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Cellular analyses of sea urchin metamorphosis

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Larvae of marine invertebrates undergo metamorphosis in response to environmental cues (Chia & Burke, 1978). In sea urchins, free fatty acids (Kitamura *et al.*, 1993), dibromomethane (Taniguchi *et al.*, 1994), pheromonal peptides (Burke, 1984) and L-glutamine (Yazaki & Harashima, 1994; Yazaki, 1995) have been known as metamorphosis-inducing substances. The mechanisms by which cells respond to these cues and how the larval tissues are absorbed have not been clear, however. In the present study, we used L-glutamine (Gln) and a natural cue, green algae (*Ulvella* sp.), to induce metamorphosis of *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*, and investigated the intracellular changes during metamorphosis.

Intracellular changes in metamorphosing larvae treated with Gln

After being subjected to 10^{-5}–10^{-3} M Gln for 10–24 h, larvae cease swimming, settle, begin to retract their larval arms, extrude the primary podia and finally evert their echinus rudiment (ER). In *H. pulcherrimus*, larvae retracted their arms from 6 h to 24 h after the start of Gln treatment and then everted the ER. *A. crassispina* larvae underwent similar processes to those of *H. pulcherrimus*. The larval surface is composed of squamous epithelium and columnar epithelium. The epithelium of the ciliary bands or epaulets is columnar.

In the squamous epithelium, the nuclear chromatin in the larval arms and body, and in the oesophagus, markedly condensed after treatment with Gln for 24 h. Electron microscopy revealed swelling of both nuclei and mitochondria, while their membranes seemed to be intact. In the cytoplasm, lipid-like structures and electron-dense substances appeared. A further 24 h after Gln treatment, the chromatin condensation had progressed. Most nuclei in which chromatin had condensed were positive to the TUNEL assay, which detects DNA fragmentation. These results suggest that cell death in the squamous epithelium is apoptotic rather than necrotic. The mitotic activity of columnar cells, which was high in the control larvae (not treated with Gln), had markedly decreased after 24 h of Gln treatment (Yazaki, 1995; Sato & Yazaki, 1998). Neither chromatin condensation nor swelling of nuclei was found in these larvae. However, the swollen mitochondria, lipid-like structures and some unknown electron-dense substances were present in their cytoplasm.

Large cells (9–14 μm × 6–8 μm in diameter) of irregular shape were found in the mesenchyme in control larvae. Since the large cells in the mesenchyme had enveloped the TUNEL-positive cells or included TUNEL-positive particles in their cytoplasm after the 24 h of Gln treatment, these cells must be phagocytes. A further 24 h later the phagocytotic large cells with TUNEL-positive particles had increased in number.

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Metamorphosis induced by *Ulvella*

When larvae were transferred into a well containing *Ulvella*-growing plastic films they had everted their ER by 6 h after the start of treatment, then retracted their arms. After the 24 h of treatment, metamorphosis was almost completed. On the aboral side of the newly metamorphosed juveniles, larval tissue with columnar-like cells remained. Neither chromatin condensation nor nuclear swellings were found in these larval cells. Cameron & Hinegardner (1978) described the closely packed cells of epaulet, ciliary band and larval epithelium on the aboral hemisphere region of the newly metamorphosed juvenile. The phagocytotic cells including TUNEL-positive particles were also found in the mesenchyme near the aggregate of remaining larval cells. This suggests that apoptotic processes occurred in the metamorphosis induced by *Ulvella* rather faster than in Gln-induced metamorphosis.

Induction mechanism of metamorphosis

Degeneration of larval tissue can occur independently of the eversion of the ER. γ-Aminobutyric acid induces temporary ER eversion but never induces arm retraction (Yazaki & Harashima, 1994), while L-Dopa produces only the degeneration of larval tissues (Yazaki, this supplement). In the case of *Ulvella*-induced meta-

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morphosis, it is supposed that at least two substances are present on the *Ulvella* film to evoke ER eversion and arm retraction, respectively.

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