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Determination and morphogenesis in the sea urchin embryo

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Summary

The study of the sea urchin embryo has contributed importantly to our ideas about embryogenesis. This essay re-examines some issues where the concerns of classical experimental embryology and cell and molecular biology converge. The sea urchin egg has an inherent animal–vegetal polarity. An egg fragment that contains both animal and vegetal material will produce a fairly normal larva. However, it is not clear to what extent the oral–aboral axis is specified in embryos developing from meridional fragments. Newly available markers of the oral–aboral axis allow this issue to be settled. When equatorial halves, in which animal and vegetal hemispheres are separated, are allowed to develop, the animal half forms a ciliated hollow ball. The vegetal half, however, often forms a complete embryo. This result is not in accord with the double gradient model of animal and vegetal characteristics that has been used to interpret almost all defect, isolation and transplantation experiments using sea urchin embryos. The effects of agents used to animalize and vegetalize embryos are also due for re-examination. The classical animalizing agent, Zn^{2+}, causes developmenal arrest, not expression of animal characters. On the other hand, Li^{+}, a vegetalizing agent, probably changes the determination of animal cells. The stability of these early determinative steps may be examined in dissociation–reaggregation experiments, but this technique has not been exploited extensively.

The morphogenetic movements of primary mesenchyme are complex and involve a number of interactions. It is curious that primary mesenchyme is dispensable in skeleton formation since in embryos devoid of primary mesenchyme, the secondary mesenchyme cells will form skeletal elements. It is likely that during its differentiation the primary mesenchyme provides some of its own extracellular microenvironment in the form of collagen and proteoglycans. The detailed form of spicules made by primary mesenchyme is determined by cooperation between the epithelial body wall, the extracellular material and the inherent properties of primary mesenchyme cells.

Gastrulation in sea urchins is a two-step process. The first invagination is a buckling, the mechanism of which is not understood. The secondary phase in which the archenteron elongates across the blastocoel is probably driven primarily by active cell repacking. The extracellular matrix is important for this repacking to occur, but the basis of the cellular–environmental interaction is not understood.

There are new tools, especially well-defined specific antibodies and recombinant DNA clones, that may be applied to these problems and should help illuminate some of the underpinnings of embryogenesis in an organism that relies so heavily on cellular interactions as a develomental strategy.

Key words: sea urchin, determination, axis, gastrulation.

Introduction

The subject of this essay is to explore some selected aspects of determination and morphogenesis in the developing sea urchin embryo. Some explanation is necessary to justify this undertaking. Many readers will know that there are a number of reviews, some very recent, that consider various aspects of the development of sea urchin embryos. Although there are some papers on various aspects of sea urchin development that have just appeared or are in press and therefore do not figure in any of these accounts, most of what I shall discuss has been cited by others. Rather than presenting a review, I propose to reconsider and re-examine some important older experiments and some more recent information bearing on the same problems, in the hope of highlighting what opportunities and challenges there are for the student

of development. There are limitations of space as well as my own limitations and I shall restrict consideration to two broad issues: the determination of early lineages in the sea urchin embryo, especially the role of cell interactions in establishing the overall pattern of the embryo; and the cellular and molecular bases of gastrulation which lead to the formation of the organs of the pluteus larva.

Practitioners of molecular biology, cellular biology or embryology tend to have their own sets of biases or assumptions particular to a given field. One may occasionally detect a hint of arrogance among adherents of one approach or another. My own personal bias is that the time is ripe for a concerted application of the joint tools and approaches of cell and molecular biology and experimental embryology to problems of development. I believe that nowhere is this more apparent than in the developing sea urchin.

Though most biologists realize that the sea urchin embryo has had an important role in the history of physiology, genetics and even molecular biology, it is not so well remembered that information from these creatures played a crucial role in some present ideas about embryology, *per se*. It was here that the difference between the prospective fate and potency of a cell was clearly understood and underlined (Horstadius, 1939). Our ideas and language about the nature of determination and commitment of cells largely come from work on sea urchins and amphibians. It is well known that the ideas of gradients that led to modern ideas on positional information were first applied in a detailed way to sea urchin embryos (Runnstrom, 1929). Some of the first penetrating analyses of the cellular basis of morphogenetic movements were carried out in sea urchins (Gustafson & Wolpert, 1967).

The ability to isolate mutants in *C. elegans* and *D. melanogaster* affords great power in the analysis of development. On the other hand, development in these two organisms depends heavily on stereotyped cytoplasmic–nuclear interactions to establish lineages. This contrasts with the situation in sea urchins and amphibians where cell interactions are extensively used to found lineages and establish pattern. Sea urchins, like amphibians, are accessible for analysis of the cellular basis of morphogenesis. While standard diploid genetic analysis is not presently practical in sea urchins (Hinegardner, 1969), the power of paragenetic analysis afforded by recombinant DNA technology is now applicable (Flytzanis *et al.* 1985). It is in the area of the mechanisms of the origin of lineages and the role of cell interactions in this process, as well as the relationship of changing cell phenotype to morphogenesis, that the study of the sea urchin embryo may be able to make general contributions to the study of development.

The reader may wish to consult some excellent reviews of different aspects of development of sea urchins. In addition to Horstadius' masterly treatment of the classical embryology of the sea urchin embryo, (1939, 1973), there are excellent analyses of classical work in the book edited by Czihak (1975), especially the chapter by Okazaki (1975). The third edition of Wilson's 'The Cell in Development and Heredity' (1925) is still a valuable source. The original (1973) and new installment of the book by Giudice treats the literature in detail (1986). Solursh has written an excellent account of morphogenesis and cell motility (1986), and Gustafson & Wolpert's stimulating analysis of morphogenesis (1967) treats many problems. Spiegel & Spiegel (1986) and McClay (1987; McClay & Ettensohn, 1987) have also written recent reviews of selected aspects of morphogenesis. Eric Davidson and his colleagues have published several reviews on gene expression in early sea urchin development (Angerer & Davidson, 1984); the new third edition of 'Gene Expression in Early Development' by Davidson (1986) treats both molecular biological and embryological issues in sea urchin development in a penetrating way.

Determination

Cell interactions, embryo patterning and early determination of cell lineage

The study of cell determination and patterning of the embryo has been dominated by the double gradient theory of Runnstrom (1929). These ideas, derived in large part from experiments and theories of Child (see Child, 1940, for a review), have a strikingly modern ring, though they lack the mathematical foundation of newer theoretical work (e.g. Meinhardt, 1982) and lack the insight of Lewis Wolpert (1971) that gradients have to be 'interpreted' by the cells of the embryo if they are to amount to anything.

What Runnstrom proposed was that the early sea urchin egg and embryo were pervaded by two opposing gradients; the one was a graded potentiality to form skeleton, gut, muscular elements and coelom, the so-called vegetal characteristics. This potentiality was strongest and localized near the vegetal end of the egg, a region that could be identified in *P. lividus* because of a subequatorial pigment band in the cortex of eggs from some females of this species. The other gradient favoured a tendency to form very long cilia opposite the site of blastopore formation. Some animal halves will also form a thin epithelium typical of gastrulae, a ventral ciliary band and sometimes a stomodeum. These animal characters, especially the long cilia and lack of internal organs, are somewhat difficult to define in terms of specific differentiated

characteristics. Nonetheless, the idea of Runnstrom that the two gradients of potentiality, animal and vegetal, run in opposite directions to one another, somehow interact and are occasionally 'mutually hostile' to one another, has been the basis for interpretation of almost all subsequent experiments in this area. The brilliant transplantation experiments of Horstadius (1973) showed clearly that interactions between blastomeres may cause spectacular transformation in cell fate; they have been primarily interpreted as a result of interaction of the two gradients.

Let us briefly look again at the evidence that the egg and early embryo have an inherent organization that specifies patterns; we shall examine both the oral–aboral axis and the animal–vegetal axis. The evidence for a graded potential of development in sea urchin eggs forms one of the foundations of classical experimental embryology. If egg fragments or blastomeres derived from eggs by cleavage are separated along the animal–vegetal axis, resulting in meridional fragments, the separated parts form embryos with more or less equal and complete representation of the major organ systems of the embryo, consisting of ciliary tufts, skeleton, gut, coelomic rudiments and surface epithelium. The result of the experiment is shown in cartoon fashion in Fig. 1A.

However, the total and equal developmental potential of meridional fragments or blastomeres is not really securely established. The early experiments of this kind by Boveri (1901, 1918), Driesch (1894) and Hebst (1900) did not examine blastomeres and fragments derived from a single egg, the result only showing that a portion of an egg could give rise to a larva. In 1927, Plough reported that often one or both members of a pair of isolated blastomeres from a 2-cell stage developed poorly, slowly or abnormally. Horstadius & Wolsky (1936) also observed this; using both *Lytechinus pictus* and *S. purpuratus*, we have also frequently observed in our laboratory that both blastomeres of the 2-cell stage may not each give rise to a perfect pluteus (unpublished; Maziu, 1958). Marcus (1979) carefully analysed development of separated blastomeres of *A. punctulata* at the 2-cell stage, in which about 60% survived to pluteus as complete pairs. Of the 10% of the separated blastomere pairs that survived metamorphosis, a large proportion showed abnormalities in size and in anatomy of the ocular, genital and periproct plates of the mature urchin.

If there is any predisposition to have localized developmental potential along an oral–aboral axis, this would be revealed by development of isolated blastomeres or fragments from the 2- and 4-cell stages. However, the diagnosis of oral or aboral is difficult to make by solely anatomical criteria until the invagination of the archenteron is complete and the stomodeum forms. Manipulations that interfere with the mechanics of gastrulation will affect the interpretation of the experimental result, regardless of the effect on potential to form oral or aboral structures. Furthermore, this experiment can only assess fairly stable or irreversible biases in potential. Hence, there is in the literature no decisive analysis of localization of oral–aboral potentiality. In his masterly summary of these experiments, Horstadius (1973) concludes that the first two meridional divisions do distribute potential along the aboral–oral axis and Czihak's histochemical staining results, showing localized staining of cytochrome oxidase (1963) along the oral–aboral axis at the 16-cell stage certainly sustains this point of view. The recent lineage tracer experiments of Cameron *et al.* (1987) reveal axis specification by the 8-cell stage.

The subject of the equipotentiality of early blastomeres and fragments along the meridional axis is ripe for renewed analysis. Our understanding of gastrulation is now better, allowing design of experiments in

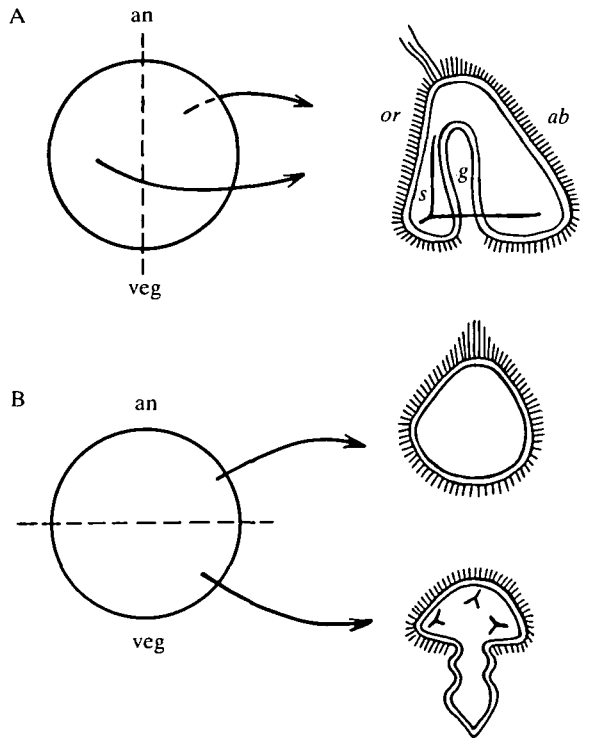

Fig. 1. The classical result of meridional (A) or equatorial (B) separation of egg fragments or blastomeres is depicted. As discussed in the text, meridional separations give rise to almost normal larvae (A). Separation of animal (an) from vegetal (veg) fragments along the equatorial plane (B) give rise to arrested blastulae from the animal half. Vegetal fragments may give rise to exogastrulae, as depicted. Vegetal fragments may also give rise to normal larvae, as discussed in the text. *or*, oral; *ab*, aboral; *s*, spicule; *g*, gut; *an*, animal; *veg*, vegetal.

which abnormalities of gastrulation caused by a limited number of cells or purely mechanical considerations may be taken into account. Even more important, there are easily applied, quantitative markers of potential along the oral–aboral axis that have recently become available. Among these markers are the *Cy III* genes of the actin family that are exclusively expressed in aboral ectoderm cells, beginning at late blastula (Cox *et al.* 1986). The family of genes termed 'spec' (Lynn *et al.* 1983), which encode troponin-like proteins, are expressed in a localized fashion in the aboral ectoderm cells. There is also an epitope recognized by a monoclonal antibody (Ecto V) isolated in McClay's laboratory (McClay & Ettensohn, 1987) that is characteristic of the ectodermal cells in the stomodeal anlagen and the foregut endoderm. The expression of any of these characters would have to be analysed during cases of abnormal morphogenesis to determine if any of them are expressed if gastrulation is defective. If any of the above indices of aboral differentiation pass this test, they will be admirable tools that may be applied to individual embryos to re-examine this old question of blastomere equivalence and determination of the oral–aboral axis.

The difference between results with the meridional fragments that we have just discussed and equatorial fragments is strikingly illustrated in recent work by Maruyama, Nakaseko & Yagi (1985). They capitalized on Schroeder's (1980*a*,*b*) important observations, prefigured in much earlier work by Boveri (1901), that there are clear indications of the animal–vegetal axis in unfertilized eggs (see Sardef & Chang, 1985). The position of the polar bodies is later revealed by a discontinuity in the jelly layer around the egg, the jelly canal, unfortunately called a micropyle by some older workers. Sperm may activate the sea urchin egg anywhere on the egg surface. The position of the first cleavage plane bears no relationship to the site of sperm activation. Rather, in over 90% of the cases, the first cleavage plane traverses the egg through the plane defined by the jelly canal and its opposite pole. The second cleavage traverses the same jelly canal axis, thus producing four blastomeres separated along the meridional axis. The third cell division is orthogonal to the first two, bisecting the jelly canal axis, resulting in an equatorial separation (Fig. 1). Thus, the jelly canal indicates the classical animal–vegetal axis; the canal can be revealed by placing unfertilized eggs in a suspension of very fine ink particles, which penetrate the discontinuity of jelly that defines the canal.

When Maruyama *et al.* (1985) fertilized meridionally bisected eggs both halves formed gastrulae whether they contained the female pronucleus or not. Though the androgenetic haploid merogons derived from anucleate halves cleaved more slowly, they were able to form imperfect plutei with the main organs present. Separation of the egg along the equatorial plane gave a very different result. In 90% of the cases, the equatorial egg fragment from the animal half (where the jelly canal is located) never undergoes gastrulation, never forms pigment, never forms mesenchyme or its derivatives and instead forms a 'permanent' blastula. The long immobile cilia, characteristic of embryos of this half, do eventually become replaced with shorter beating cilia. This is the classical 'animal' development observed by many others.

Egg halves from the equatorial fragment opposite the jelly canal formed fairly normal plutei in almost all of 160 cases; the photographs show that the resultant larvae are not quite normal, having less-extensive skeletal elements, but all major organ systems are present in the correct positions. The presence of the animal half is thus apparently not important for overall patterning or development of any of the tissues of the embryo, playing at best only a subtle modulatory role. This is in accord with numerous observations from the time of Driesch that vegetal egg halves may often develop into normal larvae (Wilson, 1925).

The results of Maruyama *et al.* (1985) are distinctly different from the usual predictions of the double gradient hypothesis. Fig. 1B shows the classical interpretation of Horstadius (1939). Animal halves give rise to permanent blastulae, like those observed by Maruyama, while vegetal halves give rise to creatures with little surface epithelium, overdeveloped or exogastrulated archenterons, lack of a defined oral–aboral axis and abnormal spicules. As pointed out by Horstadius (1939) and Wilson (1925) among others, there is considerable variation in the ability of vegetal equatorial halves to give complete larvae, although this variation is relatively consistent in eggs from a given female. In any case, it is clear that vegetal halves frequently give rise to complete larvae and the results of Maruyama *et al.* (1985) show consistent near totipotency of the vegetal equatorial half.

The idea of a double gradient, as opposed to a single centre of influence emanating from near the vegetal pole, is based on several considerations. First, the isolated animal half has accentuated development of one feature, the length of apical cilia, and so it is argued that this is an example of unrestrained animal tendency. This seems to be the main morphological index of an 'animal tendency'. The second basis for the idea comes from cell interactions revealed by transplantation. If one isolates blastomeres arising from the middle portion of a 32-cell-stage embryo, as shown in the cartoon of Fig. 2, normal embryos may

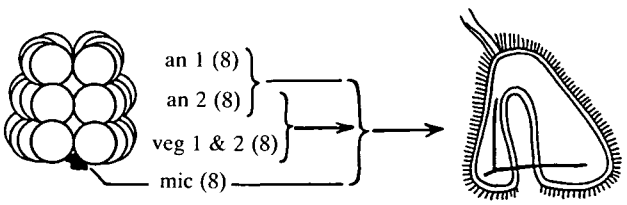

Fig. 2. The result of transplantation of blastomeres of the 32-cell stage is shown schematically. If micromeres (black) are combined with blastomeres from the animal half (an 1 + an −2), a normal larva may result. If vegetal blastomeres are combined with animal blastomeres a normal larva also may result. The cartoon is meant to reflect interpretation of transplantations found in the classical literature.

result. Or, if blastulae derived from animal blastomeres combined with micromeres of the extreme vegetal end are allowed to develop, a normal pluteus may result. The classical interpretation of these results is that the manipulations still allow the developing embryo to contain the correct ratio of animal and vegetal information.

It is very difficult to distinguish reciprocal tissue interactions and inductions by micromeres or veg 2 on the one hand, from a balance of opposing forces on the other, as recently pointed out by Davidson (1986). There are, indeed, many observations that do not conform to the double-gradient hypothesis. I have just mentioned some of these, including the development of normal larvae from vegetal halves and the inability of isolated micromeres to form anything other than skeleton. There has been no adequate way to distinguish a passive role of the animal half from an active one. One of the more provocative experiments is that isolated *animal* halves, when treated with the vegetalizing agent, Li^+, do form nearly normal larvae. Both von Ubisch (1929) and later Horstadius (1939) showed that one could obtain normal plutei from animal halves treated with Li^+. The mechanism of action of Li^+ is completely elusive, but the complex set of epigenetic events set in motion is equivalent to influencing the animal half by micromere implantation. Clearly, the animal half possesses regulative ability, may be influenced in its determination and this effect may be caused by an ionic imbalance!

It has been shown recently that Li ions also evoke appearance of dorsal and anterior structures in amphibian eggs that have been exposed to u.v. irradiation and thereby rendered incapable of forming normal dorsal–anterior structures (Kao, Masui & Elinson, 1986). This effect of Li^+ on amphibian eggs is not displayed by a large range of other cations. No theory of Li^+ action has been proposed, but recent work has shown that Li^+ interferes with the regeneration of phosphatidylinositol lipids, which are involved in the inositol phosphate secondary messenger pathway (Majerus *et al.* 1986). At any rate, the effects of Li^+ make theories of localization of morphogenetic determinants in the vegetal egg half less compelling.

Another result bearing on the importance of gradients and localized substances is a set of experiments by Nemer, Wilkinson & Travaglini (1985) in which the classical, and most effective animalizing agent, Zn^{2+}, is examined for its effects on gene expression. This is the first time an animalizing or vegetalizing agent has been examined directly for effects on expression of a particular tissue-specific gene. Nemer, using embryos of *S. purpuratus*, examined effects of Zn^{2+} treatment on expression of the gene *spec1*; *spec1* encodes a troponin-C-like protein which occurs only in the aboral ectoderm of later-stage gastrulae. Spec 1 mRNA is localized only in aboral ectoderm and thus acts as a good indication of differentiation of cells of the animal hemisphere (Lynn *et al.* 1983). Nemer found that Zn^{2+} treatment causes inhibition of transcription of *Spec1* genes and, if Zn^{2+} is removed by the chelator EDDA, normal development and *spec1* expression resume. Hence, Zn^{2+} treatment of whole embryos causes a developmental arrest, a kind of diapause, and not, apparently, a change in cell potential or fate. It will be interesting to learn if surgically isolated animal halves display the same kind of inhibition of *spec1* gene expression; the failure of *spec1* expression in isolated animal halves, were this the result, would imply an inductive effect of the vegetal half on the animal portion of the embryo and it would strongly argue against an 'animalizing' gradient.

An alternative to the double-gradient hypothesis is that separation of the animal half from materials in the vegetal hemisphere causes developmental arrest of animal cells. The animal half cells are totipotent and may be provoked, either by induction or by exposure to Li^+, to adopt new fates and form a complete embryo. The distinction between gradients of materials, somehow disposed in the vegetal half, and localized determinants may not be a useful distinction. Rather, one might emphasize the importance of early cell and tissue interactions, comparable to ideas about early amphibian development (Wylie, 1986). The use of newly available tissue-specific markers of gene activity, in conjunction with isolation, defect and transplantation experiments, may provide a good way to re-examine the cell interactions in echinoderms.

If cell interactions are important, one would predict that interruption of cell interactions would have important consequences for expression of animal

hemisphere lineage-specific markers, less so for vegetal half markers like spicule matrix proteins (Benson *et al.* 1987; Sucov *et al.* 1987) and collagen (Venkatesan, de Pablo, Vogeli & Simpson, 1986). Cameron, Hough-Evans, Britten & Davidson (1987) have recently published a careful analysis of the fate of the blastomeres of the 8-cell stage; aboral ectoderm is derived from blastomeres of both the animal and vegetal hemispheres and, therefore, aboral-ectoderm-specific markers may not be adequate indicators of animal hemisphere differentiation. Oral ectoderm is solely derived, however, from the animal half and the discovery of oral-ectoderm-specific markers would be welcome. Treatment of isolated animal half cells with Li^+ should cause appearance of vegetal-specific gene expression in animal half cells. Treatment of isolated cells or embryo parts with Zn^{2+} should suppress gene expression generally, and be reversible with EDDA. One can isolate cells of different determined types microsurgically. Micromeres may be isolated *en masse* (Okazaki, 1975), as can primary mesenchyme (Ettensohn & McClay, 1986). It should be possible using these isolated cell types to study mechanisms of their interactions. For example, the extracellular components synthesized by these cells may be released into the medium or may coat the culture substrate. Once these molecules are characterized, their functions may be evaluated by utilizing the medium or culture substrate as a component of the culture of either isolated cells or entire embryos.

Another consideration is a closer examination of the inductive and differentiative capacities of the cells derived from the vegetal half of the egg. The classical view assigned a single potentiality, the vegetal influence, to this hemisphere, but this surely cannot be correct. The vegetal half normally selfdifferentiates to form part (anal plate and aboral ectoderm) of the epithelial surface of the blastula (veg 1), the archenteron, pigment cells of the surface epithelium, secondary mesenchyme, and primary mesenchyme and its derived spicules. In addition, a special population of cells in the coelomic sacs that resume division at metamorphosis are derived from the original micromeres (Pehrson & Cohen, 1986). Furthermore, in embryos devoid of micromeres, skeletons may form and, in this instance, Horstadius (1973) reports that it is secondary mesenchyme cells (that normally form muscle) that transdifferentiate into skeletal elements. Others have noticed that in the absence of primary mesenchyme, the secondary mesenchyme will descend and assume the role of primary mesenchyme, forming a skeleton (von Ubisch, 1939; Langelan & Whiteley, 1985; Ettensohn & McClay, 1986). In an especially interesting study apparently unknown in the Occident, Fukushi (1962) showed that, if one removed the primary mesenchyme cells from a mesenchyme blastula, the secondary mesenchyme cells (derived from veg 2 tier) will change their morphogenesis and differentiation and form a normal skeleton. While cells from veg 2 and perhaps even veg 1 have a wide potency, the extreme vegetal end, the micromeres, are limited in their potency, forming only skeletal elements and cells destined for the echinus rudiment. There are no reported examples of change in micromere fate. Micromeres also have morphogenetic influence on neighbouring cells, which will be discussed later.

There are indications that the fourth cleavage leading to separation of micromeres from macromeres in the vegetal hemisphere is important in establishment of lineages, thereby setting up participants for the interactions that follow. For example, one may upset the normal time schedule of division in a number of ways, and thereby cause the third division to form an 8-cell embryo which has an animal quartet of very large cells and a vegetal quartet of small cells. Such small cells, so-called precocious micromeres, behave as micromeres from a 16-cell embryo and may form spicules autonomously, in culture (Kitajima & Okazaki, 1980).

The normal cell division of the fourth cleavage is unusual in the vegetal quartet and has been described by Dan and his associates (Dan, 1979; Dan, Endo & Uemura, 1983). First, the nuclei of the vegetal quartet of the 8-cell stage move toward the vegetal pole prior to prophase and the spindles that then assemble are very close to the cortex of the vegetal pole, even apparently pushing some granules of the cortex in this region to one side (Dan *et al.* 1983). It has been suggested that this very close association of the spindle pole with the cortex may be important for formation of the micromere lineage. In support of this idea, the asymmetric fourth division may be suppressed with different agents; notably, treatment with SDS prevented nuclear migration and resulted in embryos that did not develop primary mesenchyme or spicules (Tanaka, 1976). Nonetheless, since this is a negative result, one must be cautious, especially since there are numerous examples in the classical literatures to which I have already alluded that support the idea that the peculiar cleavage plane at the fourth division is dispensible (Horstadius, 1939). Hence, the paper of Langelan & Whitely (1986) extending the experiments of Tanaka, using both sand dollar and sea urchin embryos, probably represents a more secure interpretation. They found that treatment with SDS does suppress micromere formation, but treated embryos were able to form primary mesenchyme and had normal spicules. Indeed, they were also able to suppress the asymmetric fifth cleavage and in a large number of cases completely

suppressed ingression of primary mesenchyme into the blastocoel. Nonetheless, these embryos underwent archenteron formation and secondary mesenchyme cells descended from the archenteron tip, formed a syncytial array, and a larva replete with gut and skeleton formed in a high percentage of cases. Clearly, the unusual asymmetric divisions at the fourth and fifth cleavages are not essential in these species, and any theory which posits that some special character of this division is essential for determination seems incorrect, both on comparative grounds (Raff, 198) and on the basis of the experiment of Langelan and Whiteley. The formation of spicules from cells emanating from the archenteron tip is very likely the same phenomenon observed in embryos surgically deprived of micromeres.

Manipulations of blastomeres of the early sea urchin embryo show that some cells have very great potentialities and cell interactions probably play a role in determination and origin of the normal pattern of the embryo. The results do not seem really to require a double gradient of opposing potentialities. In fact, because of the extremely wide differentiation potential of the cells in the vegetal half, it is difficult to know if there is a gradient at all. What is required to make an embryo is some cells from veg 2 and some other material from the animal half (or even veg 1). The cells in veg 2 or micromeres probably cause responding cells to adopt particular fates. It is likely that both localized determinants in the vegetal hemisphere and cellular interactions serve to define patterns of determination and differentiation.

How early and how stable is cell lineage specification?

The micromere lineage is apparently autonomous in most of its further development from the moment of its origin at the fourth cell division. Indeed, many cells derived from this lower vegetal portion of the egg may give rise to a cell lineage endowed with properties of primary mesenchyme, as I have discussed in the preceding section. There have been extensive investigations to catalogue the biochemical and cellular differences between micromeres and the remainder of the embryo, including histone types (Senger & Gross, 1978), complexity of RNA (Mizuno, Lee, Whiteley & Whitely 1974; Ernst, Hough-Evans, Britten & Davidson, 1980; Rodgers & Gross, 1978) and kinds of proteins synthesized (Tufaro & Brandhorst, 1979; Harkey & Whiteley, 1983; Kitajima & Matsuda, 1982); these findings have been reviewed and analysed extensively by others on several occasions (Wilt, Benson & Uzman, 1985; Harkey, 1983). The difficulty with the interpretation of a catalogue of differences between different cells is that it is impossible in the absence of a functional test to know which differences, if any, are developmentally relevant. There is good evidence, though, that the factors of the egg that result in the eventual formation of the primary mesenchyme are not easily dislodged by stratification of organelles using centrifugation (Wilson, 1925). Hence, some structural basis for the origin of the lineage may reside in the plasma membrane or the stiff submembraneous cortical region. DeSimone & Spiegel (1985) have recently labelled cell surface proteins of micromeres from *A. punctulata* and *S. drobachiensis* with ^{125}I. The pattern of labelled cell surface proteins is different in the two species, but in both cases there are distinct labelled bands in micromere cell surface proteins that are not present in surface proteins of the other blastomere types. Presumably, these labelled proteins exist in the egg and are distributed to the micromeres, though this has not yet been directly shown. Sano (1977, 1980) has shown that micromeres possess a different agglutinability by Con A during cleavage and also that these cells have a somewhat different surface charge, as measured by their electrophoretic mobility. It has been reported that micromeres will 'cap' lectins, while mesomeres and macromeres will not (Roberson, Neri & Oppenheimer, 1975).

The stability of specification of the micromere lineage is probably strict, though this issue has not been adequately tested. By specification, we mean the bias to form a given tissue when development occurs in isolation. By determination, we mean the bias to form the same phenotype as the normal fate when cells develop in an ectopic location. The fact that micromeres, when isolated or transplanted, form skeleton does not entirely settle the issue, for if their descendants form other tissues, this could not have been detected. In a sense, changes in specification or determination can only be demonstrated, but not the converse. One way to examine the stability of determination is the dissociation–reaggregation experiment using marked cells. In this instance, micromeres, or any other blastomeres, may be exposed to a new environment and the stability of early specification examined. Of course, transplantation is another alternative, and one might use [^{3}H]thymidine or vital lineage tracer dyes to check the results of earlier work.

It is not even known if the properties of the micromere lineage are a necessarily coherent set of behaviours. Micromeres do give rise to another group of cells that divide once, then remain quiescent, and later resume division in the rudiments that will form the adult urchin (Pehrson & Cohen, 1986). The regulation of the formation of this sublineage is not understood. Furthermore, primary mesenchyme precursors are entrained in a different rhythm of cell division from their siblings (Dan, Tanaka, Yamazaki

& Kato, 1980; Masuda & Sato, 1984). It is claimed that the micromeres provide some mitotic pacemaker for waves of cell division during the latter half of cleavage (Filosa, Andreuccetti, Parisi & Monroy, 1985). The importance of this pacemaker phenomenon is not clear, especially since micromereless embryos may form plutei. The progeny of micromeres do change their affinities for one another and for the hyalin layer and basal lamina (McClay & Fink, 1982; Fink & McClay, 1985). And they become motile and display a complex set of behavioural qualities, including fusion, ectopic archenteron formation and induction, as discussed before (Solursh, 1986).

The timing and stability of determination of other cells in addition to micromeres in the early embryo is also not known. Presumably all layers other than micromeres have some lability in their specification and determination during mid and late cleavage. When Horstadius transplanted various tiers of cells to ectopic locations, the experiments were usually carried out on cells from the 32- to 64-cell stage. The results showed cells from the animal and vegetal layers participated in forming patterns of various types in a regulative fashion. It is not so clear what the cellular basis for this regulation of pattern might be. Was there a respecification of cell fate, or were there modifications of the rate and pattern of cell divisions so that some cells reformed a pattern by differential cell division, or were there changes in morphogenetic movements that somehow led to regulation. These are, of course, not mutually exclusive possibilities. Probably the clearest case is the transplantation of micromeres to an isolated animal half in which invagination and archenteron formation take place in animal half cells. This constitutes a change in cell fate of animal cells from surface epithelium to archenteron lining, mesenchyme, etc. Data shown in line drawings of the embryos formed from these transplants look like normal plutei; one presumes they have functional tissues and that lineage-specific genes are expressed, but this has not been definitively shown. It appears there is a genuine specification of animal cell fate due to induction by micromeres. Transplantation experiments that include any of the veg 1 or veg 2 tiers in the transplant recipient or donor are very difficult to interpret because isolated vegetal halves that contain these cells may give rise to normal plutei.

Thus, even though the brilliant line of experimenters beginning with Boveri, Driesch and Herbst, and continuing with Horstadius and von Ubisch were seminal, their investigations were limited, as are we all, by the tools of the time. Modern micromanipulators, stable lineage-tracer dyes, monoclonal antibodies and cell-lineage-specific genes that can be used as markers open up new possibilities to investigate lineage stability and transformation in a rigorous way. For example, animal half cells may be marked with tetramethylrhodamine isothiocyanate (Ettensohn & McClay, 1986). Unstained micromeres may be combined with such an animal half and the resultant regulated embryo examined by *in situ* hybridization for type IV collagen genes (Venkatesan *et al.* 1986) or cell surface proteins (Leaf *et al.* 1987) expressed in primary and secondary mesenchyme to see if there has been a respecification of the marked animal half cells.

One method for testing stability of cell determination mentioned previously is the dissociation and reaggregation of cells, a method easily carried out with echinoderm embryos at a number of different developmental stages, as shown by the work of Giudice and his colleagues. This work has been reviewed extensively by others (Giudice, 1973, 1986; Spiegel & Spiegel, 1986). In some cases, even when dissociation is carried out at blastula stages, embryos will form that have a surface epithelium, skeleton and gut. Experiments have not yet been performed to see if these reconstructed tissues are correctly expressing lineage-specific genes. Surprisingly, there has been very little work to distinguish cell type transformation from sorting out. Nor has the influence of small nests of incompletely dissociated cells been evaluated. Melvin & Evelyn Spiegel (1975) have demonstrated that a mixture of dissociated cells from *Arbacia* and *Lytechinus* sort out and reconstitute larva-like structures. Spiegel (1978) also reaggregated micromeres stained with Nile blue sulphate with mesomeres and macromeres from unstained embryos and showed that the micromeres sort out within 4 h and go on to form primary mesenchyme. One could also test the determination of mesomeres, macromeres and other cell types in this kind of experiment, but it has not yet been done. Jenkinson (1911) cut mesenchyme blastulae and gastrulae into large pieces and found very limited regulative abilities at these stages, though this type of experiment might be useful if modern markers could be used.

There is some evidence that suggests that the blastomeres of the early embryo possess a stable apicobasal polarity. Perhaps the original egg plasmalemma defines a polarity for the blastomeres; new membrane may appear on the blastocoel sides of the blastomeres and thereby provide a stable cell polarity marker, much as is the case in amphibian blastomeres. Marina Dan-Sohkawa and her co-workers (Kadokawa, Dan-Sohkawa & Eguchi, 1986) have recently shown that dissociated starfish blastomeres display an inherent apical–basal polarity and that when the aggregates form a gastrula this polarity is intact. As cell division stops, septate desmosomes appear in the epithelium of the blastula of sea

urchins, as well as tight and gap junctions. Amemiya, Akasaka & Terayama (1982) examined the polarity of archenteron cells in the gut formed by exogastrulation. They found that the ciliated prospective luminal side, which is now the superficial side exposed to sea water in the exogastrula, still retained its original polarity. Spiegel & Howard (1985) have shown that the morphology of septate desmosomes in the gut of exogastrulae have normal morphology and tissue distribution in an exogastrula. It may be very informative to learn what properties of cells are autonomous when maintained as dissociated cell populations and what properties require cell associations. Though the stability of determination is difficult to discern from the available evidence, it is likely that use of new molecular markers will be useful in interpretation of embryological experiments.

Morphogenesis

The events resulting in specification of cell lineage are a necessary prelude to the actual morphogenesis that forms the pluteus. The activities of the individual cells and their responses to cues that transcend domains of single cells drive the formation of the embryo. These morphogenetic activities in turn entrain further refinements in the definition of the pattern of differentiation. It is appropriate to inquire how determination in early stages brings about morphogenetic movements and how these movements in turn lead to further pattern specification and differentiation. I shall discuss the morphogenetic movements of primary mesenchyme cells and then turn to gastrulation.

Morphogenetic movements of primary mesenchyme

Since the behaviour of primary mesenchyme cells has been so well described in recent reviews, I wish to re-examine only a limited number of issues that seem especially puzzling or ripe for analysis. The morphogenetic movements of the sea urchin embryo were the subject of a provocative review by Gustafson & Wolpert in 1947. They emphasized the role of cell adhesion and changes in cell shape and motility as driving forces in morphogenesis. The changing adhesive properties of primary mesenchyme cells have been the subject of many studies since then and there are several recent reviews (McClay & Ettensohn, 1987; Solursh, 1986; Spiegel & Spiegel, 1986; Watanabe, Bertolini, Kew & Turner, 1982). New ways of testing the affinities of primary mesenchyme cells, and other types of cells, are consistent with the view that there are changes in the affinities of cells for one another, for hyalin and basal lamina, and that these changes help explain the ontogeny of behaviour of primary mesenchyme. Proof of these speculations will come when the molecular basis of cell affinity and adhesion is better understood. It might then be possible to carry out experiments aimed to change the adhesive properties of cells by judicious use of inhibitors, antibodies, anti-sense RNA of sequences encoding putative adhesive components, or DNA-directed transformation of cells with sequences encoding adhesive molecules fused to inappropriate promoters.

Once the primary mesenchyme cells have ingressed into the blastocoel, the behaviour of the cells and their relationships to the extracellular environment is very complex. The developmental autonomy of cells derived from micromeres seems clear; the general sequence and relative timing of cell division and of changes in adhesivity and motility all occur on schedule in isolated micromeres (Okazaki, 1975). However, it is not known what effect cell associations have on all these later behaviours of the micromere lineage. Do cells have to remain associated in order for motility to ensue or for spicule matrix genes to be transcribed? In other words, what, if any, are the homotypic cell associations necessary for the morphogenesis of this lineage? The goal of research in this area has been to describe the details of motile behaviour of primary mesenchyme cells, to learn what molecules of the cell surface are important in motility and in interactions of the cells with each other and with the environment, and to describe the changing nature of the extracellular environment in the blastocoel. Solursh's (1986) recent review clearly describes the history and present status of work in this area. The experimental tasks are very difficult and we are only at the very beginning. Though primary mesenchyme cells are extremely motile (Karp & Solursh, 1985*a*,*b*), neither the internal machinery nor the external molecules involved in motility or adhesion are known. The blastocoel and basal lamina contain sulphated glycosaminoglycans, collagen, fibronectin and laminin (Solursh & Katow, 1982; Spiegel, Burger & Spiegel, 1983; Katow & Hayashi, 1985). The proportions and nature of the molecules change during gastrulation as primary mesenchyme cells undergo their motile, then skeletogenic phases (Karp & Solursh, 1974). Interference with biosynthesis and processing of the various components results in changes in the matrix molecules and inhibits primary mesenchyme morphogenesis and differentiation. Reversal of treatments with sulphate-free sea water or β-xylosides may allow development to resume. Since most of the treatments cause negative effects, e.g. cessation of morphogenesis, it is difficult to draw rigorous conclusions. There are some interesting recent studies that illustrate the complexity of this whole area.

Blankenship & Benson (1984) studied the formation of skeletal spicules from isolated, cultured

micromeres in the presence of inhibitors of collagen metabolism. They found, as had others (Mintz, DeFrancesco & Lennarz, 1981), that exposure of the cultured cells to analogues of proline or inhibitors of post-translational modifications of collagen, resulted in inhibition of spicule formation. If, however, the culture dishes were coated with rat tail tendon collagen before use in the experiment, the same inhibitors had very little effect on the ability of micromeres to form spicules. This finding suggests that primary mesenchyme cells may synthesize collagen and that collagen then becomes part of a permissive environment favouring differentiation into spicules. In line with this finding, Venkatesan *et al.* (1986) have isolated a portion of a collagen gene from a genomic library of *S. purpuratus* using a mouse alpha 1 (IV) cDNA probe. The mRNA for this collagen in the embryo is 9 kb, appears first at the morula stage and becomes prominent on RNA blots by the mesenchyme blastula stage. This mRNA for a putative type IV collagen is found localized in primary mesenchyme cells (personal communication). It is possible that primary mesenchyme cells synthesize some collagen, which then forms part of the fibrous environment of the blastocoel (Crise-Benson & Benson, 1979) or even part of the basal lamina. Wessel & McClay (1985) have discovered an antigen (meso 1) of the plasma membrane that appears on the surface of primary mesenchyme cells just as they ingress and that later become evident in the basal lamina along the blastocoel. Kitajima & Matsuda (1982) have followed the synthesis of different proteins by cultured micromeres, as have Harkey & Whiteley (1983). More recently, Kitajima (1986) carried out some of their experiments with micromeres on an agarose surface, which almost totally prevents attachment of cells to the substrate; the isolated micromeres differentiate while floating in the medium in grape-like clusters. Though definite proof is lacking, the idea that primary mesenchyme cells contribute in an important, though perhaps permissive, way to their own microenvironment is in accord with the facts.

As the primary mesenchyme cells undergo morphogenesis *in vivo*, the composition and morphology of the extracellular material in the blastocoel changes (Akasaka, Amemiya & Terayama, 1980, 1982). Katow & Amemiya (1986) describe the appearance of 15 nm fibrils during gastrulation (Kawabe, Armstrong & Pollock, 1981). Both the surface of the primary mesenchyme cell and the blastocoel side of the basal lamina are associated with granules (15–30 nm) that stain with ruthenium red and may be proteoglycan. Treatment of embryos with sulphate-free sea water inhibits sulphation of the sulphated glycosaminoglycans (predominantly chondroiton-6-sulphate, dermatan sulphate and heparin sulphate) and results in a reduction of the amount of the granular component (Katow & Solursh, 1979; Solursh & Katow, 1982). Treatment of sea urchin embryos with β-D-xylosides, which interfere with synthesis of glycosaminoglycan attached to proteoglycan cores and may result in accumulation of free glycosaminoglycans, also results in depression of appearance of the granular component (Solursh, Mitchell & Katow, 1986). Both xylosides and sulphate-free sea water allow ingression of primary mesenchyme cells into the blastocoel, but migration of the ingressed mesenchyme is inhibited. Katow (1986) has studied the mobility of isolated primary mesenchyme cells *in vitro* on different substrates and gels in plastic dishes. Fibronectin, laminin, type IV collagen and dermatan sulphate were all tested as substrates for migration; only combinations including fibronectin and collagen allowed migration, and a gel composed of fibronectin, collagen and dermatan sulphate was superior. Venkatasubramanian & Solursh (1984) and Katow & Solursh (1981) also studied migration of primary mesenchyme cells *in vitro* and found that mesenchyme cells from embryos treated with sulphate-free sea water had an inherently lower capacity to migrate, even on competent substrata. The effects of sulphate deprivation may, therefore, affect the migratory ability of primary mesenchyme cells as much as or more than affecting the suitability of the substrate for motility. It is appropriate to remember that during this period of primary mesenchyme cell morphogenesis there is intense synthesis of tunicamycin-sensitive *N*-glycosylations of protein, studied by Lennarz and his associates (Heifitz & Lennarz, 1979; Lau & Lennarz, 1983; Schneider, Nguyen & Lennarz, 1978). Inhibitors of glycosylation interfere with primary mesenchyme cell differentiation and with gastrulation. Once again, all the observations are consistent with the importance of a multicomponent extracellular material, some components of which may arise, in part, from the mesenchyme cells themselves.

Morphology of spicules

The spicules that form in the living embryo often have a very complex morphology and the details of the arrangements of hooks, spurs, fenestrations and shape are species specific (Okazaki, 1975). The regulation of the formation of these beautiful patterns has been a topic of interest to students of morphogenesis for a long time. This topic has been studied by several methods and has been reviewed by Solursh (1986). The focus of attention has been directed to finding out whether the primary mesenchyme cells, the extracellular material or the epithelial wall contribute to the final detailed pattern. Of course, these roles are not mutually exclusive and the accumulated evidence suggests that all three of these participants

play some role. Von Ubisch (reviewed in 1939) showed this clearly in chimaeras formed from transplantation of micromeres of one species to embryos or animal halves of another species. Whether the spicule is simple or fenestrated is a property of the primary mesenchyme cell. The overall position of the spicule and placement of rods seems to conform more to the source of the epithelial wall. When primary mesenchyme cells of two species are enclosed in an epithelium from only one of the species, the result is often so complex it is difficult to interpret. In some ways, the interpretation of experiments on spicule pattern depend on which observations one chooses to emphasize. For example, in Okazaki's (1975) pioneering work on development of isolated micromeres, she pointed out that frequently rather normal-looking spicules would form in the absence of blastocoel walls (but not of extracellular material). On the other hand, Harkey & Whitely (1980) concluded from studies of development of spicules in isolated bags of basal laminae that the presence of epithelium was important to the final pattern. Undoubtedly both views are correct and the terminal differentiation of spicule from the cells may represent an excellent opportunity to study how different tissues, cells and extracellular materials may interact to realize a complex pattern. Kinoshita & Okazaki (1984) carried out an important study in which details of the morphology of the spicules that form *in vitro* were studied. They found that if micromeres adhere to the substrate, needle-like spicules form. Under conditions in which the micromeres formed cell aggregates the resultant spicule was three dimensional and similar to the spicule formed *in vivo*.

Ettensohn & McClay (1986) have recently devised a way to vitally stain primary mesenchyme cells, then to inject them into the blastocoel of another embryo of the same species. Using this technique, they were able to show that the migration and disposition of the mesenchyme cells is dependent upon the stage of the host. Interactions of primary mesenchyme with the blastocoel environment of the recipient embryo are important until the late gastrula stage. For instance, after placement of primary mesenchyme into the blastocoel of an early prehatching blastula, the implanted mesenchyme cells remain quiescent until the host cells begin their ingression, then the implanted cells assume their typical position in the blastocoel and integrate with the host primary mesenchyme cells. The availability of culture methods and our better understanding of the nature of the extracellular material and cell surface makes molecular studies of these phenomena now possible.

DeSimone & Spiegel (1986*a*,*b*) have carried out several studies characterizing the effects of injection of concanavalin (con A) and wheat germ agglutinin (WGA) into the blastocoel. WGA binds only after mesenchyme cell ingression and binding sites are especially prominent where primary mesenchyme cells contact the basal lamina. Con A binds more generally to the basal lamina and causes the epithelial cells to round up. Treatment of embryos with sulphate-free sea water alters the pattern of lectin binding. Iwata & Nakano (1985*a*) have extracted a polysaccharide from sea urchin embryos that binds to sea urchin fibronectin. Treatment of embryos or cultured micromeres with antibody directed against the fibronectin-binding polysaccharide enhances spicule formation and alters the morphology of the spicules that form. Iwata & Nakano (1986) have recently isolated a protein from sea urchin embryos that may be involved with Ca^{2+} binding, as have Carson *et al.* (1985). These proteins are distinct, however, from the structural protein of the spicule matrix recently characterized by Benson, Benson & Wilt (1986).

Primary mesenchyme and gastrulation

The micromere progeny supposedly play a role in gastrulation, though clearly primary mesenchyme cannot be essential. There are many reports in the literature in which sea urchin embryos deprived of micromeres gastrulate and form perfectly normal plutei (Horstadius, 1973). In cleavage-stage embryos deprived of micromeres, new micromeres are not formed, nor is primary mesenchyme formed by ingression. In fact, the primitive order of urchins, the Eucidaroidea, have no micromeres or primary mesenchyme, yet they gastrulate and form a skeleton (Raff, 1987). Embryos of the Asteroidea also have no micromeres or primary mesenchyme (Kume & Dan, 1968; Reverberi, 1971). It is curious, therefore, that transplantation of sea urchin micromeres to a lateral aspect of a cleaving embryo supposedly induces a secondary archenteron (Horstadius, 1939). There are even some cases where movement of a micromere to the animal pole results in a small secondary archenteron opposite to the host archenteron. These secondary structures often fuse with the main invagination of the embryo. This suggests that primary mesenchyme cells might be normally responsible for the initial first phase of gastrulation. If so, the mechanisms by which they act is completely unknown. Or is the power of the micromeres to induce an archenteron an erroneous interpretation and perhaps there is a polyingression of descendants of the transplanted micromeres? Modern microscopic and cell marking methods ought to resolve this question.

When micromeres are removed there is no primary mesenchyme; the embryo gastrulates normally, the secondary mesenchyme issues from the tip of the archenteron and then some secondary mesenchyme

cells move toward the blastopore region and assume the behaviour of primary mesenchyme, subsequently differentiating and forming skeletal spicules (Horstadius, 1973; Fukushi, 1962; Langelan & Whiteley, 1985). This finding implies that secondary mesenchyme cells, derived from the vegetal 2 tier, have skeletogenic potential, completely concordant with Horstadius' isolation and transplantation experiments. It also implies that the pluripotential nature of secondary mesenchyme persists through the secondary stages of gastrulation. It is interesting that a cytoplasmic actin transcript, Cy IIa, is present in secondary mesenchyme cells between blastula and gastrula stages, a time when secondary mesenchyme still possesses skeletogenic potential (Cox *et al.* 1986). Secondary mesenchyme pluripotentiality seems to persist rather long. Furthermore, the observation implies that primary mesenchyme somehow imposes a suppression of the skeletogenic potential of secondary mesenchyme cells (Ettensohn & McClay, 1986). This seems to be an excellent situation to study long-range cell interactions; primary mesenchyme may be isolated (Ettensohn & McClay, 1987) and it might not be too difficult to isolate populations of secondary mesenchyme as a population of outwandering cells from archenterons (McClay & Marchase, 1979). Good markers of gene expression characteristic of both primary and secondary mesenchyme are available and the influence of primary mesenchyme on skeletogenic potential of secondary mesenchyme could be studied *in vitro*.

Gastrulation

An attempt to describe and understand gastrulation in terms of the behaviour of individual cells has been a long-standing effort for sea urchin development. Since Moore & Burt (1939) showed that isolated vegetal halves could undergo gastrulation and Gustafson & Wolpert (1967) described changes in cell shape, motility and adhesivity, work on sea urchin gastrulation has served as a model for attempts to understand gastrulation in other animals.

New advances have been made, both on the biochemical and cellular fronts. Gastrulation proceeds in two distinct phases: first, there is a buckling of a thickened vegetal plate of the blastocoel wall, from which primary mesenchyme cells have already emigrated. Ettensohn (1984) repeated the older experiment of Moore showing that this occurs in isolated vegetal halves. The process does not involve rounding up of cells, as formerly predicted (Gustafson & Wolpert, 1967), nor is cell division or DNA synthesis important in either this or the second phase of gastrulation (Stephens, Hardin, Keller & Wilt, 1986). My associates and I incorrectly attributed the hypothesis that cell division might play a role in gastrulation to Wolpert and we regret this incorrect attribution. Ettensohn (1984) showed that less than 100 cells of the approximate 500-cell mesenchyme blastula are involved in the invagination, and there is no further contribution of the epithelium of the blastula to the archenteron after the original vegetal plate has buckled. The signals for the beginnings of invagination, the role of primary mesenchyme in invagination and the mechanics of the primary invagination process are still a mystery.

The second phase of gastrulation, the elongation of the buckled plate across the blastocoel, is now better understood. Ettensohn (1985) showed that elongation was accompanied by repacking of cells. A short, squat cylinder with many cells along its perimeter is converted into a longer, thinner cylinder with a much smaller number of cells in the circumference. The archenteron epithelium behaves like a fluid, cells repacking and changing neighbours during elongation. There are junctional complexes between the cells; different regions of the archenteron actually possess slightly different kinds of desmosomes, and these desmosomes persist through gastrulation. Whether these junctional complexes undergo mechanical and structural change, or form and reform at new positions during gastrulation, is unknown. Hardin & Cheung (1986) confirmed the conclusions on cell repacking as a mechanism of archenteron elongation. They carried out quantitative morphological analysis and computer simulations and showed that active cell repacking was probably a major influence in elongation. They also adduced evidence against an extensive role of filopodial contraction pulling the archenteron across the blastocoel. They pointed out that pulling should also deform the apex of the blastula roof and produce shape changes at the blastopore end of the archenteron, but these are not observed. Slight apical deformation is seen in some species of sea urchins from Japan, but not to the extent required by mechanical theory. More telling is a reanalysis of exogastrulation in *S. purpuratus* and *L. Pictus* by these workers. Perfectly normal but everted archenterons may form during exogastrulation and these produce normal subdivision of the gut and elaborate esterases typical of gut. These guts are extended and as long as those in normal complete gastrulae. In this instance, there can be no filopodial pulling. These exogastrulae do not form by eversion of normal invaginated archenterons, but develop gradually with the same tempo of secondary gastrulation as in the controls. Hence, even though filopodial exploration may be important in location of the stromodeum or play a subservient role in some species, cell repacking in the archenteron is probably the primary driving force for elongation. It is interesting to note that cell repacking is also proposed as the

dominant motive force in amphibian gastrulation (Gerhart & Keller, 1986).

Hardin (1987) has recently shown that the primary and secondary phases of gastrulation can take place in the presence of sufficient levels of nocodazole to disrupt microtubules, while the colchicine analogue, β-lumi-colchicine, stops gastrulation but does not disrupt microtubules. The microtubular assembly is probably not involved in invagination or archenteron elongation. The way now seems open to examine changes in cell shape, adhesivity and motility, and how these properties bring about primary invagination and cell repacking.

Another aspect of gastrulation that deserves comment is the role of the extracellular environment of the blastocoel and cell-surface-associated molecules in the process. Just as in the case of studies with primary mesenchyme cells, the changing composition of basal lamina and the blastocoel contents are relevant to gastrulation and are described in detail by Solursh (1986; see also Welply, Lau & Lennarz, 1985; Wessel, Marchase & McClay, 1984; Yamaguchi & Kinoshita, 1985). As in primary mesenchyme cell ingression, interference with metabolism of molecules of the extracellular matrix often arrests gastrulation. It would be helpful to know if such inhibition stops both primary and secondary gastrulation, or just secondary elongation, but this is not usually noted. I have noticed that embryos in sulphate-free sea water often undergo a limited form of invagination; a small invagination may appear, resulting in a kind of button-like appearance, which then, however, progresses no further. Tunicamycin, which inhibits dolichol-phosphate-mediated *N*-linked glycosylations, and β xylosides, which inhibit processing and elaboration of polysaccharide components of glycoproteins, both stop gastrulation (Schneider *et al.* 1978; Solursh *et al.* 1986).

Several lines of recent evidence implicate collagen in gastrulation, though the description is incomplete. The essential finding is that interference with enzymes that form hydroxyproline or hydroxylysine, also interfere with gastrulation (Mizoguchi & Yasumasu, 1982*a*,*b*, 1983*a*,*b*). Prolyl hydroxylase, an Fe^{2+}-requiring enzyme, may be inhibited by the chelator, α,α-di-pyridyl, which also inhibits gastrulation. Zn^{2+} also inhibits prolyl hydroxylase and the enzyme is stimulated, according to these authors, by ascorbate and α-ketoglutarate. Ascorbate and α-ketoglutarate partially reverse effects of Zn^{2+} on gastrulation. But Zn^{2+} has many effects on cellular metabolism, and the specificity of ascorbate and α-ketoglutarate action have not been satisfactorily determined. Hence, the evidence for involvement of proline hydroxylation is only suggestive.

Evidence for involvement of collagen cross-linking is somewhat stronger. Both Wessel & McClay (1987) and Butler, Hardin & Benson (1987) report that the lathyrogen β-aminoproprionitrile (BAPN) prevents collagen cross-linking by inhibition of lysyl oxidase in sea urchin embryos and prevents gastrulation. The effect is reversible, even after 96 h of developmental arrest. Upon removal from BAPN, gastrulation is completed. Both groups of workers present evidence that BAPN has little general effect on metabolism in the embryo and that the BAPN actually does prevent collagen cross-linking. The completion of gastrulation after 96 h arrest in the lathyrogen is remarkable, implying that the process that drives cell repacking is very stable. There is no explanation, however, for why collagen might be necessary. When BAPN exposure begins after gastrulation starts, secondary elongation continues, but the archenteron is flaccid and kinked, rather than turgid. Perhaps collagen on the basal, blastocoel surface of the archenteron serves to stabilize consequences of other morphogenetic manoeuvers.

Conclusion

There are opportunities to re-examine determination, differentiation and morphogenesis in sea urchin embryos using modern tools of cellular and molecular biology. Many of the questions and paradoxes that I have discussed are ready for experimental attack. In the process of analysis many old ideas may fall by the wayside, but we should soon gain a more complete understanding of sea urchin development. This will undoubtedly shed some light on how other embryos, like vertebrates, carry out development. I should like to briefly list some of the questions that have arisen for me as I have prepared this essay, as a way of summarizing some of the conclusions.

When is the oral–aboral axis first specified and how stable is this specification?

Does the expression of genes in animal half characteristic of surface epithelium depend upon interactions with cells from the vegetal hemisphere?

Can animal half cells be respecified by interactions with micromeres or with lithium when modern molecular markers are used as indices of differentiation?

Do all animalizing agents work by causing developmental arrest or is there a variety of mechanisms?

Is the vegetalizing influence seen in classical experiments a coherent response to some localized determinant(s) or can the various behaviours associated with 'vegetal' determinants be separated from one another?

Do dissociated cells from blastula and older stages sort out from one another to reconstruct the pluteus, or is respecification of cell fate involved?

How do primary mesenchyme cells exert a negative influence on secondary mesenchyme cells that suppresses the skeletogenic potential of the latter?

Do primary mesenchyme cells truly play a role in primary gastrulation?

What is the molecular mechanism by which cell repacking drives archenteron elongation?

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