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Regional specification in the early embryo of the brittle star *Ophiopholis aculeata*

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Abstract

Early embryogenesis has been examined experimentally in several echinoderm and hemichordate classes. Although these studies suggest that the mechanisms which underlie regional specification have been highly conserved within the echinoderm + hemichordate clade, nothing is known about these mechanisms in several other echinoderm classes, including the Ophiuroidea. In this study, early embryogenesis was examined in a very little studied animal, the ophiuroid *Ophiopholis aculeata*. In *O. aculeata*, the first two cleavage planes do not coincide with the animal–vegetal axis but rather form approximately 45° off this axis. A fate map of the early embryo was constructed using microinjected lineage tracers. Most significantly, this fate map indicates that there is a major segregation of ectodermal from endomesodermal fates at first cleavage. The distribution of developmental potential in the early embryo was also examined by isolating different regions of the early embryo and following these isolates though larval development. These analyses indicate that endomesodermal developmental potential segregates unequally at first, second, and third cleavage in *O. aculeata*. These results provide insight into the mechanisms of regional specification in *O. aculeata* and yield new material for the study of the evolution of echinoderm development.

Keywords: Echinoderm; Early embryogenesis; Regional specification; Fate map; Blastomere isolation; Evolution

Introduction

The phylum Echinodermata is composed of five extant classes—Echinoidea, Holothuroidea, Asteroidea, Ophiuroidea, and Crinoidea—and is a sister-group to the phylum Hemichordata (Cameron et al., 2000; Hyman, 1955). Many echinoderm and hemichordate species develop indirectly via free-swimming planktonic larvae (Fig. 1). Experimental work conducted on sea urchins has broadly defined the mechanisms that underlie embryogenesis and larval developmental in the echinoderm class Echinoidea (Hörstadius, 1973). Recent investigations have built upon this knowledge by uncovering many of the molecular mechanisms that underlie early development in echinoids. This work has led to the identification of several intracellular signaling pathways, cell–cell interactions, and gene regulatory networks known to play fundamental roles in echinoid embryogenesis (for reviews, see Angerer and Angerer, 2003; Davidson et al., 2002; Ettensohn and Sweet, 2000). Despite the wealth of knowledge regarding the mechanisms of early development in echinoids, very little is known about the mechanisms which underlie embryogenesis in the other groups of the echinoderm-hemichordate clade. A good deal of what is known about embryonic mechanisms in these other groups is from work examining blastomere fates and the distribution of developmental potential in early asteroid and hemichordate embryos.

The larval fates of blastomeres have been examined in a number of species within the echinoderm-hemichordate clade. Fate-mapping analyses have been conducted on the embryos of multiple echinoid species (Cameron and Davidson, 1991; Cameron et al., 1987; Henry et al., 1992; Hörstadius, 1973; Wray and Raff, 1990), one asteroid

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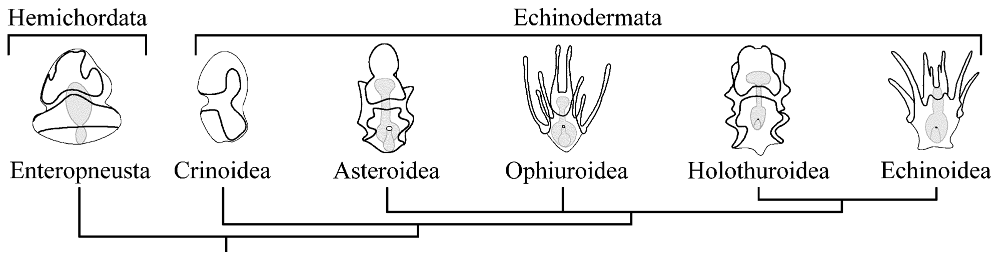

Fig. 1. Larval forms of extant echinoderm classes with an enteropneust hemichordate out-group. Crinoids represent the most basal extant echinoderms and echinoids and holothuroids are sister-taxa; placement of asteroids and ophiuroids within this group is less certain. Echinoids and ophiuroids are the only groups which develop via the production of a pluteus larva (which bear long ciliated arms supported by a larval skeleton). This and all ensuing phylogenies are based on Littlewood et al. (1997) and Cameron et al. (2000).

species (Kominami, 1983), and two hemichordate species (Colwin and Colwin, 1951; Henry et al., 2001). Comparative analyses of these fate maps indicate that some cell lineage-specific features are variable between taxa; the orientation of the first embryonic cleavage plane with respect to the plane of larval bilateral symmetry is one such feature. These analyses also suggest that other cell lineage-specific features appear to have been highly conserved throughout the evolution of the clade (Raff, 1999). Conserved features include the distribution of larval cell fates along the animal–vegetal axis (A–V axis) and the orientation of the first three embryonic cleavage planes with respect to the A–V axis.

The distribution of developmental potential has also been examined in the echinoderm-hemichordate clade. If blastomeres are separated at the 2-cell stage in the indirect-developing echinoid *Paracentrotus lividis* or the asteroid *Asterina pectinifera*, both blastomeres are capable of producing a small but normal larva (Dan-Sohkawa and Satoh, 1978; Hörstadius, 1973). Similar experiments in the hemichordates *Saccoglossus kowalevskii* and *Ptychodera flava* are consistent with these results (Colwin and Colwin, 1950; Henry et al., 2001), indicating that in these three taxa the potential to form ectoderm, endoderm, and mesoderm segregates equally into both daughter cells at first cleavage. If the same experiment is conducted in the direct-developing echinoid *Heliocidaris erythrogramma*, on the other hand, one isolate gives rise to more endodermal and mesodermal derivatives than the other, indicating that the potential to form endoderm and mesoderm segregates unequally at first cleavage in this embryo (Henry and Raff, 1990).

If blastomeres are separated along the third cleavage plane at the 8-cell stage in the echinoids *P. lividis* or *H. erythrogramma* or in the asteroid *A. pectinifera*, the vegetal half develops into a small but normal larva while the animal half produces an ectodermal vesicle (Henry and Raff, 1990; Hörstadius, 1973; Maruyama and Shinoda, 1990). These results indicate that while the potential to form ectoderm is present in both the animal and vegetal halves of the embryo at the 8-cell stage, the potential to form endoderm and mesoderm segregate exclusively to the vegetal half of the embryo at third cleavage in these species. These isolation experiments suggest that the distribution of developmental potential has also been highly conserved throughout the evolution of the echinoderm-hemichordate clade; the only exception may be a consequence of a switch in developmental mode. Without the appropriate data from the ophiuroids, holothuroids, and crinoids, however, it is difficult to draw any firm conclusions on the matter.

The purpose of this study was to determine whether the mechanisms which underlie regional specification in the ophiuroid *Ophiopholis aculeata* are similar to or different from those which take place in echinoids, asteroids, and hemichordates. An ophiuroid was chosen for this study primarily because many ophiuroids, like many echinoids, develop indirectly via the production of a pluteus larva; no holothuroids, crinoids, or asteroids develop via a pluteus larva (Fig. 1). Moreover, cladistic analyses indicate that the pluteus larva may have arisen independently in these two classes through a process of convergent evolution (Littlewood et al., 1997; Smith, 1988). It is not known, however, whether these two groups employ similar or divergent developmental mechanisms to construct a pluteus larva. Therefore, the embryonic axial properties and orientation of early cleavage planes were examined, a fate map of the early embryo was constructed and a series of experiments were performed on the early *O. aculeata* embryo to elucidate the mechanisms which underlie the process of regional specification in this embryo. This work represents the first piece of experimental embryology conducted on any member of the echinoderm class Ophiuroidea.

Materials and methods

Animals and embryos

O. aculeata adults were collected intertidally on San Juan Island, WA, and were maintained at 10–12°C in aquaria with running sea water. To induce spawning, animals were exposed to a combination of bright light, heat, and physical perturbation (shaking, swirling, and/or inversion) for 1–2 h. Animals were then placed in filtered