3. The Sea Urchin

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Introduction

The sea urchin embryo has been used for more than a century to study many problems central to developmental biology. During the latter part of the nineteenth century, marine stations in Italy, France, and the United States flourished, and the embryos of marine organisms were found to be favourable material for investigating early embryonic development. The study of echinoderms and, in particular, of sea urchins, that was carried out at these marine stations was influential in the formation of many seminal ideas in developmental biology (for reviews, see the classic texts of Wilson, 1925; Morgan, 1927). Later, in the early part of the twentieth century, the experiments performed on sea urchin embryos using chemical agents and the classic blastomere recombination experiments performed by Hörstadius (1939; 1973) paved the way for ideas about graded distributions of morphogenetic substances in the embryo. The sea urchin also provided useful material for studying many aspects of nucleic acid structure, complexity, and function in the early days of molecular biology (reviewed by Davidson, 1988). The reader interested in the historical role played by sea urchin embryos in the emergence of developmental biology, and the importance and relevance of such experiments today is referred to Wilt (1987), Davidson (1989; 1990) and Livingston and Wilt (1990).

More recently, the sea urchin embryo has been used as a convenient system for studying morphogenetic movements and cell interactions during gastrulation, the changes in gene expression associated with the establishment of tissue territories along the embryonic axes, and phylogenetic variability and associated modifications in early development. In summarizing such work, this chapter provides a brief overview of normal development in the sea urchin embryo and a few case studies illustrating modern uses of this system for studying early developmental events. Where possible, the reader is referred to reviews that treat individual topics in more detail than is possible here.

For methods of maintaining adults, obtaining gametes, and culturing embryos, see Hinegardner (1967; 1975a) and Leahy (1986). General methods for culturing and experimentally manipulating embryos can be found in Harvey (1956) and Hörstadius (1973), while more up-to-date methods are in Schroeder (1986).

Normal development

Sperm, eggs, and fertilization

Sea urchin and sand dollar gametes can be obtained in large numbers by intracoelomic injection of 0.5M KCl or by electrical stimulation; this leads to the shedding of gametes into sea water (in the case of eggs) or 'dry' into a dish (in the case of sperm). Depending on the species, several millilitres of ripe eggs or sperm can be obtained from a single animal and the embryos can be conveniently reared in finger bowls or in stirring cultures. The major stages of early development in the sea urchin are shown in **3.1–3.15**, and each stage will be discussed in turn in the following sections. Mature sea urchin eggs, unlike eggs from many other animals, have completed meiosis and the extrusion of polar bodies in the ovary to produce a haploid gamete (**3.1**). Immediately apposed to the egg plasma membrane is the *vitelline envelope* which contains the glycoproteins essential for species-specific fusion of sperm and egg, while freshly shed eggs are surrounded by a *jelly coat*. Marking the jelly coat with small ink particles reveals the jelly canal, a marker for the animal pole of the egg first described by Boveri (1901) and more recently re-investigated by Schroeder (1980b; see also Maruyama *et al.*, 1985). Another marker of polarity in the unfertilized egg is the subequatorial concentration of orange pigment in some batches of eggs of the Mediterranean sea urchin, *Paracentrotus lividus*, especially those obtained from Villefranche in France (Boveri, 1901; Hörstadius, 1973; Schroeder, 1980a).

Unfertilized eggs possess several other kinds of distinct granules with different distributions within the egg. *Cortical granules* lie immediately beneath the egg surface and are released upon fertilization. These are lamellar structures which contain components necessary for the construction of the *fertilization envelope* (**3.2**) and the *hyaline layer*, an extracellular matrix layer which lies on the outside of the embryo. *Pigment granules* are particularly prominent in species such as *Arbacia punctulata* from America. Other granules, which release their contents following fertilization, but on a much slower time course than the cortical granules, contain extracellular matrix proteins. Some of these granules can be redistributed by centrifuging eggs suspended in sucrose

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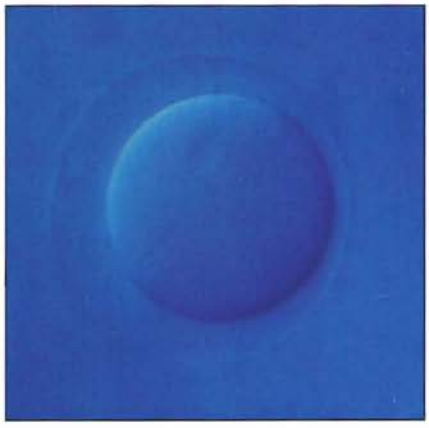

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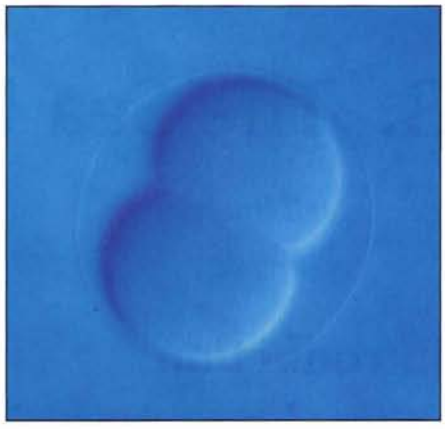

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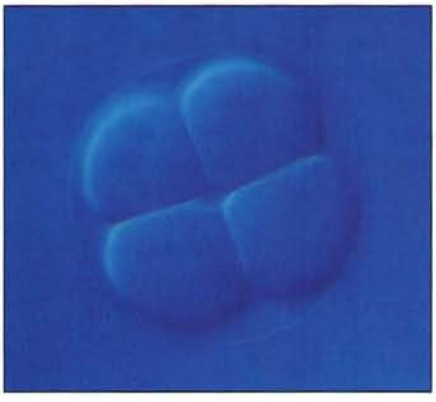

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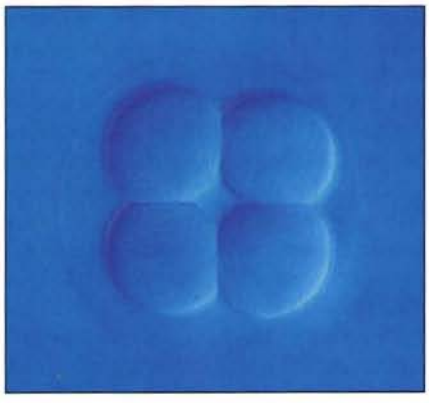

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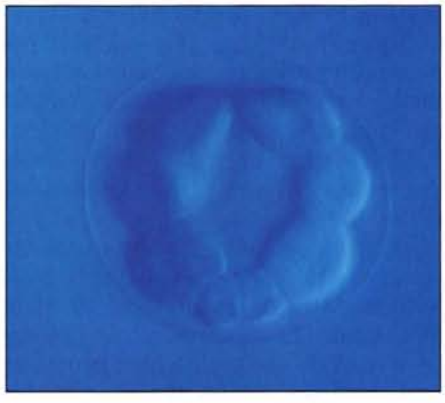

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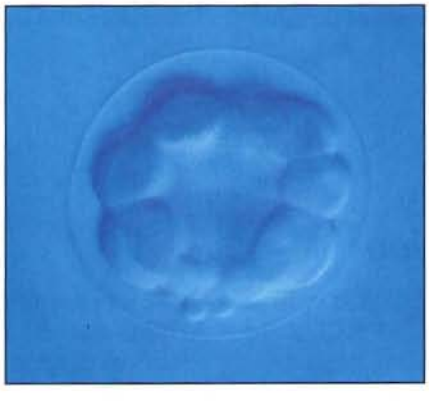

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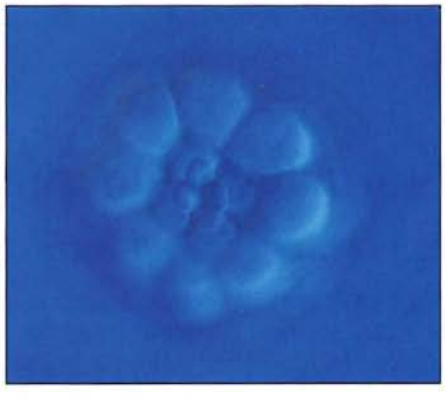

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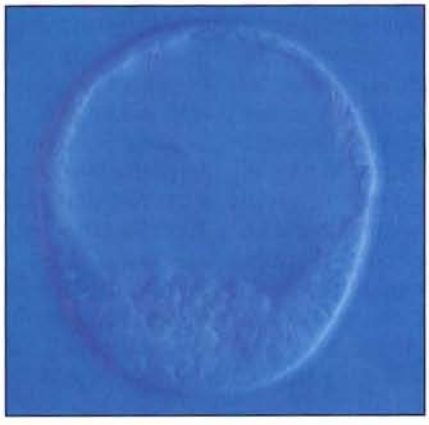

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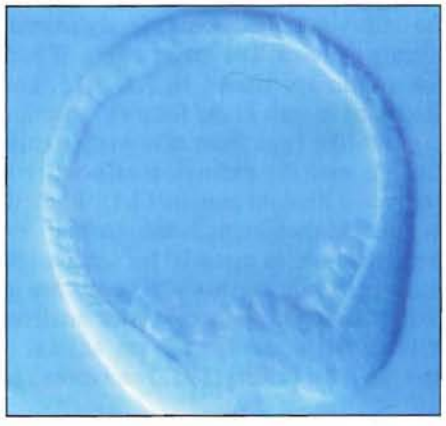

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3.1–3.15 Characteristic stages during early development in *Lytechinus variegatus*. The animal pole, when evident, is at the top unless otherwise indicated. **3.1**, unfertilized egg; **3.2**, recently fertilized egg (note the fertilization envelope and the fertilization cone, at the top of the egg); **3.3**, two-cell; **3.4**, 4-cell; **3.5**, 8-cell; **3.6**, 16-cell (the micromeres are at the bottom); **3.7**, 16-cell embryo viewed from the vegetal pole to show the micromeres; **3.8**, 32-cell embryo (the small micromeres are visible at the extreme vegetal pole); **3.9**, vegetal pole view of a 32-cell embryo showing the small micromeres and the larger micromere derivatives immediately above them; **3.10**, blastula stage; **3.11**, mesenchyme blastula; **3.12**, early gastrula (courtesy of C. Ettensohn); **3.13**, late gastrula; **3.14**, prism stage embryo (note the coelomic pouches beginning to form); **3.15**, pluteus (×450).

density gradients with little effect on patterns of development (reviewed in Harvey, 1956; McClay *et al.*, 1990; **3.16**).

Under normal circumstances, sea urchins simply shed gametes directly into the marine environment. Three mechanisms appear to help ensure that interactions between sperm and egg are species-specific. First, in *Arbacia punctulata*, the peptide *resact*, which is contained in the egg jelly, appears to be a species-specific chemo-attractant for sperm (Ward *et al.*, 1985). Second, activation of sperm by egg jelly is also species-specific in some species so that contact between them results in a rapid *acrosome reaction*. The *acrosomal process* which contains proteolytic enzymes, fuses with the sperm plasma membrane and extends dramatically, driven by the rapid polymerization of actin microfilaments. Third, the adhesion of activated sperm to the vitelline layer is mediated by the acrosomal protein bindin, whose binding to the vitelline envelope also appears to be mediated by a species-specific ligand-receptor interaction (Glabe and Vacquier, 1978; Moy and Vacquier, 1979). All regions of the egg surface support sperm attachment and fusion in the sea urchin.

At the site of sperm-egg fusion, localized polymerization of actin filaments in the egg cortex produces the *fertilization cone* (**3.17**). The *fast block to polyspermy*, a rapid depolarization of the egg mediated by an influx of sodium ions, provides an initial barrier to penetration of the egg by more than a single sperm. A slower but more *permanent block to polyspermy* results from *cortical granule exocytosis* and elevation of the *fertilization envelope* (**3.2**) which is triggered by release of calcium ions in a wave that sweeps across the egg. Following its entry into the egg, the sperm nucleus decondenses to form the *male pronucleus*. Microtubules polymerize away from the sperm centriole in the direction of the *female pronucleus*, the two pronuclei migrate towards one another, and ultimately they fuse to form the diploid, zygote nucleus (Bestor and Schatten, 1981). Ultimately, the release of calcium responsible for cortical granule exocytosis results in *activation* of the egg, a complex series of events leading to the initiation of protein and DNA synthesis. Egg activation can be mimicked by a number of treatments which lead to *parthenogenetic activation* and include treatment with butyric acid and hypertonic sea water and calcium ionophores (reviewed by Weidman and Kay, 1986).

Cleavage and the blastula

Sea urchins undergo synchronous, radial, holoblastic cleavages until the blastula stage. The first two cleavages are meridional and perpendicular to one another, passing through the animal and vegetal poles to produce first two and then four cells of equal size (**3.3–3.5** and **3.18**). The third cleavage is equatorial, separating the embryo into an animal and vegetal quartet of cells (**3.5**). At the fourth cleavage, the cells of the animal tier divide equally to produce eight *mesomeres*, while the cells in the vegetal tier divide unequally, producing four large *macromeres*, and four small *micromeres* (**3.6, 3.7** and **3.19**). In

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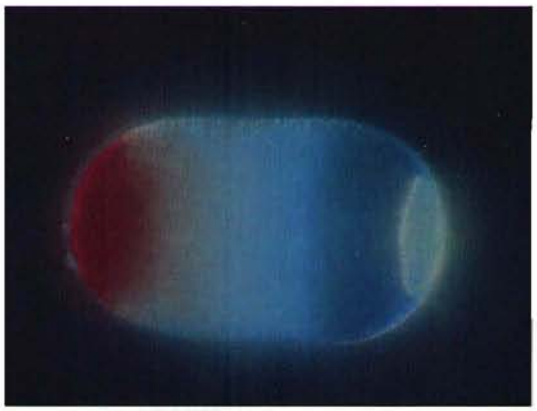

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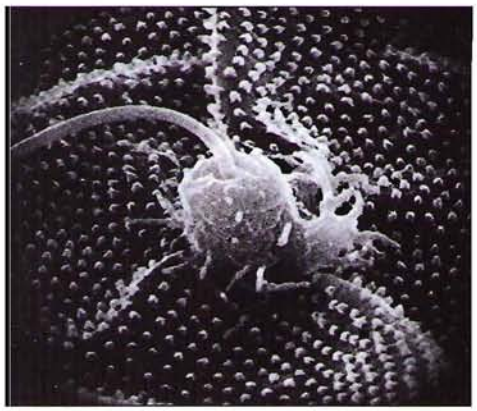

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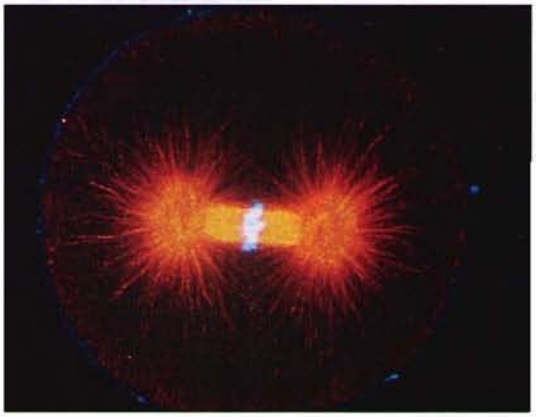

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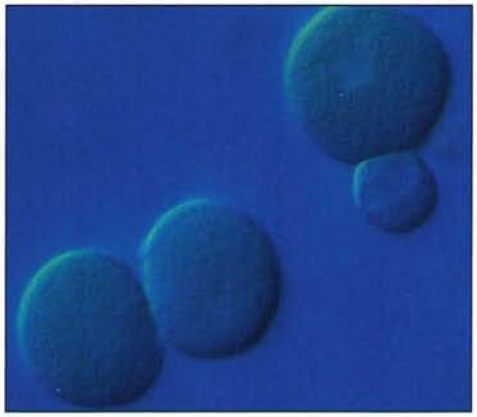

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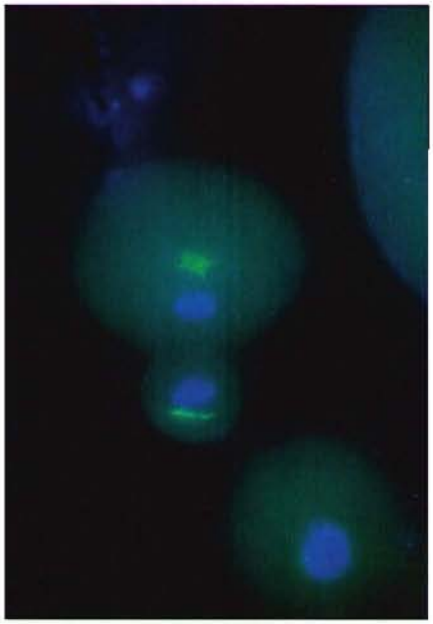

3.16–3.20 An *Arbacia punctulata* egg stratified by centrifugation in a sucrose gradient is seen in **3.16** (×500). Red pigment granules stratify at one end of the egg. **3.17** (×20,000) shows a scanning electron micrograph of a sperm entering a *Lytechinus pictus* egg. The vitelline layer, with microvilli protruding into it, is also visible (courtesy of G. Schatten). **3.18** (×1500) shows first mitosis visualized by double labelling of microtubules in the spindle (orange) and DNA (blue) in *Lytechinus pictus* (courtesy of J. Holy). **3.19** (×600) shows asymmetric division of an isolated vegetal pole blastomere into a macromere and a micromere at fourth cleavage. **3.20** (×700) shows a macromere/micromere pair double labelled for microtubules (green) and DNA (blue) to show the asymmetric placement of nuclei and spindles during fourth cleavage (courtesy of J. Holy).

the vegetal blastomeres of the 8-cell embryo, the nucleus moves to an eccentric, vegetal position, and the mitotic spindle is subsequently assembled eccentrically as well, with the result that the aster is flattened and shortened (**3.20**). The asymmetric fourth cleavage is the first sign that cells distributed along the animal-vegetal axis of the embryo are different and only the micromeres will go on to form primary mesenchyme cells (pmc) that produce the larval skeleton (see below).

At the fifth cleavage, the mesomeres divide equatorially to produce two animal tiers (denoted by Hörstadius as $\mathbf{an}_1$ and $\mathbf{an}_2$), and the macromeres divide meridionally to produce a tier of eight 'half-macromeres'. The micromeres divide asymmetrically to produce a tier of *small micromeres* at the extreme vegetal pole of the embryo, and a tier of larger micromere derivatives immediately above them (**3.8, 3.9**). At the sixth cleavage, all cells divide equatorially to produce a 64-cell embryo with five tiers: the daughters of the $\mathbf{an}_1$ and $\mathbf{an}_2$ cells lie at the animal pole, the $\mathbf{veg}_1$ and $\mathbf{veg}_2$ tiers, derived from the macromeres, lie in the vegetal hemisphere, and the micromere descendants lie at the vegetal pole. At the seventh cleavage, all cells divide meridionally to

produce a 128-cell blastula. During the blastula stage, cells no longer cleave synchronously: as development proceeds, divisions of local groups of cells remain synchronous, but these regions gradually decrease in size, and eventually the cell cycle lengthens and becomes largely randomized (Dan *et al.*, 1980).

The early blastula is an epithelial monolayer enclosing a central, spherical *blastocoel* (**3.10**) whose cells develop septate junctional contacts between one another (Spiegel and Howard, 1983) and begin to produce the *basal lamina* lining the blastocoel. The exterior, apical ends of the epithelial cells possess numerous microvilli, which are embedded in the hyaline layer, and *apical lamina* (Hall and Vacquier, 1982). The cells of the blastula eventually seal off the internal, embryonic environment from the external environment, with the epithelium becoming impermeable to small sugar molecules by the midblastula stage (Moore, 1940). The forces responsible for formation of the blastocoel remain unknown, although osmotic influx of water into the blastocoel and attachment to the hyaline layer have been suggested as possible factors (Dan, 1960; Dan and Inaba, 1968; Gustafson and Wolpert, 1962). At the midblastula stage, each cell produces a cilium, the embryo begins to rotate within the fertilization envelope, and a *hatching enzyme* is synthesized that is secreted into the space between the embryo and the fertilization envelope. Here it digests the envelope and allows the embryo to hatch and become a free-swimming blastula.

Establishment of the major tissue territories of the embryo

What changes in the shape of the embryo take place during gastrulation, and what consequences do they have for subsequent tissue-specific differentiation? Analyses of cell fate and cell movements both shed light on these questions. By staining individual blastomeres with Nile blue at the 32- and 64-cell stages, Hörstadius (1935) constructed a fate map for the *Paracentrotus lividus embryo* (**3.21**). According to Hörstadius, the $\mathbf{an}_1$ tier of cells, which is distinct at the 32-cell stage, gives rise to the ectoderm of the animal pole, the $\mathbf{an}_2$ tier gives rise to equatorial ectoderm, the $\mathbf{veg}_1$ tier of the 64-cell embryo gives rise to the vegetal pole ectoderm and the $\mathbf{veg}_2$ tier generates the cells of the archenteron and a group of mesenchymal cells, the *secondary mesenchyme*, which form at the tip of the archenteron, while the large micromere derivatives give rise to the primary mesenchyme which forms the skeletal structures of the larva.

3.21–3.24 Organization and fates of cells in the early sea urchin embryo according to Hörstadius (for *Paracentrotus lividus*; **3.21**) and Cameron and co-workers (*Strongylocentrotus purpuratus*; coloured diagrams; from Davidson, 1988, with permission). The coloured regions refer to the following presumptive tissue territories. (Key: red = skeletogenic cells, yellow = aboral (dorsal) ectoderm, green = oral (ventral) ectoderm, magenta = small micromere derivatives, blue = archenteron and associated structures.)

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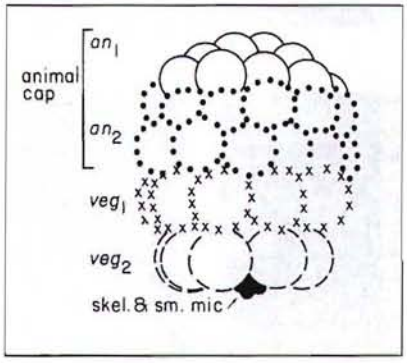

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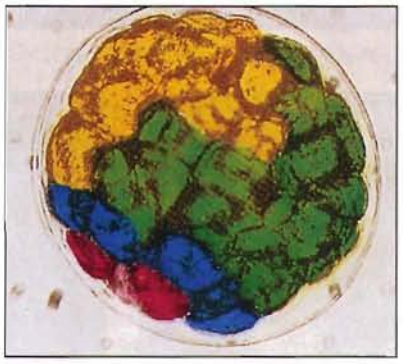

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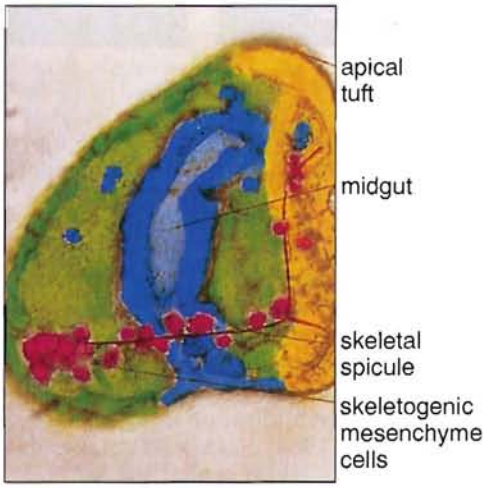

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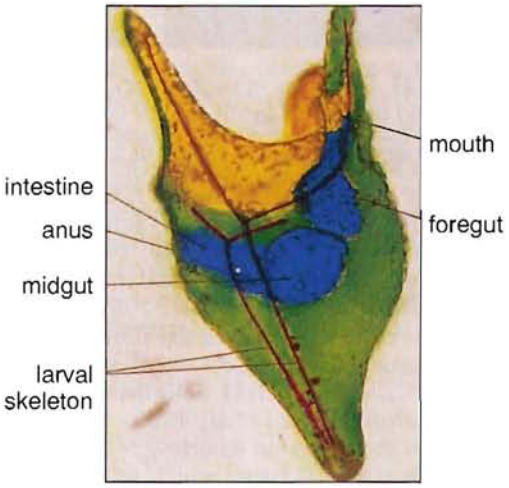

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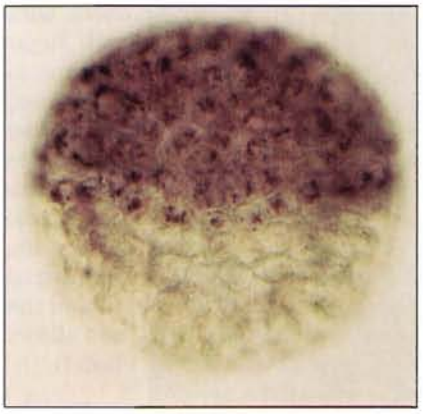

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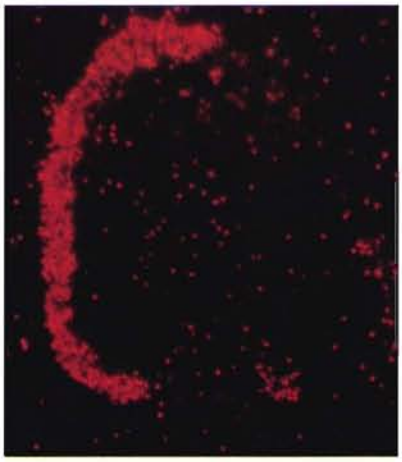

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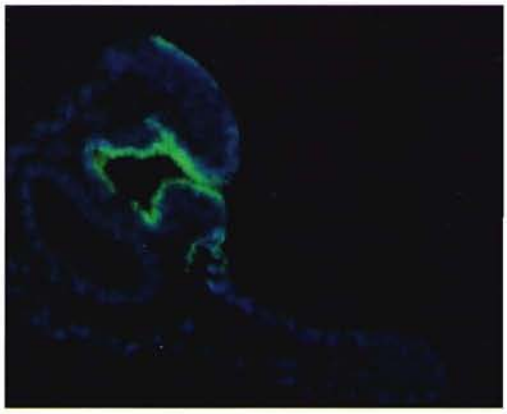

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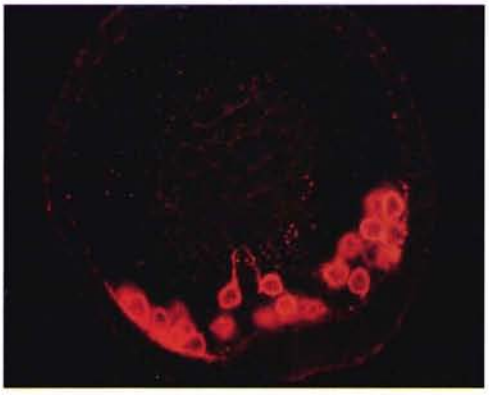

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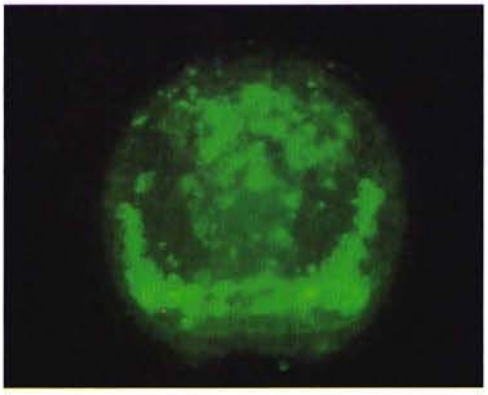

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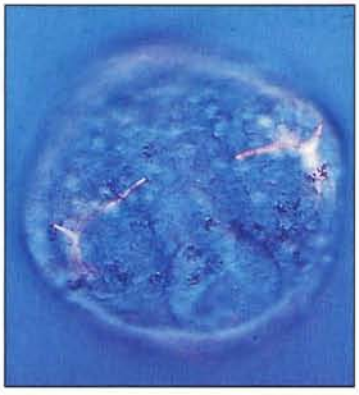

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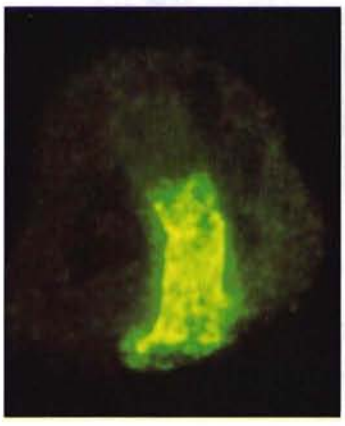

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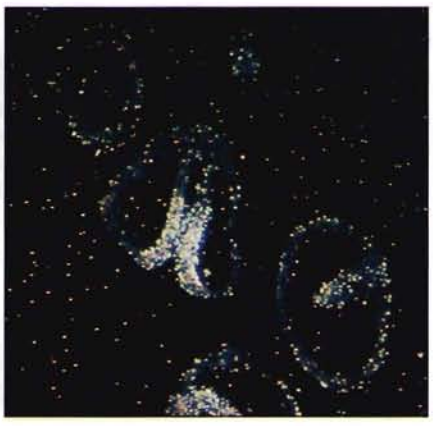

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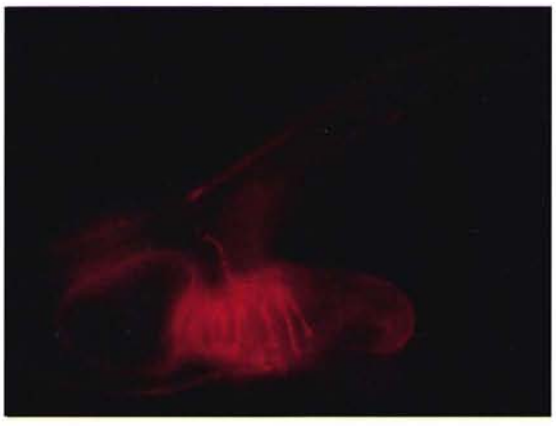

3.25–3.33 Examples of tissue-specific gene expression in the sea urchin embryo. The probes described here are representative, but by no means exhaustive. **3.25** (×450) is a whole mount *in situ* hybridization using an anti-sense BP-10 RNA (courtesy of T. Lepage; Lepage *et al.*, 1992). The boundary of expression sharply demarcates future ectoderm from endoderm and mesoderm. **3.26** (×420) shows an *in situ* hybridization of a *Lytechinus variegatus* embryo using an anti-sense probe for LvS1 (Wessell *et al.*, 1987), a member of the Spec 1 family of genes (Lynn *et al.*, 1983). **3.27** (×350) shows a section of a *Lytechinus variegatus* pluteus immunostained for the Ecto V antigen (V for **v**entral); staining is complementary to aboral markers (courtesy of D. McKlay; Coffman and McClay, 1990). **3.28** (×450) shows whole mount immunostaining for the msp130 homologue in *Lytechinus variegatus*, a cell surface antigen expressed by primary mesenchyme cells and one of a host of probes specific to these cells (e.g., Benson *et al.*, 1987; Drager *et al.*, 1989; Leaf *et al.*, 1987; Wessell and McClay, 1985). **3.29** (×350) shows pigment cells localized to the presumptive arm buds of a *Lytechinus variegatus* prism stage embryo. **3.30** (×350) shows immunostaining for Meso 1, a cell surface epitope present on the surfaces of primary and secondary mesenchyme cells (Wray and McClay, 1988).**3.31** (×400) shows immunostaining for Endo 1, an antigen expressed by the mid- and hindgut of the archenteron (Wessell *et al.*, 1985). **3.32** (×200) shows *in situ* hybridization using an anti-sense probe for LvN1.2, which also localizes to this region (courtesy of G. Wessell; Wessell *et al.*, 1989). **3.33** (×500) shows rhodamine-phalloidin staining for the esophageal muscle bands which surround the foregut (Ishimoda-Takagi *et al.*, 1984; Wessell *et al.*, 1990).

More recently, these lineage studies have been refined and extended to account for distinctions along the dorsoventral axis of the early embryo. Based on unique patterns of gene expression, cell lineages, and one or more characteristic differentiated cell types, sea urchin embryonic cells can be classified into five major tissue territories (**3.22–3.24**; Cameron and Davidson, 1991; Davidson, 1989). These are:

- The *aboral* (or *dorsal*) *ectoderm*, which forms a simple, squamous epithelium.
- The oral (or ventral) ectoderm, which forms the epithelium of the mouth region and the *ciliated band*, a structure that lies at the boundary between oral and aboral ectoderm.
- The *vegetal plate*, which gives rise to the archenteron and its derivatives.
- The *primary mesenchyme cells*, which will produce the larval skeleton.
- The *small micromeres*, which have been reported to contribute to the coelomic pouches (Pehrson and Cohen, 1986).

Each of these tissue territories derives from a specific group of founder cells whose lineages become distinct during cleavage. The lineages of cells lying within these domains can be distinguished by the completion of the sixth cleavage, i.e. when there are approximately 64 cells in the embryo (**3.22–3.24**). The four animal blastomeres of the 8-cell embryo contribute progeny to either oral or aboral ectoderm, and they are termed **Na** and **No** cells (**a**nimal, **o**ral and **a**boral). Some cells from Hörstadius' $\mathbf{veg_1}$ tier, derived from the macromeres of the 16-cell embryo, form aboral ectoderm, while others contribute to oral ectoderm. The $\mathbf{veg_2}$ tier of Hörstadius gives rise to the structures of the archenteron: the larger (animal) progeny of the micromeres generate skeletogenic mesenchyme, whereas the small (vegetal) progeny of the micromeres contribute to the coelomic pouches.

The clonal boundaries described by Cameron and co-workers are established through invariant cleavages and these boundaries seem to coincide very closely with spatially restricted patterns of gene expression (**3.25–3.33**). That clonal boundaries and patterns of gene expression are co-extensive suggests that the reliability of cleavage may be important in establishing patterns of differentiating tissue within the *unperturbed* embryo. The sea urchin embryo is, however, well known for the ability of its cells to adopt new fates when placed in unusual environments, indicating that the establishment of reliable clonal boundaries does not reflect an underlying 'mosaic' quality of the early cells of the sea urchin embryo, in contrast to some other invertebrate embryos (e.g., nematodes and ascidians).

Gastrulation and post-gastrula development

The various territories of the embryo also differ in their patterns of motility as the embryo is transformed during gastrulation. The study of sea urchin gastrulation has been influential in shaping ideas about general mechanisms of morphogenetic movements, and the work of Gustafson and co-workers in particular demonstrated the power of time-lapse microscopy in elucidating morphogenetic processes (reviewed by Gustafson and Wolpert, 1963; 1967). Just prior to gastrulation, there is a dramatic decrease in the overall rate of cell division, and the embryo comprises about 1000 cells. At the animal pole, a thickened region of epithelium, the *apical plate*, or *acron*, appears with a tuft of cilia that are longer than those found on the rest of the embryo (**3.11**), while the epithelium at the vegetal pole of the embryo flattens and thickens to form the *vegetal plate*.

The onset of gastrulation is marked by the ingression of *primary mesenchyme cells* (pmc) into the blastocoel (**3.11** and **3.34–3.36**), an event accompanied by alterations in cell polarity and loss of the epithelial phenotype (Anstrom and Raff, 1988) and the appearance of new cell-surface determinants and transcripts (see above). When they begin to ingress, pmc become bottle-shaped in profile as the surface area of their apical ends is reduced (Katow and Solursh, 1980), and they eventually detach from the hyaline layer. These cells use bristle-like filopodia to move (reviewed by Solursh, 1986) and require sulphated proteoglycans on their surfaces and/or in the blastocoel for their migration (Lane and Solursh, 1988; Lane and Solursh, 1991; Solursh *et al.*, 1986). Primary mesenchyme cells migrate away from the vegetal plate, but eventually form a ring in the vegetal pole region of the embryo (**3.35**). Ultimately, two clusters of pmc form in the ventrolateral ectoderm and give rise to the *spicule rudiments* of the larva (**3.36**; for further details, see Solursh, 1986, and Decker and Lennarz, 1988).

Following the ingression of pmc, *pigment cells* depart from the vegetal plate (Gibson and Burke, 1985; **3.30**). The vegetal plate then begins to bend inward to form a short, squat cylinder, the *archenteron*. During this initial phase of invagination (*primary invagination*), the archenteron extends $^1/_4$–$^1/_2$ of the way across the blastocoel (**3.12**). A short pause follows primary invagination, after which the archenteron resumes its elongation (*secondary invagination*). At about the time secondary invagination begins, cells at the tip of the archenteron (*secondary mesenchyme cells*) become protrusive, extending long *filopodia* into the blastocoel (**3.13**). Eventually the archenteron elongates across the blastocoel, and its tip attaches to the ventral ectoderm near the animal pole.

By the time the archenteron completes its elongation, pmc have localized into two major clusters in the ventrolateral ectoderm to form spicule rudiments. At the tip of the archenteron, two bilateral outpocketings, the *coelomic pouches*, appear (**3.14**). Ultimately the tip of the archenteron

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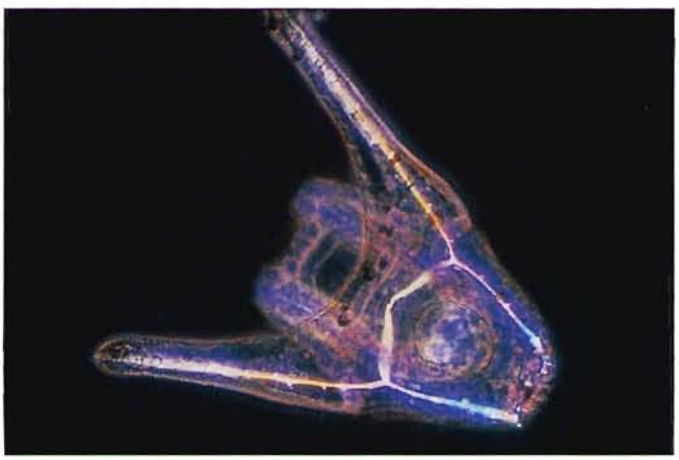

3.34–3.36 Primary mesenchyme cell behaviour during early development. **3.34** (×500) is a scanning electron micrograph of a *Lytechinus variegatus* mesenchyme blastula during ingression of primary mesenchyme cells (courtesy of J. Morrill; magnification. **3.35** (×500) is an *L. variegatus* gastrula viewed from the vegetal pole, showing the aggregation of primary mesenchyme cells into two ventrolateral clusters (magnification. **3.36** (×250) is an *L. variegatus* pluteus viewed with darkfield optics (courtesy of M.A. Alliegro; magnification.

fuses with the ectoderm to form the larval mouth. As the *pluteus* larva develops (**3.15**), the archenteron becomes tripartite, and a host of differentiated tissues appear that include nerve cells in the ectoderm (Bisgrove and Burke, 1987). The left coelomic pouch ultimately forms the hydrocoel which, together with the ectodermal vestibule, gives rise to the *echinus rudiment*, the imaginal structure which generates the juvenile urchin during metamorphosis (Czihak, 1971; Okazaki, 1975a). The eversion of the echinus rudiment is a geometrically complex process and the interested reader is urged to consult the beautiful drawings found in Czihak (1971) and Okazaki (1975a).

The sea urchin as an experimental system

Mutational analysis is not practical in the sea urchin because of the long generation times and the difficulties of rearing embryos through metamorphosis in the laboratory (but see some interesting mutants produced by Hinegardner, 1975b). In contrast to many of the commonly used experimental systems in which genetics is feasible, however, the early embryonic development of the sea urchin can be manipulated directly, thereby allowing epigenetic influences on development to be examined. In particular, the sea urchin system is an excellent system in which to study the role of cell interactions during early development.

Cell interactions in the early embryo

The landmark experiments of Driesch (e.g., 1891) in which he separated early blastomeres of the sea urchin embryo demonstrated that cells derived from the 2- and 4-cell embryo could *regulate* to produce a small embryo possessing most of the normal larval tissues. Since that time, the sea urchin embryo has been used to study how cells become committed to various pathways of differentiation. In particular, some of the most remarkable experiments in the history of embryology were those

performed by Hörstadius (1939, 1973) investigating the properties of cells along the animal-vegetal axis of the early sea urchin embryo. By separating unfertilized eggs and early embryos into animal and vegetal halves, separating tiers of cells at the 32- and 64-cell stages, and by juxtaposing various tiers of cells originating at different locations along the animal-vegetal axis, Hörstadius found that a graded influence on differentiation existed along the animal-vegetal axis. These experiments led to the idea that a 'vegetalizing gradient', originating in the unfertilized egg, exerts effects along the embryonic axis. More recently, micromeres have been shown to interact with mesomeres at the level of single pairs of cells, so that eventually the mesomeres form an array of mesodermal and endodermal structures (reviewed by Livingston and Wilt, 1990). The effects of this vegetalizing influence can be mimicked by the *vegetalising agent*, lithium chloride, which confers progressively more vegetal qualities to blastomeres which would not normally possess them (see Hörstadius, 1973). More recent experiments using molecular assays have confirmed that lithium induces alterations in gene expression that include overproduction of endoderm in whole embryos (Nocente-McGrath *et al.*, 1991) and the appearance of mesodermal and endodermal markers in the descendants of single mesomeres (Livingston and Wilt, 1989).

Cell contact also seems to be important in regulating gene expression and differentiation in the early embryo. When mesomeres are isolated as an intact group of eight cells from a 16-cell embryo, they produce a ciliated ball, or *Dauerblastula* (Hörstadius, 1939); when, however, mesomeres are dissociated and recombined, thereby altering cell-cell contacts, they produce spicules and archenterons (Henry *et al.*, 1989). Pervasive alterations in zygotic gene expression also result when cell contacts are continuously prevented from forming by stirring blastomeres in calcium-free sea water (Hurley *et al.*, 1989; Stephens *et al.*, 1989). Cell contact and cell interactions thus seem to be important in specifying cell fate in experimentally treated blastomeres.

In contrast to most cells in the early sea urchin embryo, however, micromeres appear to be committed to a spiculogenic pathway as soon as they appear at the 16-cell stage. This conclusion is largely based on the work of Okazaki (1975b), who developed culture conditions allowing micromeres to differentiate *in vitro*. Cultured micromeres divide, become motile, migrate, and the cells later associate into syncitia and produce spicules (Okazaki, 1975b; see **3.41**). They also undergo several simultaneous adhesion changes at the time that pmc would ordinarily ingress into the blastocoel; these include:

- A loss of an affinity for the hyaline lamina proteins hyalin and echinonectin.
- A loss of an affinity for neighbouring cells.
- An increased affinity for the basal lamina (Fink and McClay, 1985; Burdsal *et al.*, 1991).

That these changes can occur in cultured micromeres suggests that this transformation is entirely autonomous. The fourth cleavage can be equalized by treatment of eggs with low concentrations of sodium dodecyl sulphate and, here, sixteen cells of equal size are produced, and, in some but not all cases, formation of pmc is suppressed (Langelan and Whiteley, 1985).

Cell interactions and the extracellular matrix

In the forming sea urchin embryo, cells interact not only with each other, but with the extracellular environment. The epithelial tissues of the sea urchin embryo are in contact with several extracellular matrix layers: the apical lamina, and hyaline layer, on their apical surfaces, and the basal lamina on their basal surfaces (**3.37, 3.38**). In addition, Dan (1960) has suggested that the hyaline layer is important as a structural support and mechanical integrator of epithelia. Consistent with this idea, treatment of sea urchin embryos with monoclonal antibodies specific for the protein hyalin (a major component of the hyaline layer) results in visible delamination of the hyaline layer from the epithelium, abnormal thickening of the epithelium, and blockage of invagination (Adelson and Humphreys, 1988; **3.39**). If antibody-treated embryos are removed from the antibody, development resumes, and a normal pluteus larva results (Adelson and Humphreys, 1988). These results suggest that the antibody interferes with the mechanical and structural integrity of the epithelium, but also with more general requirements for the initiation of gastrulation. Incubation of *Strongylocentrotus purpuratus* embryos from hatching through to gastrulation in F_{ab} fragments of antibodies which recognize a fibrous apical lamina protein results in disruption of the normal epibolic movements preceding gastrulation, and failure of invagination (Burke *et al.*, 1991), again pointing to an important integrating role for these apical layers during sea urchin embryogenesis.

Treatments affecting the basal lamina also block gastrulation. Incubation of embryos from fertilization onward in β-aminoproprionitrile (BAPN), an inhibitor of lysyl oxidase (an enzyme involved in collagen crosslinking), results in embryos which develop normally to the mesenchyme blastula stage, but fail to progress further. If the drug is removed, even after the embryos have been arrested at the mesenchyme blastula stage for more than 24 hours, the embryos begin to gastrulate and complete development normally (Butler *et al.*, 1987; Wessell and McClay, 1987). In the normal embryo, a fibrillar meshwork is present in the blastocoel along which mesenchyme cells appear to migrate (**3.40**). BAPN treatment results in a poorly constructed basal lamina and poor motility of mesenchyme cells (Butler *et al.*, 1987; Hardin, 1987) and, in addition, antigens normally expressed in the archenteron fail to appear as long as the embryos are incubated with the drug (Wessell and McClay, 1987). The similarity of the effects observed when either extracellular matrix layer is disrupted suggests that a critical period precedes gastrulation during which normal contact with both the basal lamina and the hyaline layer is required in order for gastrulation to begin.

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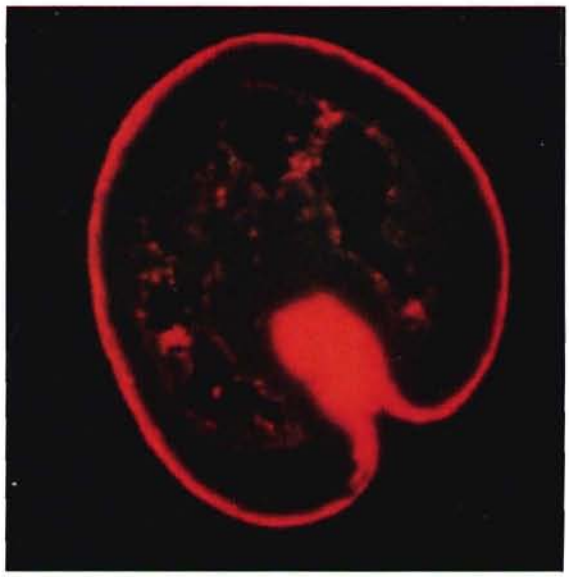

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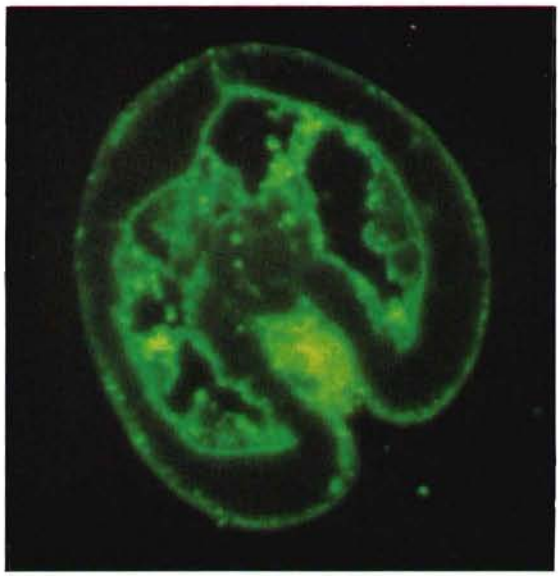

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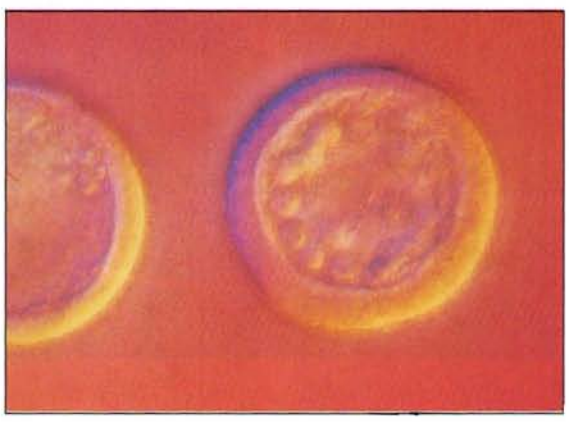

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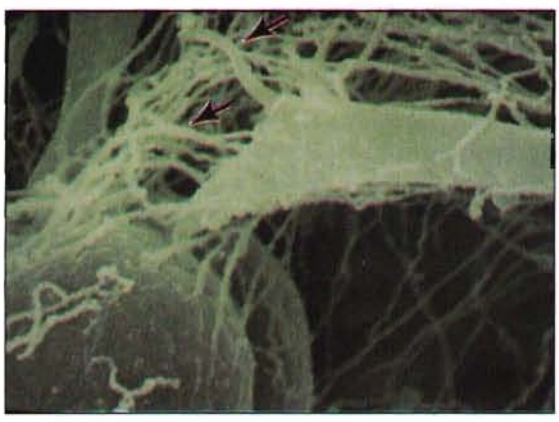

3.37–3.40 The role of the extracellular matrix during sea urchin gastrulation. **3.37** and **3.38** show immunofluorescent localization of the hyaline layer component hyalin and a basal lamina antigen, Ib10 (×550). **3.39** shows the effects of treating a *L. variegatus* embryo with antibodies against hyalin (×400). **3.40** is a scanning electron micrograph of a protrusion from a secondary mesenchyme cell; thin filopodia (arrows) contact fibrils of extracellular matrix (×10,000; courtesy of J. Morrill).

Cell interactions between primary mesenchyme cells and surrounding tissues

Although micromeres are committed early in development to form a pmc (**3.41**), it is also clear that the behaviour of later cells is affected by the embryonic environment. Okazaki *et al.* (1962) observed that pmc localize at sites in the ectoderm where the epithelial cells are thickened, producing an optical effect reminiscent of an oriental fan. When this belt of cells is shifted along the animal-vegetal axis in vegetalized embryos treated with lithium chloride, the pmc localize to the shifted ectoderm (Gustafson and Wolpert, 1961; Okazaki *et al.*, 1962). Likewise, when the normal differentiation of ectodermal tissues is disrupted by treatment of embryos with $NiCl_2$, pmc form spicules, but in a completely radialized pattern (Hardin *et al.*, 1992; **3.42**), even though transplantation experiments suggest that it is only the ectoderm, and not the mesenchyme cells, which are affected (Hardin and Armstrong, 1991). These experiments imply that regionally specific information is contained within the ectoderm which helps specify where pmc localize, but the molecular basis of this localized attachment is still not understood.

The pmc not only receive signals from the environment, but also appear to interact with themselves and other mesenchyme cells. When supernumerary pmc are transplanted into host embryos, the resulting skeleton is indistinguishable from the normal one, even though as many as two to three times the normal number of cells participate in skeleton formation (Ettensohn, 1990b) and it seems that some signal(s) operate which restrict both the size and location of skeletal elements. When all pmc are removed from the embryo, a skeleton arises from a subpopulation of secondary mesenchyme cells which become spiculogenic and produce a normal skeleton (Ettensohn and McClay, 1988; **3.43**), even though secondary mesenchyme cells do not normally participate in spicule production. The

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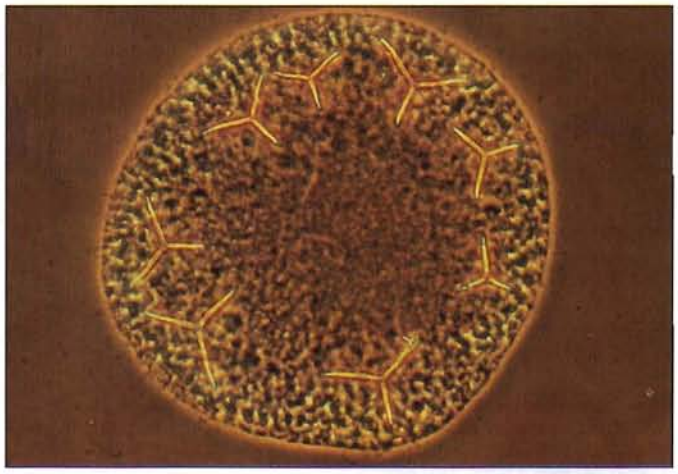

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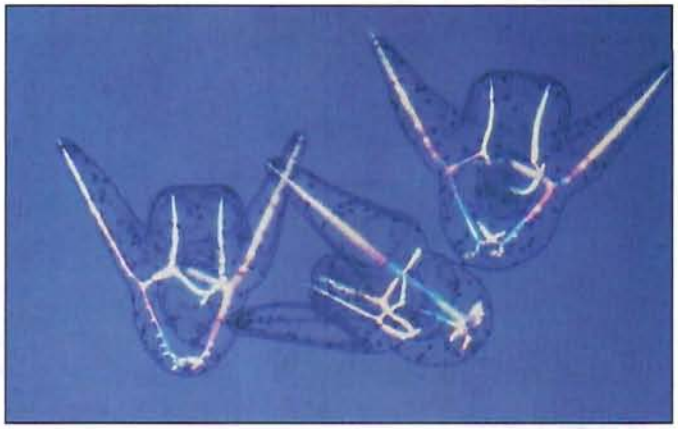

3.41–3.43 Cell autonomy and cell interaction during skeleton formation in the sea urchin embryo. **3.41** shows five picules formed by cultured *Arbacia punctulata* micromeres (×150; courtesy of R. Fink). **3.42** shows radialized skeleton formed in an embryo treated with 0.5 mM $NiCl_2$ from fertilization through the early gastrula stage (×500). **3.43** shows a skeleton produced by 'converted' secondary mesenchyme cells in embryos in which all primary mesenchyme cells were removed (×100; courtesy of C. Ettensohn).

pmc must therefore provide some restrictive signal preventing the secondary mesenchyme cells from differentiating into spicule-producing cells. This interaction displays a remarkably quantitative character for, as one removes more and more pmc from *Lytechinus variegatus*, a progressively greater number of secondary mesenchyme cells convert to compensate for the loss of spiculogenic cells (Ettensohn and McClay, 1988). This restrictive influence must operate over a distance of tens of microns since the two populations of mesenchyme are some distance from one another in the embryo. Based on studies by Ettensohn (1990a), the period during which this interaction occurs ends at about the time the archenteron makes contact with the animal pole (for possible mechanisms, see Ettensohn, 1991).

Cell interactions during archenteron formation

Surprisingly little is known about the forces which promote the inward bending of the archenteron, although many ideas have been put forward. Neither local proliferation of cells nor changes in lateral contact between cells which would ultimately result in bending seem to be involved (reviewed by Hardin, 1990). *Apical constriction* of cells in the vegetal plate has been proposed to account for primary invagination (Odell *et al.*, 1981; reviewed in Ettensohn, 1985b) and there are apically constricted cells in the centre of the vegetal plate, with adjacent cells having expanded apices, so suggesting that they are under tension (Hardin, 1989; **3.44**), but interpretable experimental disruption of this process has been difficult (see Hardin, 1990). *Swelling pressure* generated by secretion of proteoglycans into the lumen of the archenteron has also been suggested as a means by which the archenteron could invaginate (Morrill and Santos, 1985). In support of this idea, a chondroitin sulphate proteoglycan has been localized to the lumen of the archenteron (Lane and Solursh, 1991). Finally, a noticeable amount of *epiboly*, or spreading of the pre-gastrula epithelium, occurs just prior to gastrulation, and this includes tissue immediately adjacent to the vegetal plate (Ettensohn, 1984; Burke *et al.*, 1991). When these movements do not occur properly, invagination fails, so suggesting that they may be important for primary invagination (Burke *et al.*, 1991). Whatever mechanism(s) account for primary invagination, there are no forces outside the immediate vicinity of the vegetal plate required for its invagination, since the vegetal plate can be isolated several hours before primary invagination begins and it will still invaginate on schedule (Moore and Burt, 1939; Ettensohn, 1984).

Considerably more is known about the elongation of the archenteron and here *epithelial cell rearrangement* plays an

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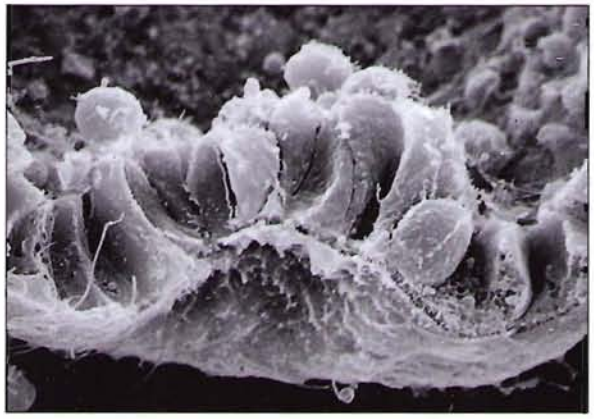

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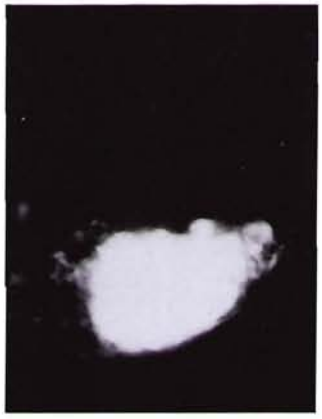

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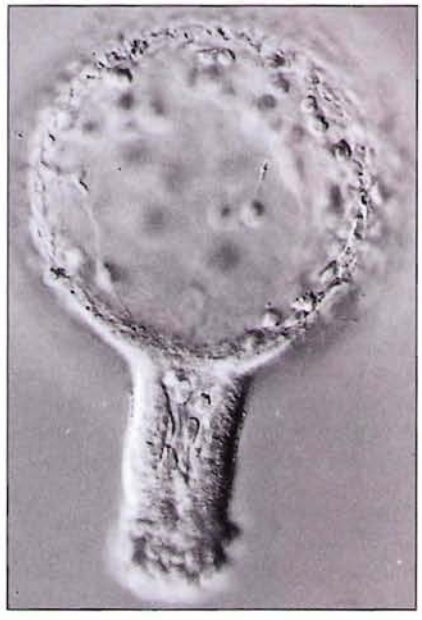

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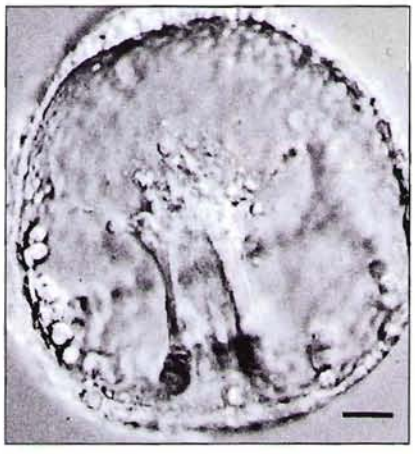

3.44–3.48 Formation of the archenteron *Lytechinus pictus*. **3.44** is a scanning electron micrograph of an early gastrula (×1500). **3.45** and **3.46** (×500) show changes in shape of rhodamine-labelled clones during gastrulation (**3.45**, early gastrula; **3.46**,late gastrula). **3.47** is an embryo is an embryo whose secondary mesenchyme cells have been ablated by a laser microbeam. Elongation of the archenteron ceases at two thirds of its normal final length (×500). **3.48** is an exogastrula produced by treatment with lithium chloride (×500).

important role. Ettensohn (1985a) deduced that, as the archenteron elongates, the number of cells around the circumference of the archenteron decreases, while more direct evidence for cell rearrangement comes from the behaviour of fluorescently labelled patches of cells within the vegetal plate during invagination: patches of such labelled cells gradually extend and narrow as cells interdigitate to lengthen the archenteron (**3.45, 3.46**). Cell rearrangement appears to be the dominant means by which the archenteron elongates, since additional material is not added by mitosis (Stephens *et al.*, 1986) or involution in *L. pictus* (Hardin, 1989). What forces drive this rearrangement? Several observations suggested that the filopodia of secondary mesenchyme cells can exert significant tension and led to the hypothesis that filopodial traction causes the archenteron to elongate (Dan and Okazaki, 1956; Gustafson, 1963; reviewed in Hardin, 1988), but two observations counter this suggestion. When filopodia are ablated with a laser microbeam or when embryos are induced to *exogastrulate*, so producing an evagination rather than an invagination, the archenteron elongates to two-thirds of the normal length, even though secondary mesenchyme cells do not attach and pull in either case (Hardin and Cheng, 1986; Hardin, 1988; **3.47, 3.48**).

The cellular processes which generate autonomous rearrangement are not understood. Direct observation of cell rearrangement in *Eucidaris tribuloides* suggests that cells 'jostle' against one another, and their basal ends display vigorous motility (Hardin, 1989), but how such motility might be translated into directed rearrangement is not known. In any case, completion of archenteron elongation requires the activity of secondary mesenchyme cells. In laser-irradiated embryos in which a few filopodia are left intact, the archenteron will continue to elongate after the two-thirds gastrula stage, but more slowly than normal (Hardin, 1988). At about the time that secondary mesenchyme cells reach the animal pole, there is a transient stretching of cells in the archenteron, apparently in response to filopodial traction (Hardin, 1989).

Cell interactions between secondary mesenchyme cells and the ectoderm

How do secondary mesenchyme cells aid the attachment of the archenteron to a specific site in the ectoderm? When observed closely, the basic behaviour of secondary mesenchyme cells involves continual extension of filopodia (**3.49**); these often remain attached for a time, but eventually detach and collapse, only to be re-extended in a cyclical fashion (Gustafson and Kinnander, 1956; Hardin and McClay, 1990). Analysis of the duration of attachments of filopodia which make contact near the animal pole (**3.50**) indicate that they attach 20–50 times longer than those making attachments at other sites (Hardin and McClay, 1990). Several lines of evidence indicate that secondary mesenchyme cells respond uniquely to this region (Hardin and and McClay, 1990). First, when the animal pole region is pushed towards the tip of the archenteron so that contact is allowed earlier than it would normally occur, the cyclical behaviour of the secondary mesenchyme cells ceases early, and the archenteron stably attaches ahead of schedule (**3.51**). Second, when embryos are squeezed into narrow diameter capillary tubing so that secondary mesenchyme cells cannot attach to the animal pole, they continue their cyclical extension for a longer period of time than in normal embryos. If the embryo is held in such a tube for several hours, some secondary mesenchyme cells eventually detach from the archenteron, migrate to the animal pole, and undergo the change in behaviour seen in normal embryos (**3.52**). If the embryo is released from the tube, it regains a spherical shape, and, as it does so, the tip of the archenteron rapidly attaches to the animal pole. Finally, archenterons attach to the nearest available apical plate region in fused multiple embryos. All of these experiments point to the existence of localized information in the animal pole region which elicits this specific change in the motility and behaviour of secondary mesenchyme cells (Hardin and McClay, 1990).

3.49–3.52 Behaviour of secondary mesenchyme cells during gastrulation in *L. variegatus*. **3.49** (×750) shows a laser scanning confocal image of a midgastrula stained with rhodamine-phalloidin. Filopodia radiate away from the archenteron, producing a 'spray' of protrusions which make contact with the ectoderm. **3.50** (×350) shows a late gastrula; secondary mesenchyme cells cease their protrusive activity when they make contact near the animal pole. **3.51** (×450) shows an embryo imprisoned in nylon cloth; secondary mesenchyme cells attach prematurely at the animal pole. **3.52** (×500) shows an embryo imprisoned in capillary tubing for 2hr. Some secondary mesenchyme cells have detached from the archenteron and congregated at the animal pole (arrow).

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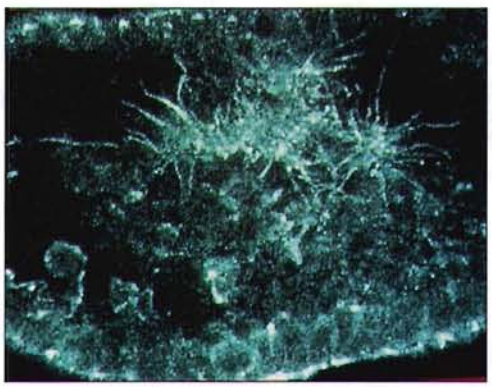

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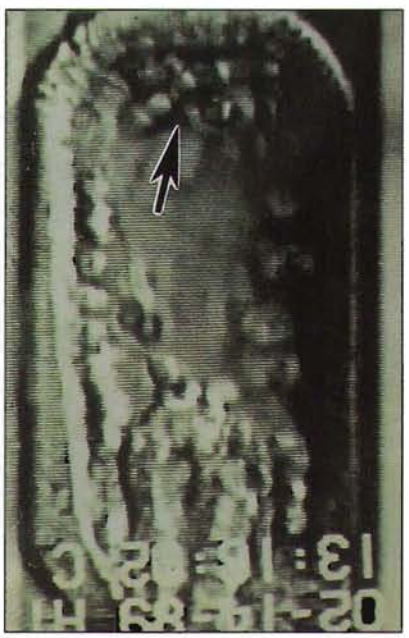

Phylogenetic differences in modes and timing of development

The sea urchin embryo has recently received attention as a system for examining phylogenetic diversity during early development (Raff, 1987; Raff and Wray, 1989; Wray and Raff, 1990). In different sea urchin species, for example, there seem to be several ways in which the archenteron can elongate, all apparently accounted for by differences in embryonic shape and placement of the future oral region (Hardin and McClay, 1990). In addition to variations in embryonic shape, there are also variations in the timing of developmental events with respect to one another when different species are compared (*heterochronies*), with ingression of spiculogenic cells being a good example of this sort of variation. Spicule-producing cells ingress at the mesenchyme blastula stage in the euechinoid sea urchin embryos with a typical larval mode of development, but ingression of spiculogenic cells in *Eucidaris* occurs many hours after invagination of the archenteron has begun, even though these cells are derived from the micromeres (Wray and McClay, 1987; Urben *et al.*, 1988). Even more radical alterations are evident in direct-developing sea urchins where the production of a functioning larval gut does not occur and whose metamorphosis is exceedingly rapid compared to sea urchins which pass through a true larval phase (**3.53–3.56**). Here, features of early development which appear to be devoted to the production of exclusively larval structures are often completely lost (reviewed by Raff, 1987; Raff and Wray, 1989; Wray and Raff, 1990). Other alterations include dramatic differences in cell lineages, mechanisms of cell fate determination along the dorsoventral axis, and mode of gastrulation (reviewed in Raff, 1992)).

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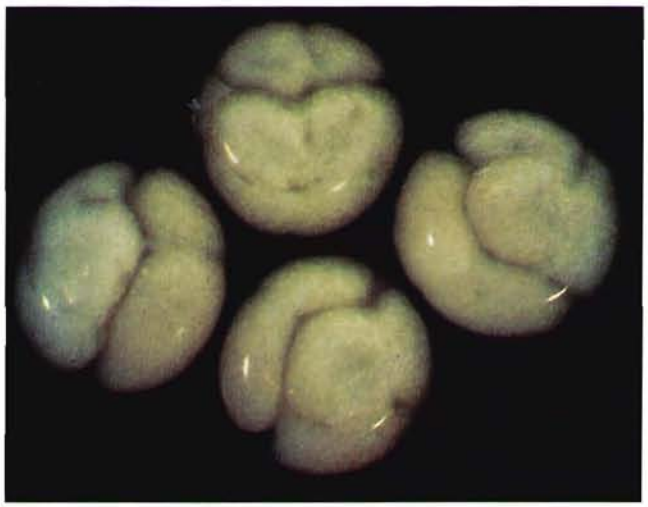
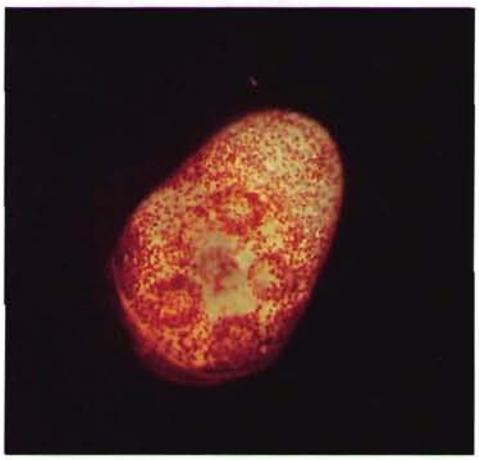
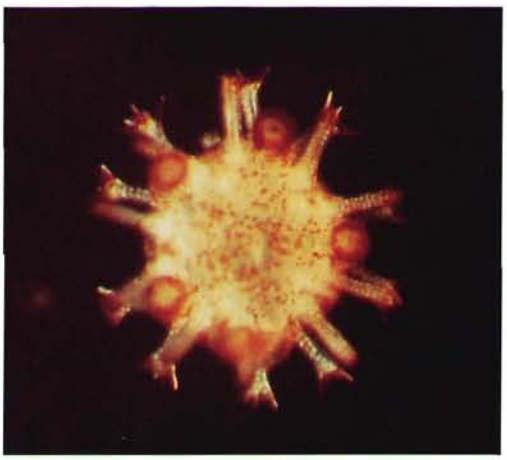


3.55



3.56



3.53–3.56 Direct development of the Australian sea urchin, *Heliocidaris erythrogramma*. **3.53** shows an unfertilized egg immersed in orange ink to visualize the jelly coat. **3.54** shows the wrinkled gastrula stage. **3.55** shows a four-day-old embryo immediately before metamorphosis, oral view. Tube feet and vestibule are visible. **3.56** shows a juvenile shortly after metamorphosis (×50; courtesy of L. Herlands).

The future

The sea urchin embryo occupies an important place in the history of developmental biology, and continues to be a useful experimental system today. As a model system for uniting experimental embryology and observation with the modern tools of molecular and cell biology, the sea urchin embryo continues to provide unique opportunities for studying early development. Current work is focusing on the roles of cell adhesion during gastrulation, of cell-cell interactions in altering the expression of specific genes, and of DNA binding factors in the regulation of tissue-specific gene expression, a particularly prominent feature of sea urchin development (reviewed by Davidson, 1989). As the tools of modern biology become increasingly refined and powerful, the simplicity of the sea urchin embryo and the ease with which it can be manipulated will provide a rich context in which to study the molecular basis of early development.

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