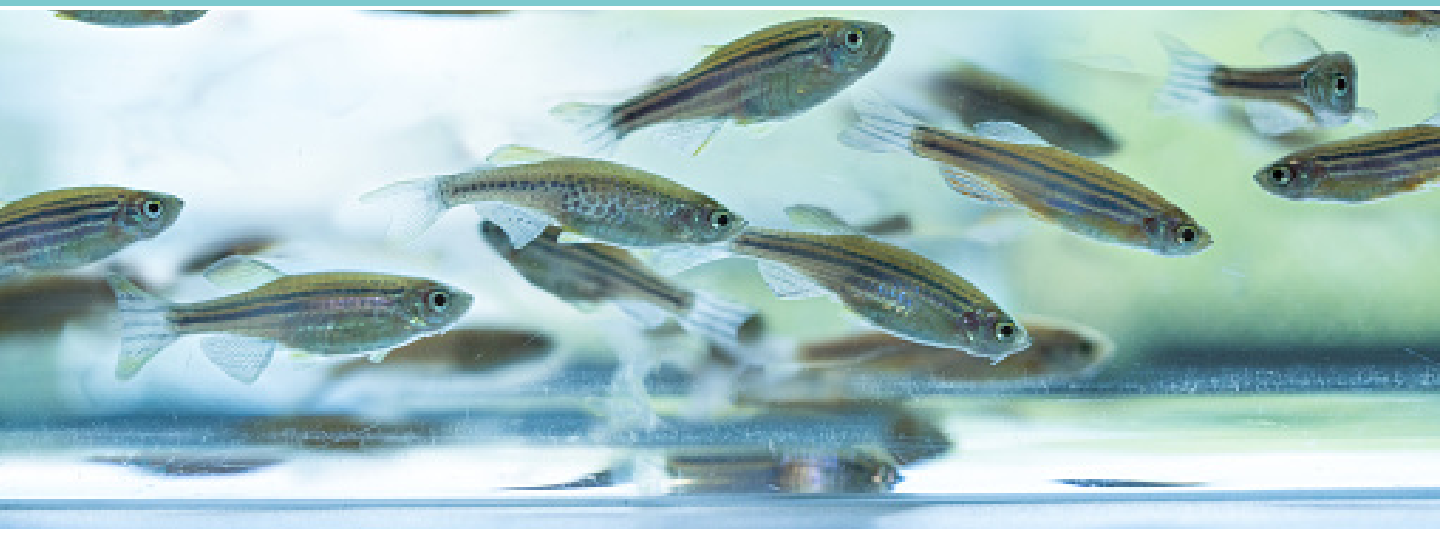
CENAKVA

South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses

INVITATION to LUNCH SEMINAR

Katsutoshi ARAI, Rafael Henrique NÓBREGA



Friday, September 16, 2022
11:00 - 13:00

Campus of the University of South Bohemia, ZR building,
Na Sádkách 1780, České Budějovice

Lunch sandwich for all registered participants.
Free admission.
Capacity max. 50 persons.

REGISTER HERE

Please confirm your participation by September 14, 2022

Lecture

„Hybridization, Polyploidization and Cloning in Fishes“

Katsutoshi ARAI

Visiting Professor, CENAKVA, Faculty of Fisheries and Protection of Waters, University of South Bohemia.

Lecture

„Fsh regulates the proliferation of embryonic-like germ stem cells in adult zebrafish testes“

Rafael Henrique NÓBREGA

Visiting Professor, CENAKVA, Faculty of Fisheries and Protection of Waters, University of South Bohemia.

ABSTRACTS, CV

Fakulta rybářství a ochrany vod
Faculty of Fisheries and Protection of Waters

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

Curriculum Vitae

Katsutoshi ARAI

Present Title:
Professor Emeritus, Hokkaido University

Education

1969 April—1972 March: Hokkaido Sapporo Asahigaoka High School

1972 April – 1976 March: Hokkaido University, Faculty of Fisheries

1976 March: B.Sc. in the field of Biology and Aquaculture

1976 April – 1978 March: Hokkaido University, Graduate School of Fisheries, Master Program

1978 March: M.Sc. in the field of Biology and Aquaculture

1978 April – 1980 April: Hokkaido University, Graduate School of Fisheries, Doctor Program

1984, June: Ph.D. in the field of Biology and Aquaculture, by the Dissertation, Hokkaido University, Graduate School of Fisheries

Academic Career

1980 May – 1984 Sept.: Research Associate, Kitasato University, School of Fisheries Science

1984 Oct. – 1989 Oct. : Lecturer, Kitasato University, School of Fisheries Science

1989 Nov. – 1999 March: Associate Professor, Hiroshima University, Faculty of Applied Biological Science

1999 April – 2017 March: Professor, Hokkaido University, Faculty and Graduate School of Fisheries Sciences

2017 April: Professor Emeritus, Hokkaido University

2017 April – 2018 March: Specially Appointed Professor, Hokkaido University, Faculty and Graduate School of Fisheries Sciences

2018 April – 2022 March: Specially Appointed Professor, Hokkaido University, Institute for the Advancement of Higher Education

Present Address:
Sapporo city, Hokkaido, Japan

Abstract

Hybridization, Polyploidization and Cloning in Fishes

Hybridization means results of the heterospecific fertilization between different species. Thus, the resultant progeny have a new combination of genomes (chromosome sets) from different species. Chromosome manipulation is a system of techniques to control numbers and constitution of conspecific and heterospecific chromosomes. Elevation of ploidy status can be achieved by inhibiting polar body release just after fertilization and cleavage in early embryogenesis. Induction of uniparental development, i.e., gynogenesis (all-female inheritance) and androgenesis (all-male inheritance) can be triggered by fertilization with genetically inactivated gametes. These techniques were investigated in the early 20th century by embryologists mainly with amphibians, but fish biological and aquaculture-oriented studies began in late 1970's and early 1980's, although pioneer works existed in 1950's and 1960's.

Survival and reproductive capacity of hybrids varied according to the combination of parental species and the direction of cross-breeding. Generally, hybrids between closely related species showed viability, while those between remotely related species died in embryonic and/or larval stages. However, viable hybrids often exhibited various levels of sterility from retarded gonadal development (gonadal sterility) or aberrant gametogenesis (gametic sterility) to aneuploid gametes which produced inviable progeny (zygotic sterility). Inviability of certain hybrids was explained by chromosome elimination and aberrant expression of genes. Unfortunately, mechanisms of hybrid sterility have not been well disclosed in fishes so far.

In induced sterile triploids, energy reallocation from maturation occasionally gave rise to growth outperformance. Sterility in induced triploids can be explained by aberrant meiosis including bivalents and univalent. Configurations comprising both bivalents and univalent often induce abnormal gonadal development and production of aneuploid (1.5n) gametes. In some inviable hybrids, triploidization often induced better survival, i.e., recovery of developmental potential. Induced gyno- and androgenesis were useful to produce unisexual (mostly all-female) aquaculture population as well as to estimate sex-determination system in species without morphologically distinct sex chromosomes. Since gyno- and androgenetic doubled haploids were completely homozygous to produce isogenic gametes, clonal strains could be established by the second cycle of gyno- or androgenesis of gametes of doubled haploids, followed by chromosome duplication. However, production of tetraploids was very difficult except for a few successful examples.

In dojo loach Misgurnus anguillicaudatus (Cobitidae; Teleostei), Japanese wildtype is gonochoristic diploidy (2n = 50), but natural tetraploids are frequently found in market samples because they have an origin in China. Gynogenetic progeny induced from diploid loach exhibited inviable abnormalities (haploid syndrome), but those induced from tetraploid loach were viable without any chromosome duplication. Thus, they are not concluded to be evolutionary tetraploids, but genetic tetraploids with four sets of chromosomes (4n = 100). Bisexual tetraploids generate fertile 2n gametes, thus cross-breeding followed by inhibition of 2nd polar body release (PBI) produced fertile hexaploid progeny (6n = 150). Cross breeding between tetraploid females and diploid males (4n x 2n) produced triploids (3n = 75). Triploid females laid 3n eggs and 1n eggs, while males were sterile. Triploid unreduced eggs were likely generated by genome duplication before meiosis, i.e., premeiotic endomitosis, while haploid eggs was presumably produced by meiotic hybridogenesis (MH), eliminating one haploid set before meiosis. PBI after 4n x 2n produced pentaploids (5n = 125), which females laid 2n eggs, while males had aneuploidy 2.3n sperm. Diploid eggs were presumably formed by MH and aneuploidy likely resulted from meiotic configurations including univalents.

In certain area of Japan, clonal lineages exist and they are considered hybrid origin between genetically different ancestors. These clonal diploids produce isogenic 2n eggs which develop by spontaneous gynogenesis to maintain clonal lineages, but such 2n eggs accidentally incorporate 1n sperm nucleus to develop triploids. Unreduced 2n eggs (2n sperm in sex-reversed clonal males) with isogenic genotypes are produced by premeiotic endomitosis to assure sister chromosome pairing. Clone-origin triploid males are sterile, whereas triploid females lay 1n eggs by MH. Altered constitution of chromosome sets due to hybridization and polyploidization are supposed to trigger expression of atypical reproduction such as unreduced gametogenesis, spontaneous gynogenesis and meiotic hybridogenesis.

Experimental results from artificially induced and naturally occurred hybrids, polyploids, and clones suggest us new reproductive, developmental, genetic and evolutionary insights into basic and applied biology of fishes and other aquatic organisms.

Curriculum Vitae

Rafael Henrique NÓBREGA

Associate Professor at Cellular and Molecular Biology · UNESP - Universidade Estadual Paulista.

Education

2014 – Habilitation à Diriger des Recherches (HDR). License to supervise PhD and master students in Genetics and Aquaculture Post-Graduate Programs, São Paulo State University, São Paulo State, Brazil

2014 - Ph.D. in Cancer Genomics and Development, Department of Developmental Biology, Faculty of Science, Utrecht University, Utrecht, the Netherlands - supervised by Dr. Rüdiger W Schulz (Utrecht University, the Netherlands) and Dr. Luiz Renato de Franca (Federal University of Minas Gerais, Brazil).

2006 - Master in Cellular and Structural Biology, University of Campinas, Campinas, São Paulo State, Brazil.

2003 - Bachelor in Biological Sciences, São Paulo State University, Botucatu, São Paulo State, Brazil.

Academic Career

Associate Professor (Permanent position), São Paulo State University, Botucatu, São Paulo State, Brazil (2019 - until now).

Assistant Professor (Permanent position), São Paulo State University, Botucatu, São Paulo State, Brazil (2014 - 2019).

Professional experiences

Visiting Professor/Researcher, Faculty of Fisheries and Protection of Waters, University of South Bohemia, Czech Republic (01/06/2022 – 30/11/2022)

Visiting Researcher, INRAE, l'Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, Rennes, France (04/10/2021 – 31/05/2022) (8 months)

Visiting Researcher, Physiologische Chemie Julius-Maximilians - Universität Würzburg, Würzburg, Germany (2015) (3 months)

Postdoctoral fellow, Department of Developmental Biology, University of Utrecht, Utrecht, the Netherlands (2014) (3 months)

E-mail: rafael.nobrega@unesp.br or biorhn@yahoo.com.br
Tel: +55 (14) 3880-0466 (Office)
Cell phone: + 55 (11) 99797-0134

Abstract

Fsh regulates the proliferation of cells in adult zebrafish testesembryonic-like germ stem

Rafael HenriqueNOBREGA1,2, Lucas Benites DORETTO1, Manon THOMAS2, Nathalie CHENAIS2, Jean-Jacques LAREYRE2

1Reproductive and Molecular Biology Group, São Paulo State University, Botucatu, Brazil; rafael.nobrega@unesp.br; lucas.doretto@unesp.br

2Laboratory of Fish Physiology and Genomics, INRAE, 35042 Rennes cedex, France, nathalie.chenais@inrae.fr; jean-jacques.lareyre@inrae.fr

A novel subpopulation of pluripotent stem cells, named embryonic-like stem cells (ELs), has been recently reported among spermatogonial stem cells in humans and mice. Furthermore, it has been shown that ELs in testes and ovaries express FSHR, and FSH has a direct effect on these cells. In this study, we sought to investigate whether ELs were present in zebrafish testes. To address our aims, expression analyses (RT-qPCR) of genes involved in pluripotency were carried out during zebrafish embryonic stages and the culture of testicular explants incubated or not with recombinant zebrafish Fsh (rzfFsh). To further identify the pluripotent markers and Fshr, immunofluorescence, western blot or flow cytometry were employed on wild-type or Fshr::eGFP zebrafish reporter lines. Finally, RNAseq libraries were produced from total RNA extracted from zebrafish testicular explants cultivated with trilostane (an inhibitor of sexual steroid production) in presence or absence of rzfFsh (100 ng/mL). We first demonstrated that the selected pluripotent genes, pou5f3, nanog and nanos3, showed higher expression levels at the blastula stage,and later, mRNA levels were significantly down-regulated over the gastrulation. Furthermore, we showed that Pou5f3, Nanog and Nanos3 were found among the different generations of spermatogonia although their staining pattern varied depending on the spermatogonial development in adult testes.

The pluripotent markers were expressed at higher levels in early spermatogonia (type A undifferentiated (Aund) and differentiated (Adiff) spermatogonia) compared to type B spermatogonia, and no longer detected in meiotic and post-meiotic germ cells. Using a specific antibody, we observed that Fshr was expressed in somatic cells and in Aund and Adiff. We further evaluated whether the selected pluripotent genes were regulated by Fsh. Similar to mammals, we found that Fsh increased pou5f3 mRNA levels, while nanog and nanos3 were down-regulated after 7 days of Fsh exposure. RNAseq libraries also showed a deregulation for many mediators of the stem cell signaling pathway in the testes cultivated with Fsh. Finally, we observed that a transgenic zebrafish line carrying the GFP reporter gene under the control of a proximal promoter fragment of the fshr gene showed high GFP expression levels not only in somatic cells, but also in Aund and Adiff. Altogether, our data indicate the existence of Fsh-dependent proliferating ELs in adult zebrafish testes.

Keywords: stem cell; pluripotency; germinal niche; endocrine regulation; Fsh.

Acknowledgements: FAPESP (20/03569-8).