2019 年臺法雙邊研討會

海洋生物神經胜肽與激素研討會

Taiwan-France Bilateral Conference on

Neuropeptides and Hormones in Marine Organisms

Monday July 15 - Friday July 19, 2019

National Taiwan Ocean University

Keelung, Taiwan

Welcome

On behalf of the NTOU, I am pleased to invite you to join us for “Taiwan-France Bilateral Conference on Neuropeptides and Hormones in Marine Organisms”. It will be held at the National Taiwan Ocean University, Keelung, Taiwan from July 16 (Monday) to July 17 (Wednesday), 2019. This Summer Workshop will provide fantastic opportunities not only to exchange and share research information, discoveries and advancements, but also provide us the chance to network with our colleagues and build new friendships.

This Summer Workshop covers the areas of development, reproduction and evolution in marine organisms. The symposium will provide a great opportunity for our students to share their ideas/experiences and to discuss the various challenges that are being faced in this field. It is also a good opportunity to see how various collaborations can make significant contributions to our science field.

The Summer Workshop being held in Taiwan will provide an excellent chance not only to participate symposium but also to visit our NTOU campus and enjoy the cultures that is the essence of Taiwan. You will be able to tour the city of Keelung and Taipei as well as visit the historical town of Taiwan amongst other interesting things.

We appreciated the financial supports from Ministry of Education and The Center of Excellence for the Oceans of National Taiwan Ocean University and the financial support from ANR, France. Thank for the Director Dr. Sylvie DUFOUR and participants from the BOREA (Biology of Aquatic Organisms and Ecosystems) Research Unit, CNRS, Museum National d'Histoire Naturelle and University of Caen, France.

We look forward to seeing you here in Taiwan and anticipate a great scientific interaction and joyful stay.

Ching-Fong Chang, Ph. D.
President & Professor, National Taiwan Ocean University (NTOU)
2, Pei-ning Rd., Keelung 20224, Taiwan
B0044@email.ntou.edu.tw

2019年臺法雙邊研討會 海洋生物神經胜與激素研討會

Taiwan-France Bilateral Conference on Neuropeptides and Hormones in Marine Organisms

Monday July 15 - Friday July 19, 2019

Schedule

Monday	**July 15th,**	**2019**	Arrive Taiwan 06:30 BR0088 stay in K-Hotel (Keelung) 18:30 dinner in K-Hotel (Keelung)
Tuesday	**July 16th,**	**2019**	08:30 meet at K-Hotel lobby Conference day1 Visit campus Dinner seafood restaurant 友信海產 (新北市瑞芳區深澳路189-7號)
Wednesday	**July 17th,**	**2019**	08:30 meet at K-Hotel lobby Conference day2 Visit NTOU G.L Aqua center水生生物研究暨保育中心 Ling Jiou Mountain靈鷲山 ***Religious holy places, please wear suitable clothes*** Yinyang Sea & Golden Fall 陰陽海&黃金瀑布 Jiufen九份 Dinner-Keelung City Indigenous Cultural Hall原住民文化會館
Thursday	**July 18th,**	**2019**	09:00 meet at K-Hotel lobby Taipei city National Palace Museum故宮博物院 Lunch Bopiliao Historic Block剝皮寮歷史街區 Chiang Kai-shek Memorial Hall中正紀念堂 Dinner-Miaokuo Night Market基隆廟口夜市
Friday	**July 19th,**	**2019**	09:00 meet at K-Hotel lobby Visit Heping Island Park和平島公園 National Museum of Marine Science and Technology海洋科技博物館 Lunch back to K-Hotel (Keelung) Free time 16:30 Check out (Lauggages leave in K-Hotel) Visit Zhongzheng (Jhongjheng) Park & Zhuputan Temple中正公園&主普壇 18:30 Dinner- Red Kitche紅廚 20:00 meet at K-hotel lobby -> to Airport 23:50 BR0087
Saturday	**July 20th,**	**2019**	Arrive Paris

2019年臺法雙邊研討會 海洋生物神經胜肽與激素研討會
Taiwan-France Bilateral Conference on Neuropeptides and Hormones in Marine Organisms
Monday July 15 - Friday July 19, 2019

July 16th, 2019 (Tuesday) Auditorium 2

09:00-09:15	Opening Remarks (Ching-Fong CHANG & Sylvie DUFOUR)			
	Chairman: CHANG Ching-Fong 張清風 (National Taiwan Ocean University, Keelung, Taiwan)			Page
09:15-09:40	DUFOUR Sylvie	Research Director, CNRS, MNHN, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, Paris, France	Evolutionary history of gonadotropin-inhibiting hormone (GnIH) with new insights from basal teleosts	1
09:40-10:05	FAVREL Pascal	Professor, University of Caen-Normandy, Research Unit BOREA, Biology of Aquatic	The pacific oyster *Crassostrea gigas* : a Lophotrochozoan model to investigate the evolution of neuroendocrine signaling systems	3
10:05-10:30	SOURDAINE Pascal	University of Caen-Normandy, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, Caen, France	Endocrine *versus* paracrine control of spermatogeneis in Elasmobranchs: new insights from shark genomes	5
10:30-10:40	Photo time			
10:40-11:00	Break		Snack & hot tea	
	Chairman: WU Guan-Chung 吳貫忠(Department of Aquaculture, National Taiwan Ocean University,			
11:00-11:25	CHUNG Bon-chu 鍾邦柱	Academician, Academia Sinica, Taipei, Taiwan	Action of neurosteroid pregnenolone in zebrafish brain	7
11:25-11:45	HWANG Pung-Pung黃鵬鵬	Distinguished Research Fellow, Inst. of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan	New actions of neuropeptides on body fluid ionic and acid-base homeostasis in fish	8
11:45-12:05	HUANG Yung-Sen 黃永森	Associate professor, Department of Life Science, National University of Kaohsiung, Kaohsiung, Taiwan	Sensitivities of Japanese eel primary ovary on exogenous androgen – correlated with ovarian neuropeptides and their receptors?	10
12:05-12:20	LIN Chien-Ju 林倩如	Postdoc, Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan	Activation of the brain-pituitary-gonadotropic axis during gonadal differentiation and testis development in protandrous black porgy, *Acanthopagrus schlegelii*	12
12:20-13:30	Lunch box		Auditorium 2	
	Chairman: CHUNG Bon-chu 鍾邦柱 (Academician, Academia Sinica, Taipei, Taiwan)			
13:30-13:55	LAFONT Anne-Gaëlle	Researcher, Sorbonne University, Hopital Saint Antoine, Biological Resource Center & MNHN, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, Paris, France.	Evolutionary history of neuropeptide and receptor super-families in vertebrates, with a special focus on a basal teleost, the eel	13
13:55-14:20	MIRABEAU Olivier	Research Engineer, Institut Curie, Paris, France	Ancient coevolution of neuropeptides and their receptors in bilaterian genomes	15

14:20-14:45	LEPRINCE Jérôme	Researcher, INSERM, Manager of Peptide Synthesis and Functional Screening facilities of PRIMACEN Cellular Imaging Platform the University of Rouen-Normandy, Rouen, France	Differences and Similarities between Mammalian and Fish Endozepinergic System	18
14:45-15:10	REALIS-DOYELLE Emilie	Lecturer, University of Caen-Normandy, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, Caen, France	Characterization of neuroendocrine signalling systems during gametogenesis in the Pacific oyster, *Crassostrea gigas*	21
15:10-15:30	Break		Bubble tea (Auditorium 2)	
	Chairman: HWANG Pung-Pung 黃鵬鵬(Inst. of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan)			
15:30-15:50	WU Guan-Chung 吳貫忠	Associate professor, Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan	How protandrous black porgy to determine their sexes through the HPG axis?	24
15:50-16:10	JENG Shan-Ru 鄭絢如	Professor, Department and Graduate Institute of Aquaculture, National Kaohsiung University of Science and Technology, Kaohsiung, Taiwan	Expression of sex-specific genes during testicular differentiation in Japanese eel	25
16:10-16:30	CHEN Chieh-Jhen 陳倢蓁	Postdoc, Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan	The plasticity of gonad development of sexual reproduction in a scleractinian coral, *Porites lichen*	26
16:30-16:45	NAGARAJAN Aruna	Basic sciences department, PYD, King Faisal University, Saudi Arabia	Differential expression of brain arginine vasotocin and isotocin in response to osmotic change in black porgy, *Acanthopagrus Schlegelii*	27
16:45-18:00	NTOU Founding Park, NTOU History Museum, Ocean Center			
18:30-20:00	Dinner		Shen'ao Fishery harbor, seafood 友信海產	

2019年臺法雙邊研討會 海洋生物神經胜肽與激素研討會
Taiwan-France Bilateral Conference on Neuropeptides and Hormones in Marine Organisms
Monday July 15 - Friday July 19, 2019

July 17th, 2019 (Wednesday) Auditorium

				Page
	Chairman: Pascal SOURDAINE (Research Unit BOREA, University of Caen - Normandy, Caen, France)			
09:00-09:25	TOSTIVINT Hervé	Professor, Muséum National d'Histoire Naturelle, Research Unit Molecular Physiology and Adaptation, Paris, France	Peptides of the urotensin II family: evolutionary and functional perspectives	28
09:25-09:45	NAGARAJAN Ganesan	Assistant Professor, Biology PYD (Molecular Biology & Neuroendocrinology), King Faisal University, Al Hasa, Kingdom of Saudi Arabia	Neuropeptide AVT and E2 system stimulates neurogenic activity and early brain development in the orange-spotted grouper	30
09:45-10:10	COLLET Bertrand	Research Director, INRA, Unit of Molecular Virology and Immunology, Jouy en Josas, France	Gene editing in fish cell lines – perspectives for the functional study of peptides with immunological functions	31
10:10-10:35	BONDON Arnaud	Research Director, CNRS, University of Rennes, 1, Institute of Chemical Sciences, Rennes, France	Three dimensional structure determination by NMR of neuropeptides in membrane mimic models	33
10:35-11:00	Break and Photo time		Snack & hot tea	
	Chairman: JENG Shan-Ru 鄭絢如 (Department and Graduate Institute of Aquaculture, National Kaohsiung University of Science and Technology Kaohsiung, Taiwan)			
11:00-11:20	LEE Chi-Ying 李奇英	Professor, Department of Biology, National Changhua University of Education, Changhua, Taiwan	Regulation of metabolism by crustacean hyperglycemic hormone with pathological implications	35
11:20-11:40	LIU Yi-Wen 劉蕙雯	Professor, Department of Life Science, Tunghai University, Taichung, Taiwan	Deciphering the molecular and cellular mechanisms that regulate organogenesis of the interrenal gland	36
11:40-12:05	ZALTINY-GAUDIN Céline	Associate Professor, University of Caen-Normandy, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, Caen, France	Identification of neuropeptides in marine organisms by in silico analysis and peptidomic approach	37
12:05-12:25	SHIKINA Shinya 識名信也	Associate professor, Institute of Marine Environment and Ecology, National Taiwan Ocean University, Keelung, Taiwan	Neuropeptides in a scleractinian coral *Euphyllia ancora*	38
12:25-12:30	Closing			
12:30-13:15	Lunch box		Auditorium 2	
14:00-15:00	NTOU G.L Aqua center (New Taipei city)			
15:30-18:00	Ling Jiou Mountain靈鷲山 Yinyang Sea & Golden Fall 陰陽海&黃金瀑布 Jiufen九份			
18:30-20:00	Dinner		Hepin Island Keelung City Indigenous Cultural Hall	

Evolutionary history of gonadotropin-inhibiting hormone (GnIH) with new insights from basal teleosts.

S. Dufour [a], G. Maugars [a], A.-G. Lafont [a], J. Pasquier, C. Atkinson[a], A. Campo [a], N. Kamech [a], B. Lefranc [b], J. Leprince [b], K. Rousseau [a]

[a] *Muséum National d'Histoire Naturelle, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, CNRS, IRD, SU, UCN, UA, Paris, France*
[b] *Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, INSERM U1239, Normandy University, 76000 Rouen, France*

sylvie.dufour@mnhn.fr

In 2000, a novel RF-amide neuropeptide was discovered by Tsutsui and coworkers in birds, inhibiting gonadotropin release from cultured quail pituitaries, and named gonadotropin-inhibitory hormone (GnIH). *Gnih* gene (also named *npvf* gene) was then identified in non-avian vertebrates, including mammals, amphibians, fish and agnathans. The *gnih* gene encodes multiple homologous peptides reflecting ancestral intragenic duplication of peptidic sequence: three peptides in birds and reptiles (GnIH and two GnIH-related peptides, GnIH-RP1 and 2), two in mammals (named RFRP), four in amphibians (named GRP, GRP-RP-1 to -3 or LPXRF-1 to 4), two to three peptides in teleosts (named LPXRF, LPXRF-like or GnIH). An homologous gene encoding three PQRF-peptides was identified in a cephalochordate, amphioxus. It that may represent the common chordate ancestor to both vertebrate *gnih* and *npff* (neuropeptide FF) genes, which would have arisen through the whole genome duplications (WGD 1R/2R) in early vertebrates.

In the present study, we identified a single *gnih* gene in the eels (*Anguilla species*), representative species of a basal group of teleosts (Elopomophs). We also retrieved a single *gnih* gene in another basal group of teleosts, Osteoglossomorphs, as well as in representative of the various groups of more recently emerged teleosts, Clupeocephala. Phylogeny and synteny analyses allowed us to infer that one of the two *gnih* paralogs issued from the teleost-specific whole genome duplication (TWGD or 3R), would have been lost shortly after the 3R, before the emergence of the basal groups of teleosts. This gene loss led to the presence of a single *gnih* gene in extant teleosts, the same situation as in other vertebrates despite the teleost 3R. Two *gnih* paralogs were still found in some teleost species, such as in salmonids, but resulting from the additional whole genome duplication that specifically occurred in this lineage (4R).

Cloning of European eel *gnih* cDNA confirmed the genomic sequence of the GnIH precursor, encoding three putative mature GnIH amidated peptides, named aaGnIH-1, aaGnIH-2 and aaGnIH-3, and presenting a characteristic C-terminal RF amide sequence LPLRF, LTRRF and LPQRF, respectively. The sequences of the three GnIH peptides were fully conserved in the European eel sister species, the American eel, *Anguilla rostrata*. GnIH-1 sequence was also identical in the Japanese eel, while GnIH-2 and -3 differed by a few amino acids but with conserved C-terminal sequences.

Gnih transcripts were exclusively expressed in the diencephalon part of the brain of the European eel, as analyzed by quantitative real-time PCR. This is in agreement with the location of *gnih*-expressing neurons in the nPPV (nucleus preopticus parvocellularis) and projecting to the pituitary, as shown in some Clupeocephala by various research groups, using *in situ* hybridization and immunocytochemistry.

The three European eel putative GnIH- amidated peptides were synthesized and tested tested for their direct effects on eel pituitary cells *in vitro*. Eel GnIH peptides inhibited the

expression of gonadotropin subunits (*lhβ, fshβ,* and common *a*-subunit) as well as of GnRH receptor (*gnrh-r2*), with no effect on *tshβ* and *gh* expression. This revealed a dual inhibitory action of GnIH on eel gonadotropic function, by inhibiting the expression of gonadotropins and also reducing the responsiveness of gonadotropic cells to GnRH. In addition to dopamine and some other neuropeptides such as kisspeptins and tachykinins (as shown by our previous studies), GnIH may thus represent one of the major brain actors involved in the prepubertal blockade of eel sexual maturation. Similarly to their role in birds, GnIH peptides have been shown to exert an inhibitory action on gonadotropins in mammals and some clupeocephalan teleosts. In contrast, stimulatory effects of GnIH were reported in agnathan and some other clupeocephala, leading to contradictory hypotheses on the ancestral role of GnIH. The inhibitory effect of GnIH on gonadotropic function in a basal teleost is in favour of an ancestral inhibitory role of GnIH in the neuroendocrine control of reproduction in vertebrates.

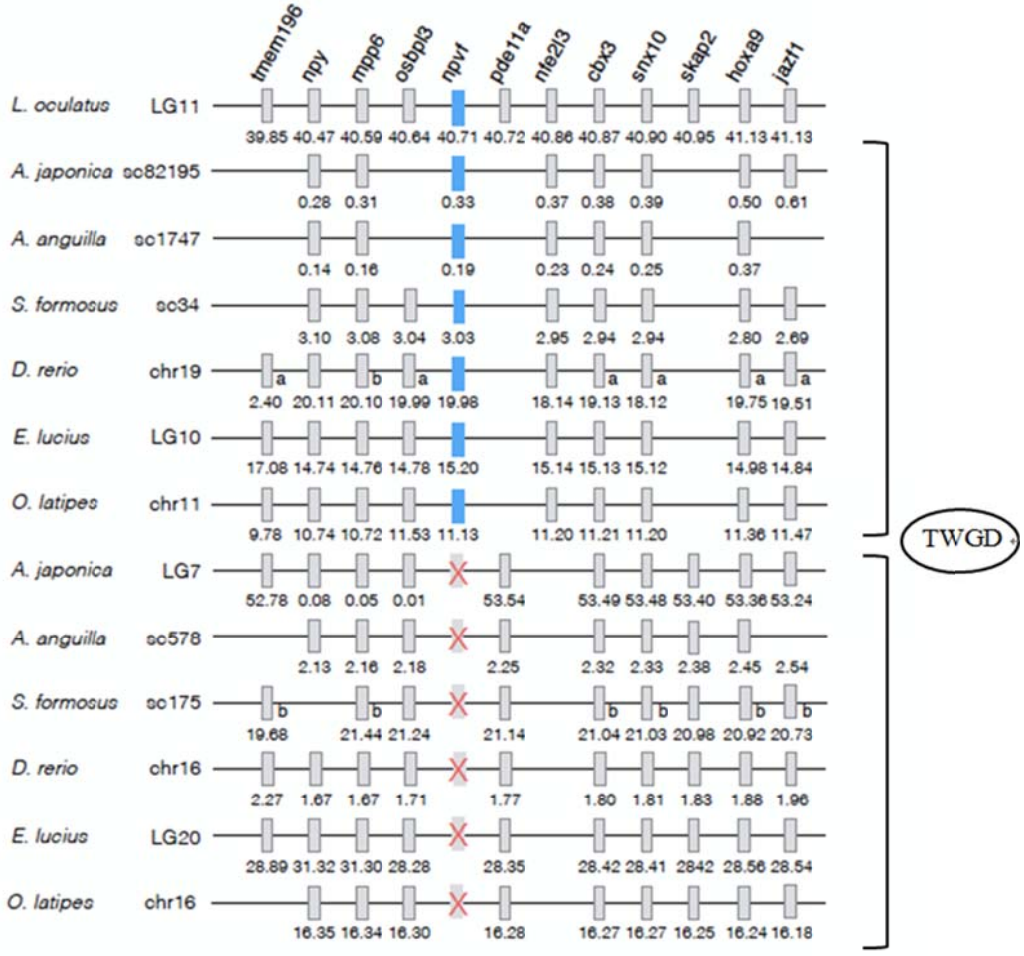


Figure: Synteny analysis of *gnih* (*npvf*) genomic region in actinopterygians
TWGD: teleost-specific whole genome duplication.

The pacific oyster *Crassostrea gigas*: a Lophotrochozoan model to investigate the evolution of neuroendocrine signaling systems

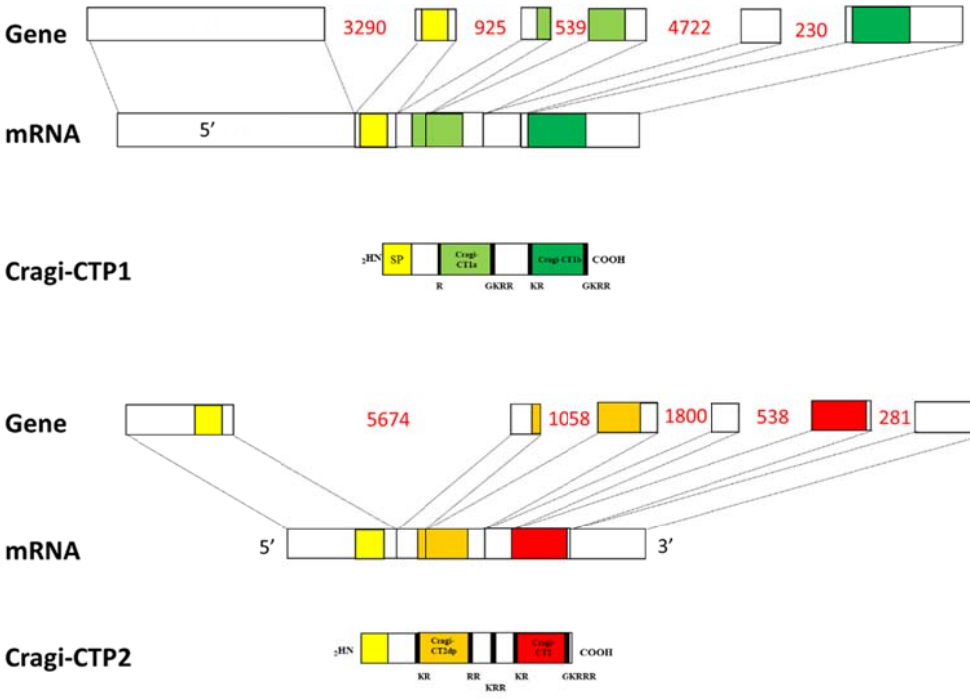
Pascal Favrel[1], Julie Schwartz[1], Jeremy Pasquier[1], Emile Réalis-Doyelle[1], Benjamin Lefranc[2], Marie-Pierre Dubos[1], Benoit Bernay[3], Anne-Gaëlle Lafont[4], Jérôme Leprince[2] and Arnaud Bondon[4]

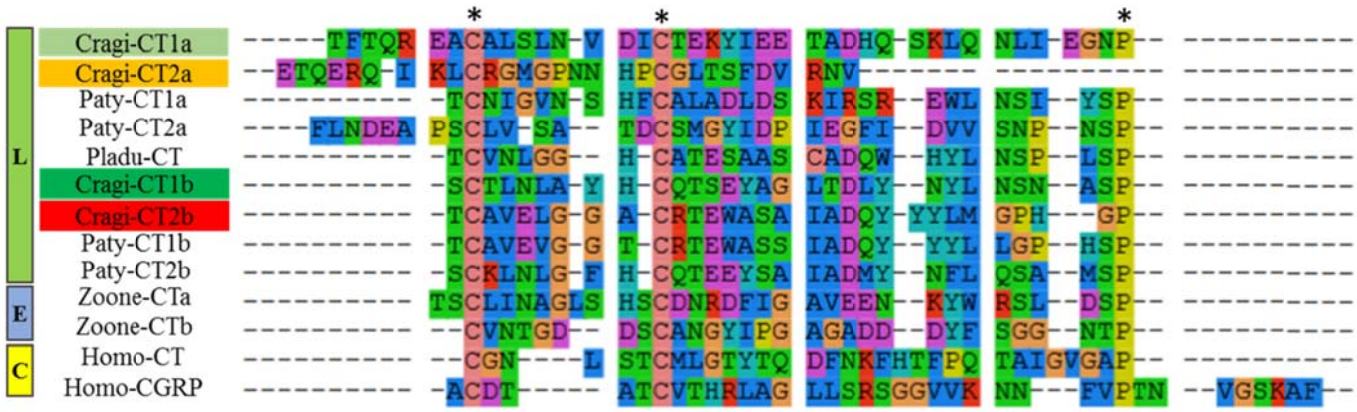
[1.] *Normandie Université, UNICAEN, Sorbonne Université, MNHN, UPMC, CNRS 7208, IRD 207, UA, Biologie des Organismes et Ecosystèmes Aquatiques (BOREA), CS14032, 14032 CAEN, Cedex 5, France.*
[2] *Normandie Université, UNIROUEN, INSERM, U1239, Laboratoire Différenciation et Communication Neuronale et Neuroendocrine, F-76000 Rouen, France.*
[3] *Normandie Université, UNICAEN, SF4206 ICORE, PROTEOGEN Esplanade de la Paix, 14032 CAEN cedex.*
[4] *Université de Rennes, UMR CNRS 6226, PRISM,, Equipe CORINT, CS 34317, Campus de Villejean, , F-35043 Rennes, France*

pascal.favrel@unicaen;fr

In Eumetazoa, neuropeptides regulate a majority of biological processes and play a crucial role in the elaboration of adapted physiological and behavioural responses to environmental constrains. Until recently, knowledge on neuropeptide signalling systems was chiefly limited to well-studied vertebrate species and to ecdysozoan model species. The tremendous expansion of molecular resources in Lophotrochozoa, one of the most diverse and evolutionarily highly successful bilaterian lineage, opens up the opportunity to refine our knowledge on the origin and the evolution of neuroendocrine signalling systems via the introduction of new models at a key phylogenetic position between Ecdysozoa and Deuterostoma. In this context, the pacific oyster *Crasssostrea gigas,* one of the most important aquaculture shellfish resource worldwide, has emerged as an attractive model due to the identification, using combined data mining and/or peptidomic approaches, of extended repertoires of both neuropeptides and G protein-coupled receptors (GPCRs).

Using examples of newly characterized signalling systems in oyster: Egg Laying Hormone (ELH) / Corticoliberin (CRH) / Diuretic hormone 44 (DH44) signalling system and the Calcitonin / Diuretic hormone 31 (DH31) signalling system, we illustrate how pairing of receptors and ligands, determination of the spatiotemporal and the stimulus induced patterns of expression of their encoding genes provide, in a comparative context, interesting insights about evolutionary paths of some neuroendocrine systems.





Schematic representation of the genes encoding the two Calcitonin precursors (Cragi-CTP1 and Cragi-CTP2) in *Crassostrea gigas* and alignment of oyster calcitonin mature peptides with some calcitonin family members from Ecdysozoa (E) and Chordates (C). Pladu: *Platynereis dumerilii*, Trica: *Tribolium castaneum*, Drome:, Paty: *Patinopecten yessoensis*, Zoone: *Zootermopsis nevadensis*, Homo: *Homo sapiens*.

Endocrine *versus* paracrine control of spermatogeneis in Elasmobranchs: new insights from shark genomes

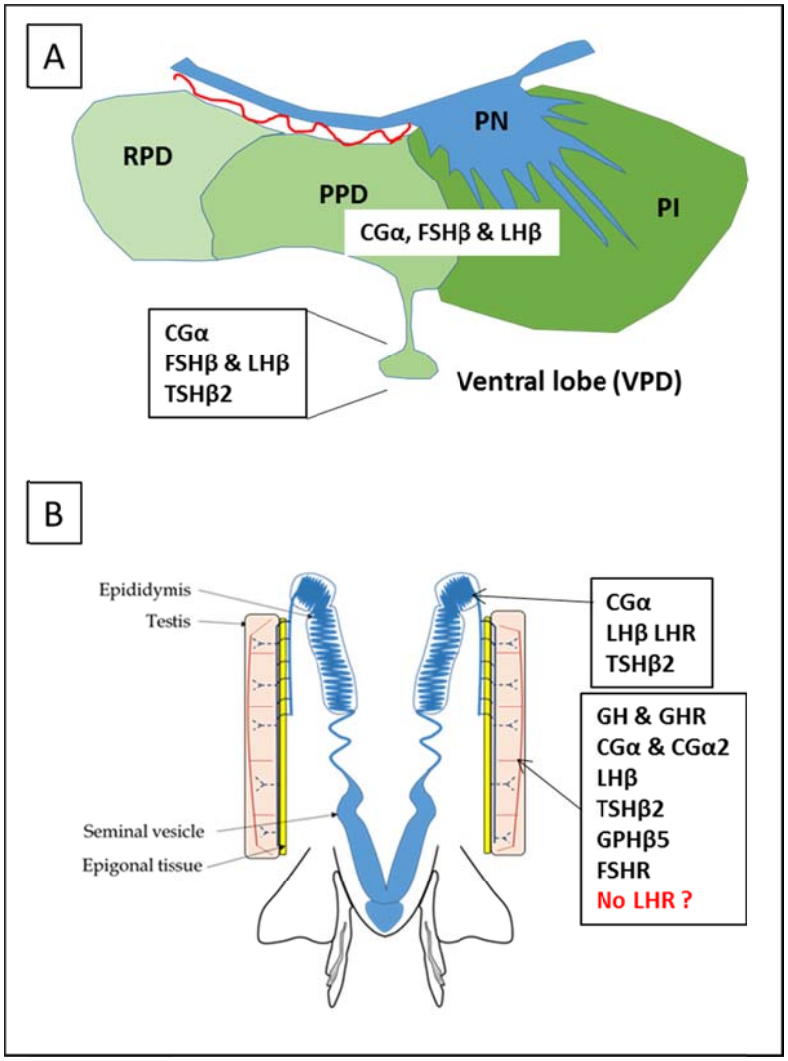
Pascal Sourdaine

BOREA Research Unit, CNRS-2030, IRD-207, MNHN, SU, UCN, UA.
University of Caen Normandy, CS 14032, 14032 CAEN, FRANCE.

pascal.sourdaine@unicaen.fr

The hypothalamic-pituitary system is considered as an evolutionary innovation leading to the neuroendocrine control of numerous biological functions by the gradual emergence of a new level of control (glycoprotein hormones and their receptors) concomitant with the development of a distinct anatomical structure, the pituitary. This evolution was accompanied by a specialization of peptides (including GnRHs) that could have a tissue distribution and broader functions in invertebrates. These peripheral functions have persisted in vertebrates but have been poorly studied because of the predominance of the hypothalamic-pituitary axis. In an evolutionary context, it seems important to us to include Chondrichthyans as a sister group of Osteichthyans (bony vertebrates) to better understand the evolution of GnRH systems and of their regulatory neuropeptides in the endocrine and paracrine control of gonadic functions, (i.e. spermatogenesis). The increasing number of Chondrichthyan genomes presently available offer us an opportunity to investigate the role of neuropeptides on gonadotropins secretion and on testicular function. The recent and noticeable work made by Hara et al. (2018) will be reviewed here in order to highlight new data on the reproductive endocrinology in Chondrichthyans as well as on candidate factors involved in paracrine regulation of spermatogenesis.

Analyses of genome of *Scyliorhinus canicula* by Gaillard *et al.* (2018) and of *Rhincodon typus*, *Chiloscyllium punctatum* and *Scyliorhinus torazame* by Hara *et al.* (2018) have shown that GnRH gene repertorie was conserved in Selachians and consist of GnRH1 (QHWSFDLRPG), GnRH2 (QHWSHGWYPG) and GnRH3 (QHWSHGWLPG). Four GnRH-receptor subtypes have also been identified (GnRHR1a, GnRHR1b, GnRHR2a, GnRHR2b) and are expressed in the ventral lobe of the *pars distalis* (VPD) which is the main site expressing polypeptides of the glycoprotein hormone alpha (CGα), LHβ, FSHβ and TSHβ2. This is also in agreement with a GnRH control of gonadotropin biosynthesis. However, the absence of portal vessel toward the VPD rise the debate on the systemic circulation way used by GnRH to stimulate gonadotrope cells as well as on the direct endocrine function of GnRH on testis which express, at least, GnRHR1b. Interestingly, several glycoprotein hormone polypeptides (CGα and its paralog CGα2, LHβ, TSHβ2 and GPHβ5) are expressed in testis, suggesting paracrine functions of glycoprotein hormones even if more studies are needs due to apparent contradictions, such as lack of LH receptor testicular expression. Otherwise, testicular expression of genes coding peptides and corresponding receptors have been noticed such as vasopressin, oxytocin, orexin and peptides of the spexin/galanin/kisspeptin family. By taking into account old studies on the endocrine regulation of spermatogenesis and steroidogenesis in Elasmobranchs, the autonomy of the testicular function with respect to the hypothalamic-pituitary axis will be discussed.



Glycoprotein hormone and glycoprotein hormone receptor expressions in pituitary and in the male reproductive tract in *Scyliorhinus sp*.. A: diagram of the pituitary with the rostral pars distalis (RPD), the proximal pars distalis (PPD), the pars intermedia (PI), the ventral lobe of the pars distalis (VPD) and the pars nervosa (PN). B: schematic representation of the male reproductive tract including testes associated to the epigonal tissues, epididymis, seminal vesicles and claspers.

References:

Gaillard AL, Tay BH, Pérez Sirkin DI, Lafont AG, De Flori C, Vissio PG, Mazan S, Dufour S, Venkatesh B, Tostivint H. 2018. Characterization of Gonadotropin-Releasing Hormone (GnRH) Genes from Cartilaginous Fish: Evolutionary Perspectives. Front Neurosci. 12:607.

Hara Y, Yamaguchi K, Onimaru K, Kadota M, Koyanagi M, Keeley SD, Tatsumi K, Tanaka K, Motone F, Kageyama Y, Nozu R, Adachi N, Nishimura O, Nakagawa R, Tanegashima C, Kiyatake I, Matsumoto R, Murakumo K, Nishida K, Terakita A, Kuratani S, Sato K, Hyodo S, Kuraku S. 2018. Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates. Nat Ecol Evol. 2:1761-1771.

Action of Neurosteroid Pregnenolone in the Brain

Bon-chu Chung

Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

mbchung@gate.sinica.edu.tw

Steroids have been widely used in treating inflammation, hormonal imbalance and disorder of reproduction. They are popular drugs because of their small size and the ease of administration. A new class of steroids, neurosteroids, has recently gained interest because of their roles in the brain. Pregnenolone (abbreviated as P5) in neuronal tissues improves memory and cognition and helps neurological recovery. In addition, it is required for zebrafish embryonic epiboly migration and microtubule abundance. However, the detail mechanism of P5 functions is unknown. Here we explore the possibility of using zebrafish as a model to study the action of P5. We showed that P5 decreases zebrafish anxiety and increases learning/memory in behavior tests. We also showed that P5 promotes cell migration and microtubule polymerization by binding to a microtubule plus end protein Cytoplamic Linker Protein 1 (CLIP-170). We captured CLIP-170 from zebrafish embryonic extract using a P5 photoaffinity probe linked to diaminobenzophenone. Upon binding P5, CLIP-170 became more extended, increased its interaction with $p150^{Glued}$ and LIS1, and promoted microtubule polymerization more efficiently. CLIP-170 was essential for P5 to promote epiboly migration, and over-expression of P5-binding domain $CLIP\text{-}170_{890\text{-}990}$ in zebrafish embryos caused epiboly delay. P5 was also required for microtubule growth and he directional migration of mouse adrenocortical Y1 cell. Our results elucidate the mechanism of P5 action in promoting cell migration and microtubule polymerization. The interaction of P5 and CLIP-170 may be a therapeutic target for the treatment of memory loss, or neurodegenerative or psychiatric disorders.

New actions of neuropeptides on body fluid ionic and acid-base homeostasis in fish

Pung-Pung HWANG

Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

pphwang@gate.sinica.edu.tw; zophwang@ccvax.sinica.edu.tw

Aquatic fish and mammals have developed similar sophisticated homeostatic mechanisms for the transport of various ions or acid equivalents, which generally involve hormonal control of body fluid homeostasis when systemic ionic and acid-base status is disturbed by metabolic or environmental stressors. Aquatic environments are much more diverse than terrestrial environments in terms of ion composition and pH levels. To cope with such fluctuant aquatic environments, fish have developed their iono/osmoregulation mechanisms with higher plasticity than those in mammals. To overcome the research limitations of traditional model species, zebrafish have recently become emerging models to study the molecular physiology of ionic and acid-base regulation and the control of related hormones. In the present study, we identified some new actions of isotocin and vasotocin (the teleost homologs of oxytocin and vasopressin, respectively) on ion uptake and acid secretion functions.

Oxytocin is known to act on the atrial natriuretic peptide-mediated water and sodium transport in the kidney, thereby regulating blood pressure (Haanwinckel et al. 1995). Isotocin was also proposed to play some roles in fish osmoregulation mainly based on the expression of isotocin or isotocin receptors in fish exposed to different salinities Cao et al. 2018). In zebrafish, isotocin expression was stimulated by ion-deficient or low-pH environments, which then led to compensatory enhancement of ion uptake and acid secretion (Chou et al. 2011). This action of isotocin appears to be exerted by stimulating the proliferation of epidermal stem cells and differentiation of ionocyte progenitors via the p63 and Foxi3a transcription factors. As such, isotocin enhances the differentiation of ionoytes expressing H+-ATPase, epithelial Ca2+ channel, and Na+-Cl--cotransporter (NCC) and consequently stimulates their functional activities (H+ secretion and uptake of Ca2+, Na+, and Cl-) (Chou et al. 2011).

In mammals, vasopressin is well known as a short-term regulator for water and salt transport mainly through the post-translational regulation and trafficking of acquaporin (Deen et al., 1994; Suzuki et al., 2015) and NCC (Mutig et al., 2007; Pedersen et al., 2010) in the kidney. Vasotocin was also reported to regulate body fluid homeostasis in fish and amphibians (Lema et al., 2019; McCormick and Bradshaw, 2006; Warne, 2002); however, the molecular physiological mechanisms behind these regulatory pathways including the target transporters and ionocytes remain largely unknown. In zebrafish, vasotocin was found to show similar actions of isotocin with subtle difference. Isotocin expression was stimulated by ion-deficient environment. Loss-of-function experiments demonstrated vasotocin as a positive regulator of Cl- uptake and H+ secretion functions by adjusting the expressions of the related transporters (NCC, Cl- channel, H+-ATPase, anion exchanger) through controlling the proliferation of epidermal stem cells and differentiation of ionocyte progenitors.
In summary, isotocin and vasotoin exert their actions on regulation of the proliferation of epidermal stem cells and differentiation of ionocyte progenitors to increase ionocyte densities and ultimately regulate the functions of ionocytes for body fluid ionic and acid-base

homeostasis. These novel findings not only enhanced our understanding hormonal control of fish osmoregulation but also provided new insights into vertebrate endocrinology.

Sensitivities of Japanese eel primary ovary on exogenous androgen – correlated with ovarian neuropeptides and their receptors?

Yung-Sen Huang[a*], Chung-Yen Lin[b]

[a] *Department Life Science, National University of Kaohsiung, Kaohsiung, Taiwan.*
[b] *Institute of Information Science, Academia Sinica, Taipei, Taiwan.*

[*] yshuang@nuk.edu.tw

In mammals, the ovary and the central nervous system through the autonomic pathways is connected, the evidences highlight the role of the superior ovarian nerve (SON) in the ovarian phenomena in rats. Furthermore, effects on the ovary of neurotransmitters and neuropeptides have been found in this organ. If the development of eel ovary was more like to organogesis, the role of angiogenic growth factors in organogenesis has been reviewed, angiogenesis and neurogenesis are coupled processes, VEGF stimulates neurogenesis, *vice versa*, the vascular cells are also responsive to neurotrophins. In the eel (*Anguilla spp*.), the positive effect of androgens on the primary ovarian development is established in the last years, but there are few data to elucidate how is this stimulatory effect to exerting. Indeed, by decreasing ovarian *PTEN*/PTEN levels or by stimulating follicular *FSH receptor* expression has been documented in the eel by exogenous androgens. In the Mammal, androgens have been shown to stimulate ovarian granulosa cell proliferation, to increase FSHR, and to decrease AMH, furthermore, to down-regulate TGFβ ligands as well as the receptors has also been reported. Actually, in the eel, the variations on exogenous androgen-induced effect in a population is obvious.

The aims of this study are: (1) to explore what caused the variation on exogenous androgen-induced effect; (2) to understand the correlation between gene background pattern and positive effect of exogenous androgen-induced effects based on expression patterns of ovarian neuropeptide receptors, growth factor receptors, and angiogenic as well as neurogenic factors; (3) to find plausible biological markers to predict the results of eel artificial maturation-induce process.

Pond-cultured Japanese eels with similar body weight (c.a. 650 g, n= 7) were operated to sample ovarian tissues before androgen (MT) implant-treatment (to minimalize the injection-procedure-related stress), then, after 4 weeks, ovarian tissues were collected. Sampled ovarian tissues were stored in the liquid nitrogen. The effect of MT on ovarian development was evaluated based on the increase of egg diameter before and after the treatment in the same individual. The ones with a significant difference on egg diameter were selected, and the paired sampled tissues RNAs were sequenced, transcriptome was made of total RNA-Seq analyzes both coding and multiple forms of noncoding RNA. By the way, the Japanese eel genome has been assembled from the blood of female yellow one. By taking 1,211 scaffolds in length larger than 100Kb, these scaffolds can composite 92% of Japanese eel genome. The transcriptome information of 16,104 annotation genes from three paired ones was implemented into a web database (http://molas.iis.sinica.edu.tw/jpeel).

The results indicated that: (1) The positive effects of MT might be in vain if, in the begin, the egg diameter was small than 0.10 mm. (2) By cluster analysis and based on heatmap method, the ones in the same clade had a similar phenotype, although the pattern of heatmap was not identical, and the certain genes seemed to correlate to the androgen stimulatory effects by Venn diagram method. (3) correlation between the patterns of gene expressions of those growth factors as well as their receptors and the phenotype were not totally coincided with.

Our report based on the exploring data from a transcriptome leads arguments that: (1) the importance of various gene isoforms on a biological process, especially in the Teleost; (2) the importance of basal gene expression levels, of induced gene expression ones, or that of gene expression fold-changes; (3) the importance of the understanding of whole genome on NGS and on gene annotation; (4) the importance of new mathematics or/and statistics, and bio informatics methods on biological studies. (5) the importance of complexity in a small, compact, high efficient cell/tissue.

Activation of the brain-pituitary-gonadotropic axis during gonadal differentiation and testis development in protandrous black porgy, *Acanthopagrus schlegelii*

Chien-Ju Lin[1,2], Guan-Chung Wu[1,2], Sylvie Dufour[3], and Ching-Fong Chang[1,2]

[1]*Department of Aquaculture, National Taiwan Ocean University, Keelung 20224, Taiwan*
[2]*Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 20224, Taiwan*
[3]*Laboratory Biology of Aquatic Organisms and Ecosystems (BOREA), Muséum National d'Histoire Naturelle, CNRS, IRD, Sorbonne Université, Université de Caen Normandie, Université des Antilles, 75231 Paris Cedex 05, France*

cathy30257@yahoo.com.tw; D98330005@ntou.edu.tw

Black porgy, *Acanthopagrus schlegelii*, is a hermaphrodite protandrous teleost, and is a natural mono-sex male fish population for first 2 years as a functional male. An estradiol (E2) -dependent peak in brain activity, including neurosteroidogenesis and neurogenesis, was found in the black porgy during the gonadal differentiation period. The brain-pituitary-gonadotropic (BPG) axis is a key regulator of reproduction and may also be involved in gonadal differentiation, but its activity and potential role in black porgy during the gonadal differentiation period is still unknown. The expression of regulatory factors involved in the BPG axis were analyzed at the time of gonadal differentiation (90, 120, 150 days after hatching [dah]) and subsequent testicular development (180, 210, 300 dah). Gene expression of brain aromatase *cyp19a1b* peaked at 120 dah, and this was followed by a gradual increase during testicular development. The expression of gonadotropin subunits increased slightly but not significantly during gonadal differentiation and then increased significantly at 300 dah. In contrast, the expression of brain *gnrh1* and pituitary *gnrh receptor 1* (*gnrhr1*) exhibited a pattern with two peaks, the first at 120 dah, during the period of gonadal differentiation, and the second peak during testicular development. Gonad *fshr* and *lhcgr* increased during gonadal differentiation period with highest transcript level in prespawning season during testicular development. These results support the possibility of the activation of the axis of brain *gnrh1*, pituitary *gnrhr1* and gonadotropin subunits, and gonad *gthrs* at the time of gonadal differenciation. It might be just a correlation, but also that the activation of this axis may be involved in the activation of gonadal differentiation in black porgy. E2 treatment increased brain *cyp19a1b* expression at each sampling time, in agreement with previous studies in black porgy and other teleosts. E2 also significantly stimulated the expression of pituitary gonadotropin subunits at all sampling times, indicating potential E2-mediated steroid feedback. In contrast, no significant effect of E2 was observed on *gnrh1*. Furthermore, treatment of AI or E2 had no statistically significant effect on *gnrh1* transcription levels during gonadal differentiation. This indicated that the early peak of *gnrh1* expression during the gonadal differentiation period is E2-independent and therefore not directly related to the E2-dependent peak in brain neurosteroidogenesis and neurogenesis also occurring during this period in black porgy. Both E2-independent and E2-dependent mechanisms are thus involved in the peak expression of various genes in the brain of black porgy at the time of gonadal differentiation.

Keywords: teleost, *cyp19a1b*, *gnrh*, estradiol, gonadal differentiation

Evolutionary history of neuropeptide and receptor super-families in vertebrates, with a special focus on a basal teleost, the eel

Anne-Gaëlle Lafont[1], Aurora Campo[1], Nédia Kamech[1], Benjamin Lefranc[2], Jérôme Leprince[2], Hervé Tostivint[3], Karine Rousseau[1] and Sylvie Dufour[1]

[1] *Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, Muséum National d'Histoire Naturelle, CNRS, IRD, SU, UCN, UA, Paris, France*
[2] *Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, INSERM U1239, Normandy University, 76000 Rouen, France*
[3] *Molecular Physiology and Adaptation, UMR 7221 CNRS, Muséum National d'Histoire Naturelle, Sorbonne Universités, Paris, France.*

anne-gaelle.lafont@mnhn.fr

G protein-coupled receptors (GPCR), represent one of the largest families of proteins in metazoan. Some GPCR are activated by small signaling molecules, named neuropeptides, and together play a crucial role in major physiological functions, such as growth, development and reproduction. In vertebrates, rounds of whole genome duplications, followed by potential loss events, have generated the number of paralogs present in extant species. The European eel, *Anguilla anguilla*, as a representative species of a basal group of teleosts (Elopomorphs), represents a good model for studying the impact of the teleost-specific third round of whole genome duplication (3R) on gene diversity and functionality in teleosts. As a model of physiological, ecological and economical relevance, the eel represents also a key species in the study of the evolution of neuroendocrine systems and their role in the regulation and the plasticity of biological cycles and reproduction.

In this study, we focused on GPCR and neuropeptides related to the RF-amide super family and likely relevant for reproductive functions. *In silico* analysis of the European eel genome has led to the characterization of 16 RF-amide receptors and 51 RF-amide related receptors in the eel. The corresponding sequences have been retrieved from the genomes of human and spotted gar, a representative of non-teleost actinopterygians, holosteans, that emerged before 3R. Phylogeny analysis of these genes has led to the nomenclature identification and classification of these receptors and suggested various impact of 3R on the number of eel paralogs. Different scenarii have been proposed according to gene investigated, such as (1) no impact of 3R, (2) conservation of all 3R-duplicated paralogs, (3) additional losses in the eel after 3R. In order to refine the RF amide related family classification and nomenclature, an additional phylogeny analysis has been performed including orthologous genes from various vertebrates. This analysis has revealed the existence of a new well-supported (bootstrap of 100) sub-group of RF-amide related receptors. This sub-group was probably not yet identified, due to the absence of a paralog in human and the inexact automatic annotations of genes in numerous genomes.

Further analyses of RF-amides related neuropeptides and receptors have been performed in families involved in reproduction, such as Tachykinins and Tachykinin receptors. Phylogeny analyses in vertebrates have shown the impact of 1R, 2R, and 3R on these gene families. The investigation in the eel allowed us to retrieve the full complement of tachykinin peptides and receptors for the first time in a teleost. Likely resulting from teleost 3R, the eel presents a double set of tachykinin genes (six tachykinin genes: two *Tac1,* two *Tac3* and two *Tac4*) as well as a double set of tachykinin receptor genes (six), as compared to three tachykinin and three tachykinin receptors in the spotted gar and human. A special focus has then been made on the evolutionary history of *Tac3* and its potential function in the

European eel. In mammals, neurokinin B (NKB) is a short peptide encoded by the *tac3* gene involved in the brain control of reproduction. Two *tac3* paralogous genes (*tac3a* and *tac3b*) were identified in the eel genome, each encoding two peptides (NKBa and NKBb, and NKB-related peptides: NKB-RPa and NKB-RPb, respectively). Phylogeny and synteny analyses have suggested that the duplicated *tac3 g*enes in teleosts likely result from the teleost-specific whole genome duplication (3R). Among teleosts, TAC3b amino acid precursor sequences are more divergent than TAC3a, and a loss of *tac3b* gene may have occurred independently in some teleosts. Tissue distribution of eel *tac3a* and *tac3b* mRNAs, as analyzed by real time qPCR, showed major expression of both transcripts in the brain. The four eel peptides have been synthesized and tested *in vitro* on primary culture of eel pituitary cells. They all inhibited the expression of both luteinizing hormone beta subunit, *lhß* and gonadotropin-releasing hormone receptor, *gnrh-r2*. This reveals a potential dual inhibitory role of the four peptides encoded by the two *tac3* genes in eel reproduction, exerted at the pituitary level on both luteinizing hormone and GnRH receptor.

Ancient coevolution of neuropeptides and their receptors in bilaterian genomes

Olivier Mirabeau

Institut Curie, Paris, France

olivier.mirabeau@gmail.com

Neuropeptides are small secreted proteins that signal through G protein-coupled receptors (GPCR) to modulate the activity of behavior and physiology-controlling neurons in the brain of animals. These ligand-receptor pairs form peptidergic systems (PS) that are found in large numbers in the genome of all animals with bilaterian symmetry (bilaterians). During my postdoc I have tried to uncover the evolutionary relationships between these different systems.

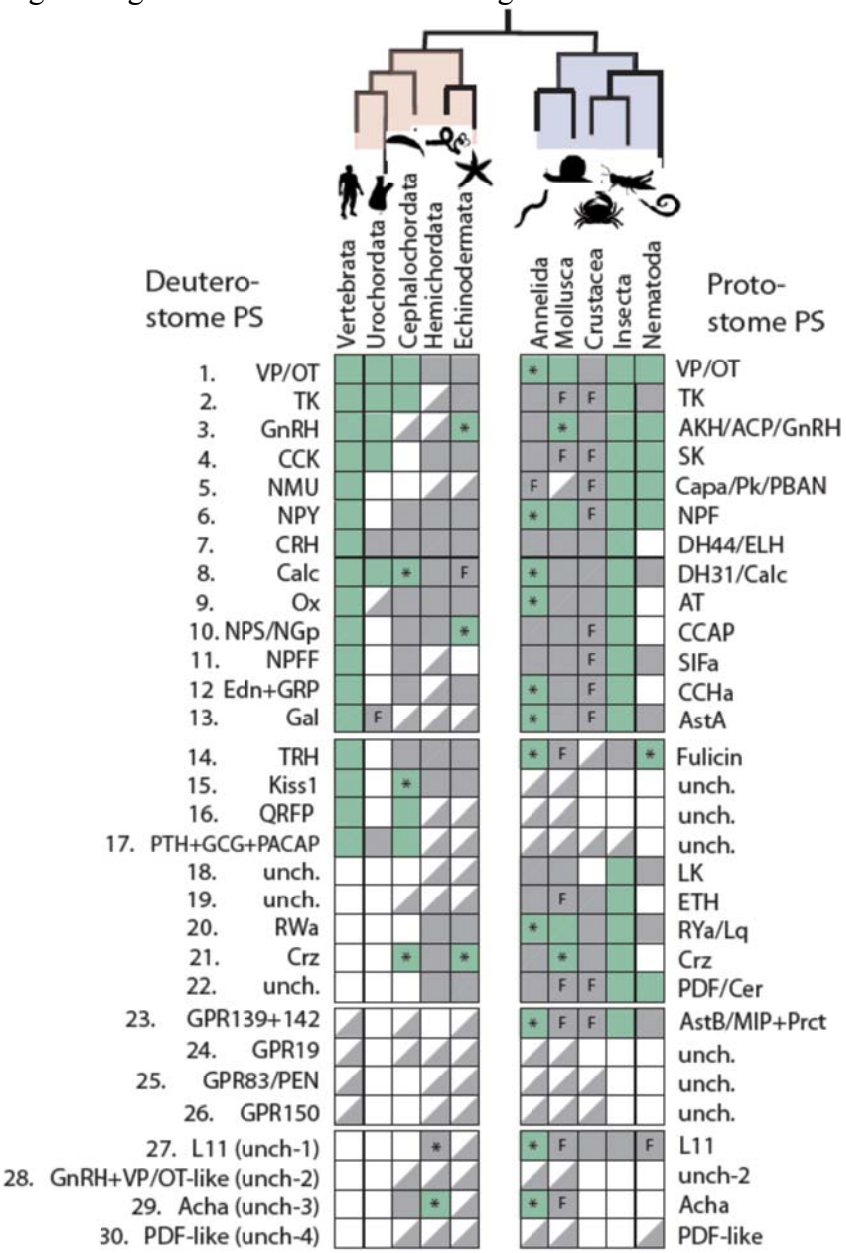
By probing publicly available genomic databases I constructed alignments of GPCR sequences from multiple species and used state-of-the-art phylogenetic reconstruction methods to infer orthology relationships between receptors from mollusks, insects and vertebrates. I saw an unexpected large number of groups of orthologous receptors (29) containing sequences from both deuterostomian (echinoderms and chordates) and protostomian (insects, nematodes and mollusks), a number which was updated to 30 in a recent review (Elphick, Mirabeau and Larhammar 2018). The results obtained on receptor sequences were used as a canvas to understand the evolutionary relationships among the peptide precursors that were predicted from proteome sequences from bilaterian species. It is possible, using hidden Markov models, to predict neuropeptide precursors from primary sequence alone because they have characteristic features including a short sequence, a secretory signal peptide, and dibasic cleavage sites which delimitate mature peptides buried inside the precursor.

Strikingly the analysis of known peptide-GPCR pairs, originally mostly from biochemical studies in mammals and insects, and of predicted GPCR and peptide pairs found either by looking at the phylogenetic trees of GPCRs or by visual inspection of peptide precursor alignments built incrementally by trial-and-error, led to the conclusion that the evolutionary relationship of peptide precursors was consistent with that of their cognate receptors. This consistency argues for a large-scale long distance coevolution between neuropeptides and their receptors.

We can now ask ourselves what is the implication of these orthology relationships and how it affects our understanding of the function of these ligand-receptor systems. Can this conservation of molecular function established through orthology be extended to other levels of functional conservation, including the neuronal circuit level (participation in the same neuronal circuits), the expression regulation level (eg expression controlled through the same factors or mechanisms, expression in the same tissues) and global behavioral and physiological levels (eg feeding behavior or energy homeostasis). Some recent studies point to a possible conservation of function between protostomian and deuterostomian at the homeostatic level (Van Sinay et al., 2017), but these studies are sparse and need to be further substantiated.

Elphick, M.R., Mirabeau, O., and Larhammar, D. (2018). Evolution of neuropeptide signalling systems. J. Exp. Biol. 221, jeb151092.

Van Sinay, E., Mirabeau, O., Depuydt, G., Van Hiel, M.B., Peymen, K., Watteyne, J., Zels, S., Schoofs, L., and Beets, I. (2017). Evolutionarily conserved TRH neuropeptide pathway regulates growth in Caenorhabditis elegans. Proc. Natl. Acad. Sci. 201617392.



Legend: Occurrence and characterisation of neuropeptide signalling pathways in bilaterians (modified from Elphick, Mirabeau and Larhammar 2018).
The occurrence of 30 bilaterian neuropeptide signalling systems in different taxa is shown, with deuterostomian phyla or sub-phyla (pink) shown on the left side, and protostomian phyla/classes shown on the right side (light blue). PS, peptidergic systems. A square half-filled with grey indicates that only the receptor of a neuropeptide-receptor signalling pathway has been identified in a taxonomic group. A full grey square indicates that both a peptide ligand and a receptor for a neuropeptide signalling pathway has been identified in a taxonomic group. A full green square indicates that, for at least one member of that taxonomic group, binding between a peptide and its receptor has been demonstrated experimentally. Inclusion of an asterisk in a green full square indicates that binding between a peptide and its receptor has been reported since publication of Mirabeau and Joly (2013). An

empty (white) square indicates that a neuropeptide signaling pathway has been lost in a taxonomic group. Inclusion of the letter F in a grey square indicates that experimental insights into the physiological function(s) of the neuropeptide have been obtained.

Differences and Similarities between Mammalian and Fish Endozepinergic System

Jérôme Leprince,[a,b] Koukei Matsuda,[c] Benjamin Lefranc,[a] Marie-Christine Tonon,[a] and Hubert Vaudry,[b]

[a] *Normandy University, Neuronal and Neuroendocrine Differentiation and Communication, Inserm U1239, Rouen, France*
[b] *Normandy University, Regional Plateform for Cell Imaging of Normandy (PRIMACEN), Institute for Research and Innovation in Biomedicine, Rouen, France*
[c] *Toyama University, Laboratory of Regulatory Biology, Graduate School of Science and Engineering, Toyama, Japan*

jerome.leprince@univ-rouen.fr

The term "endozepines" designates a family of peptides that were originally isolated from the rat brain for their ability to interact with benzodiazepine receptors. All endozepines originate from the processing of diazepam-binding inhibitor (DBI), an 86-amino acid polypeptide whose sequence is well conserved in mammals, notably in regions corresponding to the sequences of the triakontatetraneuropeptide TTN (DBI_{17-50}) and the octadecaneuropeptide ODN (DBI_{33-50}), suggesting that endozepines exert vital functions. Indeed, there is currently clear evidence that endozepines are involved in the pathophysiology of anxiety and mood disorders, affect energy homeostasis, exert neuroprotective effects, and control steroid and peptide hormone secretion.[1]

Mainly documented in mammals, the endozepinergic system has also been identified in the central nervous system of non-mammalian vertebrates showing that evolutionary pressure has acted to preserve the DBI sequence in the vertebrate phylum. These comparative studies have highlighted strong conservation of the functional properties of endozepines during evolution but have also revealed substantial differences notably in the expression pattern of the DBI gene. The aim of the present work is to summarize the current knowledge concerning endozepines in fish and more specifically in the goldfish (*Carassius auratus*), and to compare their actions to those reported in rodents.

Immunohistochemical studies using antibodies against rat or human ODN have shown that, in teleost fish, endozepine immunoreactivity is present in hypothalamic neurons located in the lateral part of the nucleus lateralis tuberis, in trout and goldfish.[2,3] The occurrence of endozepines in fish neurons is in marked contrast with the glial expression of DBI in mammals and amphibians.[1,4] In the trout as in the goldfish, ODN-positive neurons send processes in the pars intermedia and proxima pars distalis of the pituitary.[2,3]

The full-length DBI cDNA has been cloned in the goldfish.[5] It encodes an 87-amino acid polypeptide that displays more than 80% similarity with zebrafish and chicken DBI, and 61% with the human and rat counterparts. Using a scototaxis protocol (white/black background preference test), it was found that intracerebroventricular (icv) injection of ODN (10 pmol/g BW) or FG-7142, a central-type benzodiazepine receptor inverse agonist (9 pmol/g BW), increased the time to move from the black to the white background area indicating that, in fish as in mammals, ODN exerts anxiogenic-like effects. Moreover, preinjection of the central-type benzodiazepine receptor antagonist flumazenil totally prevented the ODN-induced psychomotor activity.[5]

Previous studies have shown that very low doses of ODN inhibit food intake in rodents.[6,7] Since conspicuous differences have been reported in the effect of several neuropeptides on energy homeostasis in fish and in mammals,[8] the central effect of ODN on

feeding behavior was investigated in fish. Icv injection of ODN induced a dose-dependent inhibition of food intake in goldfish. Flumazenyl did not modify the ODN-evoked effect, while cyclo[DLeu^5]OP, a selective antagonist of a still unknown GPCR for ODN, reversed its inhibitory action.[9]

Finally, consistent with the occurrence of ODN-immunoreactive fibers in the goldfish pars intermedia where somatolactin-secreting cells are located, ODN stimulated somatolactin release from cultured goldfish pituitary cells. The hypophysiotrophic action of ODN was blocked by preincubation with cyclo[DLeu^5]OP, indicating that the ODN-induced activation of somatolactin release is mediated through the endozepine GPCR.[3]

In conclusion, these studies provide evidence for a role of endozepines in the control of emotional behavior and energy homeostasis in sub-mammalian vertebrates as well as the role of ODN as a hypophysiotrophic neurohormone. Taken as a whole, our data show that not only the anxiogenic and anorexigenic actions of endozepines, but also the type of receptor mediating these effects and its pharmacological properties have been fully conserved during evolution, from fish to mammals.

This work was supported by the Agence Nationale de la Recherche (grant NEMO; ANR-14-CE02-0020 to JL), the Institut National de la Santé et de la Recherche Médicale (Inserm) and the Normandy University (Rouen).

1. Tonon M.-C., Vaudry H., Chuquet J., Guillebaud F., Fan J., Masmoudi-Kouki O., Vaudry D., Lanfray D., Morin F., Prevot V., Papadopoulos V., Troadec J.-D., Leprince J. Endozepines and their receptors: structure, functions and pathophysiological significance. *Pharmacol. Ther.* (in revision).

2. Malagon M., Vallarino M., Tonon M.-C., Vaudry H. (1992) Localization and characterization of diazepam-binding inhibitor (DBI)-like peptides in the brain and pituitary of the trout (Salmo gairdneri). *Brain Res* **576**, 208-214.

3. Azuma M., Wada K., Leprince J., Tonon M.-C., Uchiyama M., Takahashi A., Vaudry H., Matsuda K. (2013) The octadecaneuropeptide stimulates somatolactin release from cultured goldfish pituitary cells. *J. Neuroendocrinol*. **25**, 312-321.

4. Malagon M., Vaudry H., Vallarino M., Gracia-Navarro F., Tonon, M.-C. (1992) Distribution and characterization of endozepine-like immunoreactivity in the central nervous system of the frog Rana ridibunda. *Peptides* **13**, 99-107.

5. Matsuda K., Wada K., Azuma M., Leprince J., Tonon M.-C., Sakashita A., Maruyama K., Uchiyama M., Vaudry H. (2011) The octadecaneuropeptide (ODN) exerts anxiogenic-like action in goldfish. *Neuroscience* **181**, 100-108.

6. Garcia de Mateos-Verchère J., Leprince J., Tonon M.-C., Vaudry H., Costentin J. (2001) The octadecaneuropeptide [diazepam-binding inhibitor (33-50)] exerts potent anorexigenic effects in rodents. *Eur. J. Pharmacol.* **414**, 225-231.

7. Do-Régo J.-C., Orta M.-H., Leprince J., Tonon M.-C., Vaudry H., Costentin J. (2007) Pharmacological characterization of receptor mediating anorexigenic action of the octadecaneuropeptide: evidence for an endozepinergic tone regulating food intake. *Neuropsychopharmacology* **32**, 1641-1648.

8. Matsuda K., Kang K.S, Sakashita A., Yahashi S., Vaudry H. (2011) Behavioral effect of neuropeptides related to feeding regulation in fish. *Ann N Y Acad Sci*. **1220**, 117-126.

9. Matsuda K., Wada K., Miura T., Maruyama K., Shimakura S.I., Uchiyama M., Leprince J., Tonon M.-C., Vaudry H. (2007) Effect of the diazepam-binding inhibitor-derived peptide, octadecaneuropeptide, on food intake in goldfish. *Neuroscience* **150**, 425-432

Mass spectrometry as a tool for Neuropeptides identification

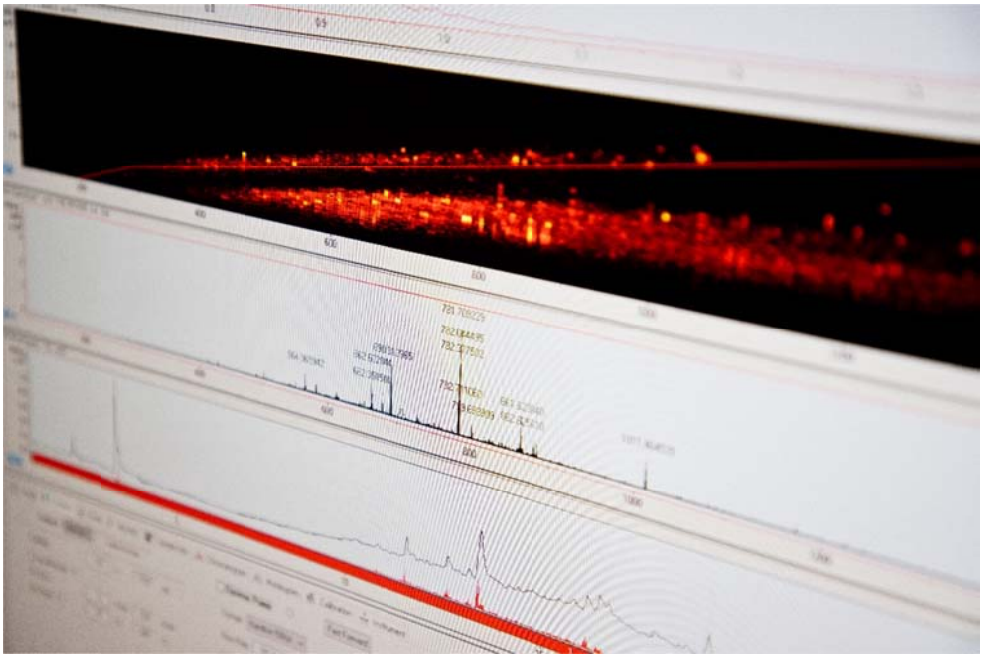
Benoît Bernay[1]

[1] *Normandie Université, UNICAEN, SF4206 ICORE, PROTEOGEN Esplanade de la Paix, 14032 CAEN cedex*.

benoit.bernay@unicaen.fr

Neuropeptides are ones of the most diverse classes of signaling molecules and are implicated in a wide range of physiological processes. However, there are unique challenges associated with their studies stemming from the highly variable molecular sizes of the peptides, low *in vivo* concentrations, high degree of structural diversity and large number of isoforms. In this context mass spectrometry (MS) has proven to be useful for detecting small amounts of peptides in complex biological samples, making it a high-throughput and versatile technique ideal for the study of endogenous neuropeptides. This advancement has enabled the studying the entire neuropeptide complement as a whole either by comparing spectra to a database of known neuropeptides or *de novo* sequencing to discover new neuropeptides.
Using examples of characterized neuropeptides in marine organisms, MS method will be detailed as a tool for:

- Peptides identification
- Peptides sequencing
- PTM characterization
- Peptide quantitation



LC-IMS-MS spectrum of a complex sample analysis.

Characterization of neuroendocrine signalling systems during gametogenesis in the Pacific oyster, *Crassostrea gigas*

Emilie Realis-Doyelle[1], Julie Schwartz[1], Cedric Cabau[2], Christophe Klopp[2], Marie-Pierre Dubos[1], Guillaume Rivière[1], Pascal Favrel[1].

[1] *UMR BOREA, Normandie Université, UNICAEN, MNHN, CNRS-7208, IRD-207, Sorbonne Université, Esplanade de la Paix, 14032 CAEN cedex.*
[2] *INRA, Sigenae UR875 Biométrie et Intelligence Artificielle, BP 52627, Castanet-Tolosan Cedex, 31326, France.*

emilie.realis-doyelle@unicaen.fr

Mollusca with more than 85,000 species represents one of the most diverse group of animals on the planet. In the marine environment they represent approximately 23% of the 230,000 known animal species. Despite the major commercial and ecological importance of some species, mollusks remain like a majority of Lophotrochozoan species, an under-described phylogenetic group with respects to the functional knowledge of their large genomic and transcriptomic resources. Their adaptation to various environments could be explained by their mode of reproduction. Indeed, the reproduction maintains a diversity of traits, adapts to labile environments, and contributes to the evolution by mixing genes. As for a majority of biological processes, reproduction is physiologically regulated by the neuroendocrine system which also participates to the elaboration of adapted responses to environmental constraints.

To gain insight into the role of the neuroendocrine system in the regulation of reproduction in a mollusc, we investigated the molecular bases of this system in the Pacific oyster *Crassostrea gigas*.

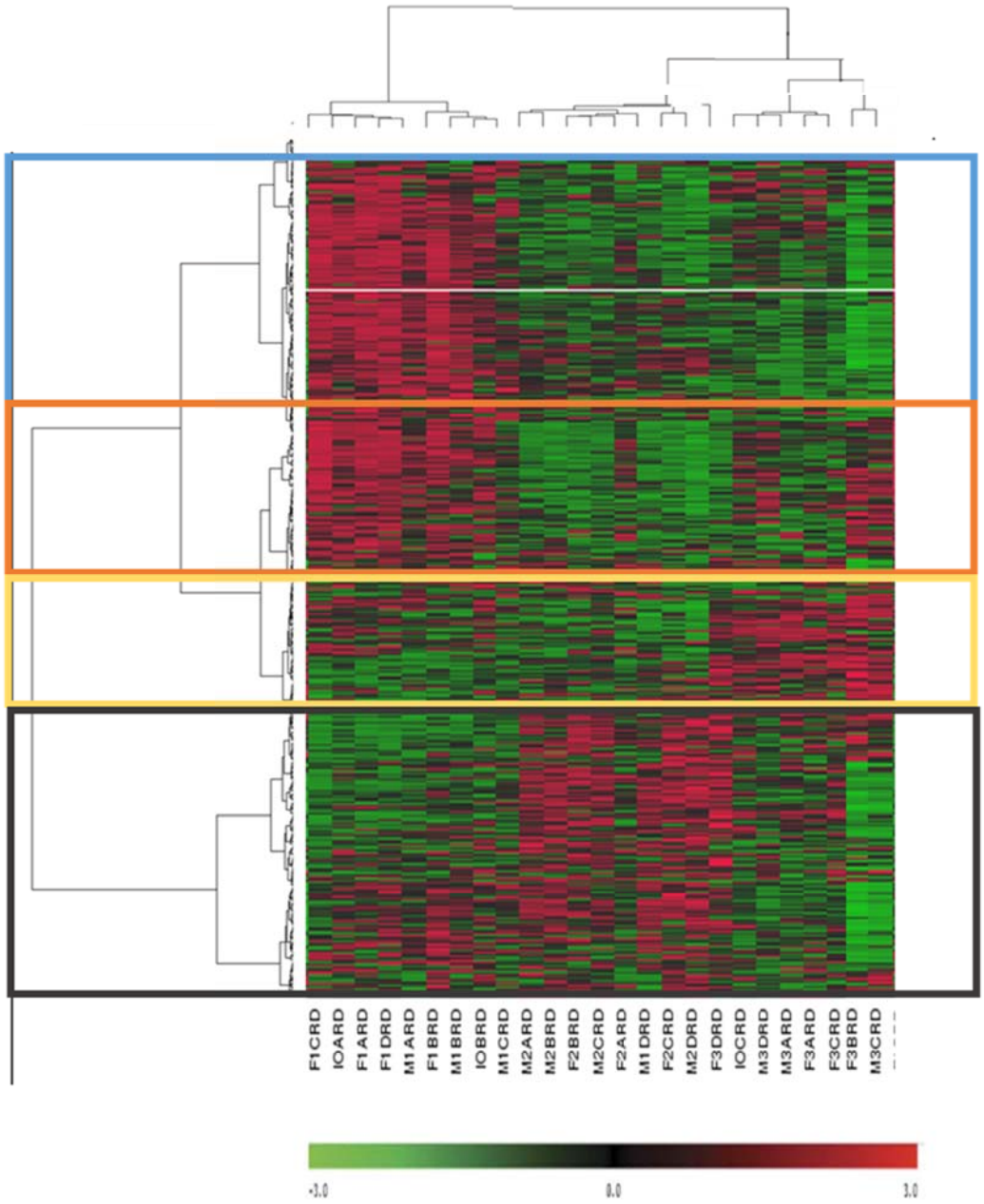
A genome wide expression profiling of visceral ganglia at different stages of gametogenesis was conducted using quantitative RNAseq data. Statistical analysis using student's t tests ($p<0.01$ with adjusted Bonferroni's correction) found no difference between males and females but identified, among 37518 transcripts, 416 differentially expressed genes distributed into 4 clusters corresponding to distinct expression patterns: gene expression decreasing as gametogenesis proceeds, gene expression increasing along the gametogenetic cycle, genes with a high expression at the maturation stage. Among them, 35 genes encoding G-protein coupled receptors (GPCR) (dopamine receptor, neuropeptide F receptor, FMRF amide receptor …) and 13 genes encoding neuropeptide precursors (Allatostatin B, GnRH, Calcitonin, Allatostatin C–like and allatotropin-like and NKY …) were identified suggesting their involvement in the regulation of reproduction or associated processes such as energy allocation.

Although statistically not significantly differentially expressed in the VG during reproduction, we were interested in a GPCR that was shown to be expressed in the gonads in GigaTON, a *C. gigas* comprehensive transcriptomic database. BLAST analysis showed homology of the *C. gigas* receptor with arthropod Crustacean Cardioactive peptide receptors and was named (Cragi-CCAPR). This receptor displays 42% identity with Drosophila CCAPR and 34% human neuropeptide S receptor (NPSR). A calcium mobilization assay was used to identify the cognate ligands of Cragi-CCAPR. Transiently transfected HEK293T cells expressing the oyster receptor and the promiscuous G protein Gα16 were challenged with putative oyster CCAP synthetic peptides (Cragi-CCAP1: VFCNGFFGCSN-amide,

Cragi-CCAP2: LFCNTGGCF-amide) and with the structurally related oyster putative oxytocin (Cragi-Oxy1: CFIRNCPQG-amide and Cragi- Oxy2: GCFIRNCPPG-amide). These peptides have previously been characterized as part *of C. gigas*' repertoire of neuropeptides. Cragi-CCAPR was activated by Cragi-CCAP1 peptide in presence or absence of the promiscuous Gα16 protein, a dose-dependent activation of Cragi-CCAP1 was recorded by omitting the Gα16 protein. Half maximal effective concentration (EC50) was of $1{,}47.10^{-7}$ M for Cragi-CCAP1 peptide. In contrast, Cragi-CCAP2 and Cragi-Oxy1/2 were ineffective even at high doses.

The expression of Cragi-CCARP and of Cragi-CCAP1 genes was investigated by RT-qPCR in adult tissues. We found that Cragi-CCAPR gene was significantly more expressed in gills and to a lower level in a majority of adult tissues especially the digestive gland and the gonads. The gene encoding the precursor of Cragi-CCAP was mainly expressed in the visceral ganglia and to a lesser extent in the labial palps and the gills.

Expression profiles of Cragi-CCAPR and Cragi-CCAP genes were then investigated in the VG and the gonads along the reproductive cycle. Both genes showed a peak of expression at stage 1 (gonial multiplication stage and energy storage stage) in both male and female gonads. In VG, the Cragi-CCAPR gene expression pattern paralleled that in the gonad although the Cragi-CCAP gene expression was shifted. These results suggest a role of this signalling system in the neuroendocrine control of reproduction and underline congruent results in other molluscs (cuttlefish) and in Locusts.



Heat map of gametogenesis specific genes, Hierarchical clustering obtained using Pearson's correlation on the 416 genes differentially genes distributed into 4 clusters corresponding to distinct expression patterns: gene expression decreasing as gametogenesis proceeds, gene expression increasing along the gametogenetic cycle, genes with a high expression at the maturation stage (T test, p,0.01, adjusted Bonferroni's correction, rows) and on all individual VG samples (columns).

How protandrous black porgy to determine their sexes through the HPG axis?

Guan-Chung Wu[1,2], Hau-Wen Li[1], Chien-Ju Lin[1], Ching-Fong Chang[1,2]

[1]*Department of Aquaculture, National Taiwan Ocean University, Keelung 20224, Taiwan*
[2]*Center of Excellence for the Ocean, National Taiwan Ocean University, Keelung 20224, Taiwan*

gcwu@mail.ntou.edu.tw

Sex steroids are widely used for controlling the sexes of fish. However, hermaphroditic fish have a plastic sex, and a stable sex is difficult to maintain with sex steroids. We used the black porgy (*Acanthopagrus schlegelii*) as a model to understand the possible mechanism of sexual fate decision. Exogenous E2 (6 mg/kg of feed) induced the regression of the testis and the development of the ovary and resulted in an unstable expression of femaleness (passive femaleness, with the primary oocytes). The removal of testicular tissue by surgery could result in a precocious femaleness. Our data demonstrated that natural sex change is blocked by the maintenance of male function with gonadotropin-induced dmrt1 expression in the testis. However, our data also showed that sexual fate not maintenance in maleness through a long-term LHRHa treatment. Our finding revealed that transition of sexual fate from male to female is determined by the status of the testicular tissue.

Expression of sex-specific genes during testicular differentiation in Japanese eel

Shan-Ru Jeng[1*], Guan-Chung Wu[2], Wen-Shiun Yueh[1], Yi-Ting Pen[1] Sylvie Dufour[3] and Ching-Fong Chang[2, 4]

[1] Department of Aquaculture, National Kaohsiung University of Science and Technology,, Kaohsiung 811, Taiwan; [2]Department of Aquaculture, National Taiwan Ocean University, Keelung 202, Taiwan; [3] CNRS, MNHN, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, Paris, France; [4]Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 202, Taiwan

srjeng@nkust.edu.tw

The process of gonadal development and molecular mechanism involved in sex differentiation in eels are still unclear. The objectives were to investigate the gonadal development and expression pattern of sex-related genes during sex differentiation in the Japanese eel, *Anguilla japonica*. For control group, the elvers of 8-10 cm were reared for 8 months; and for feminization, estradiol-17β (E2) was orally administered to the elvers of 8-10 cm for 6 months. Only males were found in the control group, suggesting a possible role of environmental factors in eel sex determination. In contrast, all differentiated eels in E2-treated group were female. Gonad histology revealed that male eels seem to differentiate through an intersexual stage, while female eels (E2-treated) would differentiate directly from an undifferentiated gonad. The transcripts of *vasa*, *wt1*, *sox9a*, *nr5a1*, *dax1*, *dmrt1*, *amh*, *amhr2* and *gsdf-like* were significantly increased in males during testicular differentiation and development. Dmrt1 proteins were abundant expressed in spermatogonia B cells, spermatocytes, spermatids but not in spermatozoa, spermatogonia A or Sertoli cells. Therefore, Dmrt1 might play vital roles at the specific stages during spermatogenesis from spermatogonia B cells to spermatids in the Japanese eel. Furthemore, we found that *gsdf-like* was expressed in somatic cells during testicular differentiation. *Gsdf-like* may play a crucial role in testicular differentiation. The *figla* and *sox3* transcripts in ovary were significantly increased during sex differentiation, and were related to ovarian differentiation. The *cyp19a1* transcripts were significantly increased in differentiating and differentiated gonads, but did not show a differential expression between the control and E2-treated eels. This suggests that *cyp19a1* is involved both in testicular differentiation and development in control males, and in the early stage of ovarian differentiation in E2-treated eels.

Key words: sex differentiation; dmrt1; Japanese eel

The plasticity of gonad development of sexual reproduction in a scleractinian coral, *Porites lichen*

Chieh-Jhen Chen

Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 20224, Taiwan

coralerin2933@gmail.com

Metazoans have evolved a wide array of reproductive patterns. Sexuality in the reproductive biology of scleractinian corals is not well understood, and this study aimed to address the sexuality and other reproductive characteristics in *Porites lichen*, a common species in the Indo-West Pacific. This study represents the first description of this species' sexual characteristics, which were determined by histological analysis of the samples collected in northern Taiwan. In addition, female and hermaphroditic colonies were separately cultured in aquarium to further monitor the release of eggs/planulae and thereby confirm the mode of reproduction. The results demonstrate that *P. lichen* is a polygamodioecious brooder and displays seasonal gametogenesis and embryogenesis that ends in late summer. In hermaphroditic colonies, male polyps are predominant and hermaphroditic polyps make up a very small percent (1% to 19.3%). In addition, two new gametogenic features were observed from the histological analysis: 1) oocytes developed within the spermaries in hermaphroditic polyps during the early stage of gametogenesis and 2) melanin granular cells were clustered in spermaries in both male and hermaphroditic colonies. These unique features provide insights into the comparative biology of metazoan reproduction.

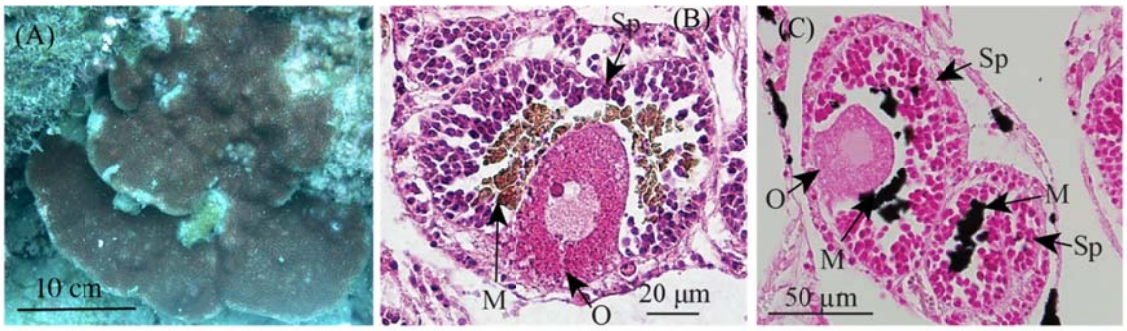


Figure (A) a colony of *Porites lichen*, (B) oocyte appeared in the spermary and (C) melanin granular cell appeared in the spermary.

Differential expression of brain arginine vasotocin and isotocin in response to osmotic change in black porgy, *Acanthopagrus Schlegelii*

Aruna Nagarajan[1*], Nagarajan Ganesan [2], Ching Fong Chang[1]

1. *Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan*
2. *Basic sciences department, PYD, King Faisal University, Saudi Arabia*

n.aruna@yahoo.com

Our studies for the first time demonstrate a differential expression of arginine vasotocin (*avt*)/ isotocin (*it*) system in brain and pituitary of black porgy (*Acanthopagrus schlegelii*) in the time course of osmotic stress. The expression of *avt*, *avt*-receptor (*avt-r)* and *it* receptor (*it-r)* transcripts at day 1 (an immediate response) and *it* and *it-r* transcripts at day 7 and 30 (a dilatoray response) in the brain and pituitary of freshwater (FW) fish revealed that the *avt* and *it* transcripts were differentially sensitive to the external salinity as well as different exposure time. The transcript of *it-r* is the most sensitive one and had the big increase in fish exposed to the environmental change. The expression of *it-r* after in FW transfer could be increased by 10-fold in telecephalon (Tel), 20-fold in diencephalon (Dien) and 7-fold in pituitary at day 7. In the present study, decreased osmolality and increased cortisol in serum were observed in FW black porgy compared to seawater (SW) fish. Furthermore, *in situ* hybridization demonstrated a spatially differential expression of *avt*, *avtr*, *it* and *itr* transcripts were expressed in the nucleus Preopticus parvocellularis of pars gigantocellularis (PMgc), magnocellularis (PMmc) and parvocellularis (PMpc) of the hypothalamus. And, the *avt*, *it* and *itr* neurons were highly abundant in PMpc by osmotic stress highlighted that the localization of *avt*, *it* and *it-r* neurons in PMpc may controlling homeostasis.

Key words: avt, avt-r, it, it-r, salinity stress

Peptides of the urotensin II family: evolutionary and functional perspectives

Tostivint H.[1]**,** Xu B.[2], Gaillard A.L[1]., Mirabeau O. [3], Mohamad T[1]., Bergqvist C.A.[2], Bertrand S.[4], Leprince J.[5], Escriva H.[3], Larhammar D.[2], Pézeron G.[1]

[1] *Molecular Physiology and Adaptation, UMR 7221 CNRS, Muséum National d'Histoire Naturelle, Sorbonne Universités, Paris, France.*
[2] *Department of Neuroscience, Science for Life Laboratory, Uppsala University, Uppsala, Sweden.*
[3] *Genetics and Biology of Cancers Unit, INSERM U830, Institut Curie, PSL Research University, Paris, France.*
[4] *Biologie Intégrative des Organismes Marins, UMR 7232 CNRS, Observatoire Océanologique, Université Pierre et Marie Curie, Sorbonne Universités, Banyuls-sur-Mer, France.*
[5] *Neuronal and Neuroendocrine Differentiation and Communication, INSERM U982, Institute for Research and Innovation in Biomedicine (IRIB), Regional Platform for Cell Imaging of Haute-Normandie (PRIMACEN), University of Rouen, Mont-Saint-Aignan, France*

htostivi@mnhn.fr

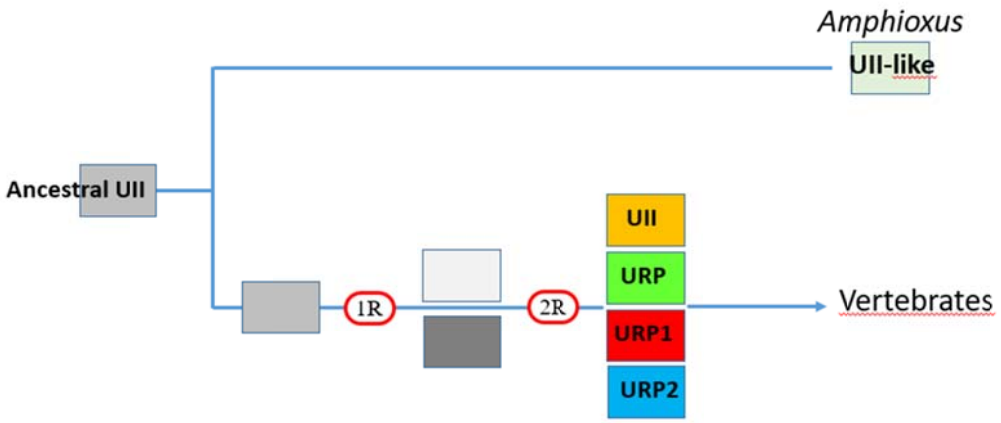
Urotensin II (UII) is an evolutionarily conserved neuropeptide initially isolated from teleost fishes in 1980 on the basis of its smooth muscle-contracting activity. Subsequent studies have demonstrated the occurrence of several UII-related peptides (URPs) in vertebrates. The UII family is now known to include at least four paralogue genes called *UII*, *URP*, *URP1* and *URP2*. Our results suggest that these genes probably arose through the two rounds of whole genome duplication (2R) that occurred during early vertebrate evolution. *UII* has been found in all vertebrates, while the occurrence of *URP* has only been reported in osteichthyans. For their part, *URP1* and *URP2* were initially observed only in actinopterygians but our recent studies revealed their existence also in chondrichthyans and sarcopterygians, respectively.

UII and URPs exhibit the same cyclic hexapeptide core sequence (CFWKYC) while their N- and C-terminal regions are more variable. All these peptides exert their action through G-protein-coupled receptors called UTRs. We showed that the vertebrate ancestor possessed five UTRs (UTR1-5) genes that also originated in 2R.
In teleosts, as in chondrichthyans, UII mRNA and/or peptide has been mainly found in Dahlgren cells of the caudal neurosecretory system. In contrast, in tetrapods, the UII gene is primarily expressed in motoneurons of the brainstem and the spinal cord. For its part, URP mRNA is mainly located in motoneurons in both tetrapods and teleosts. Finally, URP1 and URP2 mRNAs were principally observed in the cerebrospinal fluid- contacting neurons of the spinal cord.

The functional properties of UII have been extensively studied since the 80's. UII has been shown to exert a large array of biologic activities, in particular in the central nervous system, the cardiovascular system, and the kidney. In contrast, the actions of URPs have been only poorly investigated. To gain more insight into the physiological roles of URPs, we performed loss-of-function experiments of the *URP*s

genes by using the zebrafish as a model. For this purpose, CRISPR/Cas9 *urp, urp1 and urp2* zebrafish mutants were produced, as well as *urp1/urp2* double mutants. Interestingly, *urp1/urp2* double mutants were found to exhibit severe body-curvature defects. Moreover, gene invalidation of one of the five zebrafish UTRs, called *uts2ra,* specifically expressed in dorsal somitic muscle cells, produced the same phenotype. These findings, reported independently by another team (Zhang 2018), suggest that the URP1/URP2 signaling pathway is a major determinant of vertebral axis formation during fish embryogenesis.

Our investigations in vertebrates suggest that a single UII-UTR couple was present in the chordate ancestor. In order to test this hypothesis, we searched the UII and UTR homologs in the amphioxus genome. One receptor (AmphUTR) clustered in phylogenetic analyses with vertebrate UTRs. Moreover, an UII-like cyclic peptide (amphUII) consisting of 15 amino acids and containing a WK motif was identified. AmphUII-expressing cells were found by *in situ* hybridization in the cerebral vesicle and close to the first pigment spot both in the amphioxus embryo and larva. The amphUII peptide was found to potently stimulate the receptor's ability to induce IP turnover in amphUTR-transfected human cells. Our data suggest that a functional UII-ergic system has already emerged in amphioxus, which provides insights into the evolutionary origin of UII in chordates.



Simplified model of the early evolution of the urotensin II family in chordates

Neuropeptide AVT and E2 system stimulates neurogenic activity and early brain development in the orange-spotted grouper

Nagarajan Ganesan[1,3*], Aruna Nagarajan[2], Ching-Fong Chang[1,2]

1. *Center of Excellence for Marine Bioenvironment and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan*
2. *Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan*
3. *Basic sciences department, PYD, King Faisal University, Saudi Arabia*

nadimoolam@kfu.edu.sa

Arginine vasotocin (Avt) is a basic neurohypophysial nonapeptide regulates wide aspects of vertebrate physiology in non-mammalian vertebrates. However, the functional association between Avt and neurosteroidogenesis in the early brain of teleosts remains elusive. We thus investigated the possible physiological effects of the interactions between the Avt and the E2 system in the early brain development of the orange-spotted grouper (*Epinephelus coioides*). We found that *Avt* and *Avt-r$_{v1}$* mRNAs (in the telencephalon and diencephalon) and localization (in the parvocellular, magnocellular and gigantocellular preoptic neurons) were abundant at 110 dah. In addition to this, dual fluorescence *in situ* hybridisation analysis revealed that the *Avt* and *Avt-r$_{v1}$* genes were highly co-expressed with key steroidogenic genes and estrogen receptors, which indicates their potential for functional association. *In vivo* Avt caused a significant increase in the cellular and gene levels of steroidogenic enzymes and estrogen receptors (ers), whereas Avt- rv1 antagonist caused a decrease in the expression of both steroidogenic enzymes and ers genes in the brain. Exogenous estradiol (E_2) strongly up-regulated *Avt* mRNAs in the grouper brain. Therefore, this Avt-E2 system may have a central effect on the early brain development through the up-regulation of neurosteroidogenic enzymes and estrogen receptors during gonadal differentiation. The possible physiological effects of the interactions between the Avt and the E2 system in the brain neurogenesis and development, osmoregulatory processes or cardiovascular regulation through neurogenic or neuroendocrine pathways are still not clear but could be interested to further study.

Key words: brain development, steroidogenic enzymes, estrogen receptors, Avt, gonadal differentiation, fish

Gene editing in fish cell lines – perspectives for the functional study of peptides with immunological functions

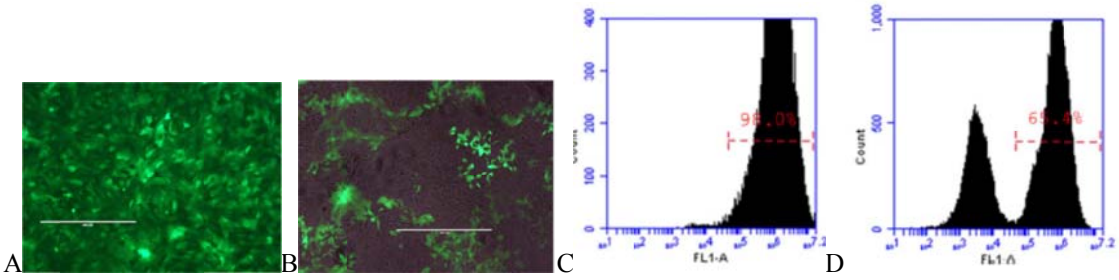
[1]**Bertrand Collet**, [1,2]Catherine Collins, [1]Pierre Boudinot, [2]Sylvie Dufour.

[1]*Virologie et Immunologie Moléculaires, Institut National de la Recherche Agronomique (INRA), Université Paris-Saclay, Jouy-en-Josas, France*
[2]*Muséum National d'Histoire Naturelle, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, CNRS, IRD, SU, UCN, UA, Paris, France*

bertrand.collet@inra.fr

The fish research community is small in comparison to its medical research counterpart and deals with a high number of evolutionary distant animal models. As a consequence, the development of traditional research tools, such as monoclonal antibodies, well-characterized cell lines or knock-out models, lags behind (Collet et al., 2018). However, the situation is different in genomics where the available data is comparable between the two research communities. Nevertheless, there is still the need for tools allowing for robust identification of gene function. We have recently developed a genome editing platform for salmonid cells facilitating loss-of-function experiments *in vitro*. The platform has been used successfully to characterize the function of immune-related genes and has the potential to be used for other disciplines including fish neuroendocrinology.

The platform is based on the isolation of a transgenic cell line, CHSE-EC, derived from the Chinook salmon *Oncorhynchus tshawytscha* CHSE-214, expressing constitutively a monomeric form of EGFP (mEGFP) and a nuclear localized Cas9 (nCas9n) (Dehler et al., 2016). This cell line was used for genome editing through its transfection with custom-made single guide RNA (sgRNA) against target genes. The methodology was firstly established by editing its mEGFP transgene (see figure below).



Oncorhynchus tshawytscha modified cell line CHSE-EC before (A, C) or after (B, D) transfection with a sgRNA targeting mEGFP. Fluorescent microscopy images of a confluent monolayer after 4 passages (A, B) and flow cytometry analysis of the corresponding cell suspension (C, D). mEGFP deficient cell population can be seen on left in D. From Dehler et al., 2016.

Endogenous CHSE genes were subsequently successfully edited. Genes involved in the immune response were chosen as targets. Interferons (IFN) are secreted factors involved in the defense against viral infections. Type I IFN (IFN1) includes many different subtypes and is responsible for the innate early antiviral mechanisms. Most nucleated cells respond to IFN1 by inducing a panel of Interferon Stimulated Genes (ISGs) encoding molecules with regulatory function or with direct or indirect antiviral properties.

IFN1 binds to membrane-bound receptors resulting in phosphorylation and subsequent association of complexes that translocate into the nucleus and activate ISGs promoter regions. Members of an important family of transcription factors, the Signal transducer and activator

of transcription (STATs), such as STAT1 and STAT2, are components of these complexes. We edited the genome of CHSE-EC cells and isolated two knock-out cell lines EC-GS1A and EC-GS2, with a disrupted *stat1a* and *stat2* gene, respectively.

The stimulation of these cell lines with recombinant IFN1 and the measure of downstream ISGs expression levels (by qPCR or RNAseq) demonstrated that the signaling pathways for IFN1 in EC-GS1A and EC-GS2 were obliterated (Dehler et al., 2019; Langevin et al., 2019).

The transcriptome analysis of the CHSE-EC cell line revealed that receptors for the neuropeptides Pituitary adenylate cyclase-activating peptide (PACAP) and neuropeptide Y (NPY) are expressed. This offers the potential to use CHSE-EC to investigate precise mechanisms of action of neuropeptides in salmonid cells through the generation of pertinent knock-out cell lines. Similar genome editing platforms are currently under development in eel (*Anguilla anguilla*, *A. rostrata*), fathead minnow (*Pimephales promelas*) and seabass (*Dicentrarchus labrax*) cell lines and will, in turn, allow study of evolutionary aspects of immune and endocrine functions in early vertebrates.

Collet B, Collins C, Lester K. 2018 Engineered cell lines for fish health research. Dev Comp Immunol. 80:34-40. 10.1016/j.dci.2017.01.013

Dehler CE, Boudinot P, Martin SA, Collet B. 2016. Development of an Efficient Genome Editing Method by CRISPR/Cas9 in a Fish Cell Line. Mar Biotechnol 18(4):449-452. 10.1007/s10126-016-9708-6

Dehler CE, Lester K, Della Pelle G, Jouneau L, Houel A, Collins C, Dovgan T, Machat R, Zou J, Boudinot P, Martin SAM, Collet B. Viral Resistance and IFN Signaling in STAT2 Knockout Fish Cells. J. Immunol. In press. 10.4049/jimmunol.1801376

Langevin C, Boudinot P, Collet B. 2019. IFN Signaling in Inflammation and Viral Infections: New Insights from Fish Models. Viruses. 11(3):302. 10.3390/v11030302

Three dimensional structure determination by NMR of neuropeptides in membrane mimic models

Anne-Gaëlle Lafont[1], Sandrine Pottier[1], Liza Mouret[1], Abderrafek El Harras[1], Benjamin Lefranc[2], Jérôme Leprince[2], Pascal Favrel[3], Maxime Endress[3], Céline Zatylny-Gaudin[3], Joël Henry[3,4] and **Arnaud Bondon**[1]

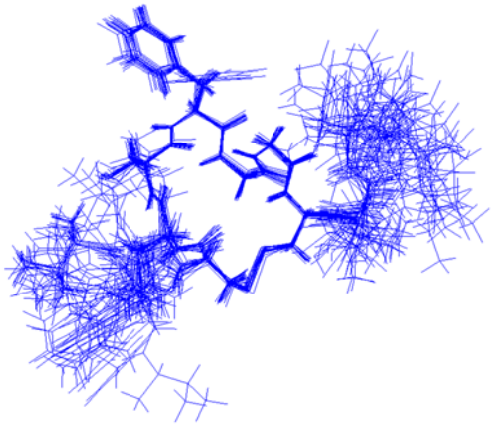
[1] *Université de Rennes, UMR CNRS 6226, PRISM, Equipe CORINT, CS 34317, Campus de Villejean, F-35043 Rennes, France.*
[2] *Normandie Université, UNIROUEN, INSERM, U1239, Laboratoire Différenciation et Communication Neuronale et Neuroendocrine, F-76000 Rouen, France.*
[3] *Normandie Université, UNICAEN, Sorbonne Université, MNHN, UPMC, CNRS 7208, IRD 207, UA, Biologie des Organismes et Ecosystèmes Aquatiques (BOREA), CS14032, 14032 CAEN, Cedex 5, France.*
[4] *Normandie Université, UNICAEN, SF[l]206 ICORE, PROTEOGEN Esplanade de la Paix, 14032 CAEN cedex.*

arnaud.bondon@univ-rennes1.fr

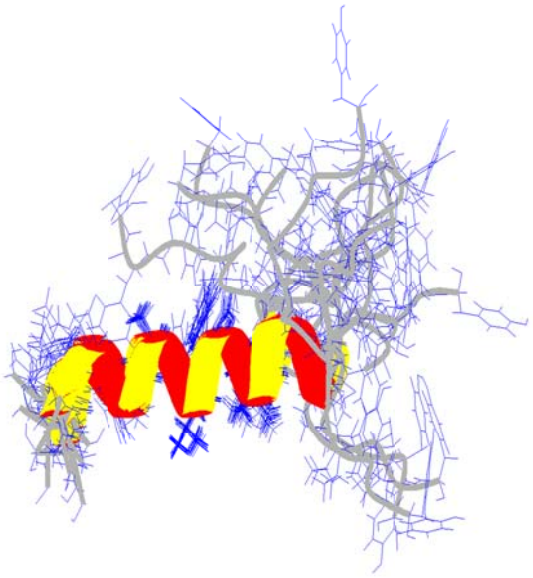
Three dimensional structure of neuropeptides is a key parameter for the interaction with their membrane receptors. Nuclear magnetic resonance (NMR) is a method of choice to perform such structural determination. However, neuropeptides are often relatively small peptides and they do not display a defined secondary structure in water. They acquire their defined structure when close to the membrane, and then diffuse to their receptor. In order to solve the peptide structure it is necessary to use a hydrophobic environment that constitutes a membrane mimic model. Several models are used such as hydrophobic solvents, micelles or liposomes. In the case of NMR studies, the most common system is micelles. NMR is very sensitive to the molecular motion of the system. Micelles are relatively small objects and deuterated compounds are easily available. In these conditions, the NMR signals of the peptide bound to the micelles are only slightly broadened allowing the three dimensional structure determination through now classical molecular modelling under NMR distance restraints. Beside micelles, small unilamellar liposomes can also be used as long as the peptide is in a relatively fast dynamic with the vesicles.

Several peptides were isolated from the cuttlefish (Sepia officinalis) which is a cephalopod mollusk. Two sets of peptides have been obtained one belonging to the crustacean cardioactive peptides family and the other close to the orcokinin B-like peptides. A representative of each set has been analyzed by NMR.

Peptide VFCNSYGGCKSF



GAESGEAHVFDSLLGGGHVPYY



	Overlay of the 20 lowest energy structures with no violations > 0.3 Å from the 100 structures calculated with AMBER software for neuropeptide SoCCAP2 in SDS micelles. Overlay was performed using the backbone atoms of residues 3–8.	Superimposition of the 18 lowest energy conformers of Pept6 in DPC micelles, fitted on segment 4-14 backbone atoms (rmsd: 0.301).

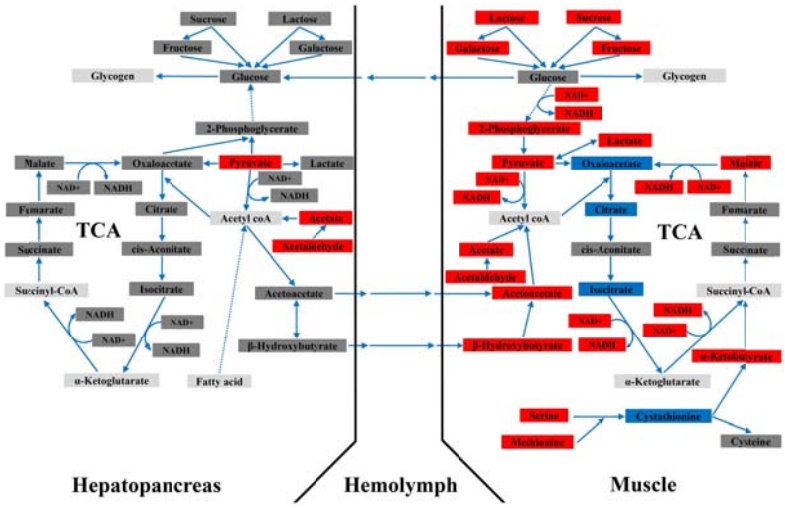
Regulation of metabolism by crustacean hyperglycemic hormone with pathological implications

Chi-Ying LEE

Department of Biology & Graduate Program of Biotechnology, National Changhua University of Education, Changhua, Taiwan

bicylee@cc.ncue.edu.tw

Crustacean hyperglycemic hormone (CHH) is a polypeptide hormone originally identified in a crustacean neuroendocrine tissue − the X-organ/sinus gland (XO/SG) complex. Available data indicate that CHH is involved with regulation of carbohydrate metabolism and it is considered a stress hormone that elicits stress responses. To functionally characterize the metabolic roles of CHH, its gene expression was silenced by *in vivo* injection of CHH double-stranded RNA (dsRNA), followed by metabolomics analysis of two CHH target tissues, the muscle and hepatopancreas, using nuclear magnetic resonance spectroscopy. Resulting data revealed that metabolic functions of CHH are more diverse than previously thought and differential between the two tissues. On the other hand, hemolymph CHH levels rapidly increased after viral challenge; the increase started as early as 3 h post-injection (hpi) and lasted for at least 2 d after the challenge, with the CHH peptide levels in the sinus glands of the virus-infected animals significantly decreased at 24 and 48 hpi. Pathological significance of the virus-elicited CHH release is currently investigated.



Metabolic profiles of the muscle and hepatopancreas 24 h after CHH dsRNA treatment. Red rectangle: significantly decreased from saline-injected levels; blue rectangle: significantly increased from saline-injected levels; dark gray rectangle: not significantly changed; light gray rectangle: not detected. Data obtained at 24 hpi were used for constructing the metabolic networks relating to carbohydrate and energy metabolism. Dotted lines indicate multiple metabolic steps are involved that are not individually specified. PLoS ONE 12(2): e0172557. doi:10.1371/journal.pone.0172557

Deciphering the molecular and cellular mechanisms that regulate organogenesis of the interrenal gland

Yi-Wen LIU

Department of Life Science, Tunghai University, Taiwan

dlslys@thu.edu.tw

My lab is using the zebrafish model to explore gene, development and disease of the kidney and the interrenal organ. We are particularly interested in investigating how various signaling transduction pathways orchestrate to regulate specification, growth, differentiation and tissue architecture of the interrenal gland, the teleostean conterpart of the adrenal gland. We have exploited a diversity of genetic tools to dissect how developing vessels provide signals and form the microenvironment that regulates migrations of the interrenal cells, and influences the interplay between interrenal and chromaffin cells.

Recently we have also addressed the role of Notch pathway for the development and steroidogenesis of interrenal tissue, the teleostean equivalent of the adrenal cortex. Although the Notch ligand JAG1 has been found up-regulated in the adrenal cancer, how Notch signaling functions during the adrenal gland development has remained unclear. Using zebrafish, we have been able to demonstrate how global and Jagged-mediated Notch pathways regulate stemness, differentiation and steroidogenesis of the interrenal organ. Furthermore, we are exploring how the Notch pathway cross-talks with the Wnt pathway during parallel development of the kidney and the interrenal gland, based on findings in characterizing functional roles of Low-density lipoprotein receptor-related protein 4, which negatively regulate activities of both Wnt and Notch signaling pathways.

Identification of neuropeptides in marine organisms by *in silico* analysis and peptidomic approach

Zatylny-Gaudin C.[1,2*,] Corre E.[4], Zannutini B. [5], Bernay B.[3] , and Henry J. [1,2,3]
[1]*Université de Caen -Normandie, F-14032 Caen, France.*
[2]*FRE 2030 BOREA CNRS, IRD, MNHN, US, UNICAEN, UA Esplanade de la Paix, 14032 Caen cedex, France*
[3]*Post genomic platform PROTEOGEN, Université de Caen Basse-Normandie, F-14032 Caen, France.*
[4] *ABiMs Platform, Station biologique de Roscoff (UPMC-CNRS), F-29688, Roscoff, France.*
[5] *Université de Caen Normandie, GREYC, UMR 6072, UNICAEN, CNRS, ENSICAEN, F-14032 Caen, France.*

celine.gaudin@unicaen.fr

In unconventional models, recent advances in *next-generation sequencing* have made it possible to obtain large-scale molecular data validated by mass spectrometry identification of mature products. In the cuttlefish *Sepia officinalis*, transcriptomics associated to peptidomics and proteomics led to the identification of the neuropeptidome [1] and several functional proteomes[2,3].
On the basis of studies carried out on this cephalopod, we will present the tools and methodologies developed (1) to analyse inaccessible data like sequences without annotated or badly annotated, (2) to characterize a peptidome and (3) to study potential activities of this peptidome.

In silico analysis of transcriptomes is essential, however the free bioinformatic softwares (Expasy) or CLC Main Workbench6 (CLC BIO) are not sufficient. A homemade software "Peptraq" has had to be developed to allow peptide precursor identification including a research from several or multiple structural criteria specific to peptide precursor like a peptide signal, a glycine residue prior to a mono or a dibasic cleavage site (C-terminal amidation), a glutamine following a dibasic cleavage site (N-terminal pyroglutamination) or a sequence tag repetition. To confirm *in silico* analysis, peptidomic approach is required. Since no method of analysis is sufficient on its own, several complementary approaches must be carried out in parallel. Finally, to evaluate the role of these peptides sometimes seen through the analysis of transcript expression, mapping of peptides and activity studies of peptide can be performed.

References

1. Zatylny-Gaudin, C. *et al.* Neuropeptidome of the Cephalopod Sepia officinalis: Identification, Tissue Mapping, and Expression Pattern of Neuropeptides and Neurohormones during Egg Laying. *J. Proteome Res.* **15,** 48–67 (2016).
2. Cornet, V. *et al.* Dual role of the cuttlefish salivary proteome in defense and predation. *J. Proteomics* **108,** 209–222 (2014).
3. Cornet, V. *et al.* How Egg Case Proteins Can Protect Cuttlefish Offspring? *PLoS One* **10,** e0132836 (2015).

Neuropeptides in a scleractinian coral *Euphyllia ancora*

Shinya Shikina[1, 2], Yi-ChenYao[3], Tai-Yu Liu[3], Ching-Fong Chang[2,3]

[1]*Institute of Marine Environment and Ecology, National Taiwan Ocean University (NTOU), Keelung, Taiwan*
[2]*Center of Excellence for the Oceans, NTOU*
[3]*Department of Aquaculture, NTOU*

shikina_s@yahoo.co.jp; shikinash@gmail.com

In various animals, neuropeptides has been shown to act as modulator and hormones, and play important roles in various biological and physiological processes. In corals, the presence and the functions of neuropeptides, including the distribution of neuronal cells in coral body remain largely unexplored in corals. To gain a better understanding of neuronal functions in coral physiology, we firstly established a transcriptome database of a stony coral *Euphyllia ancora*, and then explored the genes possibly encoding the precursor proteins of neuropeptides. Through the course of the data mining, we identified a cDNA sequence encoding Glycine-Leucine-Tryptophan-amide family neuropeptides (GLWamides) that have been shown to induce muscle contraction (myoactivity) and larval metamorphosis in several cnidarians. By 5' and 3' RACE-PCR, we successfully elucidated the full-length of GLWamide precursor cDNA in *E. ancora* (named EaGLW precursor). The deduced amino acid sequence of EaGLW precursor possessed 7 repeats of GLW motifs. Quantitative PCR analysis demonstrated that *EaGLW* precursor transcripts were highly expressed in the mouth and tentacle of *E. ancora* polyp of both sexes. In agreement with distribution patterns of the transcripts, immunodetection with an anti-GLWamide monoclonal antibody revealed that the neurons exhibiting immunoreactivity of GLWamide (GLWamide neurons) were mainly distributed in the epidermis of mouth and tentacle. *In vitro* treatments of the isolated pieces of mouth and tentacles with GLWamides were shown to induce the deformation of these isolated tissues. Treatments of *E. ancora* polyps with GLWamides were shown to induce the polyp contraction. These results suggested that GLWamides were involved in the muscle contraction of the coral.

MEMO

MEMO

MEMO