

Target Recognition by the Archenteron during Sea Urchin Gastrulation^{*1}

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During sea urchin gastrulation filopodia are sent out by secondary mesenchyme cells (SMCs) at the tip of the archenteron in continual cycles of extension, attachment, and retraction. Eventually the archenteron ceases its elongation and its tip localizes to the animal pole region of the embryo (Gustafson and Kinnander, 1956, *Exp. Cell Res.* 11, 36-57; Dan and Okazaki, 1956, *Biol. Bull.* 110, 29-42). We have investigated the mechanisms and specificity of this localization by analyzing filopodial behavior and by experimental manipulation of the interaction of the archenteron with the animal pole region. When the tip of the archenteron nears the animal pole, some filopodia make contact with a well-defined locus within this region. Filopodia that make contact with the locus remain attached 20-50 times longer than attachments observed at any other site along the blastocoel wall. The SMCs bearing the long-lived filopodia eventually change their phenotype by flattening and spreading onto this region. Several lines of experimental evidence indicate that contact with the animal pole locus, or "target" region, is crucial for the change in phenotype of the SMCs: (1) the phenotypic change can be induced precociously by bringing the animal pole region within reach of the tip of the archenteron early in gastrulation. Precocious contact with other regions of the blastocoel wall does not induce a similar change. (2) The phenotypic change can be delayed by placing the animal pole out of reach late in gastrulation, resulting in artificial prolongation of exploratory behavior by filopodia. (3) Ectopic combinations of animal pole ectoderm and archenterons in fused multiple embryos and chimaeras result in attachment of archenterons to the nearest available target, and (4) freely migrating SMCs are observed to migrate randomly within the blastocoel, then stop at the animal pole and undergo the change in phenotype. Filopodia rapidly attach to the animal pole when the shape of early gastrulae is altered such that the animal pole is <35 μm from the tip of the archenteron, even though such attachments only occur in normal embryos at the $\frac{3}{4}$ - $\frac{4}{4}$ gastrula stage. Since it has previously been shown that the archenteron elongates autonomously to $\frac{2}{3}$ of its final length (Hardin, 1988, *Development* 103, 317-324), it appears that autonomous extension of the archenteron is required to place filopodia close enough to the animal pole to allow them to interact with it. Examination of modes of gastrulation in sea urchin species within a number of genera indicates that the processes of autonomous extension of the archenteron, random filopodial exploration, and target recognition are sufficient to account for successful completion of gastrulation in all of these species, despite a seemingly wide range of morphologies. Target recognition by SMCs thus appears to serve two important functions: it provides an epigenetic signal marking the end of random exploration by filopodia, and it positions the archenteron in preparation for mouth formation. © 1990 Academic Press, Inc.

INTRODUCTION

The interaction of migratory cells with invaginating and/or involuting tissue sheets is a common feature of gastrulation in many organisms (for example, see Trinkaus, 1984a; Keller, 1986). In particular, actively motile cells are often found at the leading edge of sheets of cells that undergo spreading and elongation, including the involuting marginal zone in amphibians (reviewed by Keller, 1986), the teleost enveloping layer (Trinkaus, 1984b), and the avian epiblast (New, 1959; reviewed by Bellairs, 1982). A convenient model system for studying the interaction between mesenchymal motility and the morphogenesis of tissue sheets is the sea urchin gastrula. Following the initial invagination of the vegetal plate to form the archenteron, cells at the tip of the

archenteron (secondary mesenchyme cells; SMCs) become protrusively active at about the time the archenteron begins to elongate (reviewed by Gustafson and Wolpert, 1963, 1967; Hardin and Cheng, 1986; Hardin, 1988).

Descriptive time-lapse cinémicrographic studies by Gustafson and co-workers catalogued the behavior of these cells extensively in *Psammechinus miliaris* (Gustafson and Kinnander, 1956, 1960; Kinnander and Gustafson, 1960; Wolpert and Gustafson, 1961; Gustafson, 1963). These studies suggested that the filopodia of secondary mesenchyme cells "randomly" explore the blastocoel, undergoing continual cycles of extension, attempted attachment, and retraction. The lifetimes of filopodia were found to vary widely, from 5 min-2 hr (Gustafson and Kinnander, 1956). Because the filopodial behavior begins at the time the archenteron begins to elongate, it was proposed that their contraction is largely responsible for elongating the archenteron (Gustafson and Kinnander, 1956; Dan and Okazaki,

¹ This paper is dedicated to Dr. J. P. Trinkaus in celebration of his return to full-time research after a distinguished teaching career.

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1956). More recently, it has been shown that the archenteron undergoes extensive epithelial cell rearrangement and is capable of elongating autonomously (Ettensohn, 1985; Hardin and Cheng, 1986; Hardin, 1988, 1989). Although the early phase of elongation is autonomous, full elongation of the archenteron requires intact SMCs (Hardin, 1988).

Eventually, as gastrulation ends the cells at the tip of the archenteron undergo a change in appearance and behavior, largely ceasing their exploratory behavior, and often becoming more loosely associated with the tip of the archenteron (Dan and Inaba, 1968; Gustafson and Kinnander, 1960). Finally, several hours after gastrulation is completed in *P. miliaris*, protrusively active cells, possibly descendants of secondary mesenchyme cells, are seen to make contact with the stomodeal invagination, apparently helping to bring it in contact with the oral ectoderm to form the mouth of the pluteus larva (Gustafson and Kinnander, 1960). In other species comparatively little movement of the tip of the archenteron appears necessary to bring it into contact with the stomodeum (Dan and Inaba, 1968).

These studies raise several questions regarding archenteron morphogenesis and the successful completion of gastrulation. First, how does the tip of the archenteron *localize* to the animal pole region at the end of gastrulation? Dan and Inaba state that in several Japanese species filopodia initially extend "radially as if in search of something" (Dan and Inaba, 1968, pp. 289-290), but that the means by which they subsequently localize to the animal pole is completely obscure. In addition, Trinkaus suggested some time ago that in *Lytechinus variegatus* the filopodia initially extend laterally, and only late in gastrulation do they extend upward, thus making it difficult for them to exert appreciable tension along the axis of archenteron elongation until late in gastrulation (Trinkaus, 1965; reviewed in Trinkaus, 1984a, pp. 438-441). Trinkaus pointed out that his observations were preliminary; however, if his and Dan and Inaba's observations represent a consistent feature of filopodial behavior, then this change in orientation over the course of gastrulation must be accounted for. In addition, in light of recent evidence that autonomous extension of the archenteron does occur, is there a relation between this extension and localization of filopodial contacts to the animal pole?

Second, are there features of the blastocoel wall that provide *guidance information* for filopodial attachment? On the basis of his observations, Gustafson favored physical influences, including (1) differences in surface topography at contact sites between epithelial cells (Gustafson, 1963), (2) the proximity of nearby tissues, particularly the dorsal ectoderm and the stomodeal invagination in *P. miliaris* (Gustafson and Wol-

pert, 1967; Gustafson, 1969), and (3) differences in tissue curvature, which would presumably make nonspecific adhesion sites accessible to filopodia in some regions but not in others (Gustafson, 1963; reviewed by Gustafson and Wolpert, 1967). Such nonspecific influences were thought by Gustafson to operate both in primary mesenchyme cells (cells that ordinarily form the spicules of the pluteus larva) as well as in secondary mesenchyme cells (Gustafson and Wolpert, 1967, pp. 478-479). In addition, Gustafson suggested that the ventral ectoderm might be generally more adhesive than the dorsal ectoderm, which would result in localization of both primary mesenchyme cells and later, secondary mesenchyme cells (Gustafson, 1969). In both cases, Gustafson hypothesized that there were not specific "targets," in the chemical or molecular sense, for the two populations of mesenchyme (Gustafson, 1969). However, Gustafson readily conceded that his suggestions were made based solely on observations, and little or no attempt was made to quantify these concepts of adhesion, or to confirm these predictions by experiment (Gustafson, 1969).

More recent studies do suggest that there are regional differences in the blastocoel wall that may provide specific information to subpopulations of mesenchyme cells in the sea urchin embryo. For example, there is evidence that the ventrolateral ectoderm contains unique information that is utilized by primary mesenchyme cells during their aggregation (reviewed by Solursh, 1986). Displacement of primary mesenchyme cells, either by centrifugation of embryos (Okazaki *et al.*, 1962), or by ectopic transplantation (Ettensohn and McClay, 1986) is followed by movement of these cells to their normal, highly stereotyped location in the ventrolateral ectoderm. Older primary mesenchyme cells introduced at the animal pole of a younger host embryo migrate specifically to the vegetal plate to await the ingression and migration of the host cells (Ettensohn and McClay, 1986), again suggesting that the vegetal pole region contains specific information to which primary mesenchyme cells can respond.

In the case of secondary mesenchyme cells the evidence is less clear. Concanavalin A (con A), appears to bind preferentially to the basal lamina in the animal hemisphere at the gastrula stage in some species (Spiegel and Burger, 1982; Katow and Solursh, 1982; DeSimone and Spiegel, 1986). Furthermore, injection of con A into the blastocoel can interfere with filopodial attachment of SMCs, but not primary mesenchyme cells (Spiegel and Burger, 1982), suggesting that spatially restricted glycoproteins or proteoglycans may be involved in attachment of SMCs (discussed by Spiegel and Spiegel, 1986; Solursh, 1986). There has also been a report of an antigenic determinant restricted to the basal lamina in the vicinity of the animal pole (Wessell *et al.*, 1984). Such results suggest that there are regional differences

in the blastocoel that secondary mesenchyme cells could potentially recognize. The question remains, Are such differences indicative of *normal* targets for localization of secondary mesenchyme cells?

Secondary mesenchyme cells eventually undergo a *change in phenotype* at the end of gastrulation. Does this change reflect an intrinsically programmed behavior that occurs independently of their migratory environment, or perhaps merely close physical contact with the epithelium in the animal pole region (as suggested in passing by Gustafson, 1969), or is there *specific information* in the animal pole region that could provide a signal for this change? Clearly impressions gained from time-lapse footage, while valuable, cannot adjudicate between these possibilities.

In light of these questions, we have undertaken experimental manipulations and quantitative analyses of secondary mesenchyme cells to learn how their behavior might be coordinated with the autonomous morphogenesis that proceeds concurrently in the archenteron. We also wanted to examine experimentally the mechanisms that control the localization and ontogeny of SMCs. Our results confirm the supposition that secondary mesenchyme cells do respond to general features of the blastocoel wall. Furthermore, SMCs appear to respond to specific guidance cues within the animal pole region; contact with a well-defined zone within this region elicits a dramatic change in their phenotype. The result is cessation of filopodial activity and attachment of the tip of the archenteron to a predictable location near the animal pole. Thus this cell recognition event helps to end gastrulation and correctly positions the gut rudiment for subsequent morphogenesis.

MATERIALS AND METHODS

Procurement of embryos. Gametes of *Strongylocentrotus purpuratus* and *L. variegatus* were obtained by intracoelomic injection of 0.5 M KCl and fertilized with dilute sperm. Embryos were reared in stirring cultures in Millipore-filtered sea water (MFSW) or artificial sea water (ASW) at 16°C (*S. purpuratus*) or 19–22°C (*L. variegatus*).

Videomicroscopy. Time-lapse videomicroscopy of embryos was performed as described previously (Hardin and Cheng, 1986; Hardin, 1989). Because some visual resolution is lost in time-lapse footage, analysis of filopodial residence times was performed by marking the positions of individual filopodia on the video monitor, and following their histories in real time by continual through-focusing. Angular distributions of filopodia were obtained using the morphometrics procedures outlined below. Contingency table analysis of filopodial residence times was carried out as described by Zar (1984). Archenteron lengths were determined by measuring

embryos from the video monitor as described previously (Hardin, 1988).

Micromanipulation of embryos. Embryos were compressed along the animal-vegetal axis in one of two ways: (1) by placing a drop of embryo suspension on Nitex mesh of the appropriate mesh size, and then mounting the embryos in a standard chamber for viewing, or (2) by micromanipulation of individual embryos. For micromanipulation experiments, modified chambers were constructed by placing a 3- to 4-cm-long piece of double-stick Scotch brand adhesive tape (3M Co.) approximately 5 mm from one of the long edges of a poly-L-lysine-treated microscope slide, pipetting embryos onto the slide immediately adjacent to the tape, and then ringing the tape and the drop of embryo suspension with mineral oil. A 24 × 50-mm coverslip was then placed on the slide assemblage, resulting in a wedge-shaped opening. Additional support was given to the coverslip at the open end of the assembly by dabs of silicon grease placed on either side of the tape. This type of disposable chamber allows for insertion of large diameter pipetes for either denting or aspiration of embryos and permits sufficient gas exchange for embryos to develop normally for a period as long as 24 hr. Micropipets for compression, denting, and animal pole aspiration experiments were pulled from glass capillary tubing (o.d., 0.22 mm; i.d., 0.13 mm; Drummond Scientific Co., Broomall, PA) using either a vertical (David A. Knopf Co.) or horizontal (Narishige) pipete puller. Flat edges of ~10 μm (for compression and denting) or 40–50 μm (for aspirations) outer diameter were produced by breaking the tips of the pipetes against the edge of the microscope slide prior to their first use. Pipetes prepared in this way could be reused many times.

For elongating embryos along the animal-vegetal axis, larger diameter pipetes (70–80 μm) were produced by pulling glass capillary tubing by hand in an alcohol burner. Flat edges were produced by scribing the pulled region with a diamond pencil. Aspiration pipetes were connected to a Gilmont micrometer syringe filled with silicon oil (200, 50 dp, Dow Corning), and individual embryos were aspirated into the pipet to a depth of ~150–200 μm.

Fused multiple embryos. Fused multiple embryos were produced by placing demembrated 16-cell stage embryos in calcium-free artificial sea water (CFSW) for 10–15 min. to loosen the hyaline layer, returning them to normal sea water, and allowing them to settle as a thick monolayer in the bottom of a large finger bowl. At the gastrula stage the embryos were collected and inspected for fused embryos.

Scanning electron microscopy. *S. purpuratus* embryos were fixed in 1% glutaraldehyde in cacodylate-buffered sea water, postfixed in 1% OsO₄, and processed for

scanning electron microscopy as described previously (Hardin, 1989).

Morphometrics. Morphometrics were performed using a Summagraphics MacTablet connected to a Macintosh II computer and software written in this laboratory (see Hardin, 1989, for further details). Lengths of filopodia were measured in the midsagittal optical plane of section. Angular deflections of filopodia were measured over a range of 0–180° with respect to the animal-vegetal axis: 0° corresponds to a filopodium pointing straight up toward the animal pole; 180° corresponds to a filopodium pointing straight down toward the vegetal pole. Statistics were performed on the angular data according to Zar (1984) and Batschelet (1981). ANOVA and pairwise statistical comparisons of filopodial residence times were performed according to Zar (1984).

RESULTS

The Archenteron Attaches to a Specific Target Site near the Animal Pole

Is there a signal that is provided to the filopodia of secondary mesenchyme cells that leads to the attachment of the tip of the archenteron near the animal pole? One possibility is that the epithelium in this region (and/or its associated basal lamina) functionally differs from more lateral ectoderm, resulting in filopodial attachments of longer duration and greater stability. Detailed time-lapse analysis indicates that this is indeed the case. Figure 1 shows a gastrulating *L. variegatus* embryo during archenteron elongation. During most of gastrulation, filopodia undergo the classic cycles of extension-attachment-retraction described in other species (see Introduction). Figure 1B–1E shows a cell (designated cell 1) undergoing such cyclical behavior. Initially, the cell extends a protrusion that attaches to the lateral ectoderm in the region where the Nitex mesh filament holding the embryo has produced a slight indentation (Fig. 1B, arrowhead). Eventually this protrusion is withdrawn, and the same cell extends a new protrusion, again laterally (Fig. 1E). However, the behavior of cells that strike the ectoderm near the animal pole is quite different. At the same time that cells making lateral attachments undergo cyclical exploratory behavior, cells that strike the animal pole region undergo a change in phenotype. Upon closer examination it is clear that this phenotypic change can occur well before the entire archenteron reaches its final destination near the animal pole. For example, cell 2 initially makes contact with lateral ectoderm (slightly out of the plane of focus, Fig. 1B). This filopodium is withdrawn, and the same cell extends a second filopodium (Fig. 1C). However, this filopodium strikes the animal pole region and does not detach. Rather, it shortens in length while it maintains continual contact with this region. Similarly, cell 3,

which has just withdrawn a lateral protrusion (Fig. 1B), extends a new filopodium which makes contact with the ectoderm very near where cell 2 made its attachment. Cell 3 undergoes a similar response, first making an extremely stable contact with the apical plate region. Eventually, cell 3 detaches slightly from the tip of the archenteron, loses its filopodial phenotype, and remains in close contact with the apical plate region, prior to the arrival of the bulk of the archenteron at this site (Fig. 1F, large arrow). In both cases, these filopodia are similar in length to other, laterally directed filopodia, and the entire archenteron tip is separated from the animal pole region by an appreciable distance (~20 μm). Eventually the entire archenteron makes contact near the animal pole, and large numbers of secondary mesenchyme cells undergo the change in phenotype described for cells 2 and 3 (Fig. 1F). Significantly, in the 20 embryos examined, the boundary of the region in which this phenotypic change is observed is fairly sharp, i.e., the stable behavior does not appear to be a spatially graded phenomenon. The border region between filopodia making stable attachments and those undergoing cyclical behavior in time-lapse films appears to be ≤10 μm wide.

Quantitative analysis of filopodial behavior further demonstrates the striking difference between attachments made in the lateral and animal pole regions (Fig. 2). When the residence times of filopodia in lateral regions (>45° off the animal-vegetal axis) and in the apical plate region (<20° off axis) were analyzed, they were found to be different (significantly different on the basis of on contingency analysis: $\chi^2 = 57.1$, $P < 10^{-4}$). The residence times of filopodia making contacts with lateral ectoderm in the animal half of the embryo are essentially normally distributed, with a mean of 3.7 ± 0.3 min ($n = 38$ filopodia), whereas virtually all filopodia making contact with the apical plate region remain there for extremely long periods of time (>10 min up to hours; Fig. 2), often 20- to 50-fold longer than they do in lateral regions.

Contact with the Animal Pole Region Is Crucial for Changing Filopodial Behavior: Precocious Contact with the Animal Pole Results in a Premature Change in Phenotype

If the random exploratory behavior of the filopodia is programmed to continue until contact with the animal pole region is achieved, then several predictions following from this hypothesis can be tested. First, if filopodia are permitted to encounter the animal pole earlier than usual, then they should undergo a precocious change in phenotype. We have been able to produce such encounters by indenting the animal pole of a midgastrula with a micropipet so that the tip of the archenteron is

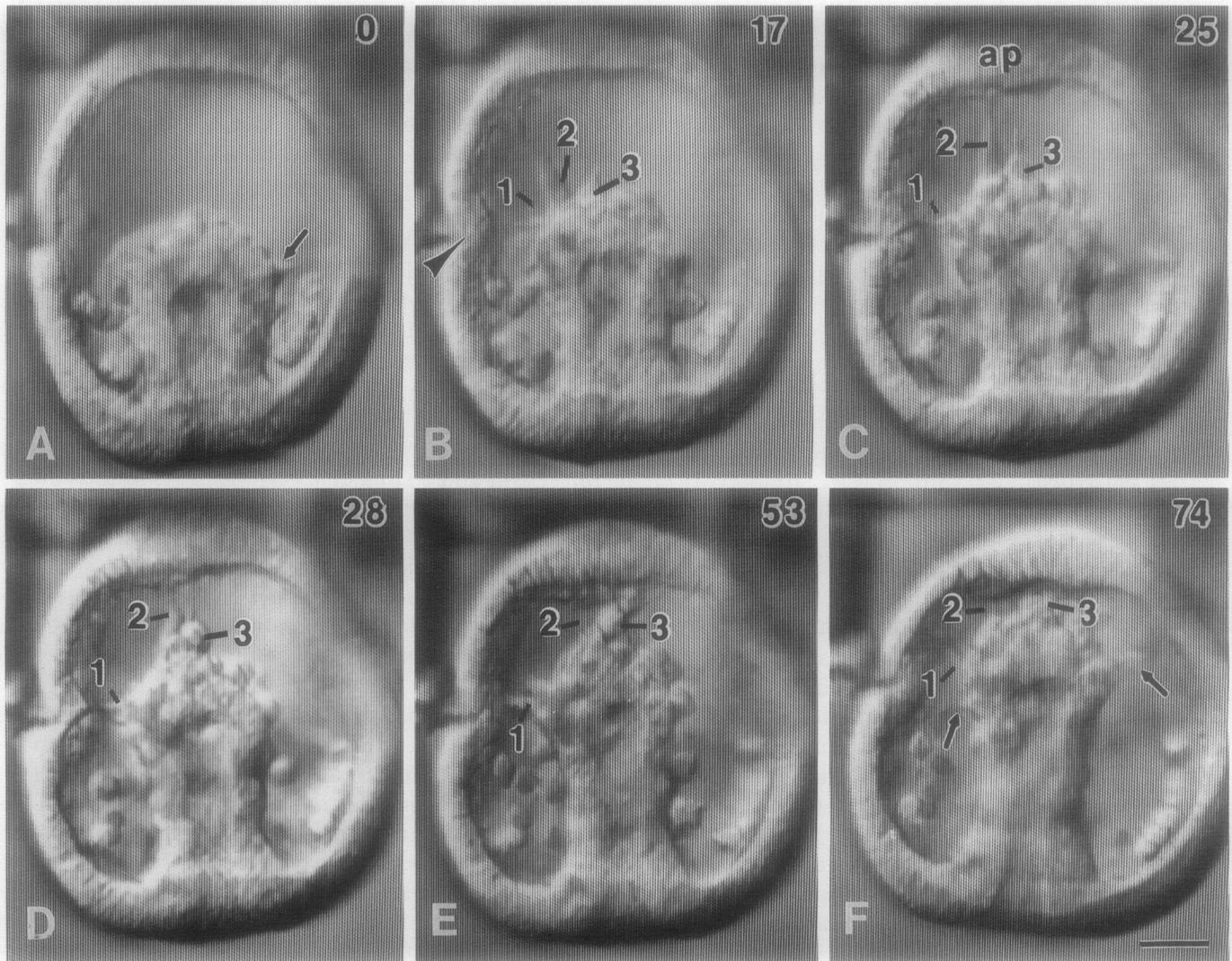


FIG. 1. Filopodial behavior during gastrulation in *Lytechinus variegatus*. (A-F) Successive time-lapse video frames of a single embryo. Times are indicated in minutes. (A) Onset of filopodial activity. A laterally directed filopodium is visible (small arrow). (B) Archenteron elongation has commenced. Cell 1 has made contact with an indentation produced by the Nitex mesh (arrowhead). Cell 2 has made a lateral attachment in the animal hemisphere. Cell 3 has just retracted a protrusion. (C) Cell 2 has produced a new protrusion in contact with the animal pole region (ap). Cell 3 has extended a new protrusion just making contact with the animal pole. (D, E). Cells 2 (slightly out of focus) and 3 maintain attachment; cell 1 detaches from the indentation. (F). The tip of the archenteron arrives at the animal pole. Cell 1 continues cyclical protrusive activity, as do other laterally directed filopodia (arrows). Bar = 25 μ m.

brought within 30 μ m of the apical plate region (Fig. 3A). When such indentations are produced, filopodia precociously attach to the dented ectoderm within 2-5 min (Fig. 3B), and undergo precociously the same change in phenotype seen in the normal embryo (Fig. 3C). In the six embryos examined, filopodia in contact with the apical plate region underwent identical behavior, furthermore, the phenotypic change was quantitatively similar as well (residence time greater than 30 min for all filopodia examined, $n = 7$ filopodia). In two cases examined, enough filopodia made contact with the animal pole such that when the pipet was removed the archenteron remained in permanent contact with the animal pole ectoderm (Fig. 3C). The precocious attach-

ment is apparently quite stable, since the embryos maintained an artificially flattened shape in order to preserve contact with the animal pole (Fig. 3C). Eventually, the archenteron underwent additional elongation, as the embryo assumed a spherical shape (Fig. 3D). In other cases, when fewer SMCs had made contact with the indentation, or when the archenteron was apparently too short to allow permanent attachment, the secondary mesenchyme cells remained attached to the animal pole region, and pulled out of the tip of the archenteron (data not shown).

Only those filopodia that make contact with the animal pole region exhibit the change in phenotype shown in Figs. 3A-3D. In all six cases in which an indentation

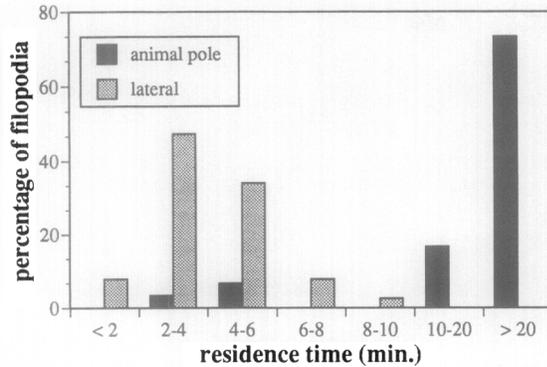


FIG. 2. The response of cells at the tip of the archenteron to the animal pole region. Histogram comparing percentages of filopodia of various lifetimes for laterally directed filopodia (angle of deflection $> 45^\circ$, $n = 38$; gray) and filopodia making contact with the animal pole region (angular orientation $< 20^\circ$, $n = 30$; black).

was produced with a micropipet in an area lateral to the animal pole, the filopodia made contact with the indentation, but continued past it until they reached the animal pole (Figs. 3E-3H), as they do when the indentation is produced by a filament of Nitex mesh (cf. Fig. 1). Ultimately the archenterons of embryos with lateral indentations localize to the animal pole (Figs. 3G, 3H). The residence times of filopodia making contact with lateral indentations are slightly greater than residence times for filopodia making contact with undeformed lateral ectoderm (7.1 ± 1.7 min, $n = 8$ filopodia; significantly different from lateral ectoderm in normal embryos at the 95% confidence level by Dunnett's two-tailed test; Zar, 1984). However, in all cases they are an order of magnitude less than filopodia making contact with the animal pole region.

Filopodia Localize to the Animal Pole Region when Given a Choice of Surfaces

In the experiment described above, lateral regions of ectoderm tend to bulge out even further laterally, making them less accessible to the filopodia. Do midgastrula stage filopodia preferentially localize to the animal pole region or do they prefer to attach to lateral ectoderm if given the choice? Nitex mesh was again used to alter the available attachment sites for filopodia, but this time with the intent of placing all ectodermal surfaces in the animal $\frac{1}{2}$ - $\frac{3}{4}$ of the embryo at roughly the same distance from the archenteron (Fig. 4). In all 20 embryos examined, although attachments were made to all regions, only those made in the animal pole region (the center of which is indicated by broad arrows in Fig. 4) were found to be highly stable; others were eventually eliminated as more and more filopodia attached to this region (Fig. 4B-4D). Although residence times for lateral filopodia were again slightly longer than in controls (significantly different at the 95% confidence level), the resi-

dence times for filopodia in lateral vs animal pole regions were different (lateral filopodia: 6.3 ± 1.4 min, $n = 8$; animal pole region: residence time > 30 min for all filopodia examined, $n = 8$). Thus when given a choice, filopodia of secondary mesenchyme cells preferentially attach to the animal pole region.

Delayed Contact with the Animal Pole Delays the Change in Phenotype

The above experiments show that early contact with the animal pole region induces stable attachment of filopodia and ultimately a phenotypic change in the secondary mesenchyme cells. However, it is possible that this response simply reflects a signal that SMCs *can* use to cease motility ahead of schedule, but that they *need not* use this signal under normal circumstances; i.e., they might autonomously undergo this phenotypic change according to some intrinsic program of differentiation. If contact provided a truly instructive signal to the filopodia, then delaying the interaction of the archenteron with the animal pole should likewise delay the change in filopodial behavior, at least well beyond the time during which it would normally occur. Delayed contact with the target can be achieved by placing an embryo at the $\frac{2}{3}$ - $\frac{3}{4}$ gastrula stage into narrow capillary tubing, thereby elongating the embryo and placing the tip of the archenteron out of reach of the animal pole (Fig. 5A). In this case SMCs do not exhibit their normal change in phenotype, but continue their cyclical explorations. If the embryo is left in the tube for 2-3 hr, eventually some SMCs leave the tip of the archenteron on their own and migrate within the blastocoel. Those that migrate to the vicinity of the animal pole undergo the characteristic change in phenotype, and a number of SMCs eventually occupy this region (Fig. 5A). All 10 embryos deprived of contact with the animal pole in this way did not complete archenteron elongation. Their archenterons stopped elongating at $\frac{2}{3}$ - $\frac{3}{4}$ of the normal final length if placed in the tube prior to the $\frac{2}{3}$ - $\frac{3}{4}$ stage, or stopped further elongation if placed into the tube after this time (Fig. 5A). These results are consistent with previous laser ablation studies (cf. Hardin, 1988).

If an elongated embryo is removed from the tube after 30-60 min., it gradually regains a normal shape (Figs. 5B-5E), and the tip of the archenteron eventually reaches the apical plate region, where contact is rapidly made (Fig. 5E). The archenterons of sibling embryos immediately adjacent to the capillary tubing complete attachment to the animal pole in 10-15 min, so attachment to the animal pole has been delayed significantly in these embryos. In contrast to embryos held in capillary tubing, the archenterons of released embryos showed further elongation (cf. Figs. 5D and 5E; archenteron length in 5D: $70 \mu\text{m}$; length in 5E: $100 \mu\text{m}$; also cf. Figs. 6A, 6D). In the three embryos examined in detail,

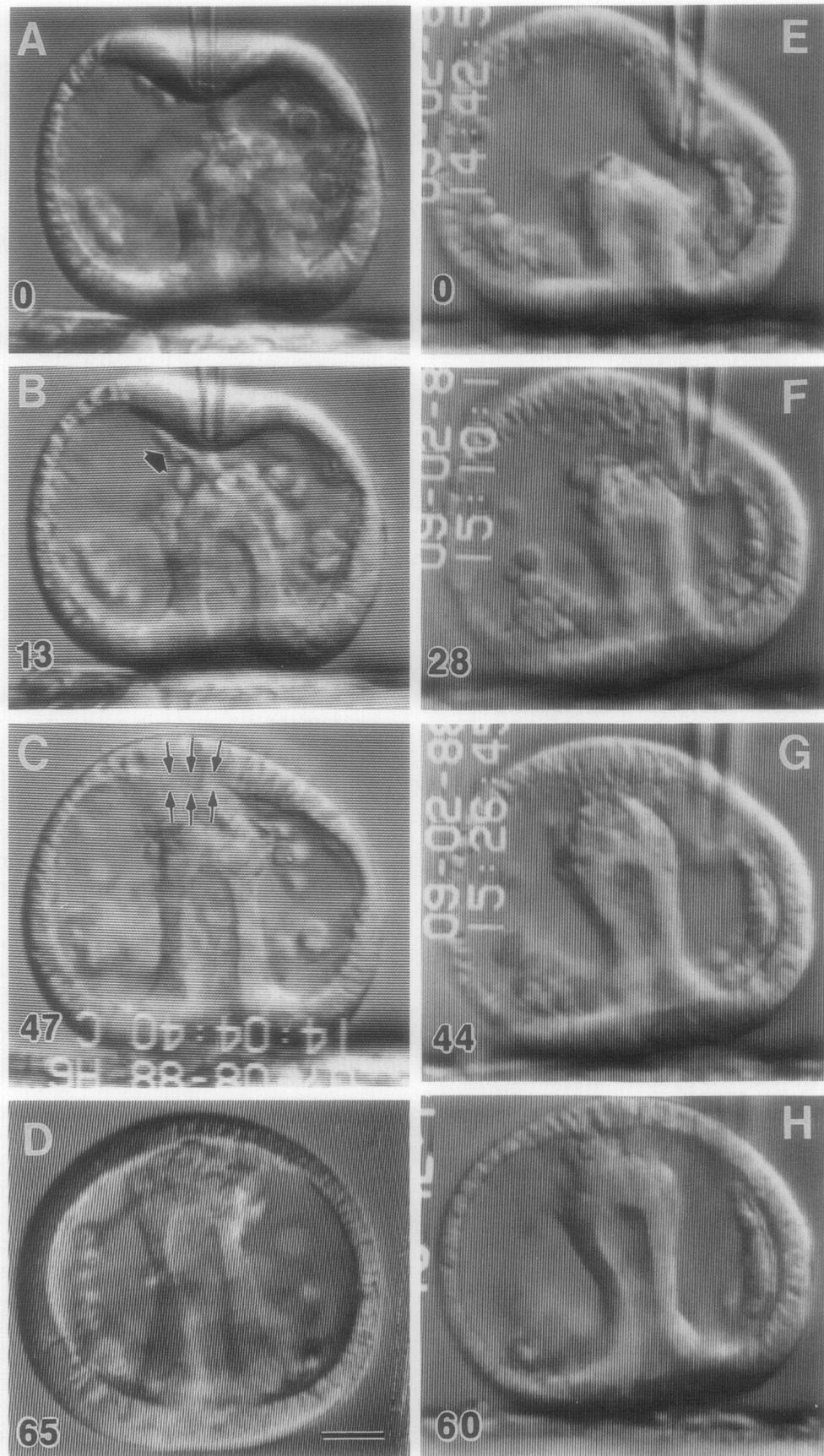


FIG. 3. Precocious attachment of the archenteron to the animal pole region. (A-D) *L. variegatus* midgastrula in which the animal pole has been indented toward the tip of the archenteron with a micropipet. Note the premature attachment of the archenteron to the animal pole (C, arrows) temporarily resulting in an abnormally flattened embryo. (D) The embryos has resumed a normal shape, and the archenteron has elongated somewhat further. (E-G) Midgastrula in which an indentation has been made in lateral ectoderm (E). The archenteron bypasses the indentation (F), eventually making contact with the animal pole (G, H). Bars = 25 μ m.

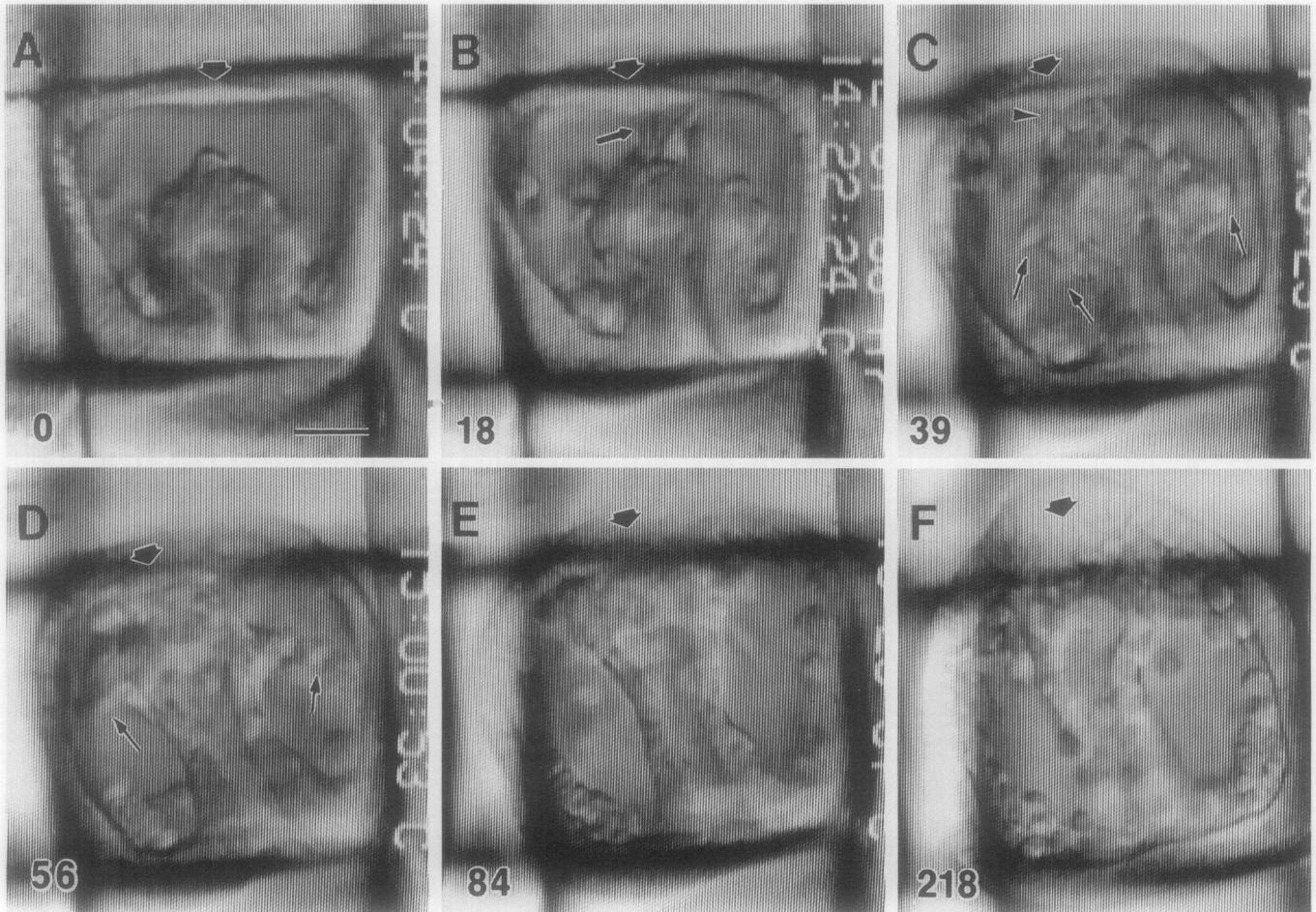


FIG. 4. Filopodia localize to the animal pole when provided with a choice of surfaces. Tracings of a *L. variegatus* embryo trapped in 100- μ m mesh Nitex cloth are shown over the course of gastrulation (~ 3 hr). Filopodia that make contact with the animal pole region remain attached and undergo a change in phenotype (B,C, arrowheads). In contrast, lateral attachments (small arrows in C) are ultimately withdrawn as more and more cells contact the animal pole region (cf. C, D, and E). The animal pole region is denoted by a wide arrow for reference; the embryo rotates slightly during filming. Bar = 25 μ m.

until contact with the animal pole was made filopodial residence times were similar to those made by laterally directed filopodia in the other situations already described (6.5 ± 1.2 min, $n = 8$). Those striking the animal pole region again had extremely long lifetimes (lifetimes all greater than 30 min, $n = 8$). If embryos were imprisoned in the tube after contact with the animal pole was made, it was quite difficult to dislodge the archenteron: the embryo had to be elongated almost to the breaking point, or extruded in and out of the tube vigorously. In contrast, if embryos were placed into the tube just as filopodia were making their first contacts with the apical plate region, it was relatively easy to dislodge the tip of the archenteron from the animal pole.

In the elongated and released embryos archenteron elongation and contact with the animal pole can occur by a different pathway than in normal *L. variegatus* embryos (Fig. 6): rather than remaining upright within the blastocoel, the archenteron can crawl up one side of

the blastocoel until it reaches the animal pole region (Figs. 6A–6C). This occurs because the archenteron is pushed off to one side if the embryo is drawn into the tube in an oblique orientation; after release, the opposite side of the embryo is simply too far away for contact to be made (confirmed by time-lapse observations of filopodia; Fig. 6A), and thus an alternate route of migration is used. Once contact with the animal pole region is made, attachment of the tip of the archenteron occurs quickly (within 10 min.), accompanied by the phenotypic changes associated with attachment in normal embryos (Fig. 6C–6E).

Archenteron Elongation and Filopodial Exploration Interact to Facilitate Contact with the Animal Pole: The Distribution of Filopodia Is Governed by the Shape of the Embryo

Secondary mesenchyme cells employ cycles of filopodial extension–attachment–retraction as their basic

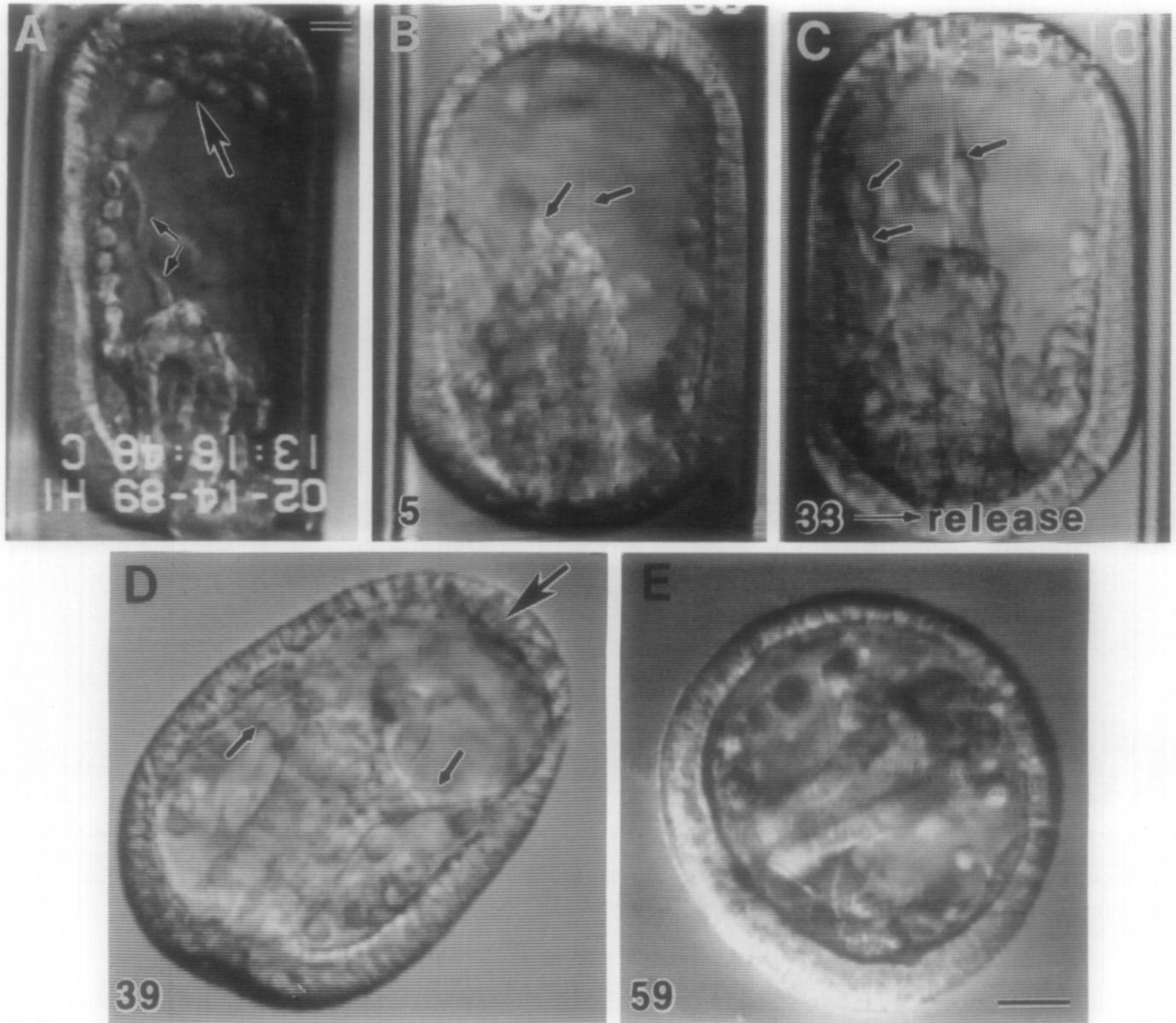


FIG. 5. Experimental delay of contact between the archenteron and the animal pole. (A) *L. variegatus* embryo trapped in glass capillary tubing, resulting in elongation of the embryo along the animal-vegetal axis. Secondary mesenchyme cells leave the tip of the archenteron to migrate within the blastocoel (small arrows). Those that reach the animal pole region stop moving (large arrow). (B-E) Elongation of a 2/3 gastrula followed by release. (B) Embryo after 5 min in the tubing. (C) Same embryo after 30 min. Many active filopodia are visible (B, C, arrows). Sibling embryos adjacent to the capillary tubing completed gastrulation by 10-15 min after observation commenced. (D) Same embryo 5 min after release from the tube. Filopodia attach to lateral surfaces (small arrows). The animal pole is denoted by a large arrow. (E) 25 min after release. The embryo has resumed a spherical shape, and attachment to the animal pole has been made. Bar = 25 μ m.

motile behavior, and the experiments described above indicate that this behavior can be changed by contact with the animal pole region. But how do filopodia normally reach this region, and how is this recognition event integrated with other aspects of archenteron morphogenesis? A systematic examination of the angular distribution of filopodia that *successfully* attach in *L. variegatus* confirms the supposition of Trinkaus (1965): filopodia do initially attach laterally (Fig. 7A). As the archenteron elongates, the filopodia progressively be-

come localized to the animal pole region (Fig. 7B). This change is well correlated with archenteron length ($r^2 = 0.63$, $n = 66$ filopodia, where r is the circular-linear correlation coefficient; significance of correlation: $P < 10^{-6}$).

What causes the gradual change in location of successful filopodial attachments? When filopodial length is plotted against archenteron length, it becomes clear that the average length of a filopodium changes little during most of gastrulation (Fig. 7C). At the end of gas-

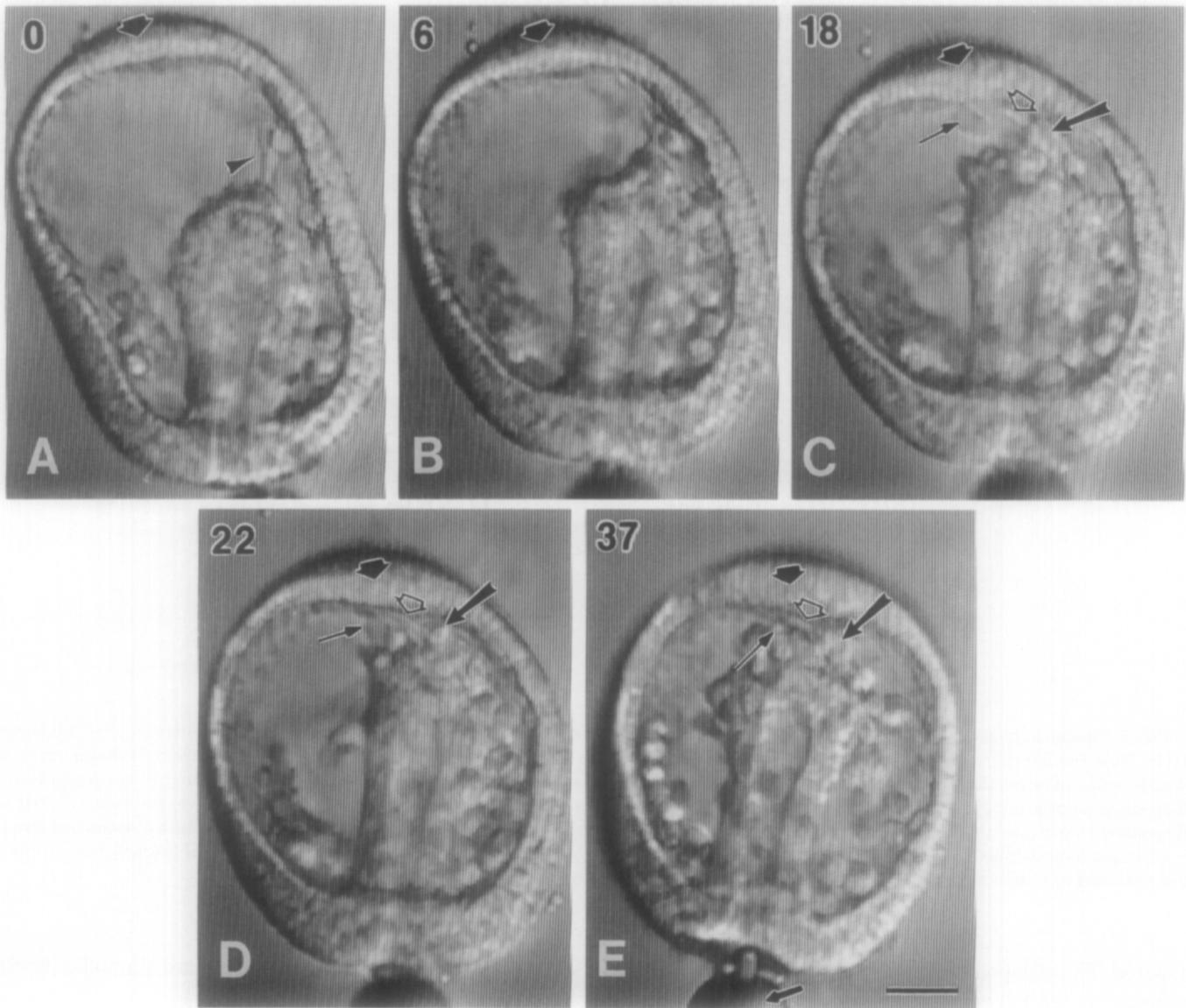


FIG. 6. Time-lapse frames of an embryo immediately after release from capillary tubing in which the archenteron has been pushed off to one side. Filopodia initially attach to the lateral blastocoel wall (arrows, A,B). Filopodia that attach to the animal pole maintain attachment (C-E; large arrow, cell body; open arrow: filopodium extended by the same cell). A cell arriving at the animal pole early undergoes the change in phenotype (C-E, small arrow). A drop of silicon oil adheres to the vegetal pole region (small bold arrow). Bars = 25 μm .

trulation filopodia become shorter, but only when the archenteron is in contact with the animal pole. Furthermore, these measurements reveal that the *maximum* length that filopodia attain is $\sim 35 \mu\text{m}$ (Fig. 7C). The implications of this maximum length become clear when the shape of the embryo is taken into consideration (Fig. 7A): early in gastrulation the animal pole appears to be out of reach of the filopodia, and the only accessible surfaces are in the lateral ectoderm. If this simple length limit can account for the initially lateral attachments of the filopodia, then one would expect that “*metastable*” filopodia (i.e., those that extend and successfully attach) would only be found laterally, but that

unstable filopodia (i.e., those that form but cannot find a suitable surface for attachment) would attempt to extend in all directions, including upward. A real-time analysis of the dynamic “life-histories” of individual filopodia reveals that this is precisely what happens. At the onset of archenteron elongation, filopodia are sent out in a cyclical fashion. Only those filopodia that reach a substrate can make metastable (lifetime 2–8 min) attachments (mean angle $71.6 \pm 9.5^\circ$, $n = 25$ filopodia; Fig. 7D). Those filopodia that extend upward early in gastrulation are unstable (lifetime < 90 sec; mean angle $22.7 \pm 9.0^\circ$, 95% confidence limits, $n = 20$; Fig. 7D); they cannot reach a suitable surface before they are re-

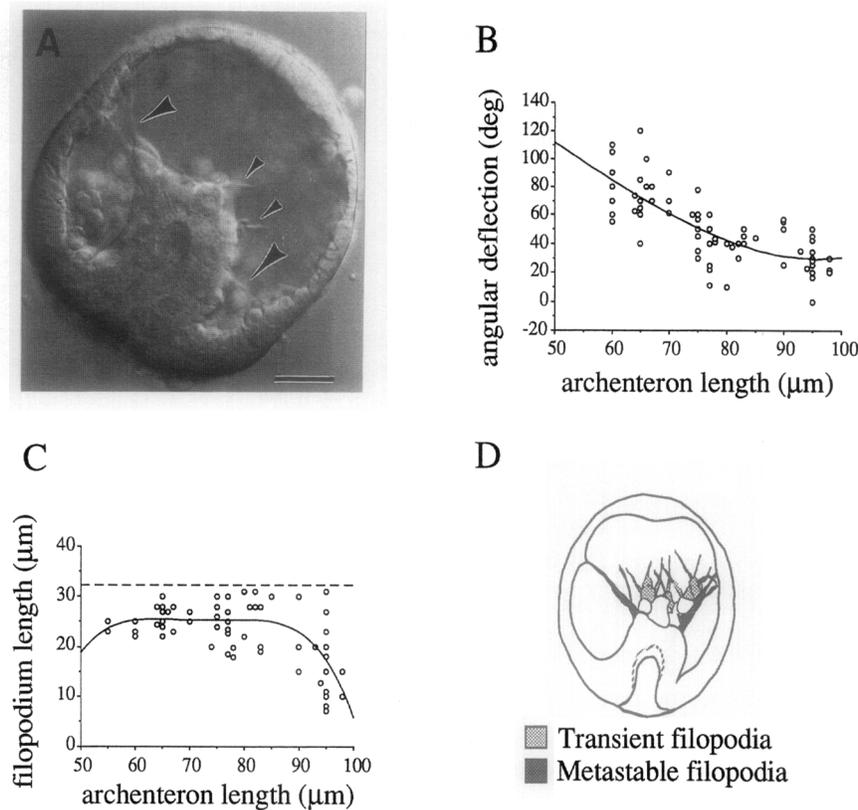


FIG. 7. Change in filopodial orientation during gastrulation in *L. variegatus*. (A) Differential interference contrast micrograph of a midgastrula. Note the lateral orientation of attached filopodia (large arrows). More animally directed filopodia in the process of collapse are also visible (small arrows). (B) Relation between archenteron length (μm) and filopodial orientation (deg) during gastrulation. 0° corresponds to a filopodium pointing straight up toward the animal pole; 180° corresponds to one pointing straight down toward the vegetal pole. (C) Plot of filopodium length (μm) vs archenteron length (μm) for various stages of gastrulation in *L. variegatus*. The maximum length filopodia achieve is $\sim 32\text{--}34 \mu\text{m}$ (dotted line). (D) Tracings of individual filopodia extended by a midgastrula over a 10-min period. Metastable (dark gray, lifetime 2–8 min) and unstable (light gray, lifetime ≤ 90 sec) filopodia are indicated.

tracted. Thus filopodial attachments are consistently restricted to lateral regions early in gastrulation (distributions significantly different, $P < 10^{-6}$).

If the only successful attachments that filopodia initially make are in the lateral ectoderm because the animal pole is out of reach, then one would also predict that altering the shape of the embryo should cause a change in the distribution of metastable filopodia. This can be accomplished reproducibly by placing embryos in Nitex cloth of varying mesh sizes (Fig. 8A). When embryos are placed in $100 \mu\text{m}$ mesh cloth, so that the animal pole is $\leq 35 \mu\text{m}$ from the tip of the archenteron, the distribution of attached filopodia is dramatically affected (Fig. 8B): many filopodia attach to the animal pole well before they would in normal embryos (mean angle of deflection from the animal-vegetal axis: $23.2 \pm 5.5^\circ$, 95% confidence limits, $n = 52$ filopodia). In contrast, when embryos are placed in $125\text{-}\mu\text{m}$ mesh cloth, so that the animal pole is just outside the $35\text{-}\mu\text{m}$ limit, filopodia only attach laterally, in a manner indistinguishable from controls (mean angle of deflection $59.9 \pm 5.5^\circ$, $n = 42$, Fig. 8B). By the $\frac{2}{3}$ – $\frac{3}{4}$ gastrula stage the animal pole be-

comes accessible to the filopodia, and filopodia begin making attachments to this region.

Ectopic Juxtaposition of Archenterons and Animal Pole Ectoderm Results in Anomalous Guidance of the Archenteron

If the interaction of the archenteron with the animal pole is governed by proximity of the tip of the archenteron to this region, then ectopic juxtaposition of animal pole ectoderm and archenterons would be expected to result in anomalous attachment of archenterons when the juxtaposed tissues are sufficiently close to allow interaction. This sort of juxtaposition can be accomplished by constructing multiple embryos with confluent blastocoels that contain multiple archenterons and/or apical plates. In the 10 such embryos examined, archenterons attached without fail to a nearby apical plate region, if one was within $35 \mu\text{m}$, even if this meant wholesale bending of the archenteron (Figs. 9A, 9B). In these embryos SMCs undergo a change in phenotype if

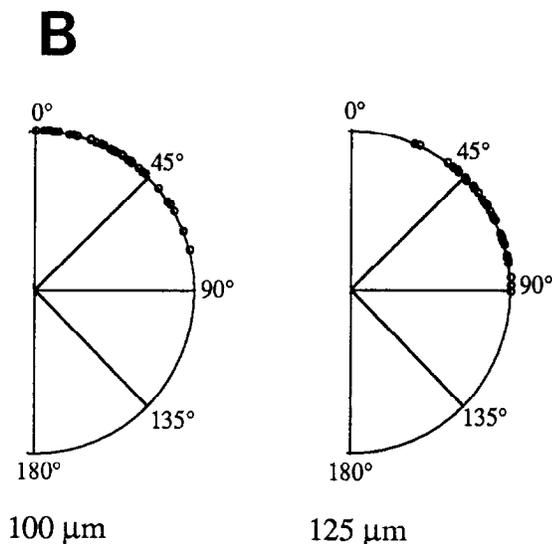
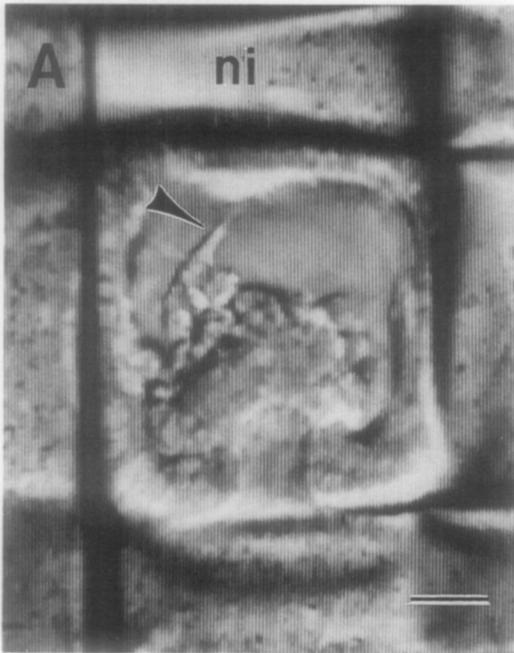


FIG. 8. The effects of altering embryonic shape on the location of filopodial attachments. (A) *L. variegatus* early gastrula compressed in 100- μm mesh Nitex cloth (ni). Within the time required to prepare the slide mount (1-2 min), filopodia make attachments to the animal pole, which is $\leq 35 \mu\text{m}$ from the tip of the archenteron (arrow). Bar = 25 μm . (B) Angular distribution of attached filopodia for embryos placed in 100- μm mesh cloth, in which the animal pole is $\leq 30 \mu\text{m}$ from the tip of the archenteron, and 125 μm mesh cloth, in which the animal pole is 40-45 μm away. The distributions are quantifiably different ($P < 10^{-4}$).

they are in contact with a region marked by apical plate ectoderm (Fig. 9B, arrows).

Phylogenetic Differences during Gastrulation Reflect Differences in Embryonic Shape

In contrast to the genus *Lytechinus*, in which the archenteron is placed centrally within the blastocoel, the

archenteron of *S. purpuratus* shows a distinct bias for migration along the ventral side of the embryo (Figs. 10A, 10B). Scanning electron microscopy (Fig. 10B) and time-lapse studies (data not shown) reveal that filopodial contacts are made with all regions of the ectoderm that are within $\sim 35 \mu\text{m}$ of the archenteron, but due to the eccentric placement of the archenteron within the blastocoel, it is only the ventral side that is within this distance. Furthermore, the apical plate is often located asymmetrically atop the flattened ventral side of the embryo, and invariably the tip of the archenteron makes its way to this region by moving along the ventral ectoderm. Thus although the motile processes employed in *S. purpuratus* appear to be the same as in *Lytechinus*, the route taken by the archenteron is different, apparently due to embryonic shape.

DISCUSSION

As pointed out by Trinkaus (1984a), four basic questions can be posed concerning cell movement during

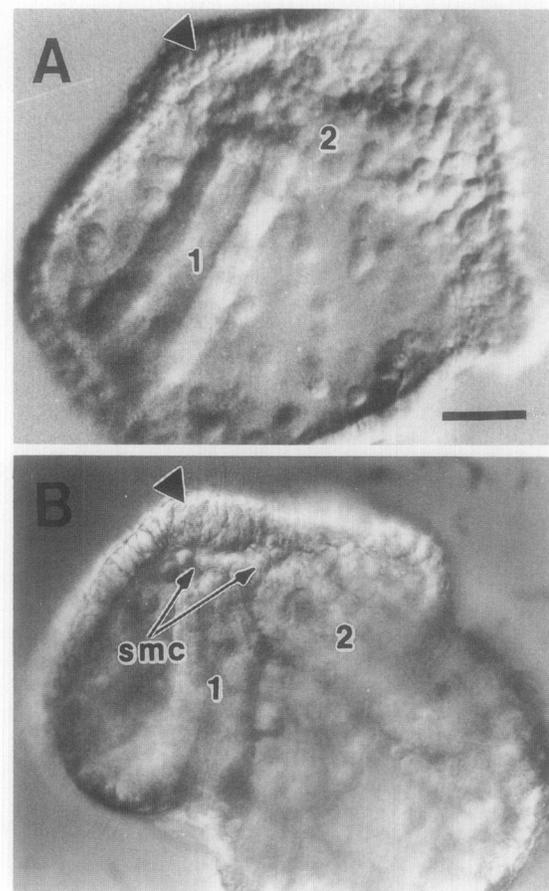


FIG. 9. Ectopic juxtapositions of archenterons with animal pole ectoderm. (A) Fused double embryo with a single thickened apical plate region (large arrow) and two archenterons (small arrows). (B) A second fused embryo containing two archenterons and a single apical plate region (large arrow). Secondary mesenchyme cells (smc) attach to the apical plate region and cease filopodial exploration (small arrows). Bar = 25 μm .

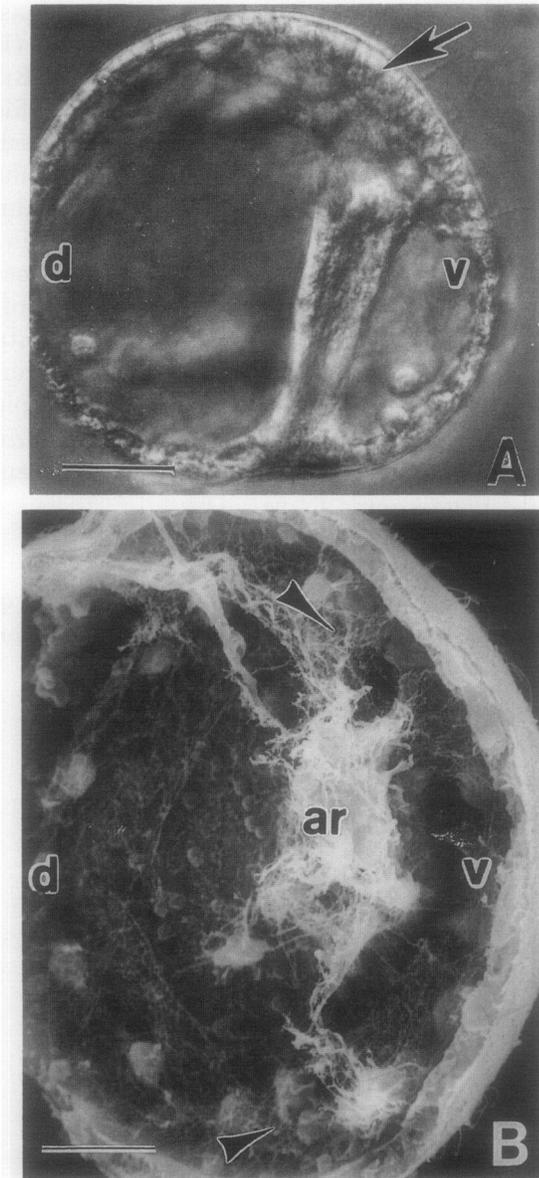


FIG. 10. Completion of gastrulation in *Strongylocentrotus purpuratus*. (A) Differential interference contrast micrograph of a *S. purpuratus* late gastrula showing the distinct ectodermal flattening on the ventral side and the eccentric position of the apical plate closer to the ventral side (arrow). d, dorsal; v, ventral. Bar = 25 μm . (B) Scanning electron micrograph of a *S. purpuratus* late gastrula viewed from the animal pole. Filopodia are restricted to the flattened ventral side of the embryo (v). The arrows indicate the positions of the ventrolateral clusters of primary mesenchyme cells. Bar = 10 μm .

morphogenesis: What makes cells start moving? What keeps them going? What gives them directionality? And what makes them stop? The mechanisms that lead to the onset of filopodial motility at the tip of the archenteron in the sea urchin embryo are unknown, although both intrinsic and environmental cues are probably involved (McClay *et al.*, 1990). The experiments presented here indicate that once it begins, the exploratory behavior of filopodia continues as they extend and attempt to

attach to any available surface. As it eventually comes within reach of the animal pole, the archenteron is guided by preferential attachments of filopodia to a locus within this region. Contact with this locus is followed by a change in the phenotype of secondary mesenchyme cells and firm anchoring of the tip of the archenteron.

Target Recognition Helps to Complete Gastrulation

The animal pole region of the embryo serves to focus filopodia to a specific anatomical site, and appears to provide an epigenetic signal for cessation of filopodial motility. Such recognition thus provides a simple mechanism for stopping a morphogenetic event only after its successful completion. Since filopodia continue to explore until they reach the correct site, such a mechanism provides temporal flexibility to the process of gastrulation. Recognition of the appropriate target also positions the tip of the archenteron at the correct location for its eventual fusion with the oral ectoderm. The embryo changes shape after gastrulation, and in the process the apical plate region and firmly adherent archenteron are shifted toward the ventral (oral) side of the embryo (Gustafson and Kinnander, 1960; Dan and Inaba, 1968). The result is that the tip of the archenteron is brought into close proximity with the eventual site of the stomodeal invagination.

Archenteron Extension and Filopodial Attachment Are "Robust" Processes

The processes of random filopodial exploration and target recognition we have characterized are a good example of how simple rules of cell behavior and epigenetic interaction can lead to impressive developmental flexibility. Archenteron elongation can be successfully completed despite considerable variations (both naturally occurring and experimentally induced) in embryonic shape, location of attachment sites of filopodia, and the timing and location of filopodial contact with the animal pole. Gustafson suggested that embryonic shape might play a role in restricting the locations of attachments to the dorsal ectoderm of *P. miliaris*, since the dorsal ectoderm is flatter than the ventral ectoderm in this species during gastrulation (e.g., Gustafson, 1964). A more extensive survey of a number of sea urchin species, including *P. miliaris*, reveals several distinct modes of archenteron elongation, all apparently accounted for by simple considerations of embryonic shape in conjunction with varying combinations of the processes of autonomous extension of the archenteron, random exploration by filopodia, and target recognition (Fig. 11). These include "central elongators" (e.g., *L. variegatus*, *L. pictus*), "dorsal crawlers," in which the dorsal side is in close proximity to the archenteron (e.g., *Echinus mi-*

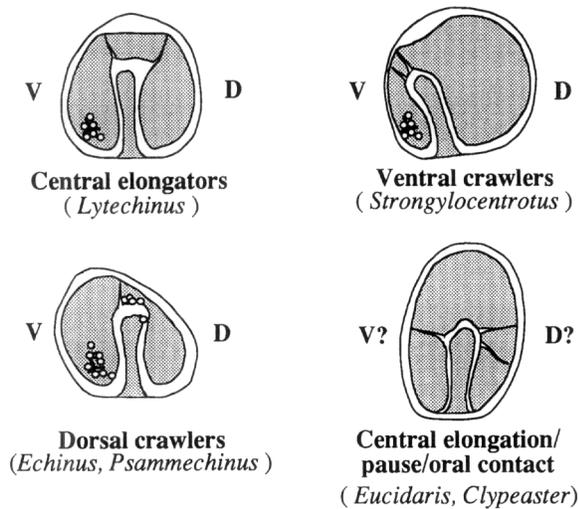


FIG. 11. A survey of species in a number of genera reveals several common ways in which the archenteron elongates. In some embryos the ventral side (V) is closer to the tip of the archenteron (ventral crawlers), in others the dorsal side (D) is closer (dorsal crawlers), and in still others the archenteron is equidistant from all ectodermal surfaces (central elongators). In embryos such as *Eucidaris* and *Clypeaster*, the archenteron never reaches the animal pole; instead the archenteron stops, pauses, and eventually bends to one side as it makes oral contact (central elongation/pause/oral contact). Sources: *Echinus*, Schmidt (1904); *Psammechinus*, Gustafson and Wolpert (1963); *Clypeaster*, Okazaki (1975); *Eucidaris*, Schroeder (1981); Hardin (1989).

crotuberculatus, Schmidt, 1904; *P. miliaris*, Gustafson and Wolpert, 1967), and “ventral crawlers,” in which the ventral side is considerably closer (e.g., *S. purpuratus*; see Results). In other species, such as the cidaroid, *Eucidaris tribuloides*, and the Japanese sand dollar, *Clypeaster japonicus*, filopodia never reach the animal pole (Okazaki, 1975; Hardin, 1989). In these cases the animal pole apparently remains out of reach. In *Eucidaris*, when the animal pole is dented down filopodia make metastable contacts with the animal pole within 2–5 min, but never any stable contacts of the kind seen in *L. variegatus* (J. Hardin, unpublished observations). In these species filopodia may extend laterally toward the stomodeal invagination directly, in a manner similar to mouth formation in asteroids (Schroeder, 1981; cf. Crawford and Abed, 1983; hence “central elongation/pause/oral contact”). That autonomous extension of the archenteron, random filopodial exploration, and target recognition can account for these different modes of gastrulation is reinforced by the results of the imprisonment of *L. variegatus* gastrulae presented here. Although they typically undergo central elongation, *L. variegatus* embryos can be induced to display either dorsal or ventral crawling behavior by altering their shape. Nevertheless, they complete gastrulation successfully (see Results).

What Is the Nature of the Target?

In many situations protrusively active cells must respond to specific guidance information if they are to find the correct targets during morphogenesis. But what does this guidance information consist of in the case of secondary mesenchyme cells? Several general conclusions can be made regarding the nature of the target we have identified. First, as Gustafson hypothesized and we have experimentally demonstrated, physical cues are important nonspecific modulators of filopodial activity. In particular, it is clear from our results that simple proximity of tissues is an important constraint on possible substrates for filopodial attachment. In addition, in those cases where we have artificially caused the curvature of a tissue to be less concave than normal (e.g., by indentation, embedment in Nitex mesh, or elongation in capillary tubing), filopodial attachment can be enhanced, in an apparently nonspecific way. It is possible that this is due to alterations of the shape and physical arrangement of the basal surfaces of epithelial cells (“opening up” of “contact points,” to use Gustafson’s terminology; Gustafson, 1964). Gustafson was not able to assess the relative importance of these physical cues on the basis of observation alone. However, we have been able to do so on the basis of our experiments, and it is clear that physical effects do not provide a sufficient explanation for the successful localization of the archenteron to the animal pole, since only a very specific region of the ectoderm is capable of supporting filopodial attachments with long-term stability in a variety of experimental situations.

Second, the information appears to be mainly contact-mediated, rather than a long-range signal. This conclusion is based on the lack of bias in orientation of filopodia toward the animal pole, even in the case of compressed embryos in which any possible long-range diffusible signals would be expected to be more intense. Furthermore, if the recognition signal were diffusible, then it must operate at very short range, since the boundary between stable filopodial attachments and merely metastable ones is quite sharp in both normal and compressed embryos. However, it should be pointed out that it is notoriously difficult to distinguish between very short-range diffusible signals and substrate-bound or cell-contact-mediated cues (Trinkaus, 1984a). Nevertheless, in light of the evidence, we favor the hypothesis that the target represents either a specialization of the cell surfaces in the animal pole region, or the associated extracellular matrix.

Although target recognition is contact mediated, it does not seem to represent a simple case of contact inhibition or increasing stability of attachment with decreasing distance from a nonspecific substrate. This conclusion is based on several lines of evidence: (1) in

unperturbed embryos as well as in indentation experiments, there are many instances in which filopodia at the tip of the archenteron are the same distance away from the animal pole as filopodia that make contact with lateral ectoderm, yet only those making contact with the animal pole region are highly stable; (2) while direct contact with an experimentally produced indentation in lateral ectoderm results in temporarily enhanced attachment, the archenteron invariably bypasses the indentation in favor of the animal pole; (3) in normal *S. purpuratus* and experimentally manipulated *L. variegatus* embryos the archenteron is very close to the blastocoel wall, yet the archenteron crawls upward to make contact with the animal pole, and (4) free SMCs are closely associated with their substrate, yet they can easily migrate along lateral ectoderm, and it is only upon contact with the animal pole region that they undergo a change in phenotype.

The third conclusion that can be drawn regarding the target region is that target recognition by SMCs seems to involve *simple trapping*, or as Weiss termed it, "selective fixation" (Weiss, 1947). Successful attachment appears to rely on chance encounters of filopodia with the target region. In this regard SMCs may be similar to primary mesenchyme cells, which may employ a related process (Gustafson and Wolpert, 1967; Solursh, 1986). In the normal embryo, the probability of a filopodium/target encounter is enhanced by the proximity of the animal pole to the tip of the archenteron at the $\frac{2}{3}$ - $\frac{3}{4}$ gastrula stage. In addition, once several filopodia have made contact with the animal pole the process of attachment becomes self-reinforcing. As stable filopodia remain attached they pull the archenteron closer toward the animal pole, thereby giving more filopodia the chance to break metastable contacts with lateral ectoderm and to make stable contacts with the animal pole region. Such trapping is particularly apparent in embryos embedded in Nitex mesh: gradually the frequency of metastable attachments decreases, as more and more filopodia are trapped by their stable attachment to the apical plate region. On the basis of our results we cannot, of course, exhaustively rule out the possibility that secondary mesenchyme cells can use long-range sensing mechanisms. For example, primary mesenchyme cells appear to display long-range, directed migration in experimentally perturbed embryos, even though they are not ordinarily presumed to do so (Gustafson and Wolpert, 1961; Okazaki *et al.*, 1962; Ettensohn and McClay, 1986). However, we have no evidence to support long-range directed migration of secondary mesenchyme cells. SMCs that detach from the tip of the archenteron and migrate within the blastocoel show persistent, but not directed, migration both *in vivo* and *in vitro* (J. Hardin, unpublished observations). Given their persistence of motility prior

to reaching the animal pole, freely migrating SMCs *need not* use such long-range cues under ordinary circumstances.

Fourth, attachment to the animal pole region appears to involve *increased adhesion*. When filopodia contact this region, they make very stable attachments to it, they spread onto it, and they maintain consistent contact with it throughout subsequent postgastrula morphogenesis. On the basis of this bioassay we cannot distinguish between simple adhesive events and other contact-mediated changes in cell behavior that might occur (e.g., see Lotz *et al.*, 1989). However, the behavior of secondary mesenchyme cells in this case is quite similar to that of many cell types that attach and then spread on a substrate (see Trinkaus, 1984a, for examples). Experimentally, it is much more difficult to dislodge the tip of the archenteron from the animal pole *after* contact has been established for a short time than at the time the first contacts with the animal pole are being made. This is further evidence that adhesive strengthening occurs as cells contact this region.

Finally, target recognition by SMCs is a *specific cell interaction*, rather than a general adhesive event. This conclusion is based on the lack of attachment of several other mesenchymal cell types to this region, despite adequate opportunities for contact. These include pigment cell progenitors (Gibson and Burke, 1985; Ettensohn and McClay, 1988) and ectopic primary mesenchyme cells (Okazaki *et al.*, 1962; Ettensohn and McClay, 1986). Although these facts do not allow the conclusion to be drawn that a specific receptor-ligand interaction mediates target recognition, at the very least the signal provided by the animal pole must be such that only secondary mesenchyme cells are competent to respond.

At present, little is known about the molecular basis of target recognition by secondary mesenchyme cells. In another well-studied case of filopodial guidance, there is abundant evidence to indicate that growth cones follow a number of cues during nerve outgrowth. These include nonspecific neuronal "highways" (e.g., see reviews by McClay and Ettensohn, 1987; Landmesser, 1988; Harrelson and Goodman, 1988; Dodd and Jessel, 1988), general negative cues (Dodd and Jessel, 1988), local positional cues within the migratory environment (Dodd and Jessel, 1988; Harrelson and Goodman, 1988; Harris, 1989), and chemotropic signals (Dodd and Jessel, 1988; Heffner *et al.*, 1990). Some of these cues might be involved in guiding secondary mesenchyme cells. In the case of sea urchin primary mesenchyme cells, there is a good correlation between the location of the mesenchymal ring and a belt of fan-shaped epithelial cells in the ectoderm under normal and experimental conditions (Okazaki *et al.*, 1962; reviewed by Solursh, 1986). However, it is not

known what molecular specialization might give rise to this anatomical association between ectoderm and mesenchyme (Solursh, 1986).

There is, at present, little clear evidence for differences in cell surface or extracellular matrix determinants specific to the apical plate region. Concanavalin A localizes to the animal hemisphere at the gastrula stage in *L. pictus*, but the size of the region recognized by the lectin relative to that of the target region is unclear (Spiegel and Burger, 1982; Katow and Solursh, 1982; DeSimone and Spiegel, 1986). There has been a report of an antigenic determinant that localizes to the animal pole region in *L. variegatus* (Wessell *et al.*, 1984), but the functional significance of this determinant is unknown. It is reasonable to suppose, however, that spatially localized molecules exist that are important for target recognition by SMCs. Thus a better notion of the molecular landscape through which these cells pass will make it possible to relate molecular events to this dramatic cell interaction that marks the end of gastrulation in the sea urchin.

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