Multiple Inductive Signals Are Involved in the Development of the Ctenophore Mnemiopsis leidyi

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Ctenophores possess eight longitudinally arrayed rows of comb plate cilia. Previous intracellular cell lineage analysis has shown that these comb rows are derived from two embryonic lineages, both daughters of the four e1 micromeres (e11 and e12) and a single daughter of the four m1 micromeres (the m12 micromeres). Although isolated e1 micromeres will spontaneously generate comb plates, cell deletion experiments have shown that no comb plates appear during embryogenesis following the removal of e1 descendants. Thus, the m1 lineage requires the inductive interaction of the e1 lineage to contribute to comb plate formation. Here we show that, although m12 cells are normally the only m1 derivatives to contribute to comb plate formation, m11 cells are capable of generating comb plates in the absence m12 cells. The reason that m11 cells do not normally make comb rows may be attributable either to their more remote location relative to critical signaling centers (e1 descendants) or to inhibitory signals that may be provided by other nearby cells such as sister cells m12. In addition, we show that the signals provided by the e1 lineage are not sufficient for m1-derived comb plate formation. Signals provided by endomesodermal progeny of either the E or the M lineages (the 3E or 2M macromeres) are also required. © 2001 Academic Press

Key Words: induction; endoderm; mesoderm; basal metazoan.

INTRODUCTION

Ctenophores are “diploblastic” pelagic marine animals thought to occupy a key position in metazoan phylogeny (Wainright et al., 1993; Ax, 1996; Collins, 1998; Zrzavy et al., 1998). The defining or apomorphic feature of the phylum Ctenophora is the presence of eight longitudinal rows of ctene or comb plates (Ctenophora = “comb bearers”) that run along the main body axis (the oral–aboral axis, Fig. 1). Each comb plate is generated by epidermal polster cells that possess thousands of laterally arrayed cilia. The coordinated beating of the comb plates within each comb row propels the animal through the water column.

Embryologically, ctenophores are known for two distinct features. They display a highly stereotyped, phylum-specific cleavage program in which each cell in the early embryo can be identified on the basis of its position and lineage history (Reverberi and Ortolani, 1963; Reverberi, 1971; Martindale and Henry, 1997a, 1999). They also exhibit a “mosaic” nature, which results from their lack of ability to regulate during the embryonic period following blastomere removal. For example, blastomeres separated at the two-cell stage each generate a juvenile adult possessing exactly one-half of the normal complement of adult pattern elements (Chun, 1880; Driesch and Morgan, 1895; Martindale, 1986; Henry and Martindale, 2000).

Early studies examining the origins of comb plates supported the mosaic nature of ctenophore development. Cell lineage experiments using chalk particles revealed that individual comb rows were generated solely by single daughter cells of the e1 micromere lineage, the e11 and e12 micromeres at the 32-cell stage (Fig. 2) (Reverberi and Ortolani, 1963). The deletion of all four e1 micromeres, but not other cells, at the 16-cell stage resulted in the complete loss of comb rows (Farfaglio, 1963; Martindale and Henry, 1986; Martindale and Henry, 1997b; Henry and Martindale, 2000).

A reinvestigation of the origins of comb plates using more refined intracellular lineage tracing techniques revealed that, in addition to the two e1 daughters, the m1 micromere lineage contributes to comb plate formation (Martindale and Henry, 1997b). However, only one of the two m1 micromere daughters, m12 that happens to be located closest to e1 derivatives, gives rise to comb plate derivatives (Fig. 2). One of the other E or M stem cell sublineages was found to generate comb plates (Martindale and Henry, 1997b). To whom correspondence should be addressed. Fax: (217) 244-1648. E-mail: j-henry4@uiuc.edu.
Because no m₁-derived comb plates are generated following e₁ removal, but removal of m₁ blastomeres has no effect on the appearance of e₁-derived comb plates, it is clear that e₁ or its derivatives generate an essential inductive signal for the m₁ derivatives (Martindale and Henry, 1997b). The inductive role of e₁ micromeres in ctenophore development is also apparent in the absence of the endodermally derived canals that normally run subjacent to each comb row, when e₁ cells are removed (Chun, 1880; Driesch and Morgan, 1895; Martindale, 1986; Martindale and Henry, 1997b). Thus, it would appear that e₁ and its derivatives interact with m₁ as well as E and M endodermal derivatives. This study uses cell lineage and cell deletion techniques to investigate the cell interactions required for m₁ comb plate formation. We show that, while m₁₂ cells are normally the only m₁ derivatives to contribute to comb plate formation, both m₁ daughters are capable of forming cten Plates. Surprisingly, the signals provided by the e₁ lineage are not sufficient to induce m₁ cells to form comb plates. Signals provided by endomesodermal progeny of either the E or the M blastomeres are also required for m₁-derived comb plate formation.

**RESULTS**

**Comb Plate Lineages**

Comb plates are generated by the derivatives of the two major stem cell lineages found at the 8-cell stage: the E ("end") and ("middle") blastomeres (Fig. 2). At the 16-cell stage, each of the four E cells and four M cells gives rise to a small blastomere at the aboral pole, the e₁ and m₁ micromeres, respectively. Subsequently, each of these micromeres divides in a meridional fashion to generate two daughters. The daughter that lies closest to the esophageal/pharyngeal plane is defined as the e₁₁ or m₁₁, and the daughter furthest from the esophagus and closest to the tentacular pole is called the e₁₂ or m₁₂. Previous cell lineage analysis indicates that e₁₁, e₁₂, and m₁₂ micromere daughters normally contribute to comb plate formation (Fig. 2) (Martindale and Henry, 1997b, 1999). The exact contributions by these cells are somewhat variable. For instance, while both
e₁₁ and e₁₂ micromeres always give rise to comb plates, m₁ cells give rise to comb plates in most (82%), but not all, of the cases (Martindale and Henry, 1997b). Furthermore, we showed that, if e₁ cells were labeled and adjacent m₁ cells were ablated, labeled comb rows formed in virtually every case. On the other hand, if m₁ cells were labeled and e₁ cells are ablated, no labeled comb rows were ever formed during embryogenesis (Table 1) (Martindale and Henry, 1997b).

**e₁ Induction**

The ctenophore embryo has no axial organization prior to first cleavage (Freeman, 1977). Thus, the distinct e₁ lineage that generates comb plates and the ability to induce comb plates from m₁ derivatives is generated as an active consequence of the cleavage program that shunts developmental potential to the tentacular ends at the aboral side of the embryo—the future site of e₁ micromere production (Fig. 2) (Freeman, 1976). Normally, the e₁₁ cell lies closest to the m₁₂ cell that also forms comb plates. To determine whether the division of the e₁ micromere asymmetrically segregates inductive ability to only one of its two daughter cells, all four either e₁₁ or e₁₂ blastomeres were deleted, following injection of diI into all four m₁ cells at the 16-cell stage. The results show that the surviving e₁ daughter cell and the m₁ derivatives make comb plates in a high percentage of cases, regardless of which e₁ daughter is ablated (Table 1, Figs. 3A–3D).

**m₁ Response**

Normally only the m₁ derivative (m₁₂) closest to the e₁ derivatives contributes to comb plates (Martindale and Henry, 1997b). We have confirmed that m₁₂ does not normally contribute to comb row formation (Figs. 3E and 3F). To determine whether both m₁ daughter cells are capable of generating comb plates, all four m₁ cells were injected at the 16-cell stage and all four either m₁₁ or m₁₂ cells were killed at the 32-cell stage. As expected, when m₁₁ cells were deleted, the m₁₂ cell

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**FIG. 2.** Early cleavage pattern of *M. leidyi* showing the segregation of comb plate-forming potential to the daughters of the e₁ and m₁ micromeres up through the 32-cell stage. Those cells that normally form comb plate cilia are colored in red. The sister cell of m₁₂, called m₁₁ (colored in green), can also contribute to comb plate formation if m₁₂ is removed (see Results and Table 1). The tentacular (T) and sagittal (S) planes are labeled in (B) and (C) and these same orientations are used for all the stages shown. Cell nomenclature follows that of Martindale and Henry (1999). Micromere identities are symmetrical across the tentacular and sagittal planes, so not all cells are labeled for the sake of simplicity. The various views are as indicated.

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went on to make contributions to one or more comb plates (Table 1). When the m12 cell was deleted, labeled comb plates derived from the m11 micromere were also observed (Table 1, Figs. 3G and 3H). Interestingly, if two adjacent m2 cells are labeled and all four m1 cells are ablated (21 cases, data not shown), comb rows are always unlabeled, indicating that unlike m1 progeny, m2 progeny are apparently unresponsive to comb plate inductive signals.

Role of Other Cell Interactions in Comb Plate Formation

The above reported experiments (see Table 1) indicate that e1 or its progeny are required for m1 progeny to form comb plates. To determine whether other cell interactions may be involved in m1-derived comb plate formation, we prepared specific combinations of blastomeres by removing the surrounding cells early during development. Isolated e1 micromeres alone spontaneously generated comb plates within 12 h of culture (Table 2, Figs. 4A and 4B), but isolated m1 micromeres never did (Table 2, Figs. 4C and 4D). Surprisingly, when labeled m1 micromeres were cultured with unlabeled e1 micromeres, comb plates formed, although none of these were labeled (Table 2, Figs. 4E and 4F). Together, these data indicate that the interactions between e1 and m1 micromeres, while required, are not sufficient to induce comb plate formation by m1. To determine which additional cell interactions are involved, we systematically included other unlabeled embryonic lineages with sets of unlabeled e1 plus labeled m1 micromeres (see combinations listed in Table 2). For instance, unlabeled e1 plus labeled m1 micromeres cultured together with all the other aboral micromeres, e2, e3, and m2 (generated by ablating the 2M and 3E macromeres) did not generate labeled comb plates (Table 2, Figs. 4G–4J). Only in those combinations that contained e1 cells together with some endomesodermal progenitors did labeled m1-derived comb plates arise (Table 2, Figs. 4K–4N).

DISCUSSION

Inductive Interactions Involved in Comb Row Formation

The results clearly show that multiple inductors are required to induce m1 progeny to form comb plates. These include the e1 micromere lineage and the endomesodermal progeny of the 2M and 3E macromeres. Although m1-derived comb plates form in the presence of either 3E or 2M derivatives (with e1 blastomeres present), at this time we cannot distinguish which progeny of these macromeres (either endodermal and/or mesodermal) are involved in
these inductive events. Both of these lineages give rise to endodermal and mesodermal (muscle) cells (Martindale and Henry, 2000). Other observations suggest that the endoderm plays an important role in comb plate formation. In a related series of experiments examining the process of postregeneration of comb rows, which takes place after the embryonic period (Martindale, 1986; Henry and Martindale, 2000), we frequently observed that endodermal tissue first extends out to lie beneath the area where comb row regeneration will eventually take place. Furthermore, the endodermal canals that normally run underneath the comb rows also fail to form following e1 micromere removal, lending support to the idea that the endoderm is involved in the appearance of m1-derived comb plates. It will be a challenge to design an approach that distinguishes which endodermal and/or mesodermal derivatives are involved in

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* From Martindale and Henry (1997b). See text for further details.

**FIG. 4.** Corresponding DIC and fluorescence light micrographs showing the formation of comb plate cilia by the progeny of e1 and m1 derivatives in embryos consisting of reduced combinations of embryonic cells. (A, B) Isolated unlabeled e1 micromere, which has formed comb plate cilia. (C, D) Isolated group of two diI-labeled m1 micromeres, which have not formed any comb plates. (E, F) Cluster of four unlabeled e1 and four diI-labeled m1 micromeres, which has formed unlabeled comb plates derived solely from the unlabeled e1 progeny. (G, H) Case in which all four 3E and all four 2M macromeres were ablated after diI labeling of all four m1 micromeres. Note that only unlabeled e1-derived comb plates have formed in the absence of the endomesoderm. (I, J) High-magnification views of the upper left set of ctenoid plates shown in G and H showing that there are no labeled comb plate cilia. (K, L) Case consisting of four unlabeled e1 and four diI-labeled m1 micromeres together with two unlabeled 1M macromeres. Note that m1 progeny have contributed to the formation of fluorescently labeled comb plates. (M, N) High-magnification view of a case consisting of four unlabeled e1 and four diI-labeled m1 micromeres together with two unlabeled 1E macromeres. Note that the m1 micromeres have generated labeled, comb plate cilia. cp, comb plates. Scale bar equals 25 μm for A–H, K and L, and 15 μm for I and J and M and N.
Embryonic comb plate induction, given that these germ lineages separate late during development. Here we have clearly shown a requirement for both e1 and the endomesodermal lineages in inducing m1 progeny to form comb plates.

The results indicate that the inductive ability to induce comb plates is not differentially segregated between the two daughter cells of e1 (the e11 and e12 cells). Either the induction occurs prior to division of e1 (at the 16-cell stage) or both daughter cells of e1 are capable of inducing m1 derivatives to form ctenoid plates. Further experiments are required to examine the exact timing of these inductive events.

In another study we discovered that e2 progeny are required for the regeneration of comb plates from m1 progeny during the adult phase (Martindale and Henry, 1999). Thus, m11 derivatives could have some opportunity to experience these inductive signals in the intact embryo; however, this may occur after the induction has occurred or when the competence to respond to these signals has been lost. The fact that m11 can form comb plates when m12 is deleted at the 32-cell stage could indicate that the inductive signals from the e1 lineage take place or persist during later stages of development. On the other hand, an alternative explanation why m11 does not normally make comb plates could be ascribed to lateral inhibition provided by m12 or its progeny. Thus, the e1 inductive signal could occur earlier, at the 16-cell stage. At this time we do not know exactly when these inductive interactions take place, though preliminary experiments indicate that comb plate induction proceeds over a prolonged period of development (data not shown).

**Evolutionary Implications: Inductive Interactions during Metazoan Development and Evolution**

Many examples of transgerm tissue induction exist among the higher metazoans—for instance, the well-known examples of inductive interactions involving the Nieuwkoop and Spemann organizers in amphibian embryos, which are involved in induction of the mesoderm via interactions between endodermal and ectodermal precursors. These events culminate in the induction of the central nervous system within the animal cap ectoderm via interactions with axial and paraxial mesoderm (Slack, 1991). Likewise transgerm-inductive interactions involving multiple inductors are thought to be involved in many forms of secondary induction as well, such as the induction of the vertebrate lens within placodal ectoderm (Grainger, 1992). In ascidians, mesodermal tissues need inductive interactions from endoderm for their formation (Nakatani and Nishida, 1994; Kim and Nishida, 1999; Kim et al., 2000), and it is likely that such inductive interactions are also involved in the development of the ascidian nervous system (Venuti and Jeffery, 1989). There are many examples among protostome invertebrates as well. For instance, in the gastropod Ilyanassa obsoleta the shell gland, derived from the ectoderm, requires endodermal inductive interactions (Cather, 1967). As mentioned above, we cannot distinguish whether mesodermal and/or endodermal derivatives of the E and M lineages are responsible for the inductive signals required for comb plate formation. However, this is the first report of multiple inductive interactions in ctenophores.
which involve two or more germ tissues. Ctenophores occupy a key basal position in metazoan phylogeny, although it is not yet clear whether the ctenophores are the sister group to the Bilateria (Ax, 1989, 1996; Zrzavy et al., 1998) or branch at a more basal position in the Metazoa (Wainright et al., 1993; Collins, 1998). While we are certainly not suggesting that any homology exists in the lineages or fates of the tissues involved in the different examples of above-mentioned metazoan induction, trans- germ tissue-inductive interactions are widespread and may represent an evolutionarily ancient developmental mechanism utilized in the formation of a variety of organ systems.

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