MECHANISM OF OOCYTE MATURATION AND OVULATION, AND ITS APPLICATION TO SEED PRODUCTION IN THE JAPANESE EEL

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Introduction:

The Japanese eel, *Anguilla japonica*, is an economically important fish for Japanese food culture and for Japanese freshwater fish aquaculturists and scientists. The grilled eel dish called "kabayaki" is one of the representative dishes of traditional Japanese food culture. However, the decreases in eel resources and the catches of glass eels as seedlings for aquaculture have been a serious concern in recent years in both Europe and East Asia. Thus, technical development to produce eel seeds for artificial cultivation is strongly desired. However, male and female eels are sexually immature under normal cultivated conditions without hormonal treatments. Moreover, silver eels that migrate down the river are also immature. Sexually mature male and females have never been obtained from the wild until recently. Therefore, fundamental information on oocyte maturation and ovulation has not been obtained from naturally matured eels. Development for artificial induction of maturation and seed production in the Japanese eel started almost half a century ago. Finally, *leptocephalus* larvae and glass eels have been produced first in the world from artificially induced mature male and female eels by elaborate and reasonable hormonal treatments [1]. In 2010, "complete culture" system (production of the next generation from broodstocks which are reared from artificially reproduced eggs) has been developed. In the presentation, basic knowledge of mechanism of oocyte maturation and ovulation, and its application to artificial induction of sexual maturation of female eels will be introduced.

Methods:

Full-grown cultivated female eels were obtained by intraperitoneal weekly injections of salmon pituitary extracts (SPE) [2] or by implantation of a single SPE-loaded osmotic pump with a long-term sustained hormone-release system [3]. Ovarian fragments containing immature full-grown oocytes or oocytes at the migratory nucleus stage were taken from a small puncture of abdomen. Matured oocytes and ovulated eggs obtained by the standard methods mentioned previously [2]. For *in vitro* experiments, oocytes were incubated with various hormones and chemicals in 24-well culture plates containing 1ml of L-15 medium.

Results and Discussion:

After a relatively long period of growth (the vitellogenic phase), oocytes undergo maturation, accompanied by several maturational processes in the cytoplasm (such as hydration, lipid coalescence, and clearing of the ooplasm) and in the nucleus (such as germinal vesicle breakdown (GVBD) followed by ovulation. The eel oocytes underwent more than three-fold increase in volume during maturation and ovulation [4]. Wet and dry weight measurements indicated that water accumulation during oocyte maturation is the major factor contributing to the follicular diameter increase. During these processes, the oocytes become buoyant which is essential for their oceanic survival and dispersal as well as for the initiation of early embryogenesis. *In vitro* experiments using inhibitors of aquaporin water permeability ($HgCl_2$), and yolk proteolysis (bafilomycin A1), also indicate that aquaporin facilitates water uptake by acting as a water channel, and yolk proteolysis is essential for water influx into oocytes via osmotic mechanisms. To elucidate the molecular mechanisms underlying hydration during oocyte maturation, we have cloned novel-water selective aquaporin 1 (AQP1b) of the eel. *In situ* hybridization studies with the eel *aqp1b*cRNA probe revealed intense eel *aqp1b* signal in the oocytes at the perinucleolus stage and the signals became faint during the process of oocyte development. Light microscopic immunocytochemical analysis of ovary revealed that the Japanese eel AQP1b was first expressed in the cytoplasm around the yolk globules of oocyte at the primary yolk globule stage and became localized around the large membrane-limited yolk masses which were formed by the fusion of yolk globules during the oocyte maturation phase. These results together indicate that AQP1b, which is synthesized in the oocyte during the process of oocyte growth, is essential for mediating water uptake into eel oocytes during the final oocyte maturation phase. *In vitro* experiments indicate that these hydration processes were first induced in oocytes at the migratory nucleus stage by the gonadotropin, LH, maybe via maturation-inducing steroid, DHP produced in the ovarian follicle. Recombinant eel LH but not FSH, which were produced using a HEK 293 cell,induced a maturational competence (the acquisition of sensitivity of the oocyte

to respond to the MIS of oocytes) in a dose-dependent manner. After acquisition of maturational competence, LH but not FSH induced GVBD and ovulation *in vitro*. SPE (or LH) did not induce *in vitro* GVBD and ovulation in oocytes at the tertiary yolk globule stage (below 700 μm in diameter) which were obtained from female eels just before SPE injection. One day after intraperitoneal SPE injection, oocytes at the migratory nucleus stage approximately 800 μm in diameter underwent GVBD and ovulation *in vitro* in response to SPE. Thereafter, oocyte over 800 μm in diameter became less sensitive to SPE afterwards. These results indicate that oocytes acquire the ability to respond to SPE at the migratory nucleus stage over 800 μm in diameter and furthermore SPE has an essential role for investment and maintenance of the ability of oocytes to respond to SPE. To induce oocyte maturation and ovulation artificially, the following hormonal treatments have been developed from information obtained from *in vitro* and *in vivo* experiments. Eels which have ovaries containing oocytes at the migratory nucleus stage (approximately 700-750 μm in diameter) were injected with SPE to induce oocyte hydration and maturational competence. Eels having competent oocytes (850-900 μm) were injected again (a priming dose) with SPE to induce and maintain maturational competence and ability to respond to gonadotropin. Final treatments of gonadotropin-releasing hormone analog (GnRHa) in combination with SPE given 24 hr after SPE-priming dose to female eels (900-950 μm in diameter) succeeded in induction of spawning in a rearing tank with spermiated male eels injected with human chorionic gonadotropin and OHP. Fertility and hatchability are approximately 80% and 50%, respectively.

Conclusion:

Egg quality obtained from the female eels induced by our hormonal treatment procedure is still not so high and fluctuates among the samples used in this study. Further studies are necessary to elucidate factors associated with egg quality and also to improve our procedures.

References:

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