Awf A. Al-Khan, Judith S. Nimmo, Mourad Taybi, Stewart D. Ryan, James Simcock, Raboola Tarzi, Charles Kuntz, Eman S. Saad, Michael J. Day, Samantha J. Richardson and Janine A. Danks

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Department of Surgery, Southpaws Veterinary Hospital, Moorabbin, Australia.
School of Veterinary Sciences, University of Bristol, Bristol, United Kingdom.
The University of Melbourne, Department of Medicine, Austin Health, Australia.

Abstract

Osteosarcoma (OS) is the most common malignant primary bone tumor in dogs and humans. The characterisation of an appropriate natural disease animal model to study human osteosarcoma is essential to elucidate the pathogenesis of the disease. Several studies have established the vital role of parathyroid hormone-related protein (PTHRP) and its receptor (PTHR1) in bone formation and remodelling. In addition, these molecules play a role in the progression and metastasis of many tumor types. Currently in anatomical pathology, no reliable prognostic indicators have yet been identified for OS. This study aimed to validate canine OS as a model for the human disease by evaluating immunohistochemically the expression of markers known to be important in human OS. The immunohistochemical panel included vimentin, alkaline phosphatase (ALP), desmin, S100, neuron-specific enolase (NSE), runt-related transcription factor 2 (Runx2) and bone morphogenetic protein 4 (BMP4). This study also investigated if the detection of PTHR1 and PTHrP in canine OS could be correlated with survival time. Immunohistochemistry was conducted on formalin-fixed, paraffin wax-embedded tissue sections from 51 dogs with confirmed primary osteosarcoma. The staining intensity of PTHR1 and PTHrP was correlated with survival time. Vimentin, ALP, Runx2 and BMP4 were highly expressed by all tumours, while desmin, S100 and NSE were expressed variably. The findings were similar to those described previously for human OS. Both PTHR1 and PTHrP were detected in all OS samples. Dogs with strongly stained tumors for PTHR1 had significantly shorter survival times (P < 0.05) when compared with OS that had moderate or weak staining. In contrast, PTHrP staining intensity did not correlate with survival time (P > 0.05). This study suggest that canine OS may represent a useful model for the study of the human disease. The results of this study also indicate that increased expression of PTHR1 antigen in canine OS is associated with poor prognosis. This suggests that PTHR1 may be useful as a prognostic indicator in canine and human OS.
Calcium regulation was required for the evolution of the skeleton

Janine Danks

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Abstract
Tight calcium regulation delineates vertebrates from invertebrates. All vertebrates have a circulating calcium level of 2.2-2.6 mmol/L (9-10.5 mg/dl). Fish and higher vertebrates have both hypocalcemic (calcitonin & stanniocalcin) and hypercalcemic (parathyroid hormone & parathyroid hormone-related protein) factors. Stanniocalcin (STC) was first found in fish and then identified in mammals. Calcitonin (CT), parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP) were all described in mammals and subsequently isolated from fish.

Some of the fish species have multiple genes for both PTH and PTHrP. There were two copies of the PTH gene in elephant sharks (Callorhinchus milii) and these have not persisted in higher vertebrates indicating that one of these PTH genes has accumulated a number of deleterious mutations and has been lost in the process. The recent sequencing of the Japanese lamprey genome (Lethenteron japonicum) (Mehta, et al. Proc Natl Acad Sci U S A 110: 16044, 2013) is instructive. Lampreys have a pivotal position in evolutionary history, having undergone two whole genome duplications when compared to invertebrates. Like sharks they have a cartilaginous skeleton but have the ability to move from seawater to freshwater. The Japanese lamprey genome database has been interrogated for the presence of PTH and PTHrP. Certainly two receptors for PTH and PTHrP (pth1r and pth2r) are present in agnathan genome (Petromyzon marinus) (Pinheiro, et al. BMC Evol Biol 12:110, 2012). Two PTH receptors have also been identified in invertebrates (Ciona intestinalis) but the ligands have not been found (Kamesh, et al. BMC Evol Biol 8: 129, 2008).

Localization and physiological studies in a number of vertebrate species have demonstrated that some, if not all, of calcium regulating factors are important in skeletal formation and maintenance. These will be discussed with regards to the latest data on the evolution of these factors.
Tuesday 10 July 2018

Symposium 9: Environmental endocrinology: responses to the environment

Chair: John Cockrem

1630 - 1800

1630 - 1700
John Cockrem
The hypothalamo-pituitary-adrenal (HPA) axis and endocrine responses of animals to changes in their environment
State of the Art Lecture 25 minutes + 5 minutes

1700 - 1720
M C Subhash Peter
Integration of interrenal and thyroid axes during stress and ease response in fish
15 minutes + 5 minutes questions

1720 - 1740
Ondi L. Crino
Reproductive plasticity in wild zebra finches: trade-offs between the HPA and HPG axes in heterogeneous environments
15 minutes + 5 minutes questions

1740 - 1800
Vishwajit S. Chowdhury, John F. Cockrem and Mitsuhiro Furuse
Neuropeptide Y plays an anti-stress role in heat-exposed chicks
15 minutes + 5 minutes questions
State of the Art Lecture

The hypothalamo-pituitary-adrenal (HPA) axis and endocrine responses of animals to changes in their environment

John F. Cockrem

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Abstract
Animals live in environments that change from moment to moment, from day to day, and between seasons. These changes can be predictable, such as the daily cycle of light and dark, or unpredictable such as the approach of another animal. When stimuli from the external environment are perceived as threatening or potentially harmful the hypothalamo-pituitary-adrenal (HPA) axis is activated and glucocorticoid hormones are secreted by the adrenal gland. The increased secretion of the glucocorticoids cortisol or corticosterone in response to a stimulus is commonly known as a stress response. However, stress is a term that has negative connotations whereas a glucocorticoid response is a natural response that helps an animal to adjust to a change in its immediate environment. The name glucocorticoid, which refers to the metabolic action of these hormones to increase blood glucose concentrations, does not encompass the wide range of actions of cortisol and corticosterone. A glucocorticoid response is a response of an animal to a change in the environment. Glucocorticoid and behavioural responses of animals to environmental stimuli are linked, with consistent individual physiological and behavioural responses known as personality. Individual characteristics of an animal determine both the environment hormone and behaviour responses; environment hormone responses do not drive behaviour responses or vice versa. The magnitude of a glucocorticoid response of an animal to a threatening stimulus is thus related to the personality of the animal. In conclusion, glucocorticoids and personality together determine how animals respond to changes in their environment.
Integration of interrenal and thyroid axes during stress and ease response in fish

M C Subhash Peter

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Abstract
Fishes have developed a network of neuroendocrine components that controls the release of stress hormones. Cortisol, the product of hypothalamo-pituitary-interenal axis, is known for its direct control on the stress response in fish. Likewise, thyroid hormones (TH) both thyroxine (T₄) and triiodothyronine (T₃), the products of hypothalamo-pituitary-thyroid axis, interact with interrenal axis and show modified response patterns particularly during stressfull challenges. We examined the pattern of inter-hormonal interference that exists between thyroid and interrenal axes during stress and recovery responses in fishes. Immunocytochemical localization of Na⁺K⁺ ATPase, its protein and transcript abundance were quantified in the osmoregulatory epithelia of these fish. We found an interactive TH-cortisol function during recovery or ease response in fish. Analysis of these hormonal titres in the test species further indicates that T₄, T₃ and cortisol show a shift in their response patterns. This appears to be physiologically relevant as the differential response would offer a fine-tuning control on the physiological processes. These functional synergistic, antagonistic and permissive interactions of these hormones would thus provide evidence for an integrative hormone-driven physiological mechanism that would offer a functional basis for the survival of fishes in challenging environment.

(Supported by the iCEIB project)
Reproductive plasticity in wild zebra finches: trade-offs between the HPA and HPG axes in heterogeneous environments

Ondi L. Crino

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Abstract
Animals continually make physiological adjustments to survive and reproduce in their ever-changing environments. Glucocorticoid hormones are a class of steroid hormones that are released via activation of the hypothalamic-pituitary-adrenal (HPA) axis and modulate physiological and behavioural responses to changing environmental conditions. In response to poor environmental conditions, glucocorticoid secretion increases, promoting animals to prioritize self-maintenance over reproduction via down-regulation of the reproductive hypothalamic-pituitary-adrenal (HPG) axis. Birds that breed opportunistically in unpredictable environments are thought to maintain partial activation of the HPG axis in order to rapidly initiate breeding when environmental conditions become suitable. However, the endocrine mechanisms that influence opportunistic breeding in response to environmental cues remains poorly understood. Drawing on studies from captive and wild birds, I discuss how corticosterone (the dominant avian glucocorticoid) modulates HPG axis activity in opportunistically breeding Australian birds.
Neuropeptide Y plays an anti-stress role in heat-exposed chicks

Vishwajit S. Chowdhurya, John F. Cockremb and Mitsuhiro Furusec

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Abstract

Neuropeptide Y (NPY) is an appetite stimulating neuropeptide in mammals and birds. Recently it was found that brain NPY mRNA expression was increased in heat exposed chickens, and that central injection of NPY reduced body temperature in fasted, but not fed, chicks at thermoneutral temperatures and at high ambient temperatures. Central injection of NPY can affect the concentrations of brain monoamines and can reduce anxiety in rats. However, the functions of brain NPY during heat stress are unknown. Chickens are a good model animal in which to study heat stress as they do not have of sweat glands and are sensitive to high ambient temperatures. Central injection of NPY decreased brain serotonin and norepinephrine concentrations in fed chicks, but increased concentrations of brain dopamine in fasted chicks. Moreover, NPY reduced plasma corticosterone concentrations in fasted, but not fed, chicks under control thermoneutral and under high ambient temperatures. NPY enhanced brain mRNA expression of heat-shock proteins and NPY sub-receptors (Y5, Y6 and Y7) in chicks under both temperatures. Furthermore, central injection of NPY decreased plasma glucose and triacylglycerol under control thermoneutral and high temperatures, and reduced the increase of plasma epinephrine that occurs in chicks in high temperatures. Central injection of an NPY Y5 sub-receptor antagonist (CGP71683) at the same time as injection of NPY attenuated the NPY-induced hypothermia. These findings suggest that NPY may act to reduce body temperature and heat stress in chickens.
Wednesday 11 July 2018

Free day: see suggestions for sightseeing in Sydney in your conference bag.

Conference Dinner “The Refectory” 1800 - 2100

Thursday 12 July 2018

Symposium 5: Marine Invertebrates

Chair: Josephine Nocillado

Session 2 1100 - 1230

1100 - 1130
Chieh-Jhen Chen*, Shinya Shikina, Ching-Fong Chang
A novel female-specific and sexual reproduction-associated dmrt gene discovered in the stony coral, *Euphyllia ancora*
State of the Art Lecture 25 minutes + 5 minutes

1130 - 1150
Shinya Shikina, Yi-Ling Chiu, Ching-Fong Chang
*De novo* transcriptome analysis of the gonads in a stony coral *Euphyllia ancora* (Anthozoa, Cnidaria) to explore the intrinsic mechanisms underlying sexual reproduction of corals
15 minutes + 5 minutes questions

1150 - 1210
Kyeong Seop Kim, Mi Ae Kim and Young Chang Sohn
Phylogenetic and functional analysis of 5-Hydroxytryptamine receptors in Pacific abalone, *Haliotis discus hannai*
15 minutes + 5 minutes questions

1210 - 1230
Chi Chen*, Yao-Tse Chung, Guan-Chung Wu, Ching-Fong Chang
The physiological adaptation to high sulfide extreme environment in hydrothermal vent crab, *Xenograpsus testudinatus*
15 minutes + 5 minutes questions

Asterisk (*) indicate student presentation
State of the Art Lecture

A Novel Female-Specific and Sexual Reproduction-Associated Dmrt Gene Discovered in the Stony Coral, *Euphyllia ancora*

Chieh-Jhen Chen\textsuperscript{a}, Shinya Shikina\textsuperscript{a,c}, Ching-Fong Chang\textsuperscript{b}

\textsuperscript{a}Center of Excellence for the Oceans, National Ocean Taiwan University, Keelung, Taiwan
\textsuperscript{b}Department of Aquaculture, National Ocean Taiwan University, Keelung, Taiwan
\textsuperscript{c}Institute of Marine Environment and Ecology, National Ocean Taiwan University, Keelung, Taiwan

Abstract

The Dmrt (doublesex and mab3-related transcription factor) family, is a name originated from a combination of two sexual-related genes, doublesex (dsx) in *Drosophila melanogaster* and male abnormal (mab-3) in *Caenorhabditis elegans*. It is well known that Dmrt gene family plays important roles in sex determination, sexual differentiation and neuron development. In this research, seven full Dmrt gene transcript sequences (\textit{EaDmrta}, \textit{Eadmrhb}, \textit{Eadmrcc}, \textit{Eadmrtd}, \textit{Eadmrte}, \textit{Eadmr uf}, \textit{Eadmrtg}, \textit{Eadmrt h}) were obtained for a gonochoric coral, *Euphyllia ancora*. These \textit{Eadmrts} were subjected to quantitative assays measuring temporal and tissue-specific expression. Results demonstrated a unique gene expression pattern for \textit{Eadmrte}, which is enriched in female germ cells during the spawning season. Based on the phylogenetic analyses performed across the homologous Dmrt genes in metazoans, we found that the female-specific \textit{Eadmrte} gene is not related to the DM1 gene of *Acropora* spp. coral nor to \textit{Dmrt1} of vertebrates, which are involved in sexual reproduction, especially in sex determination (vertebrate \textit{Dmrt1}). In conclusion, we found the first female specific Dmrt gene (\textit{Eadmrte}) in corals, which may play a crucial role in oogenesis and could be an ideal biomarker for sex identification in cnidarian.
De novo transcriptome analysis of the gonads in a stony coral *Euphyllia ancora* (Anthozoa, Cnidaria) to explore the intrinsic mechanisms underlying sexual reproduction of corals

Shinya Shikina\textsuperscript{a,b}, Yi-Ling Chiu\textsuperscript{c,d}, Ching-Fong Chang\textsuperscript{b,e}

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\textsuperscript{b}Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan.
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Abstract

During the past few decades, various aspects of coral sexual reproduction, such as broadcast spawning, gametogenesis, sexuality (hermaphrodite or gonochoristic), and the environmental factors affecting sexual reproduction, have been extensively studied in many species and location primarily from ecological perspectives. Although fundamental findings have been accumulating, molecular and cellular regulatory mechanisms underlying sexual reproduction remain poorly understood in corals. In order to get a better understanding of molecular and cellular mechanisms of coral sexual reproduction, we selected a stony coral *Euphyllia ancora* as the experimental model, and performed a transcriptomic analysis on the isolated gonads in both sexes. As the results of literature supported searching, we successfully identified many important genes involved in sex determination/differentiation and germ cell development (i.e. spermatogenesis, oogenesis, oocyte growth) in various metazoans across taxa. Some genes related to steroid hormone synthesis and stress response were also identified. Moreover, the established database was demonstrated to be a useful platform for identifying a number of genes that exhibit sexually dimorphic expressions between testis and ovary. A large number of genes that currently lack annotation were also identified in the database. These findings suggest that although sexual reproduction in corals shares common molecular characteristics with that of other metazoans, it also largely possesses characteristics that developed in the unique way during the evolutionary process of corals. Our findings here and the established dataset would be valuable resources for studying the molecular, cellular, and intrinsic mechanisms underlying sexual reproduction and sexual differences in corals.
Phylogenetic and functional analysis of 5-Hydroxytriptamine receptors in Pacific abalone, *Haliotis discus hannai*

Kyeong Seop Kim\textsuperscript{a}, Mi Ae Kim\textsuperscript{b} and Young Chang Sohn\textsuperscript{a}

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Abstract

Serotonin (5-hydroxytriptamine; 5-HT) is a neurotransmitter involved in reproductive activity in marine invertebrates across different phyla. 5-HT can induce oocyte maturation as evidenced by germinal vesicle breakdown (GVBD) and the release of eggs and sperms in mollusks. Although several physiological characterizations have been carried out on 5-HT receptors (5-HTrs) in mollusks, the attempts to classify molluscan 5-HTrs with mammalian orthologs have only been partially successful. Accordingly, the 5-HT signal transduction mechanism through 5-HTrs related to maturation and spawning of gametes is not fully understood in marine invertebrates. To identify and classify 5-HTrs in the Pacific abalone *Haliotis discus hannai*, we performed transcriptome analysis of *H. discus hannai* and further found out eight subtypes of 5-HTr-like receptors having seven-transmembrane domain. Transcripts of 5-HTr1 and 5-HTr4 were predominantly expressed in the ovarian and testicular tissues, whereas the transcript of 5-HTr2 was detected in the ovary only. In the neural tissue including pleuro-pedal ganglion, all the eight 5-HTr transcripts were highly expressed in both sexes. Mammalian orthologs 5-HTr1, 5HTr2 and 5-HTr4 are related to cAMP signal transduction pathway with Gi/Go, Gq and Gs proteins, respectively. In this study, we are now evaluating transcriptional activity of cAMP response element-mediated luciferase reporter in either 5-HTr1- or 5-HTr4-transfected HEK-293 cells. 5-HT increased the frequency of spawning and enhanced GVBD in fully grown oocytes. Thus, the phylogenetic and functional analysis of the molluscan 5-HTr-like receptors in the gonads may help to provide novel insight into the molecular basis of gonadal maturation and spawning in marine invertebrates.
The physiological adaptation to high sulfide extreme environment in hydrothermal vent crab, *Xenograpsus testudinatus*

Chi Chen\(^a\), Yao-Tse Chung\(^b\), Guan-Chung Wu\(^b\), Ching-Fong Chang\(^c\)

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Abstract

Most animals cannot survive in a high sulfide environment. However, the hydrothermal crab lives in the high sulfide (>250 m) and low pH environment. Thus, hydrothermal crab provides a unique model to understand the adaptive mechanism of sulfide detoxification. To determine the ability for sulfide tolerance in different crabs, the mortality of swimming crab, *Thalamita danae*, and hydrothermal crab was compared in the hydrothermal zone (HZ) and non-hydrothermal zone (NHZ). After 2 hrs, the mortality of swimming crab was 100% while the hydrothermal crab was 0% in HZ incubation. To clarify the mechanism of sulfide detoxification pathway, we cloned SQR, a key enzyme in sulfide detoxification, and other sulfide detoxification-related genes (*tstd, ethe1, so*). To understand the relation between sulfide detoxification-related genes and sulfide, we compared the gene expression profiles in high sulfide condition (field) and low sulfide condition (raised in normal seawater for 1 month). According to qPCR analysis and western blot of tissue distribution, *sqr1* was dominantly expressed in anterior and posterior gill. *Sqr2* was dominantly expressed in digestive gland. Furthermore, *sqr1, sqr2* and *Sqr* expression was significantly increased in high sulfide condition compared with low sulfide condition. These data seemed to have some potential endocrine communications. Furthermore, no change was found in *sqr1, sqr2* and *Sqr* expression during the incubation of HZ and NHZ. In addition, no change was observed in *hsp60, hsp70, hsp90* in high sulfide and low sulfide conditions. These results might reveal sulfide was not a stress for hydrothermal crab. However, both of the thiosulfate and sulfite concentration in hemolymph were significantly increased in HZ compared with NHZ. In conclusion, our data suggested the rate of sulfide detoxification might be regulated by the enzyme activity instead of the transcription and translation level. In future, we would like to understand the endocrine regulation in adaptive mechanism.
Thursday 12 July 2018

Symposium 10: Neuroendocrinology

Chair: Jae Young Seong

1100 - 1230

1100 - 1130
Seongsik Yun, Yoo-Na Lee, Arfaxad Reyes-Alcaraz, Jong-Ik Hwang, Jae Young Seong
Neuropeptide spexin and its receptor GALR2: A link between depression and appetite
State of the Art Lecture 25 minutes + 5 minutes

1130 - 1150
Ryo Sakanoue*, Hirofumi Ohga, Fumiko Akase, Hajime Kitano, Keishi Sakaguchi, Kohei Ohta, Michiya Matsuyama
Molecular identification and ligand activity of Kiss1 and Kiss2 core peptides in interspecies of sixteen Scombridae fish
15 minutes + 5 minutes questions

1150 - 1210
Hitomi Seike, Yi Jun Zhou, and Shinji Nagata
Effects of RF-amide peptides on the feeding behavior in the two-spotted cricket, Gryllus bimaculatus, a new model insect
15 minutes + 5 minutes questions

1210 - 1230
Kazuyoshi Ukena, Kenshiro Shikano, Masaki Kato, Megumi Furumitsu, Eiko Iwakoshi-Ukena
Identification and function of two novel small secretory proteins, neurosecretory protein GL and neurosecretory protein GM, in the chicken hypothalamus
15 minutes + 5 minutes questions

Asterisk (*) indicate student presentation
State of the Art Lecture

Neuropeptide Spexin and Its Receptor GALR2: A Link between Depression and Appetite

Seongsik Yun, Yoo-Na Lee, Arfaxad Reyes-Alcaraz, Jong-Ik Hwang, Jae Young Seong
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Abstract
Major depressive disorder often accompanies drastic changes in appetite and body weight: some depressed individuals exhibited increased appetite, while others lose their appetite. However, molecular link between depression and appetite was not explored yet. The present study shows that neuropeptide spexin and its receptor galanin receptor 2 (GALR2) are involved in the depressive and appetite behaviors. The depressed mice were generated by implanting corticosterone (CORT) pellet for 3 weeks. These mice exhibited depression- and anxiety-like behaviors with significant loss of body weight. Daily administration of a spexin-based GALR2 agonist into the lateral ventricle of the mice, significantly restored the body weight of CORT-derived depressive mice while the same treatment of sham-operated mice reduced the body weight. The GALR2 agonist significantly improved the depression- and anxiety-like behaviors as revealed by tail-suspension, sucrose preference, elevated plus maze, and open field tests. The GALR2 agonist significantly increased c-fos expression in subpopulation of serotonergic neurons in the raphe nucleus. Increased c-fos expression was also determined in the prefrontal cortex, accumbens nucleus, arcuate nucleus and paraventricular nucleus which are implicated in the pathophysiology of depression and appetite. In addition, the similar effect was observed when the GALR2 agonist was administered intranasally. Thus, the present study shows that the spexin-GALR2 system is a missing link between appetite and depression and that the spexin-based GALR2 agonist may hold a promise for treatment of the mood/appetite disorders.
Molecular identification and ligand activity of Kiss1 and Kiss2 core peptides in interspecies of sixteen Scombridae fish

Ryo Sakanouea, Hirofumi Ohga b, Fumiko Akase a, Hajime Kitano b, Keishi Sakaguchi b, Kohei Ohta a, Michiya Matsuyama a

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bFisheries Research Institute of Karatsu, Kyushu University, Saga, Japan hohga@agr.kyushu-u.ac.jp

Abstract
Kisspeptin is an RFamide peptide and causes pubertal onset in mammals. Chub mackerel (cm: Scomber japonicus) possess two kisspeptins (Kiss1 and Kiss2) and each receptors (KissR1 and KissR2); our recent study showed that GnRH1 neurons co-expressed KissR1 and KissR2 localized in vicinity of GnRH1 neurons. In addition, administration of kisspeptin peptide influenced activation of reproductive axis and gonadal development in immature fish. These results suggest that kisspeptin is important to the reproduction of cm and may be a potentially novel analog in the acceleration of maturation in Scombridae species like tunas. In this study, first, we characterized the Kiss1 and Kiss2 core sequences in Scombridae (3 Scomber, 7 Thunnus, 1 Katsuwonus, 1 Scomberomorus, 1 Euthynnus, 1 Sarda and 2 Auxis). Kiss1 core sequences are based on 15 amino acids (aa) and showed high homology (>86%) between Scombridae species. However, Scomber fishes showed an addition of histidine (H) residue at the N-terminal end. Furthermore, residues from 13 and 14 up-stream of C-terminal end showed variation in interspecies. We identified five types of peptides in 16 species. Kiss2 core sequences are 12 aa also showed high homology (>91%). Residue from 11 up-stream of C-terminal end is asparagine (N) in Scomber fishes, while other species have lysine (K). Then, we examined the binding affinity of synthetic Kiss1 and Kiss2 core peptides in 16 Scombridae species using SRE-Luc reporter system. We employed receptors from cm, Pacific bluefin tuna (Thunnus orientalis), and Japanese Spanish mackerel (Scomberomorus niphonius). All tested Kiss1 and Kiss2 core peptides showed highly and equally signal transduction potency for KissR1 and KissR2 of three species, respectively. The present study may lead to a possible development of common Kiss1 and Kiss2 analogs in artificial maturation and may contribute to the development of reproductive physiology and aquaculture of Scombridae species.
Effects of RF-amide peptides on the feeding behavior in the two-spotted cricket, *Gryllus bimaculatus*, a new model insect

Hitomi Seikea, Yi Jun Zhoua, and Shinji Nagataa

aGraduate School of Frontier Sciences, the University of Tokyo, Kashiwa-no-ha 5-1-5, Kashiwa city 277-8562, Chiba, Japan. shinjin@edu.k.u-tokyo.ac.jp

Abstract

We, here, present a new model insect, the two-spotted cricket *Gryllus bimaculatus* which has many advantageous characteristics for molecular biological experiments; adequate big size to observe, availability of an artificial diet, an omnivorous feeder convenient for investigation of decision-making or preference in feeding behavior. In particular, manipulation of transcriptional levels by RNA interference (RNAi) is remarkably effective to prepare knockdown the gene of interest. Also, we already have the automated observation system in a two-choice assay for different nutritional diets by this species (Fukumura and Nagata, 2017). In our previous reports, self-selective feeding mechanisms have been observed by knockdown targeting several endocrine regulations such as AKH-signaling in this species (Konuma et al., 2012; Fukumura et al., 2017; Zhou et al., 2018). We, therefore, aim to address the self-selective feeding mechanisms to maintain energy balance by the total endocrine controlling network in this species. RNA-sequencing analyses revealed almost all insect peptidyl endocrine factors including several neuropeptides sharing C-terminal RF-amide, which have been reported as opposing effective factors on feeding modulation from other insect species. To further address the self-selective functions through those peptides having RF-amide, we analyzed the effects of two extended RF-amide factors, Neuropeptide F and Myosuppressin, on the feeding motivation and amount of food intake in *G. bimaculatus* as the representative positive and negative factors, respectively. Administration of these chemical synthetic peptides and the transcriptional knockdown experiments of the peptides and their receptors using RNAi revealed the possibilities of weak contribution to feeding processes, which are somewhat distinctive from those in the other insect species. The present data indicates that different endocrine mechanisms exert the self-selective feeding in the omnivorous feeders and provides the evolutionary aspects of endocrine system in the feeding behavior.
Identification and function of two novel small secretory proteins, neurosecretory protein GL and neurosecretory protein GM, in the chicken hypothalamus

Kazuyoshi Ukena, Kenshiro Shikano, Masaki Kato, Megumi Furumitsu, Eiko Iwakoshi-Ukena

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Abstract
To find novel neuropeptide and/or peptide hormone precursors in the avian brain, we performed a cDNA subtractive screen of the chicken hypothalamic infundibulum, which contains instinct behavioral center. After sequencing 596 clones, we identified a novel cDNA encoding a previously unknown protein. The deduced precursor protein consisted of 182 amino acid residues, including one putative small secretory protein of 80 amino acid residues. This small protein was flanked at the N-terminus by a signal peptide and at the C-terminus by a glycine amidation signal and a dibasic amino acid cleavage site. Because the predicted C-terminal amino acids of the small protein were Gly-Leu-NH₂, the small protein was named neurosecretory protein GL (NPGL). Quantitative RT-PCR analysis demonstrated specific expression of the NPGL precursor mRNA in the hypothalamic infundibulum. Furthermore, the mRNA levels in the hypothalamic infundibulum increased during post-hatching development. In situ hybridization and immunohistochemical analysis revealed that the cells containing NPGL were localized in the medial mammillary nucleus and infundibular nucleus within the hypothalamic infundibulum. Subcutaneous infusion of NPGL in chicks increased body weight gain without affecting food intake. On the other hand, the chronic intracerebroventricular infusion of NPGL stimulated food and water intake, with a concomitant increase in body mass. Our findings indicate that NPGL participates in energy metabolism including feeding behavior in chicks. On the other hand, genome database analysis suggested that the NPGL gene has a paralogous gene in chickens, named neurosecretory protein GM (NPGM). We will also show the recent data of NPGL and NPGM in chicks.
Thursday 12 July 2018

Poster session 2

1300 - 1430

**Poster number:**

   *Resistin inhibits insulin stimulation of preadipocyte growth*

2. Masato Kitazawa*, Yusuke Matsumoto, Shinya Turuoka, Tsuyoshi Ohira
   *Localization of an insulin-like androgenic gland factor (IAG) in the kuruma prawn Marsupenaeus japonicas*

3. Mikako Shibata*, Tatsuyuki Takahashi, Takaharu Kozakai, Yoshiyuki Azuma, Yohei Kurose
   *Inhibition of intestinal glucose absorption by leptin and its intracellular signaling pathway in broiler chickens*

4. Haruna Amano, Seiichi Uno, Jiro Koyama, Naoshi Hiramatsu, Takashi Todo, Akihiko Hara
   *Development of specific enzyme-linked immunosorbent assays for multiple vitellogenins in marbled sole, Pleuronectes yokohamae*

5. Masafumi Hanazuka*, Keisuke Kaji, Yukihiro Yoshida, Naoaki Tsutsui, Tsuyoshi Ohira
   *cDNA cloning and characterization of two crustacean hyperglycemic hormone-family peptides with vitellogenesis-inhibiting hormone activity from Trachysalambria curvirostris*

6. Yusuke Matsumoto*, Shinya Turuoka, Tsuyoshi Ohira
   *Annual changes in gene expression of an insulin-like androgenic gland factor (IAG) in the Pacific grass shrimp Palaemon pacificus*

7. Yu Chen*, Haipei Tang, Le Wang, Xiaochun Liu, Haoran Lin
   *Fertility enhancement but premature ovarian failure in esr1-deficient female zebrafish*

8. Haipei Tang, Yu Chen, Le Wang, Xiaochun Liu, Haoran Lin
   *Fertility impairment with defective spermatogenesis and steroidogenesis in male zebrafish lacking androgen receptor*

9. Yuko Ambo*, Sayaka Kotaka, Tsuyoshi Ohira
   *Preparation of a recombinant crustacean female sex hormone of the kuruma prawn Marsupenaeus japonicas*

10. Huong Thi Nguyen*, Sunam Mander, Dong Hwi Kim, Yun Hee Na, Hyo Jeong Yong, Jae Young Seong, Jong-Ik Hwang
    *Real time analysis on interaction kinetics of chemokine receptors and β-arrestin*
11. Meaghan K. Smith*, Bronwyn Rotgans, Utpal Bose, Abigail Elizur, Cherie A. Motti, Scott F. Cummins
   *Brainless Matters. Neural investigations in the crown-of-thorns seastar, Acanthaster planci*

12. João CR Cardoso, Vinicius Ferreira, Rute C Félix and Deborah M Power
   *Regulation of shell formation by the mantle in bivalves*

13. Valsa S. Peter and M.C.Subhash Peter
   *Impact of nitric oxide inhibitor NAME on cortisol and thryoid hormone dynamics, gas transport and acid/base balance during hypoxia stress in air-breathing fish (Anabas testudineus Bloch)*

   *Environmental decay of faecal glucocorticoid metabolites in bare-nosed wombat (Vombatus ursinus) scats when exposed to natural climatic conditions*

15. Sunam Mander*, Dong-Joo You, Sumi Park, Dong Hwi Kim, Hyo Jeong Yong, Jae Young Seong, Jong-Ik Hwang
   *Anti-metastatic effect of Nafamostat mesilate on breast cancer cells*

*Asterisk (*) indicate student presentation*
Poster number 1

Resistin inhibits insulin stimulation of preadipocyte growth

Chia-How Chu\textsuperscript{a}, Kuo-Yu Liao\textsuperscript{a,b}, An-Ci Siao\textsuperscript{a}, Yen-Yue Lin\textsuperscript{b}, Yi-Wei Tsuei\textsuperscript{b}, Chi-Wei Liu\textsuperscript{c}, Shih-Wei Lee\textsuperscript{c} and Yung-Hsi Kao\textsuperscript{a}

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Abstract

Resistin (Rstn) and insulin have been reported to regulate fat cell activity and insulin resistance, respectively. This study investigated the pathways involved in Rstn modulation of insulin-stimulated growth in 3T3-L1 preadipocytes. Rstn inhibited insulin stimulation of preadipocyte growth in a dose- and time-dependent manner. Pretreatment with specific inhibitors of extracellular signal-regulated kinase (ERK)-1 and 2, such as U0126 and PD98059, did not reverse the anti-insulin effect of Rstn on cell growth. However, the inhibitors of both p38 mitogen-activated protein kinase (MAPK) (e.g., SB203580) and phosphatidylinositol 3-kinase/AKT (e.g., wortmannin) prevented the effect of Rstn. It was interesting that treatment with an inhibitor of c-Jun N-terminal kinase (JNK), such as SP600125, enhanced the anti-insulin effect of Rstn on preadipocyte growth. These data suggest that Rstn selectively affects particular types of MAPK family members in regulating insulin stimulation of preadipocyte growth. Moreover, treatment with an inhibitor of Janus kinase (JAK)-2, such as AG490, blocked the anti-insulin effect of Rstn. These results imply that Rstn mediates anti-insulin signaling in preadipocyte growth via the p38 MAPK, PI3K/AKT, and JAK2, but not ERK or JNK MAPKs, pathways. As Rstn was also found to inhibit insulin stimulation of preadipocyte glucose uptake, the observed anti-insulin effect of Rstn on preadipocyte growth may be mediated through modulation of energy homeostasis.
Localization of an insulin-like androgenic gland factor (IAG) in the kuruma prawn Marsupenaeus japonicas

Masato Kitazawa\textsuperscript{a}, Yusuke Matsumoto\textsuperscript{a}, Shinya Turuoka\textsuperscript{a}, Tsuyoshi Ohira\textsuperscript{a}

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Abstract
The male secondary sex characteristics of crustacean are controlled by an androgenic gland hormone (AGH), which is specifically synthesized by and secreted from the androgenic glands (AG) attached to apical part of the vas deferens. Until now, AGH has been purified and structurally determined only from the terrestrial isopod Armadillidium vulgare. AGH-like peptides have also been found in several decapod species and designated insulin-like androgenic gland factors (IAG). IAG was localized in the AG cells of the blue swimmer crab Portunus pelagicus by immunohistochemical study. In addition, IAG was expressed in the hepatopancreas of P. pelagicus by RT-PCR. Recently, a cDNA encoding the kuruma prawn Marsupenaeus japonicas IAG (Maj-IAG) has been cloned. In order to elucidate the localization of Maj-IAG-producing cells, \textit{in situ} hybridization was conducted in this study. Male reproductive organs were dissection from \textit{M. japonicas} were fixed in Bouin’s fixative for overnight at 4\textdegree{}C. The fixed samples were embedded in paraffin and sectioned at a thickness of 10 \mu{}m. The sections of apical part of the vas deferens attached with AG were subjected to \textit{in situ} hybridization. Antisense cRNA probe of Maj-IAG was hybridized with the AG cells specifically. No signals were detected in the negative control, in which the sense cRNA probe was emplyed. Next, the sections of distal part of the vas deferens were subjected to \textit{in situ} hybridization. A clear signals were observed in the cells located at the inner surface of vas deferens. This is the first result that IAG gene was expressed at vas deferens except for the AG. This result suggests that the vas deferens Maj-IAG may have different functions from Maj-IAG expressed at the AG.
**Poster 3**

**Inhibition of intestinal glucose absorption by leptin and its intracellular signaling pathway in broiler chickens**

Mikako Shibata\(^a\), Tatsuyuki Takahashi\(^a\), Takaharu Kozakai\(^b\), Yoshiyuki Azuma\(^a\), Yohei Kurose\(^a\)

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**Abstract**

Leptin, which inhibits food intake and increases energy expenditure, is primarily secreted by adipocytes in mammals. Leptin is also synthesized by stomach and inhibits glucose absorption through leptin receptors expressed in the small intestine. In avian such as chickens, however, the effects of gastric leptin on intestinal glucose absorption and its intracellular signaling pathway have yet to be clarified. So we investigated the role of gastric leptin in intestinal glucose absorption and its molecular mechanisms in 1-week-old broiler chicks. In Experiment 1, leptin receptor mRNA expression was confirmed with RT-PCR in the distal jejunum of the chicks. In Experiment 2, the chicks orally received saline plus D-glucose (2 g/kg) or murine leptin (1 mg/kg) plus D-glucose after 12-hour fasting. Then, blood samples were taken at various intervals. Blood glucose concentrations reached a peak at 20 min in both treatments, but those in leptin treatments were significantly less than those in saline treatments. In Experiment 3, using the Ussing chamber technique, the Na\(^+\)-dependent glucose absorption across isolated jejunal epithelium was evaluated as the change of short-circuit current (ΔI\(_{sc}\)) and membrane conductance with the addition of leptin (10 and 100 nM), SC79 (Akt activator), AICAR (AMPK activator), LY294002 (PI3K inhibitor) and/or Wortmannin (PI3K inhibitor) in the culture medium. The addition of D-glucose (100 μM) to the mucosal side medium clearly elevated ΔI\(_{sc}\), whereas the pre-treatment of leptin (10 nM) in the mucosal side significantly decreased glucose-induced ΔI\(_{sc}\) elevation. Moreover, glucose-induced ΔI\(_{sc}\) elevation was significantly decreased by SC79 (1 or 10 μM), but not by AICAR (2.5 mM). The inhibition of PI3K signaling by LY294002 (50 μM) or Wortmannin (1 μM) abolished the leptin-induced ΔI\(_{sc}\) reduction. In conclusion, leptin inhibits the intestinal glucose absorption via PI3K/AKT signaling elicited by jejunal leptin-receptor activation in broiler chickens.
Development of specific enzyme-linked immunosorbent assays for multiple vitellogenins in marbled sole, *Pleuronectes yokohamae*

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Abstract

Teleost vitellogenin (Vtg) is a major precursor of egg yolk proteins; lipovitellin (Lv) is one of Vtg-derived yolk proteins. Three subtypes of Vtg have been identified in highly advanced teleosts. Two of which are referred to as “complete” form (VtgAa and VtgAb). The third form that largely consists of Lv domain alone is referred to as “incomplete” form (VtgC). These Vtgs naturally appear in the blood of reproductive female fishes, while they can be produced in male and immature fish only when exposed to exogenous estrogens. Therefore, Vtgs in male and immature fish can be utilized as useful biomarkers for detecting estrogenic contamination in an aquatic environment. The marbled sole, *Pleuronectes yokohamae*, has been used as a model species for evaluation of estrogenic activities in aquatic environments. In our previous study three Lvs (LvAa, LvAb and LvC) were purified from the sole and three corresponding antisera were generated. The objective of this study is to develop enzyme-linked immunosorbent assays (ELISA) using these specific antisera for the quantification of each subtype of sole Vtgs.

The Lv antibodies were labeled with digoxigenin and used in non-competitive sandwich ELISAs with purified standard Lvs. The measurable range of all developed ELISAs was from 0.97 to 1000 ng/ml. Each ELISA was confirmed to be specific to the targeted Vtg subtype. Three Vtg subtypes were induced in immature fish by an estradiol-17β (E2) treatment. All Vtgs were induced from 1 day after E2 injection and reached to their peak levels at three days after the injection. Levels of VtgAb as LvAb equivalent were the highest among subtypes during the experiment. The present study described the first report on the development of subtype-specific Vtg ELISAs in this sole, which enabled us to perform the detection and monitoring of estrogenic contamination in aquatic environments.
cDNA cloning and characterization of two crustacean hyperglycemic hormone-family peptides with vitellogenesis-inhibiting hormone activity from *Trachysalambria curvirostris*

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**Abstract**

In crustaceans, essential physiological phenomena are regulated by neuropeptides, crustacean hyperglycemic hormone (CHH)-family peptides, which are synthesized in and secreted from the X-organ/sinus gland complex in the eyestalk. Vitellogenesis-inhibiting hormone (VIH) belongs to the CHH-family. In order to characterize multiple VIH molecules in the southern rough shrimp *Trachysalambria curvirostris*, one hundred eighty six sinus glands were dissected under stereo microscope and subsequently sinus gland peptides were extracted. Two CHH-family peptides were purified by reversed-phase HPLC and subjected to N-terminal amino acid sequencing, which identified more than 51 amino acid residues. The two CHH-family peptides showed considerable sequence similarity to CHHs characterized from other species. However, the two CHH-family peptides showed no hyperglycemic activity by \textit{in vivo} injection assay. Therefore, vitellogenesis-inhibiting activities of the two CHH-family peptides were examined using \textit{ex vivo} incubation of ovarian fragments of the kuruma prawn *Marsupenaeus japonicus*. The two CHH-family peptides reduced vitellogenin mRNA levels in the ovarian fragments. From these results, the two CHH-family peptides were designated as Trc-VIH-I and Trc-VIH-II, respectively. In order to elucidate their complete amino acid sequences, cDNAs encoding Trc-VIH-I and Trc-VIH-II were cloned by RT-PCR coupled with 5’- and 3’-RACE. The mature Trc-VIH-I and Trc-VIH-II were found to consist of 72 amino acid residues containing six conserved cysteine residues and possess an amidated C-terminus, respectively. The mature Trc-VIH-I and Trc-VIH-II showed the highest sequence similarity with a CHH-family peptide having vitellogenesis-inhibiting activity in penaeid shrimp species, \textit{Litopenaeus vannamei} VIH (Liv-SGP-G). The mature Trc-VIH-I and Trc-VIH-II shared amino acid identities 63.0% and 74.3% with Liv-SGP-G, respectively. These results indicate that Trc-VIH-I and -II regulate vitellogenesis in *T. curvirostris*. 
Annual changes in gene expression of an insulin-like androgenic gland factor (IAG) in the Pacific grass shrimp *Palaemon pacificus*

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**Abstract**

Androgenic gland hormones (AGH) have been characterized from only isopod crustaceans. Isopod AGH is synthesized by and secreted from the androgenic glands (AG) that are associated with the posterior region of the sperm duct. AGH plays a role in the control of sex differentiation. AGH-like peptides have been found in several decapod species, and designated as insulin-like androgenic gland factors (IAG). IAG was localized in the AG cells of the blue swimmer crab *Portunus pelagicus* by immunohistochemical study. Recently, IAG cDNA was cloned from the Pacific grass shrimp *Palaemon pacificus* in our previous study. In order to elucidate the annual changes in gene expression of *P. pacificus* IAG (Papac-IAG) in the AG cells, *in situ* hybridization was conducted in this study. Shrimps were collected from the sea every month. Male reproductive organs were dissected from *P. pacificus* and were fixed in Bouin’s fixative for overnight at 4°C. The fixed samples were embedded in paraffin and sectioned at a thickness of 7 µm. The sections were subjected to hematoxylin-eosin staining. AG cells of *P. pacificus* were largest in July and smallest in November. These annual changes were correlated with breeding season. Next, *in situ* hybridization was conducted. Antisense cRNA probe of Papac-IAG was hybridized with the AG cells specifically. No signals were detected in the negative control, in which the sense cRNA probe was employed. Gene expression of Papac-IAG increased in the breeding season, and decreased in the non-breeding season. These results suggest that Papac-IAG expressed in the AG cells might be involved in the Spermatogenesis.
Poster 7

Fertility enhancement but premature ovarian failure in esrl-deficient female zebrafish

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Abstract

It is well established that estrogens regulate female reproduction through estrogen receptors (ERs) in the ovary. However, the precise physiological role of estrogen/ER signaling in reproduction processes remains poorly defined in zebrafish. In this study, we successfully generated an ERα (esrl) mutant line in zebrafish via transcription activator-like effectors nucleases (TALENs). It was found in the mutant females that the fertility was enhanced and the ovarian histology was normal at 90 days post-fertilization (dpf). However, the number of fertile females decreased with age. By 180 dpf, esrl mutant females were infertile with degenerated ovaries, while the age-matching wild-type females were still fertile. Additionally, few large size of vitellogenic granules can be found in full grown (FG) follicles at 90 dpf and the expression of vtg genes were down-regulated at both 90 and 180 dpf in esrl mutant zebrafish. Moreover, steroidogenesis pathway and mTOR signaling pathway were over-activated at 90 dpf, but declined prematurely in esrl mutant zebrafish by 180 dpf. Collectively, the present study provides evidences that esrl is fundamental for ovarian maintenance in zebrafish.
Poster 8

Fertility impairment with defective spermatogenesis and steroidogenesis in male zebrafish lacking androgen receptor

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Abstract

The pivotal role of androgen receptor (AR) in regulating male fertility has attracted much research attention in the past two decades. Previous studies have shown that total AR knockout would lead to incomplete spermatogenesis and lowered serum testosterone levels in mice, resulting in azoospermia and infertility. However, the precise physiological role of $ar$ in controlling fertility of male fish is still poorly understood. In this study, we have established an $ar$ knockout zebrafish line by transcription activator-like effectors nuclease. Homozygous $ar$ mutant male fish with smaller testis size were found to be infertile when tested by natural mating. Intriguingly, a small amount of mature spermatozoa was observed in the $ar$ mutant fish. These mature spermatozoa could fertilize healthy oocytes, albeit with a lower fertilization rate, by in vitro fertilization. Moreover, the expression levels of most steroidogenic genes in the testes were significantly elevated in the $ar$ mutants. In contrast, the levels of estradiol and 11-ketotestosterone (11-KT) were significantly decreased in the $ar$ mutants, indicating that steroidogenesis was defective in the mutants. Furthermore, the protein level of LHβ in the serum decreased markedly in the $ar$ mutants when compared with wild-type fish, probably due to the positive feedback from the diminished steroid hormone levels. In summary, our results provided unequivocal in vivo evidence for the requirement of functional $ar$ in maintaining normal spermatogenesis and steroidogenesis, in ensuring normal fertility in male zebrafish.
Poster 9

Preparation of a recombinant crustacean female sex hormone of the kuruma prawn Marsupenaeus japonicus

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Abstract
Recently, crustacean female sex hormone (CFSH) has been characterized from the female eyestalk of the crab Callinectes sapidus. In the kuruma prawn Marsupenaeus japonicus, a CFSH (Maj-CFSH) cDNA has been cloned previously. Maj-CFSH gene was expressed in both female and male eyestalks. This result suggests that Maj-CFSH might not be a female sex hormone in M. japonicus. In order to characterize the function of Maj-CFSH, we tried to express a recombinant Maj-CFSH (rMaj-CFSH) using an Escherichia coli expression system in this study. A Maj-CFSH cDNA insert was prepared by PCR and subsequently ligated into an expression vector pET44a. The expression construct was designed to produce rMaj-CFSH with an N-terminally fused Nus-tag. In addition, the resulting fusion protein contained two regions of His-tag on both sides of the Nus-tag. Escherichia coli was transformed with the expression construct. The transformed bacterial cells were incubated in LB medium containing ampicillin, and then IPTG was added. After cell breakage, the soluble and the insoluble fractions were obtained and subsequently subjected to SDS-PAGE. As a result, specific band of the recombinant fusion protein was detected at 87 kDa in the insoluble fraction. The insoluble fraction containing the recombinant fusion protein was solubilized with 8 M urea and applied to a Ni-NTA column for affinity purification. A single band of the purified recombinant fusion protein was observed. After desalting by ultrafiltration, the recombinant fusion protein was digested with TEV protease. The resulting products was subjected to western blotting using an anti-Maj-CFSH antibody. Single immunopositive band was observed at 18 kDa. This value coincided well with the theoretical values of 18,168. Thereafter, only rMaj-CFSH was purified using a reverse-phase cartridge. Using this recombinant peptide, we are attempting to examine the function of Maj-CFSH at present.
Poster 10

Real time analysis on interaction kinetics of chemokine receptors and β-arrestin

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Abstract

Atypical chemokine receptor 3 (ACKR3) or C-X-C chemokine receptor 7 (CXCR7) is a member of G-protein coupled receptor family. To date, two chemokines were indentified as interacting chemokine: SDF1α/CXCL12 which also binds to CXCR4 and I-TAC/CXCL11 which also stimulates CXCR3. Unlike CXCR4 and CXCR3, which can couple and activate Gαi/o protein, CXCR7 has been proposed to solely recruit β-arrestin and acts as scavenger for the ligands. However, upstream G protein molecular and signaling events of CXCR7 remain poorly defined and controversial. Thus, in this work we tried to figure out the mechanism of CXCR7 signalling pathway. First, to evaluate the kinetic interaction of chemokine receptors and β-arrestin in living human cells, we used NanoBit technology which has been developed recently. A two-subunit system including Large Bit (LgBit) and Small Bit (SmBit) was fused to the terminal of receptors and β-arrestin. Accordingly, the GPCR-β-arrestin association results in a pair of subunits that produces a bright luminescence signal then can be measured real-time. Here we observed a much greater β-arrestin recruitment to CXCR7 after being stimulated by SDF1α or I-TAC than that of CXCR4 or CXCR3. Moreover, when CXCR4 and CXCR7 are coexpressed, CXCR7 competed against CXCR4 for binding with CXCL12. Finally, further study showed that the interaction of CXCR7 with β-arrestin was not sensitive with PTx- Gαi/o inhibitor, opposing to that of CXCR4 and CXCR3. This data confirmed that CXCR7 could not activate and make signalling through Gαi/o protein. In summary, these results advance our understanding of CXCR7 distinctive signalling properties compared to other chemokine receptors.
Poster 11


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Abstract
The Crown-of-Thorns Seastar (COTS), *Acanthaster planci* cf. *solaris* causes unprecedented damage to coral on the Great Barrier Reef, due to explosive population outbreaks. Current control methods are laborious and expensive with extrapolated estimates predicting a decline to less than 10% coral cover remaining on the reef in coming decades, unless intervened. With cyclic outbreaks of COTS a constant threat to the reef, there is a necessity to better understand COTS biological cycles and factors driving the population explosions and subsequent collapse. Lack of knowledge into molecular regulation of COTS biological processes (e.g. growth and reproduction) is one area where effective control methods are currently hindered. Exploration of the neural system and neurotransmitters in COTS is one area in critical need of research. Through this study, we present the most inclusive investigation of COTS neural system to date. The ultrastructure of COTS radial nerve cord reveals unique bulbous projections from the ecutoneral surface which appear only in *A. planci* and not in the close relative, *Acanthaster brevispinus*, nor any other seastar. Employment of -omic technologies allowed for a thorough investigation of the neuropeptide and small molecule neurotransmitters present in the radial nerve cord, specifically the bulbous structures. Neuropeptide investigations revealed a total of 48 neuropeptide precursors, including 10 novel neuropeptides, some of which elicit contractile bioactivity in COTS tube-feet. The seastar neuropeptide responsible for final oocyte maturation, Relaxin-Gonad-Stimulating Peptide (RGP) was produced in a recombinant system and successfully induced oocyte maturation and spawning, with fertilization success and larval development. This peptide is of critical relevance to COTS reproductive biology and is a strong candidate for novel biocontrol strategies. A comparative mass spectral analysis looking at small molecule neurotransmitter abundance between adult male and female COTS, and when food-deprived (~90 days) revealed (i) females contain higher levels of GABA, (ii) histamine and epinephrine increase following food deprivation, and (iii) serotonin and melatonin decrease following food deprivation. Most enzymatic biosynthesis pathway genes were identified in the COTS genome. These findings are consistent with food deprivation causing reproductive and regenerative downregulation in COTS, indicating a possible cause of the population collapse following mass outbreaks.
Regulation of shell formation by the mantle in bivalves

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Abstract
Shells are the calcareous biomineralised exoskeleton of bivalves, one of the most speciose class of the phylum Mollusca. The bivalve shell is mostly composed by calcium carbonate (CaCO₃) crystals that are trapped within an organic matrix protein layer. The mantle is a multifunctional tissue and is responsible for bivalve shell production but regulation of shell formation remains enigmatic. The objective of the present study was to determine if there is evidence of intrinsic modulation of mantle function in mussels (Mytilidae). We exploited the recent increase in the number of available mollusc genomes and transcriptomes to extend knowledge and understanding about the evolutionary origin of important metazoan endocrine gene families involved in calcium regulation. G-protein coupled receptors (GPCRs) and potential ligands were explored in available genome and transcriptome data from different species. Experiments were conducted with mussels to provoke a change in shell production or to assess the capacity of regulatory factors to modulate shell formation. Since carbonic anhydrases (CA), are a group of enzymes that play an important role in shell formation (they catalyze the rapid interconversion of carbon dioxide to calcium carbonate) we used this to assess how regulatory factors changed shell production. We identified in mollusc genomes and transcriptomes a homologue system of the human calcitonin-GPCR system. In mussel, the calcitonin-GPCR system has undergone a gene family expansion and representatives of the family are expressed in the mantle edge region. We demonstrate conserved function of the calcitonin system in calcium regulation and link this with CA enzyme activity.

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Poster 13

Impact of nitric oxide inhibitor NAME on cortisol and thyroid hormone dynamics, gas transport and acid/base balance during hypoxia stress in air-breathing fish (Anabas testudineus Bloch)

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Abstract
Nitric oxide (NO), a gasotransmitter that regulates many physiological activities, has been shown to drive ion transport functions in peripheral tissues of fishes including air-breathing fish. However, the role of NO in cortisol and thyroid hormone dynamics, gas transport and acid/base balance particularly during stress response is not yet known in fish. We, therefore, explored the response of gas and acid/base variables and thyroid hormone and cortisol status in a NO-depleted air-breathing fish (Anabas testudineus) exposed to hypoxia stress. A dose-responsive action of NAME on arterial blood PO2, PCO2, tO2 and tCO2 contents was found, which showed a modified pattern in hypoxia-induced fish. Likewise, a correlation was found between carbonic anhydrase (CA) activity in RBC lysate and blood HCO3 levels. Similarly, HCO3 levels showed a correlation with the CA activity in gills, kidney and intestine, the major acid/base-regulating epithelia. NAME treatment had little influence on the dynamics of TH but provides a modified cortisol dynamics. A differential and spatial response of NAME was found in the osmoregulatory epithelia of fish, which was not found in hypoxia-stressed fish. A reversed pattern of gas transport and acid/base variables after hypoxia stress was found in NAME-treated fish. Overall, the data indicate a role for NO in gas and acid/base homeostasis and HPI axis, which thus support the hypothesis that NO has pivotal role in driving physiological homeostasis during acclimation of air-breathing fish to hypoxia stress.

(Supported by grants from iCEIB Project, Govt. of Kerala and KSCSTE Emeritus Scientist Scheme (VSP))
Environmental decay of faecal glucocorticoid metabolites in bare-nosed wombat 
(*Vombatus ursinus*) scats when exposed to natural climatic conditions 

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Abstract
Non-invasive hormone monitoring can guide captive management and recovery programs. It is important to collect reliable samples under field conditions because steroid hormones in faecal samples are under the influence of environmental factors such as climate and bacterial degradation. Scat samples obtained in the wild are usually <12h old. Therefore, validation experiments are needed to understand whether enzyme-immunoassay done on wild scat samples will give reliable results. In this pilot study, we quantified the influence of environmental decay of glucocorticoid metabolites (FGMs) in faecal samples in a captive population of bare-nosed wombats (*Vombatus ursinus*). Fresh faecal samples were collected from bare-nosed wombats (n = 5) for two consecutive weeks from a wildlife park in Sydney during winter (April 2017-May 2017). The wombats were housed in separate enclosures and samples were collected between 8am – 9am to minimize diurnal variation. The faeces collected from each animal was mixed thoroughly and divided into six aliquots. A small aliquot (2gs) of each sample was immediately frozen at -20C in ziplocked bags. Thus, there was one control sub-sample per sample. The rest of the aliquots of each sample were kept outdoors under shaded open air enclosures for 5 days. All the samples were uniformly exposed to natural weather and was subsequently frozen at -20C in separate zip-locked bags. Daily rainfall and humidity data was recorded from Bureau of Meteorology, Government of Australia. FGM levels in wombats was quantified using a faecal cortisol metabolite enzyme-immunoassay. Our results indicate that the FGM levels in wombat scats remain stable for up to120h after sample collection in winter conditions. Further research is needed to determine how FGMs levels vary under summer conditions. In conclusion, non-invasive hormone monitoring can be a valuable biomarker to assess the stress physiology of wombats.
Poster 15

Anti-metastatic effect of Nafamostat mesilate on breast cancer cells

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Abstract
Nafamostat mesilate (NM) is a serine protease inhibitor used mostly to treat pancreatitis. It is well-recognized therapeutic agent for disseminated intravascular coagulation and systemic inflammatory responses. Recent study have shown that NM yields effective outcomes in experimental pancreatic cancer models. Importantly, its safety has been determined with a high dose. Therein, NM was examined for the anti-cancer effect in breast cancer cell. Breast cancer is one of the leading disease accounting for the large proportion of mortality in women worldwide. Despite the use of conventional chemotherapy, resistance and short duration response limits the treatment efficacy. Particularly, triple negative breast cancer subtype is aggressive with a very poor prognosis. Here, we showed that nafamostat mesilate significantly inhibits proliferation, migration and invasion in MDA-MB231 cells, induces G2/M phase arrest and inhibits the expression of cyclin dependent kinase 1. Exposure of MDA-MB231 cells to NM resulted in decreased activities of transcription factors accompanied by the regulated phosphorylation of signaling molecules and a decrease in metalloproteinases. Furthermore, we demonstrated that NM inhibits lung metastasis of breast cancer cells in NOD-SCID mice which was generated by tail vein injection of MDA-MB231 cells. Taken together, our data revealed that NM inhibits cell growth and metastasis of TNBC cells and indicated that NM is a multi-targeted drug that could be used in combination therapy for TNBC treatment.
Thursday 12 July 2018

Symposium 11: Stress Endocrinology

Chair: Edward J. Narayan

1430 - 1600

1430 - 1500
Edward J. Narayan
Assessing chronic stress in rescued wild koalas using non-invasive stress hormone monitoring
State of the Art Lecture 25 minutes + 5 minutes

1500 - 1520
Hera Maheshwari, and Koekoeh Santoso
Corticosterone response of Japanese quails exposed to prolonged heat stress from day-old quail to peak production period
15 minutes + 5 minutes questions

1520 - 1540
Takashi Bungo, Kouichi Yoshidome and Yoshimitsu Ouchi
Effects of early-life stress on stress responsiveness in later life of chicks
15 minutes + 5 minutes questions

1540 - 1600
Yada Takashi, Miyamoto Kouta and Michihisa Abe
Down-regulation of corticosteroid receptors in leucocytes of stressed rainbow trout with reference to proliferative and apoptotic conditions
15 minutes + 5 minutes questions
State of the Art Lecture

Assessing chronic stress in rescued wild koalas using non-invasive stress hormone monitoring

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Abstract

Environmental trauma and diseases are the main stressors operating in natural ecosystems and they can result in serious ecological consequences for native wildlife species. Australian iconic marsupial species, the koala (*Phascolarctos cinereus*) faces immense pressure from Chlamydia and human induced perturbation factors such as land clearing and vehicle collision, as well as feral pests (e.g. dog attacks) and bush fires. Hundreds of koalas are rescued and entered into the veterinary clinic for rehabilitation. Rescued koalas in clinical care may or may not fully recover, depending on the severity of the environmental trauma and disease that they have undergone in the wild. Conservation physiology research tools such as non-invasive hormone monitoring can be used to quantify the physiological stress response of koalas entering the veterinary clinic. The lag-time in the activation of the physiological stress response and appearance of glucocorticoid metabolites in excreta (lasting up to 10 days in koalas) could be used to determine the magnitude of stress faced by koalas at the time of rescue. By linking non-invasive fecal cortisol metabolite data with information on rescue site/location and clinical diagnosis, we can determine the physiological response of koalas to various types of environmental trauma (e.g. bush fires, urban-rural zones). Furthermore, using the reactive scope model approach we can put the stress endocrine data into perspectives of allostasis and allostatic overload (chronic stress) and apply the robust endocrinology data to make predictions of rehabilitation and recuperation koalas to clinical management interventions. Thus the outcomes of this research presentation will demonstrate how non-invasive hormone monitoring can guide the clinical care of rescued wild koalas and provide impactful research to bolster on-ground koala conservation programs.
Corticosterone response of Japanese quails exposed to prolonged heat stress from day-old quail to peak production period

Hera Maheshwari, and Koekoeh Santoso

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Abstract

Global warming is a natural phenomenon that will pose a threat to all living beings around the world, including poultry. Heat stress is a high concern in warm weather countries such as Indonesia and has high impact in decreasing avian productivity. In Indonesia, Japanese quail (*Coturnix coturnix japonica*) with the current population of 14,427,314 heads is expected to make a big contribution as a source of animal protein producer. This research was aimed to assess the hormonal response of Japanese quail by analyzing blood corticosterone level after exposing to prolonged heat from Day-Old Quail (DOQ) to the peak production period. We hypothesize that prolonged heat stressors with vary temperature will bring the birds to the stage of homeostasis, adaptation or die through natural selection. Twenty four quails were randomly allocated into 4 groups with different cage temperature. During week 1 to week 3 Group A was exposed to 39 °C, Group B to 41 °C, Group C to 43 °C, and Group D to 45 °C. At week 4, the temperature of four cages was decreased gradually by 1 °C/day. Decrease in temperature was completed until the enclosure temperature reached 25 °C for Group A, 29 °C for Group B, 32 °C for Group C, and 35 °C for Group D. Group A with the cage temperature of 25 °C was used as control. Result showed that quails maintained in high-temperature cage had higher level of blood corticosterone (Group C and D) compared to the quails maintained in thermoneutral ambient temperature (Group A). Eventhough the level of corticosterone seemed to be lower in the quails maintained in the cage with higher temperature, all data were statistically not significant different, indicated the quails might use other mechanism intracellularly in handling such a heat stress condition.

Keywords: corticosterone, prolonged heat stress, quail
Effects of early-life stress on stress responsiveness in later life of chicks

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Abstract

Stress at early life can affect the developing central nervous system, and change behavioral and physiological responses to stress later in life. In the present studies, the consequences of the exposure of chicks to brief stresses (heat or isolation) during early development were explored on the later responses to stress. Birds were assigned to one of four treatments groups. The treatments were 1) control, 2) early stress treatments at 2, 3 and 4 days old (ET), 3) late stress treatments at 5, 6 and 7 days old (LT), and 4) at 2–7 days old (ET+LT). Seven days after the treatment, all groups were challenged to the same stress condition, and recorded their behaviors and collected brain samples. In the experiment for heat stress, there were no significant effects of heat stress on thermoregulatory behaviors but respiratory rates in ET and ET+LT groups were tended to be lower than other groups after heat challenge. Significant effect of ET of heat was observed on arginine-vasotocin (AVT) where mRNA levels in ET and ET+LT groups were lower than other groups. While significant LT effect of heat was detected on the mRNA expression of corticotropin-releasing hormone (CRH) where the expression in ET+LT group was the lowest. In the isolation stress experiment, there were significant effects of LT on latency and frequency of jump whereas latency and frequency of peep in ET and ET+LT groups tended to be lower than other groups after the final isolation challenge. Moreover, significant LT effect was detected on brain AVT, and the expression levels in LT and ET+LT groups were lower than other groups. Whereas significant effect of ET was observed on brain CRH, and the expression in ET group was the lowest. These findings suggest that there are some differences between physiological and psychological stresses at early life during the critical period of development on the stress responsiveness in later life.
Down-regulation of corticosteroid receptors in leucocytes of stressed rainbow trout
with reference to proliferative and apoptotic conditions

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Abstract
Relationship between stress and immunosuppression was investigated in peripheral blood
leucocytes (PBL) in rainbow trout, with reference to the expression of corticosteroid
receptors (CRs) and responses to cortisol- and/or lipopolysaccharide (LPS)-
administrations. Confinement stress under shallow water resulted in a sustained elevation
of plasma cortisol, whereas lysozyme and immunoglobin levels were suppressed.
Significant increases in mRNA levels of caspase-6 and insulin-like growth factor (IGF)-I
were observed in the PBL isolated from stressed fish. Confinement stress also suppressed
the expression of proinflammatory cytokine, interleukin (IL)-1 beta, in PBL. There were
decreasing tendencies for the mRNA levels of CRs in the PBL of stressed fish. In vitro
treatment of LPS on isolated PBL from control trout increased both IL and CR mRNA
expression. On the other hand, in the PBL from stressed fish, LPS treatment increased
IL-1 but not CR mRNA levels. Proliferative activities estimated as in vitro incorporation
of bromodeoxyuridine (BrdU) were decreased by cortisol but increased by LPS in PBL
from both control and stressed fish groups. Ratios of apoptotic PBL quantified using
flow cytometer were increased by cortisol in both groups, however, LPS-stimulated
apoptosis only in stressed fish. These results may reflect that the immune system was
influenced by sustained stress through the cortisol receptor-mediated action.
Thursday 12 July 2018

Symposium 12: Fish Endocrinology

Chair: Kouhei Matsuda

1430 - 1600

1430 - 1450
Guokun Yang, Chaobin Qin, Caiyun Sun, WenSheng Li
*C1q/TNF-Related protein 9 in the orange-spotted grouper: molecular cloning, identification and its function*
15 minutes + 5 minutes questions

1450 - 1510
Shotaro Irachi*, Katsuhisa Uchida and Stephen D. McCormick
*Na+/K+-ATPase response to ion-deficient environment in the gill ionocytes of Atlantic salmon (Salmo salar)*
15 minutes + 5 minutes questions

1510 - 1530
Weiyi Song* and Wei Ge
*Probing the effects of bisphenol a (BPA) on gonadal differentiation and development using a novel genetic zebrafish model*
15 minutes + 5 minutes questions

1530 - 1550
Pedro F S Palma, Bruno Louro, Patrícia I S Pinto, Deborah M Power, Pedro M Guerreiro and Adelino V M Canário
*Calcitropic hormones promote gluconeogenesis and lipid metabolism in European sea bass liver*
15 minutes + 5 minutes questions

Asterisk (*) indicate student presentation
C1q/TNF-Related protein 9 in the orange-spotted grouper: molecular cloning, identification and its function

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Abstract
Adipose tissue is not only an organ of energy storage, but also an active endocrine organ. It can release a variety of cytokines, e.g. adiponectin. Adiponectin is an important adipocytokines and has been studied intensively, since adiponectin can improve sensitivity of insulin and also regulate metabolism of glucose and lipid. As the highest paralog, CTRP9 is newly identified adipokine and has the similar biological function as adiponectin. In the present study, we cloned the full-length cDNA of CTRP9 from orange-spotted grouper, Epinephelus coioides. Using real-time PCR, ctrp-9 showed relative higher expression in the kidney and brain region at normal status, while at fasting time, the expression of ctrp-9 in the liver decreased significantly compared with the normal feeding group, it will recover after refeeding. But the expression of ctrp-9 in the adipose tissue did not show obvious change during fasting or refeeding. We suggests that ctrp-9 has different expression mode at different energy status and tissues. Following successful production of recombinant CTRP9 (rgCTRP9) as well as generation of polyclone antibody of CTRP9, we have conducted pull-down assay and identified the endogenous expression of CTRP9 protein in orange-spotted grouper. The mRNA levels of adipose differentiation-related genes were affected in the adipose tissue after intraperitoneal injection of rgCTRP9. Similar observations were also found in the hypothalamus regarding the food intake-related genes. The role of CTRP9 on glucose and lipid metabolism were further examined in the primary hepatocytes culture and adipocyte culture. Interestingly, for the first time, we observed that rgCTRP9 might regulate reproduction-related genes in the hypothalamus, pituitary and mature gonad. Elucidating underlie molecular mechanisms of CTRP9 that mediate glucolipid metabolism and reproduction in teleosts requires additional studies.

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Key Words: CTRP9, clone, metabolism, reproduction, Epinephelus coioides
Na⁺/K⁺-ATPase response to ion-deficient environment in the gill ionocytes of Atlantic salmon (*Salmo salar*)

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Abstract

Na⁺/K⁺ ATPase (NKA) is thought to play an important role in maintaining ionic homeostasis of teleosts, and various isoforms have been identified. In salmonids, NKAα1a is known as the freshwater (FW) isoform and NKAα1b is known as the seawater (SW) isoform. The regulation of these two isoforms has been widely examined in FW and SW but not in low ion environments. In the present study, we evaluated the osmoregulatory responses of two-weeks exposure to de-ionized water (DW), FW, and SW in juvenile Atlantic salmon (*Salmo salar*). Plasma chloride and osmolality levels were not different in FW and DW, but were significantly higher in SW. Gill NKA activity was lowest in FW, significantly higher in DW and highest in SW. The abundance of gill NKAα1a was lowest in SW and about 30% higher in DW than that of in FW. Interestingly, the abundance of gill NKAα1b was lowest in FW, two-fold higher in DW and highest in SW. The number of NKAα1a and NKAα1b immunoreactive filamental ionocytes were not significantly different between DW and FW, but were significantly decreased and increased in SW, respectively. The number of NKAα1a immunoreactive lamellar ionocytes was about two-fold higher in DW than FW and significantly lower in SW.

These results indicate that Atlantic salmon develop NKAα1a in lamellar ionocytes and NKA α1b in filamental ionocytes in response to low ion environments, which may be involved in their capacity to maintain plasma ion levels under these conditions.
Probing the effects of bisphenol a (BPA) on gonadal differentiation and development using a novel genetic zebrafish model

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Abstract
Bisphenol A (BPA), a ubiquitous estrogenic endocrine disrupting chemical (e-EDC) present in many household products, has been reported to cause endocrine-related disorders and various reproductive malfunctions in experimental animals. Although a wide body of studies has shown adverse effects of BPA on reproductive system, there is a lack of direct evidence for its effect on sexual differentiation in vivo. For e-EDC studies, sex ratio and vitellogenin (VTG) induction are currently key endpoints to evaluate feminization in animal models exposed to potential estrogenic EDCs. However, these evaluation indexes are often complicated by high data variation and high background level (endogenous estrogen).

To address this issue, we successfully established a mutant zebrafish line without the ovarian aromatase (cyp19a1a<sup>-/-</sup>), the key enzyme for estrogen production. All mutant fish were males due to lack of endogenous estrogens, and they are therefore supposed to be sensitive to estrogenic chemical exposure. Here, the estrogen-free fish were used to test if exposure to BPA (0.01 to 10 µM) can mimic the role of endogenous estrogen in sexual differentiation and its adverse effects after long-term (20 to 150 dpf) exposure on gonadal development and function.

Strikingly, BPA(10 µM) exposure rescued the mutant phenotype with females developed, supporting the view that BPA carries estrogenic activity and therefore can mimic the role of estrogens in sexual differentiation. Further analysis showed that chronical exposure to BPA significantly delayed the development and maturation of the gonads in both sexes, resulting in infertility or decreased fertility. In addition, BPA (10 µM) exposure resulted in follicle apoptosis in females and insufficient spermatozoa in testis. Taken together, our study here provides the most direct evidence that BPA mimics the effects of estrogens on sexual differentiation and interferes with normal gonadal functions by using the novel estrogen-free mutant zebrafish line. Furthermore, the outcome of present study not only contributes to our understanding the effects of BPA on reproductive system, but also open up a powerful platform for studying any other EDCs with estrogenic activities.

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Calcitropic hormones promote gluconeogenesis and lipid metabolism in European sea bass liver

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Abstract
Stanniocalcin 1 (STC1) and parathyroid hormone-related protein (PTHrP) are known, respectively, as hypocalcemic and hypercalcemic hormones in teleost fishes. In the present study we tested the hypothesis that STC1 and PTHrP have additional functions related to metabolism in fish. Juvenile European sea bass (Dicentrarchus labrax) were injected with the pro-PTHrP treatments, i) PTHrP(1-34) or ii) a combination of PTHrP(1-34) together with STC1 antiserum, or the pro-STC1 treatments iii) PTHrP(7-34) - a PTHrP antagonist -, or iv) PTHrP(7-34) together with STC1, or v) a saline (control). Liver samples were collected at 6h and 24h after treatments. Metabolomics revealed increased concentrations of the branched-chain amino acids alanine, glutamine and glutamate in the pro-STC1 groups suggesting their mobilization from the muscle to the liver for degradation and gluconeogenesis. Only the STC1 treatment decreased the concentrations of succinate, fumarate and acetate, indicating a slowing of the citric acid cycle. In the pro-PTHrP groups the concentrations of glucose, erythritol and lactate decreased in liver, indicative of gluconeogenesis from lactate, as well as an effect on hepatic glucose export to peripheral tissues. Taurine, TMA, TMAO and carnitine changed in opposite directions in the pro-STC1 versus the pro-PTHrP groups, suggesting they have opposing effects. STC1 appears to stimulate lipogenesis and PTHrP appears to activate lipolysis/β-oxidation of fatty acids. Transcriptome analysis of differentially expressed (DE) genes in the liver revealed carbohydrate, amino acid and lipid metabolic processes were enriched in Gene Ontology. KEGG analysis of DE genes revealed enrichment with genes related to intermediary, amino acid and lipid metabolism, corroborating the metabolomics analysis indicating that PTHrP was associated with lipolysis while STC1 stimulated lipogenesis. Overall, the results from metabolomics and transcriptomics were concordant, supporting the hypothesis that PTHrP and STC1 modify liver energy metabolism promoting gluconeogenesis and potentially have an antagonistic role on lipid metabolism.

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