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**STUDIES ON THE HATCHING ENZYME AND ITS SUBSTRATE,
EGG ENVELOPE, OF *ORYZIAS LATIPES***

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Enzymatic hatching of fish embryos consists of many elemental or constitutive processes as follows: (1) Expression of hatching enzyme gene(s), (2) Formation and accumulation of hatching enzyme in association with differentiation and maturation of hatching gland cells, (3) Secretion of hatching enzyme, (4) Egg envelope breakdown by the secreted enzyme, and (5) Emergence of the embryos. Employing the hatching enzyme of the fish, *Oryzias latipes*, as material, we have analyzed some of these processes. The present paper surveys the results.

Oryzias latipes hatching enzyme was found to be an enzyme system consisting of two distinct but similar Zn-proteases, high choriolytic enzyme (HCE) and low choriolytic enzyme (LCE). By the use of polyclonal and monoclonal antibodies against each of them, cDNAs for HCE and LCE were cloned from cDNA libraries constructed from RNAs of Day 3 embryos. Analysis of the cDNAs showed that they were synthesized as proenzymes and the propeptide portions were N-glycosylated. Examination of the structure around the active site of both the enzymes strongly suggests that they belong to astacin (protease) family. Northern blotting and Western blotting analyses using their cDNA fragments and antibodies, respectively, as probes revealed concurrent expression of their genes, followed by an immediate translation of their transcripts in Day 2 embryos (stages of lens formation to retinal pigmentation). Double immunostaining of sections of the secretory granules *in situ* or in isolation with the polyclonal and monoclonal antibody

systems indicated that the proenzymes of HCE and LCE were colocalized to the same secretory granules and that HCE was located evenly in the granules, while LCE was situated at the periphery of the granules.

On secretion, the proenzymes are probably activated by some EDTA-sensitive protease(s) to change into mature forms. Although a close relation between secretory activity and respiratory activity of prehatching embryos has long been known, it seems that intracellular Ca^{++} eventually plays a crucial role in the secretion of gland cells, as Ca^{++} -ionophore induces the secretion instantaneously. The secreted and activated enzymes attack the egg envelope from the inside. It is conjectured that the major constituents (ZI-1, 2, 3) of the inner layer of egg envelope of this fish are synthesized in the form of precursors named SF-substances in the mother's liver and that they are associated to form an envelope around an oocyte. The inner layer of the complete oocyte envelope becomes tough on fertilization through hardening process, which comprises covalent cross-link formation between the associated subunit proteins. HCE and LCE exert a cooperative choriolytic action; HCE binds to the hardened inner layer and swells it remarkably through a partial proteolysis and subsequent hydration. LCE which can hardly digest the intact inner layer solubilizes the HCE-swollen inner layer very efficiently. Clarification of each of the hatching processes would provide us with some information of cellular and molecular mechanisms of reproductive and developmental phenomena.