Mesendoderm Cell and Archenteron Formation in Isolated Blastomeres from the Shrimp *Sicyonia ingentis*

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The fate map of 2- and 4-cell-stage *Sicyonia ingentis* embryos was determined by microinjection of lysyl-rhodamine–dextran into single blastomeres. Microinjected embryos were cultured to the limb bud stage, when the body plan of the nauplius larva was evident. The animal blastomere, AB, gave rise to anterior ectoderm, while the vegetal blastomere, CD, gave rise to posterior structures, including the invagination site during gastrulation. The A blastomere gave rise to mirror-image patterns of dorsal–lateral ectoderm, while the B blastomere gave rise to anterior, ventral ectoderm. The C blastomere gave rise to posterior, dorsal–lateral ectoderm, complementary to the A pattern, as well as some naupliar mesoderm. The D blastomere gave rise to mesendoderm, naupliar mesoderm, and some posterior ectoderm. To study the specification of the early blastomeres, they were microsurgically separated and cultured in isolation. Two mesendoderm cells formed in $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{16}$ blastomeres in embryos dissociated at the 2-, 4-, 8-, and 16-cell stages, respectively. CD and D blastomeres could be distinguished by their larger size and gave rise to the mesendoderm cells. Archenteron formation and elongation of the embryo occurred in CD but not in AB isolates. Isolated blastomeres were recombined in various ways to determine whether their state of commitment could be altered in different cellular environments. Duplicated mesendoderm cells and archenteron formed in CD + CD recombinations, while AB + AB recombinations formed blastulae but did not produce mesendoderm cells and did not invaginate. The normal number of mesendoderm cells and a single archenteron formed in D + AB recombinations, while C + AB recombinations remained as blastulae and did not form mesendoderm cells. The results suggest that the mesendoderm cells are autonomously specified, possibly by cytoplasmic localization at the vegetal pole. The mesendoderm may also function as a signaling region to organize other developmental events. © 1994 Academic Press, Inc.

INTRODUCTION

Although numerous descriptive studies of crustacean development have been published (reviewed in Shino, 1968; Anderson, 1973, 1979, 1982), pattern formation in this group of organisms is still poorly understood. One influential view has been that crustacean cleavage is a derived form of spiral cleavage (Shiino, 1968; Anderson, 1973). The evidence for this has come from studies of cirripeds and other crustaceans which undergo an alternation of oblique cleavages about the animal–vegetal axis (reviewed in Anderson, 1973). The interpretation that crustacean development is basically spiral is controversial, however, since the prospective fates of tissue regions are so different from those of forms with classical spiral cleavage (Weygoldt, 1979; Zilch, 1979). Furthermore, few experimental studies of crustacean blastomere specification have been undertaken (Green, 1971; Davidson, 1991). These have included centrifugation studies (in the cladocerans *Daphnia* and *Cyclops*; reviewed by Green, 1971) and isolation or ablation studies (in isopods, cladocerans, and penaeoideans; reviewed by Green, 1971). The conclusions based on these studies, e.g., that cleavage is spiral and gut, mesoderm, and germ cells are autonomously specified by cytoplasmic determinants (Davidson, 1991), have perhaps been overstated.

Specification by cell interactions during crustacean development is likely to occur, but very little work has examined this. In isopods the mesendoderm appears to be necessary for the specification of the rest of the embryo (Kajishima, 1851; Green, 1971). Recently it has been shown that during posterior segment formation in crayfish an *engrailed* homologue is expressed in a pattern similar to that in intermediate-gut band insects (Patel et al., 1989). The *engrailed* gene has been cloned in the brine shrimp *Artemia* (Manzanoares et al., 1993) and is expressed at the posterior parts of the thoracic and maxillar segments. It is still unclear whether *engrailed* expression in crustaceans depends on cell lineage or position (Patel et al., 1989; Manzanoares et al., 1993).

As an extension of our previous studies (Hertzler and Clark, 1992, 1993), we were interested in examining the specification of early blastomeres in the penaeoidean shrimp *Sicyonia ingentis*. This animal provides a favorable experimental model for fertilization and early de-