

Cell interactions and mesodermal cell fates in the sea urchin embryo

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Summary

Cell interactions during gastrulation play a key role in the determination of mesodermal cell fates in the sea urchin embryo. An interaction between primary and secondary mesenchyme cells (PMCs and SMCs, respectively), the two principal populations of mesodermal cells, regulates the expression of SMC fates. PMCs are committed early in cleavage to express a skeletogenic phenotype. During gastrulation, they transmit a signal that suppresses the skeletogenic potential of a subpopulation of SMCs and directs these cells into an alternative developmental pathway. This review summarizes present information concerning the cellular basis of the PMC-SMC interaction, as analyzed by cell transplanta-

tion and ablation experiments, fluorescent cell labeling methods and the use of cell type-specific molecular markers. The nature and stability of SMC fate switching, the timing of the PMC-SMC interaction and its quantitative characteristics, and the lineage, numbers and normal fate of the population of skeletogenic SMCs are discussed. Evidence is presented indicating that PMCs and SMCs come into direct filopodial contact during the late gastrula stage, when the signal is transmitted. Finally, evolutionary questions raised by these studies are briefly addressed.

Key words: cell interaction, mesoderm, cell fate, sea urchin.

Introduction

Intercellular signaling is a key mechanism of cell fate specification in all multicellular animals, even those that have traditionally been thought to develop in a highly mosaic manner. The sea urchin embryo has been an attractive experimental system for discerning how cell interactions control cell fates because of its optical clarity, the ease with which its cells can be isolated, transplanted and cultured, and the availability of a large collection of cell type-specific molecular markers (see reviews by Davidson, 1989; Ettensohn and Ingersoll, 1992). Blastomere isolation and recombination experiments have shown that cell interactions during cleavage are important in establishing embryonic patterns (Hörstadius, 1973; Henry et al., 1989; Khaner and Wilt, 1991). Critical cell interactions continue during gastrulation, and are involved both in regulating the extensive morphogenetic movements that reorganize the embryo during this stage of development and in specifying the fates of embryonic cells. We are investigating a key regulatory cell interaction during gastrulation that plays an important role in the specification of mesodermal cell fates and the process of skeletogenesis.

Mesenchyme cell populations in the sea urchin embryo

The mesoderm of the sea urchin embryo arises from the vegetal plate; a thickened epithelial placode at the vegetal

pole of the blastula. In most species of commonly studied sea urchins, the mesoderm consists of two distinct populations of cells, primary and secondary mesenchyme cells (PMCs and SMCs, respectively) (Fig. 1). Both cell populations are highly motile and migrate actively within the blastocoel following their release from the vegetal epithelium. The PMCs and SMCs differ, however, with respect to their time of ingression, lineage and developmental fates. (For more detailed reviews of the development of these cell populations, see Gustafson and Wolpert (1967), Okazaki (1975a), Harkey (1983), Solursh (1986), Decker and Lennarz (1988), Wilt and Benson (1988), and Ettensohn (1991a)).

The PMCs are the sole descendants of the large micromere daughter cells, four cells that arise from the unequal cleavage of the micromeres at the fifth cleavage division. These cells undergo either 3 or 4 additional rounds of cell division depending upon the species to give rise to an average of 32 or 64 PMCs per embryo, a number that remains constant throughout gastrulation and early larval development. The PMCs ingress into the blastocoel at the start of gastrulation and migrate to specific target sites on the blastocoel wall between the vegetal pole and the equator of the embryo, where they become arranged in a characteristic ring-like pattern. As the subequatorial ring pattern forms, filopodial processes of the PMCs fuse, forming thick cable-like extensions that join the cells in a syncytial network. Within these fused filopodial cables, the PMCs deposit a branched skeletal framework of crystalline rods

(spicules) composed of CaCO_3 , MgCO_3 , and several spicule matrix glycoproteins (Wilt and Benson, 1988). The skeleton serves as a structural framework for the distinctively angular pluteus larva.

The PMCs are restricted to a skeletogenic pathway of differentiation very early in embryogenesis, as micromeres isolated from 16-cell-stage embryos give rise only to skeletogenic cells when cultured in vitro, transplanted to ectopic positions in the embryo, or reassociated with other blastomeres in a variety of combinations (Hörstadius, 1973; Okazaki, 1975b; Livingston and Wilt, 1990; Khaner and Wilt, 1991). Associated with this cellular phenotype is the expression of a collection of PMC-specific gene products that have been identified by means of monoclonal antibodies and cDNAs (Carson et al., 1985; Wessel and McClay, 1985; Leaf et al., 1987; George et al., 1991; Katoh-Fukui et al., 1991).

Unlike the PMCs, the SMCs are a heterogeneous population of cells and express several different fates. In all species that have been carefully examined, a population of prospective pigment-forming SMCs ingresses relatively early in gastrulation (Gustafson and Wolpert, 1967; Gibson and Burke, 1985; Etensohn and McClay, 1988). Later in gastrulation, larger numbers of cells are released from the tip of the archenteron. Some of these cells move into the blastocoel and adopt a fibroblast-like phenotype; they have been referred to as blastocoelar cells or basal cells (Cameron et al., 1991; Tamboline and Burke, 1992), although this population might well include several distinct cell types. Shortly before the completion of gastrulation, the anterior tip of the archenteron expands bilaterally to form the two coelomic pouches. The classification of the cells of the coelomic pouches as SMCs is debatable, since most of these cells remain as part of a coherent epithelium during coelom formation despite their intense filopodial activity (Gustafson and Wolpert, 1963). After the completion of gastrulation, 10–15 cells move away from each coelomic pouch and surround the foregut; these cells form the circumesophageal musculature of the pluteus larva and express several distinctive cytoskeletal proteins (Ishimoda-Takagi et al., 1984; Cox et al., 1986; Burke and Alvarez, 1988; Wessel et al., 1990).

The SMCs are considered to be descendants of the veg2 layer of blastomeres that arises at the 64-cell stage (Hörstadius, 1973) (Fig. 1). Some cells in the coelomic pouches, however, are derived instead from the small micromeres, the siblings of the four founder cells of the PMC lineage (Endo, 1966; Pehrson and Cohen, 1986; Tanaka and Dan, 1990; Cameron et al., 1991). These cells normally undergo one additional round of division to produce eight cells that remain at the tip of the archenteron during gastrulation and later contribute to both the right and left coelomic pouches. At present, nothing is known of the mechanisms by which the descendants of the veg2 blastomeres become progressively restricted in their fate, or when such fate restrictions occur during embryogenesis.

Evidence for PMC-SMC signaling

Fate mapping studies have shown that, in the undisturbed

embryo, only PMCs express a skeletogenic fate and contribute to the larval skeleton (Hörstadius, 1973). Studies examining the development of isolated blastomeres of cleavage-stage embryos, however, demonstrate that the *potential* for skeletogenic differentiation is not restricted to cells of the micromere-PMC lineage. In particular, macromeres or their veg2 descendants, the progenitors of the SMCs and the endoderm (Fig. 1), have a significant capacity to give rise to skeletogenic cells when cultured in isolation (Hörstadius, 1973; Khaner and Wilt, 1991). Evidence that cells other than PMCs possess skeletogenic potential even at much later stages of development came from a little-known study by Fukushi (1962), who removed PMCs from embryos of *Glyptocidaris crenularis* and observed that SMCs moved to the region normally occupied by the PMCs and synthesized spicules. Langelan and Whiteley (1985) used low concentrations of SDS to inhibit micromere and PMC formation and reported that skeletal elements were nevertheless synthesized in such embryos, either by PMCs that ingressed in a delayed fashion or by SMCs. These studies suggested that in the absence of a signal transmitted by the PMCs, SMCs might alter their fate and adopt a skeletogenic phenotype. A clear demonstration of this interaction and a detailed analysis of its cellular basis has come from a combination of cell transplantation and ablation experiments, fluorescent cell marking techniques and the use of PMC-specific molecular markers, as described below. These studies show that PMCs interact with SMCs in a unidirectional manner late in gastrulation, and that the effect of this interaction is to regulate the specification of SMC fates and the process of skeletogenesis.

SMC conversion

Elimination of the entire complement of PMCs at the early gastrula stage by microsurgical or fluorescence-based methods (Figs. 2 and 8A) leads to a spectacular change in the developmental program of the SMCs. 65–75 of these cells switch fate and adopt the PMC phenotype, a process that has been termed SMC conversion (Etensohn and McClay, 1988). During this regulative event, SMCs migrate to PMC-specific target sites in the embryo, activate a PMC-specific program of gene expression, and assemble a correctly patterned larval skeleton.

Changes in directional cell movements

The normal motile activity of PMCs and SMCs is similar in two respects; both migrate as individuals within the blastocoel by means of contractile filopodia and both have a tendency to fuse with like cells. However, these two populations of cells exhibit very different directional patterns of movement. The PMCs migrate to specific target sites on the blastocoel wall and arrange themselves in a subequatorial ring pattern, while SMCs either invade the embryonic epithelium and differentiate as pigment cells or remain scattered within the blastocoel, often in the interior of the cavity or (in the case of muscle cells) along the basal surface of the foregut. Cell transplantation experiments show that even when these two cell types are microinjected into recipient embryos of the same developmental stage and are con-

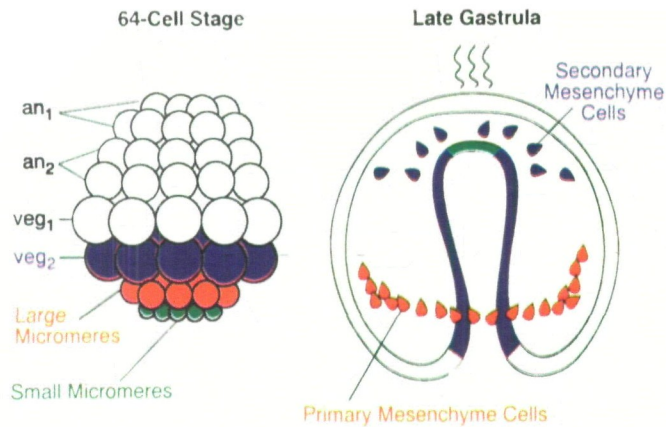


Fig. 1. Partial fate maps of the 64-cell-stage embryo and late gastrula, showing the origin of the mesoderm. The primary mesenchyme cells (PMCs) are derived from the large micromeres (red), while the secondary mesenchyme cells (SMCs) and endoderm are derived from the veg_2 layer of blastomeres (blue). The small micromeres (green), the siblings of the large micromeres, remain at the tip of the archenteron during gastrulation and later contribute to the coelomic pouches (see text for details).

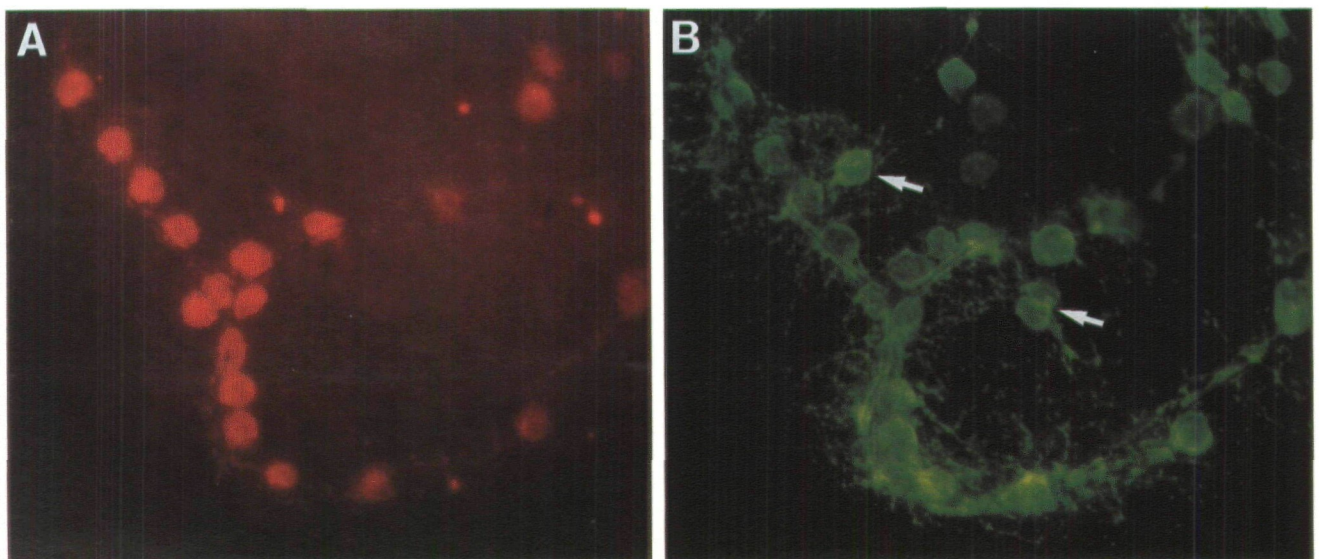


Fig. 6. SMC conversion in an embryo with an intermediate number of PMCs, as assayed by the method shown in Fig. 5. (A) Rhodamine fluorescence, showing labeled donor PMCs. (B) Immunostaining with an anti-msp protein MAb and a fluorescein-conjugated secondary antibody. All donor cells are both red and green, while converted SMCs derived from the recipient embryo (arrows) fluoresce green because they express the skeletogenic marker proteins, but are not rhodamine-labeled.

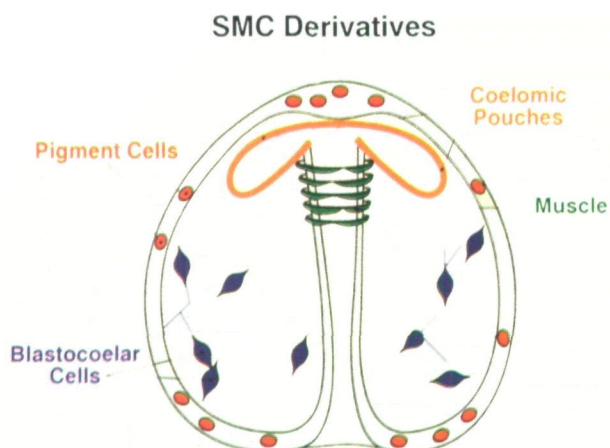


Fig. 9. SMC fates. SMCs give rise to four major derivatives. (1) Pigment cells arise from both early- and late-ingressing populations of SMCs. (2) Other SMCs (so-called basal or blastocoelar cells) that ingress late in gastrulation remain scattered in the blastocoel and have an unknown function, although they may be fibroblast-like (Tamboline and Burke, 1992). (3) Cells at the tip of the archenteron, including the small micromeres, give rise to the two coelomic pouches. (4) At the end of gastrulation, 10-15 cells migrate out of each coelomic pouch and surround the foregut, giving rise to the circumesophageal musculature (see text for details).

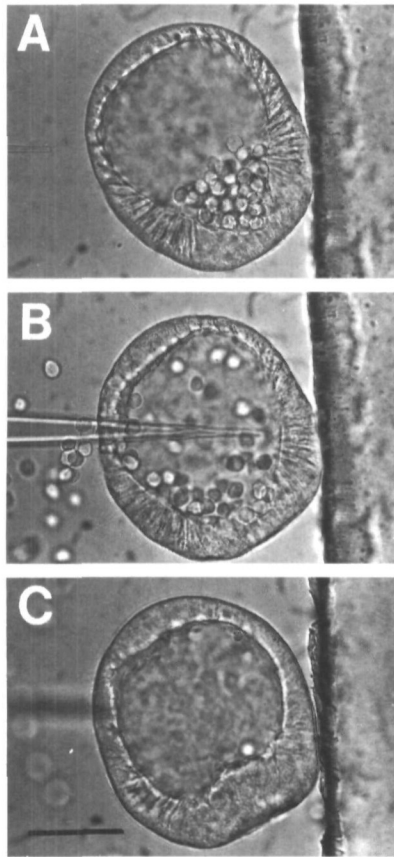


Fig. 2. PMC ablation. At the mesenchyme blastula stage, before the migration of the PMCs away from the vegetal plate, all or any fraction of the cells can be removed by directing a stream of seawater into the blastocoel from the tip of a micropipette. The PMCs flow out of the blastocoel through the wound created by the pipette. Reprinted from Ettensohn and McClay (1988), with permission from Academic Press, Inc. Scale bar = 50 μ m.

fronted with identical directional cues, each migrates in its characteristic pattern (Ettensohn and McClay, 1986). Therefore, there is a high degree of specificity in the ability of these cells to detect or respond to guidance signals in the blastocoel. During conversion, SMCs acquire the capacity to recognize PMC-specific directional cues and they arrange themselves in a subequatorial ring pattern remarkably similar to that formed by the PMCs (Fig. 3). Spiculogenesis is initiated within two clusters of cells that form along the ventrolateral aspects of the subequatorial ring, as in control embryos. Because the migration and patterning of the converting SMCs is delayed by several hours relative to the normal developmental schedule of the PMCs, the relevant directional cues must persist in PMC(-) embryos for at least that long.

Expression of PMC-specific molecular markers

The PMCs express a collection of unique cell surface glycoproteins that have been studied in several laboratories (Carson et al., 1985; Wessel and McClay, 1985; Leaf et al., 1987). The best characterized of these proteins is msp130, a PMC-specific, phosphatidylinositol-linked, cell surface glycoprotein that has been implicated in calcium transport

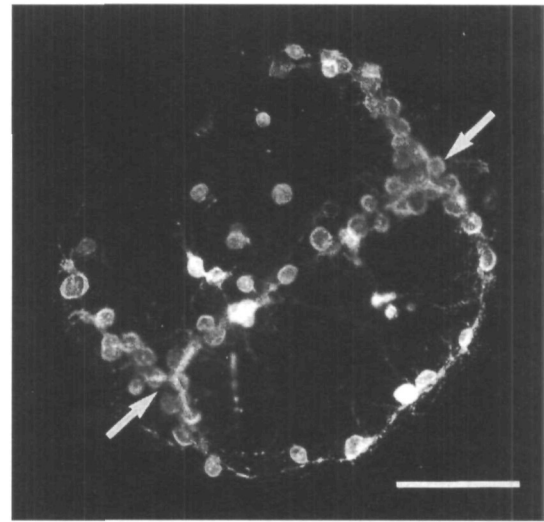


Fig. 3. Whole mount of a PMC(-) embryo after SMC fate-switching, immunostained with a monoclonal antibody (Mab) that recognizes msp proteins in order to reveal the arrangement of the converted cells. Skeletogenic SMCs form a pattern that closely resembles the subequatorial ring normally formed by PMCs. The SMC ring arises after control embryos have completed gastrulation, 8-10 hours after the PMC ring would normally form. Spicule formation begins in two ventrolateral clusters of skeletogenic cells (arrows), as in control embryos. Scale bar = 50 μ m.

(Farach-Carson et al., 1989; Parr et al., 1990). Msp130 is antigenically related to several other PMC-specific proteins (Shimizu-Nishikawa et al., 1990; Kabakoff et al., 1992). In *Lytechinus variegatus*, immunological and biochemical methods have been used to demonstrate that msp130 is one member of a family of antigenically related, PMC-specific cell surface glycoproteins ('msp proteins'). The pattern of expression of these proteins, both with respect to their overall abundance in the cell and their distribution on the cell surface, changes during the morphogenetic program of the PMCs (Fuhrman et al., unpublished observations).

The earliest indication of SMC fate-switching thus far detected is the de novo expression of the complete repertoire of msp proteins. Expression of these proteins can first be observed by indirect immunofluorescence 7-8 hours after PMC removal, at a time when many SMCs are migrating away from the tip of the archenteron but before they have accumulated in a ring pattern (Ettensohn, 1991b). Surface expression of these proteins by converted SMCs persists throughout skeletogenesis. Immunoblot analysis of msp protein expression in PMC(-) embryos shows that following conversion, skeletogenic SMCs undergo the same modulations in the pattern of msp protein expression as do PMCs. The regulated expression of these surface proteins is therefore tightly coupled to the skeletogenic program of differentiation. Skeletogenic SMCs also express surface binding sites for a lectin, wheat germ agglutinin, that normally binds specifically to PMCs (Ettensohn and McClay, 1988), although this lectin may recognize the same collection of msp proteins. The de novo expression of a different PMC-specific molecular marker, a cDNA encoding the spicule matrix protein sm50 (Livingston et al., 1991), has

also been demonstrated during SMC conversion (Ettensohn, unpublished observations). Therefore, all available evidence supports the view that the program of SMC gene expression triggered during conversion is similar or identical to the normal skeletogenic program of gene expression exhibited by PMCs. It is not known whether SMCs down-regulate SMC-specific proteins or mRNAs when they activate the skeletogenic program, as there are no molecular markers currently available that are completely SMC specific.

Spiculogenesis

Despite the delay in the initiation of skeletogenesis in PMC(-) embryos, the final pattern of the larval skeleton is the same as that of control larvae (Fig. 4). Perhaps even more surprising is the observation that when intermediate numbers of PMCs are present in the blastocoel, converted SMCs cooperate with these cells in the construction of a normal skeleton, even though the endogenous PMCs begin spiculogenesis on their own intrinsic timetable and skeletogenic SMCs are integrated into the skeletal pattern at a much later stage (Ettensohn and McClay, 1988). This regulative behavior has not yet been examined in detail, although it has been observed that when the endogenous PMCs in such an embryo are distributed predominantly in one part of the subequatorial ring pattern, converted SMCs have a tendency to fill in the deficient regions (Ettensohn and McClay, 1988). This flexibility in skeletal patterning is consistent with the fact that even when the number of PMCs is manipulated over a wide range, the cells form a normally proportioned subequatorial ring pattern and an essentially normal skeleton (Ettensohn, 1990a). Such behavior provides an especially striking example of the great flexibility of morphogenetic systems and their ability to reach a relatively constant and precise end state by means of several alternative routes (see Wolpert and Gustafson, 1961).

Signaling competence

One distinctive functional property of PMCs is their ability to suppress the skeletogenic potential of SMCs. This property can be assayed by cell transplantations (Fig. 5), and provides a different functional test of the extent to which converted SMCs adopt the PMC phenotype. To test the signaling competence of skeletogenic SMCs, fluorescently labeled converted cells were injected into PMC(-) recipient embryos and the number of endogenous SMCs that switched fate was determined by immunostaining with a monoclonal antibody that recognizes msp proteins. These studies show that skeletogenic SMCs are highly effective at suppressing the conversion of uncommitted SMCs to the skeletogenic fate. When 20-45 (mean = 32) SMCs are microinjected into recipient embryos, the numbers of SMCs in the recipients that express a skeletogenic fate is reduced by 80-85%, to an average of 10 cells/embryo ($n = 17$) (Ettensohn and Ruffins, 1992). Although the relative effectiveness of PMCs and converted SMCs in this regard has not yet been carefully analyzed, previous experiments provide some basis for comparison. When 30-40 PMCs are present in the blastocoel, an average of 7 SMCs (mean = 6.8, s.d. = 5.0, $n = 4$) switch fate (Ettensohn and McClay, 1988). Because the injection of 20-45 skeletogenic SMCs leads to the conversion of an average of 10 cells to a skele-

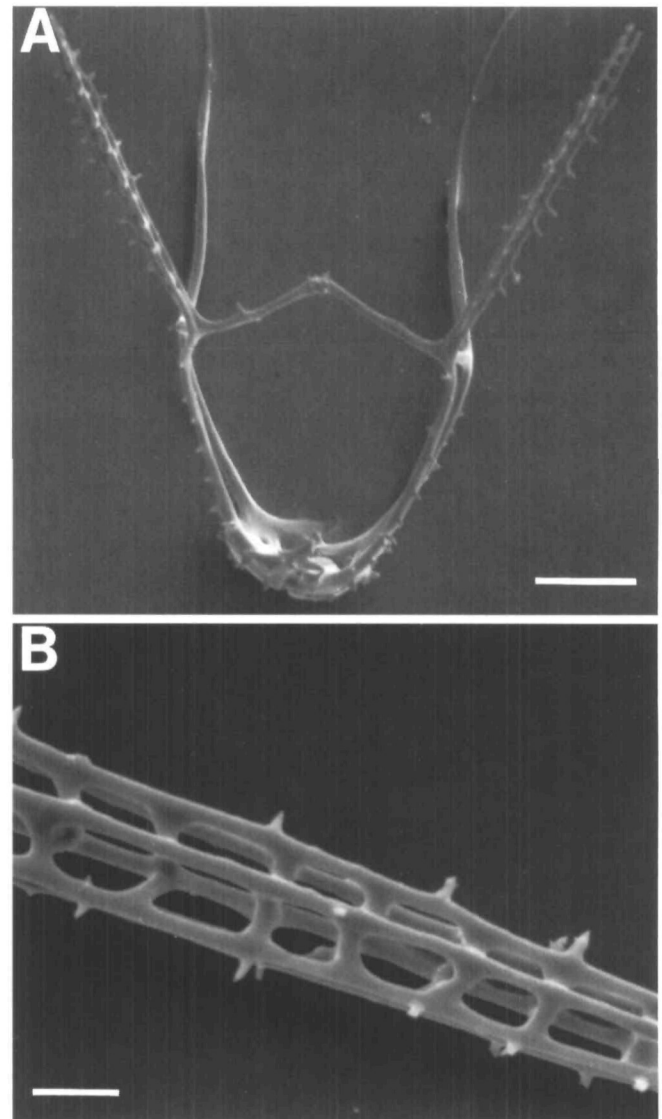


Fig. 4. Scanning electron micrographs of the skeleton formed by converted SMCs in a sand dollar, *Dendraster excentricus*. The final form of the skeleton is the same as that seen in control larvae. Species-specific differences in the skeletal pattern, such as in the fine structure of the spiculate rods, are faithfully reproduced by the skeletogenic SMCs. (A) Low magnification view showing the entire skeleton. Scale bar = 50 μ m. (B) High magnification view of the anal (postoral) rod formed by the skeletogenic SMCs. The elaborate, fenestrated structure of this skeletal rod is characteristic of *D. excentricus*. Scale bar = 10 μ m.

togenic fate, converted SMCs appear to be as effective on a per-cell basis as PMCs at suppressing SMC skeletogenesis. This assay demonstrates that when SMCs switch fate they acquire PMC-specific signaling properties.

Cellular aspects of the PMC-SMC interaction

The results of cell ablation experiments show that during normal development a signal is transmitted by PMCs that suppresses the skeletogenic potential of SMCs and shunts them into alternative developmental pathways. We have

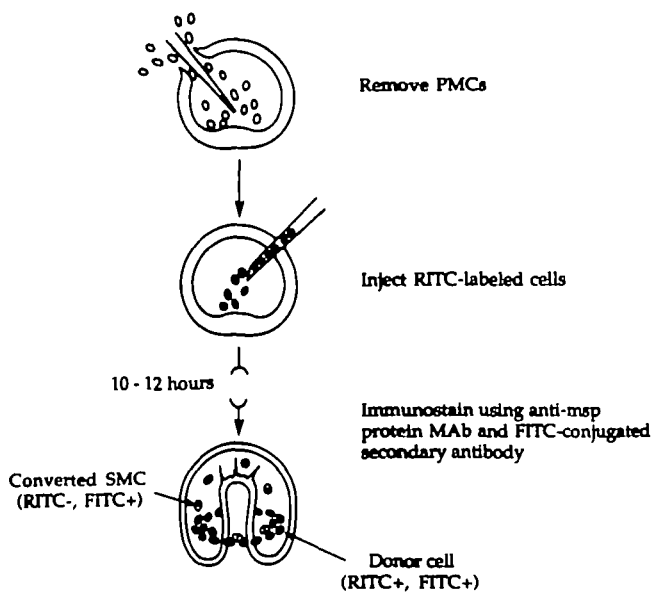


Fig. 5. Assay for mesodermal cell signaling. Rhodamine-labeled PMCs or converted SMCs are microinjected into the blastocoel of a PMC(-) recipient embryo. The number of donor cells and the developmental stage of the recipient can be varied. After SMC conversion, the recipient embryos are processed for immunofluorescence using a Mab specific for the msp proteins and a fluorescein-conjugated secondary antibody. The skeletogenic donor cells fluoresce both green and red, while any converted SMCs derived from the recipient embryo fluoresce green only. By this method the numbers of donor cells and converted SMCs in each embryo can be quantified.

analyzed this interaction at the cellular level, with the belief that such information will provide a context for a molecular analysis of the interaction.

Quantitative aspects of the interaction

One intriguing aspect of the PMC-SMC interaction is its quantitative nature. The experimental strategy shown in Fig. 5 has been used to show that the number of SMCs that express a skeletogenic fate is inversely proportional to the number of PMCs in the blastocoel (Ettensohn and McClay, 1988; Figs. 6, 7). The same approach has been used to show that there is a strict threshold for conversion; when at least 50 PMCs are present in the blastocoel, SMC fate-switching is completely suppressed. By some mechanism, the embryo 'counts' the number of PMCs in the blastocoel and regulates the number of SMCs that switch fate accordingly.

Timing of the interaction

Because ablation of the PMCs at the early gastrula stage (shortly after PMC ingression) leads to SMC conversion, it may be concluded that the SMCs are still developmentally labile at this stage and that PMCs normally interact with these cells at some time during gastrulation. To investigate the timing of PMC-SMC signaling in more detail, PMCs were eliminated at progressively later developmental stages using a fluorescence photoablation technique (Ettensohn, 1990b; Fig. 1D). These studies show that, even when the PMCs are eliminated at the late gastrula stage, the fate of the SMCs has not been irreversibly specified and these cells

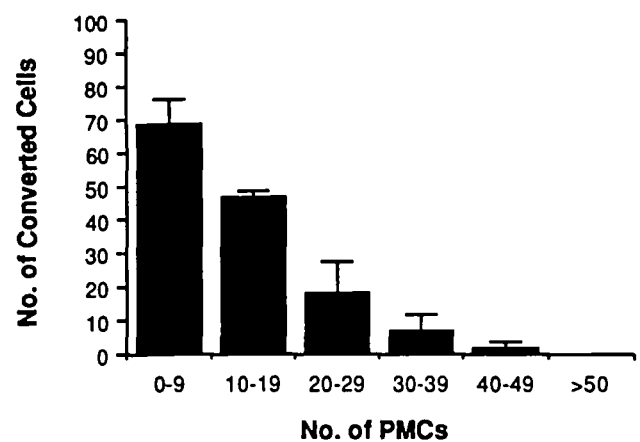


Fig. 7. Quantitative nature of the PMC-SMC interaction. Double-label experiments (Figs. 5 and 6) show that the number of SMCs that switch to a skeletogenic fate is inversely proportional to the number of PMCs in the blastocoel. SMC conversion is completely suppressed when >50 PMCs are present in the blastocoel. For each of the six classes of embryos, 4-8 specimens were scored (35 total embryos). Bars indicate standard deviations (no bar is shown for those embryos with >50 PMCs; of 6 such embryos examined, none had any converted SMCs). Reprinted with permission from Ettensohn and McClay (1988), with permission from Academic Press.

switch to a skeletogenic fate (Fig. 8). Photoablation of the PMCs at post-gastrula stages does not result in SMC conversion, suggesting that the SMCs are committed to a non-skeletogenic fate by that time. The fact that SMCs respond to PMC ablation at a late developmental stage, close to the time at which conversion begins as assayed by the expression of msp proteins, might be explained in one of two ways. Either the PMCs do not transmit a signal until the late gastrula stage, or the SMCs are insensitive to the signal until that time (or both). Cell transplantation experiments argue in favor of the latter interpretation. PMCs present in the blastocoel for a short time (3-4 hours) at the start of gastrulation will not suppress SMC skeletogenesis, but will do so effectively if placed in the blastocoel for 3-4 hours at the end of gastrulation (Ettensohn, 1990b). Assuming that the signal produced by the PMCs is the same in both cases, this result indicates that the SMCs are not competent to respond to the signal at the early gastrula stage.

Unidirectional nature of the interaction and the stability of conversion

Once the PMC-derived signal has been received, SMCs are stably committed to expressing a skeletogenic fate. When converted SMCs are microinjected into normal, PMC-containing recipient embryos, they continue to express a skeletogenic phenotype (Ettensohn and Ruffins, 1992). Therefore, although PMCs can suppress the expression of the skeletogenic fate by SMCs, they cannot reverse it once it has taken place. In addition, although PMCs regulate SMC skeletogenesis, there is no indication that they suppress their own skeletogenic differentiation in a similar manner. When large numbers of PMCs (100-150) are introduced into the blastocoel of mesenchyme blastula stage embryos, effec-

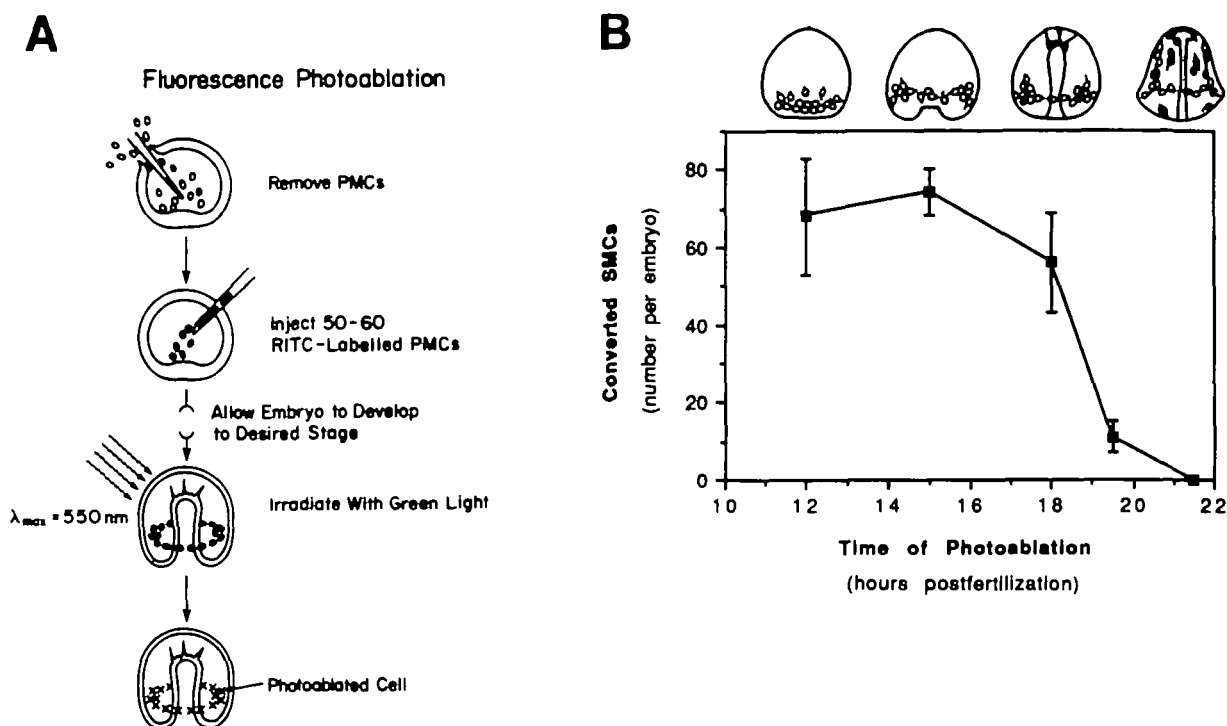


Fig. 8. Timing of the PMC-SMC interaction. (A) The fluorescence photoablation method used to eliminate PMCs after the start of their migration. The entire complement of PMCs is removed at the mesenchyme blastula stage and replaced with an equal number of rhodamine-labeled PMCs. The cells are ablated at the desired stage by prolonged excitation of the fluorochrome using a conventional epifluorescence microscope. (B) Results of fluorescence photoablation experiments. Ablation of the PMCs at or before the late gastrula stage results in SMC conversion. The number of converting cells drops sharply when PMCs are ablated at later developmental stages. Stages of embryos at the time of photoablation are illustrated by the diagrams at top. Bars indicate standard errors (95% confidence limits on the mean). For each developmental stage, 5-11 embryos were scored. Reprinted from Ettensohn (1990b), with permission from the American Association for the Advancement of Science.

tively doubling or tripling the normal complement of these cells, all the donor cells take part in skeletogenesis and none are induced to adopt alternative fates (Ettensohn, 1990a). In fact, as discussed above, no experimental conditions have yet been found that can induce cells of the micromere-PMC lineage to adopt a non-skeletogenic fate. The autonomy of PMC fate specification argues strongly that, although signals are transmitted from PMCs to SMCs, the converse is not true.

Lineage, numbers, and fates of the converting cells

Removal of the entire complement of PMCs at the early gastrula stage results in the conversion of 65-75 SMCs to the PMC phenotype. Ablation of skeletogenic SMCs does not result in the recruitment of any additional cells to a skeletogenic fate, demonstrating that by the late gastrula stage the number of SMCs with skeletogenic potential is restricted to 65-75 cells (Ettensohn and Ruffins, 1992).

Fate mapping studies using vital dyes and injected lineage tracers have shown that SMCs are derived from the veg2 layer of the macromeres (Hörstadius, 1973, Cameron et al., 1991). Two observations, however, led us to test the possibility that descendants of the small micromere daughter cells might also contribute to the population of converting cells. The small micromeres are the siblings of the large micromere daughter cells, the founder cells of the

PMC lineage, and are therefore the closest lineal relatives of the PMCs. In addition, these cells inherit the cytoplasm at the extreme vegetal pole of the unfertilized egg, and indirect evidence suggests that skeletogenic determinants are stored in the vegetal cytoplasm (Davidson, 1989). Specific labeling of the small micromere descendants with 5-bromodeoxyuridine after the method of Tanaka and Dan (1990), however, shows that these cells remain associated with the tip of the archenteron during gastrulation and do not contribute to the population of converting SMCs (Ettensohn and Ruffins, 1992). All of the skeletogenic SMCs therefore appear to be derived from the veg2 tier of blastomeres (Fig. 1).

As noted above, the SMCs give rise to four major cell types (Fig. 9). As one means of gaining information concerning the normal fate(s) of the converting cells, PMC(-) larvae were examined to determine whether specific SMC derivatives were reduced or absent (Ettensohn and Ruffins, 1992). Comparisons of the numbers of muscle cells, coelomic pouch cells, blastocoelar cells, and pigment cells in PMC(-) larvae and controls showed only one significant difference. The number of pigment cells in PMC(-) embryos was reduced from an average of 100 pigment cells in control larvae to 47 cells in PMC(-) larvae, a reduction of more than 50%. Fluorescent cell marking experiments and time-lapse videomicroscopy of gastrulation in

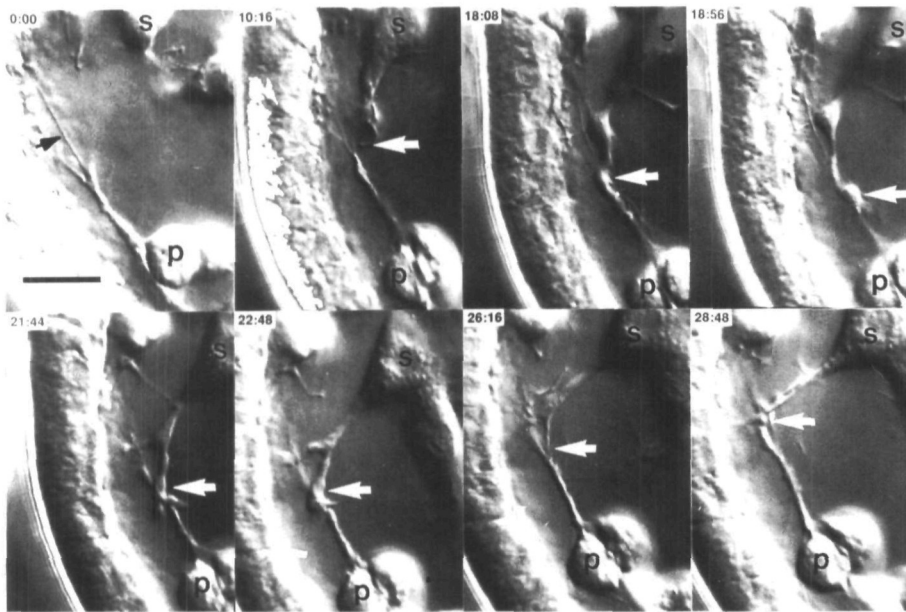


Fig. 10. Filopodial contacts between PMCs and SMCs during gastrulation. Eight frames of a time-lapse video sequence are shown. Numbers at upper left of each frame indicate the time in minutes : seconds. A filopodium of an SMC (s) makes a prolonged contact with a filopodium extending toward the animal pole from a PMC (p) in a ventrolateral cluster. The tip of the SMC filopodium (white arrow) contacts the PMC filopodium and courses down and then back up its length over a period of almost 20 minutes. The PMC filopodium (black arrowhead in panel A) remains essentially immobile during this sequence. The video recording was made using a Zeiss 63X planapochromat lens with differential interference contrast optics, a Hamamatsu Newvicon camera and Argus 10 image processor, and a Panasonic TQ-3038F optical disk recorder. Scale bar = 10 μ m.

PMC(-) embryos have shown that, in *Lytechinus variegatus*, a population of prospective pigment cells arises early in gastrulation in which fate specification is independent of PMCs (Ettensohn and McClay, 1988). Larger numbers of SMCs, some of which are also prospective pigment cells, leave the tip of the archenteron later in gastrulation (Ettensohn and Ruffins, 1992). Taken together, this indirect evidence suggests that the converting cells represent a population of late-ingressing SMCs that would otherwise differentiate as pigment cells. According to this view, the PMC-derived signal controls a developmental switch, directing SMCs to adopt a pigment cell phenotype instead of the alternative (default) skeletogenic fate.

Mechanisms of PMC-SMC signaling

PMC migration and patterning can be inhibited *in vivo* by microinjecting lectins and antibodies that agglutinate these cells into the blastocoel. SMC conversion is nevertheless suppressed, ruling out the possibility that the PMCs act by physically occupying special target sites on the blastocoel wall that promote skeletogenesis (Ettensohn, unpublished observations). Alternative signaling mechanisms include the secretion of soluble factors or extracellular matrix components by the PMCs into the blastocoel, and direct cell-cell contact between the two populations. Repeated microinjection of blastocoel fluid from normal gastrulae into PMC(-) deficient embryos fails to suppress SMC conversion, providing indirect evidence against the former model (Ettensohn, unpublished observations). On the other hand, examples of direct filopodial contact between PMCs and SMCs at the late gastrula stage are relatively easy to document by time-lapse video microscopy, particularly at the tips of the chains of PMCs that extend toward the animal pole from the subequatorial ring (Fig. 10). The duration of such filopodial contacts ranges from a few seconds to as long as 10-15 minutes. It will be important to develop means of preventing normal cell extensions by one or both

cell populations to determine whether direct filopodial contact is required to mediate the interaction. A mechanism of signaling based on filopodial extension by SMCs would be consistent with the insensitivity of these cells to the PMC-derived signal at the early gastrula stage, when SMCs exhibit little or no filopodial activity (Gustafson and Wolpert, 1967).

The molecular basis of the signaling mechanism is not unknown. Recent genetic and molecular studies of vulval development in *Caenorhabditis elegans* and photoreceptor cell development in *Drosophila melanogaster* indicate that signal transduction systems based on receptor tyrosine kinases coupled to Ras proteins regulate cell interactions in these systems (Greenwald and Rubin, 1992). Analysis of early inductive cell interactions in the amphibian embryo has provided convincing evidence that members of the fibroblast growth factor (FGF), transforming growth factor beta (TGF β), and/or Wnt gene families mediate these cell-cell signaling mechanisms (Jessell and Melton, 1992). At present, we do not know whether similar molecules play a role in PMC-SMC signaling, although they represent models for potential mechanisms.

Conclusions

The formation of the skeleton by cells of an alternative lineage following PMC ablation is an example of the well-known phenomenon of embryonic regulation. The basis of this regulative system is a unidirectional interaction between a population of skeletogenic cells committed very early in development (PMCs) and a second, more flexible mesodermal lineage (SMCs). The interaction between these two cell populations takes place late in gastrulation, a stage of development that has also been shown to be an important time in the determination of mesodermal cell fates in vertebrate embryos (Ho, this volume). In the sea urchin, although considerable information has been obtained concerning the cellular aspects of this interaction, its molecular basis remains to be elucidated.

A different question raised by this and other examples of regulative cell interactions is why they should exist at all. The selective pressures that acted during evolution to create development by cellular interactions are unknown (Wolpert, 1990). Whatever its origin, it seems unlikely that the PMC-SMC interaction has persisted because of a selective advantage to the organism. The average number of PMCs per embryo (64 cells) is considerably higher than that required to completely suppress SMC skeletogenesis (50 cells), suggesting that under natural conditions it would be extremely unlikely that an SMC would express a skeletogenic phenotype, a conclusion consistent with fate mapping studies. Nor should the quantitative nature of the interaction be viewed as an adaptive mechanism for closely regulating the number of skeletogenic cells. In fact, the overall pattern of the skeleton is unaffected by the presence of 2-3 times the usual number of PMCs (Ettensohn, 1990a), although presumably there is some minimum number of cells required for skeletogenesis. Instead, the quantitative nature of the PMC-SMC interaction probably reflects the cellular or molecular mechanism of the signaling (e.g., if cell-cell contact is involved, then the probability of contact might be proportional to the number of PMCs in the blastocoel).

Despite the above considerations, this system of cell interactions is widespread among indirect developing echinoids. PMC ablation studies carried out in *L. pictus*, *G. crenularis* and a sand dollar, *Dendraster excentricus*, show that PMC-SMC signaling takes place in these three species as well as in *L. variegatus*, the species that has been used for most studies (Fukushi, 1962; Langelan and Whiteley, 1985; Ettensohn and McClay, 1988; Ettensohn, unpublished observations). Because more 'primitive' indirect developing echinoids lack PMCs and form a skeleton from mesenchyme cells that ingress later in gastrulation (Schroeder, 1981), the evolution of an early-ingressing, skeletogenic cell population may represent an example of developmental heterochrony. Direct developing sea urchins and other classes of echinoderms show great variability in their patterns of skeletogenesis (Korshelt and Heider, 1895; Schroeder, 1981; Wray and McClay, 1988). To begin to understand how the PMC-SMC system evolved, information concerning skeletogenic lineages and mesenchymal interactions in other members of this phylum is needed, especially with respect to those species thought to most closely represent ancestral forms. Further analysis of this system might therefore shed light both on the way evolutionary processes act to modify ontogenetic programs and on the cellular and molecular mechanisms of cell interactions during development.

I am grateful to K. Guss, K. Malinda, E. Ingersoll, and S. Ruffins for valuable comments and suggestions, and to B. Livingston and G. Wessel for providing antibodies and cDNAs. This research was supported by National Institutes of Health Grant HD-24690, a Basil O'Connor Starter Scholar Award from the March of Dimes Foundation, and a National Science Foundation Presidential Young Investigator Award.

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