solid-phase approach take weeks rather than years as they would if performed by classical methods.

The refinement of the chemistry and technology of solid-phase peptide synthesis remains a major concern of the Merrifield laboratory. In recent years Professor Merrifield and his co-workers have made substantial progress in developing new protecting groups and in improving the conditions for coupling amino acids when building peptide chains. Through the analysis of the mechanisms of side reactions, they have devised ways to eliminate many of these, thus preventing the formation of undesired by-products. Merrifield has also enhanced the effectiveness of the solid-phase process by improving the separation methods for the final purification of the desired peptide chain. While the synthesis of peptides greater than 100 amino acids in length remains a difficult challenge, even by the solid-phase method, Merrifield is applying improvements developed over the last several years to the synthesis of such large molecules. With his wife, Elizabeth, who has been working with him for the past few years, he has made major strides in the synthesis of an interferon (166 amino acids in length).

Bruce Merrifield's research at Rockefeller has had the objective of understanding the relations between the chemical structures of peptides and proteins and their physical and biological properties. Through his development and use of solid-matrix synthetic methodology, he has demonstrated that many questions can best be answered by chemical synthesis of these compounds and appropriate structural variants. His "simple and ingenious" methodology not only has increased the power of basic research but has also enriched the promise of peptide chemistry for medical applications.—EMIL THOMAS KAISER

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Molecular Indices of Cell Lineage Specification in Sea Urchin Embryos

Robert C. Angerer and Eric H. Davidson

Summary. The origins of several of the differentiated cell lineages of the advanced sea urchin embryo are well defined. Cytological application of molecular probes to three lineages, those responsible for the formation of the skeleton, the gut, and the aboral ectodermal wall of the late embryo, has demonstrated expression of lineage-specific genes long before overt morphological differentiation. These observations lead to useful generalizations regarding the processes of gene regulation that underlie the molecular biology of cell lineage specification in the embryo.

In the normal development of many species each of the sets of cells that form the diverse structures of the embryo descends from a specific group of cleavage-stage blastomeres. Where the cell lineages are known, embryogenesis can be regarded as the sum of the processes by which these lineages are initially specified and then induced to express their different properties. The sea urchin embryo invites this form of cellular and molecular analysis. Thus, within a few days of fertilization the discrete cell lineages of this embryo differentiate into the larval gut, skeleton, ectoderm, and other tissues and cell types. The blastomeres from which the prominent lineages arise were long ago identified and in some, although not all, cases the number of cells constituting each lineage at every point in early development is also known, at least for certain species. In this article we use the term cell lineage to denote a set of embryonic cells of given function that descends from a restricted group of early progenitor cells. The major experimental advantages of the sea urchin embryo such as its availability, the ease of preparative fractionation of some embryonic cell types, the relatively small number of embryonic cells, and the growing repertoire of molecular probes for specific genes and gene products have been described (*1*). New and sensitive cytological methods in which such molecular probes are used are now being applied to the study of the sea urchin embryo, with consequent elucidation of the process of cell lineage differentiation.

Normal Cell Lineage Along the Animal-Vegetal Axis

One of the embryonic axes of symmetry, the animal-vegetal axis, is preformed in the egg at the time of fertilization. This was initially demonstrated by Boveri (*2*), who reported several features of the unfertilized sea urchin egg that bear an invariant relation to the future orientation of the embryo. The polar bodies are extruded at the future animal pole, and Boveri also noted at this pole a minute channel in the external coat of the egg called the "jelly canal." He studied the eggs of the Mediterranean species, *Paracentrotus (Strongylocentrotus) lividus*, and described, following earlier observers (*3*), a subequatorial band of pigment granules that is orthogonal to the planes of the first two cleavages, and to the future animal-vegetal axis of the embryo. These observations have now been confirmed and extended to other species (*4*). The preformed animal-vegetal organization of the egg is not affected by the point of sperm penetration, which may occur at any locus on the egg surface (*4*). After fertilization, the egg cytoplasm is divided equally by two successive meridional cleavages intersecting at right angles

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along the animal-vegetal axis, and then by an equatorial cleavage. The following several cleavages are unequal in some regions, and give rise to tiers of blastomeres that are the specific progenitors of most of the differentiated cell lineages of the advanced embryo. The morphogenetic contributions of the lineages descendant from each blastomere tier along the animal-vegetal axis are indicated in Fig. 1, which is derived from classical cell lineage studies and vital staining experiments of Hörstadius and earlier authors (*5*).

From the egg to the pluteus stage, the mass of cytoplasmic constituents such as ribosomes, mitochondria, polyadenylated RNA, and total protein remains almost the same. In the species in which most of the experiments that we review were carried out, *Strongylocentrotus purpuratus*, the pluteus stage is attained within 3 days after fertilization. Under natural circumstances, the larva then begins to feed and to increase in mass. Laboratory-raised larvae of *S. purpuratus* contain about 50,000 cells after 5 weeks of growth (*1*). On presentation of a suitable substratum, such a larva undergoes a rapid and complete metamorphosis, and there emerges the radially organized juvenile sea urchin, which has been assembled from imaginal rudiments during the larval growth phase. Most of the other larval tissues are destroyed (*6*). The structures of the rudiments are formed mainly from coelomic sacs that appear at the distal end of the archenteron (primitive gut). Thus the adult sea urchin is, in the end, mainly a product of the cell lineages that contribute initially to the archenteron, namely the veg_2 blastomeres, though there also may be contributions from certain offspring of the micromeres, and a few of the larval ectoderm cells.

Cytoplasmic Localization and Induction in the Early Sea Urchin Embryo

As is true for the embryos of most taxons (*7*), specification of the various sea urchin embryo cell lineages involves both determination dictated by localized factors inherited by the progenitor blastomeres from the egg cytoplasm, and intercellular induction. Although the normal embryonic fates of the various cell lineages indicated in Fig. 1 are completely predictable, the mechanism of their determination varies. The micromeres, and the primary mesenchyme cell lineage to which they give rise, are almost certainly determined by a cytoplasmic localization process. Thus, only vegetal pole egg cytoplasm can specify formation of a cell lineage that gives rise to mesenchyme cells and endows the embryo with its skeletal rods or spicules. This has been shown by culturing vegetal, as opposed to animal, half eggs, and various isolated blastomeres and combinations of blastomeres (*8*). If during early cleavage the migration of embryo nuclei into the vegetal cytoplasm is prevented, as by treatment with dilute detergent solutions, the resulting embryos lack primary mesenchyme cells and never form spicules (*9*). Furthermore, if micromeres are cultured in isolation from any other embryo cell types, they give rise under appropriate conditions to skeleton-forming mesenchyme cells in vitro (*10*). These and other earlier observations (*5*) show that the destiny of the primary mesenchyme cell lineage is fixed irreversibly by virtue of maternal cytoplasmic determinants inherited by their progenitors, the micromeres. This is not

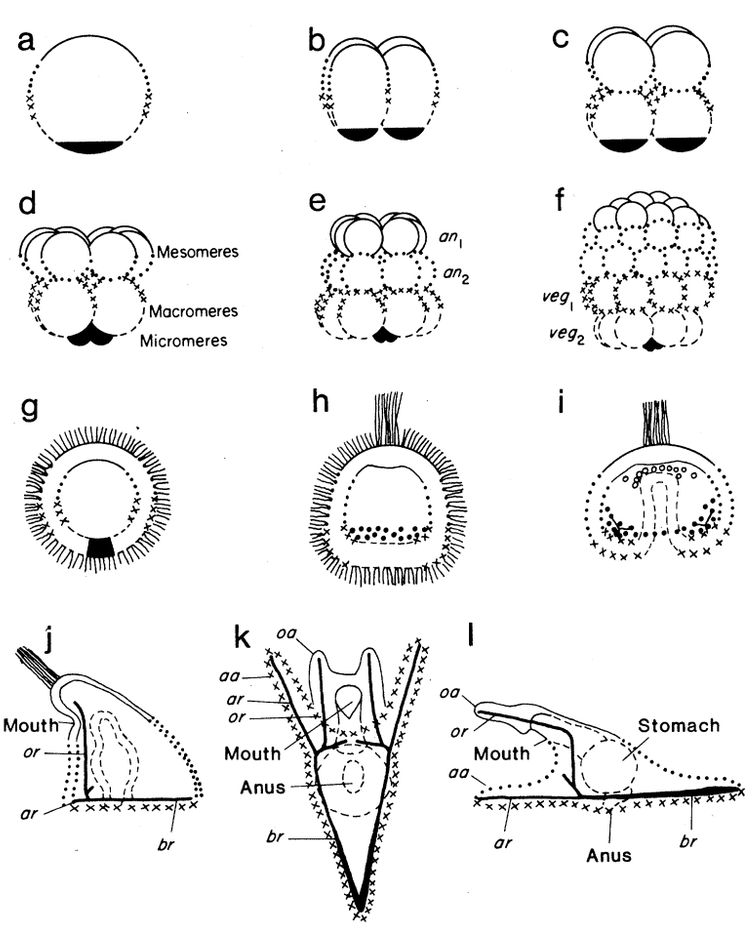

Fig. 1. Morphogenetic values of cell lineages descending from early blastomere tiers in the sea urchin embryo. Structures deriving from the animal pole tier an_1, indicated as solid lines; from an_2, as dots. These tiers are formed at the 5th cleavage, as shown in (e). They give rise to the ectoderm of the animal portion of the embryo, and ultimately to the mouth, the oral and aboral ectoderm, and the apical plate. Structures deriving from the vegetal region tier veg_1 are shown as crosses; and from veg_2, as dashed lines. These tiers are formed at the 6th cleavage [see (f)]. Veg_1 descendants form part of the ectoderm on the anal side of the pluteus, as shown in (k, l). From veg_2 originates the archenteron lineage, the secondary mesenchyme cells, and cells descendant from veg_2 progeny ultimately contribute to the coelomic sacs (definitive mesoderm) from which grow the imaginal rudiments of the adult sea urchin. The 4th cleavage micromeres and skeletal structures deriving from them are shown in black [omitting a set of eight micromere derivatives that are later incorporated in the pharyngeal region and which might also contribute to the coelomic rudiments (*13*)]. (a to f) Cleavage: (a) uncleaved egg; (b) 4-cell stage; (c) 8-cell stage; (d) 16-cell stage, with micromeres; (e) 32-cell stage; (f) 64-cell stage. (g) Hatching blastula, about 400 cells in *S. purpuratus* (*43*); 800 in *M. globulus* (*44*). Presumptive mesenchyme cells are shown in black, resident within the body wall at the vegetal pole. (h) Mesenchyme blastula. Ingressed mesenchyme cells are shown as black circles, presumptive archenteron cells of vegetal plate indicated by overlying and underlining dashed lines. The body wall is one cell layer thick. (i) Gastrula, triradiate spicules forming toward the oral side (facing outward from the page) and secondary mesenchyme cells shown as open circles. (j) Bilaterally symmetrical late gastrula, oral side to left. The archenteron is indicated by dashed lines. Mouth and parts of skeleton are indicated: or, oral rod; ar, anal rod; br, body rod. (k) Pluteus larva, from anal side, mouth, stomach, and anus indicated; aa, anal arm; and oa, oral arm. The pluteus of *S. purpuratus* contains about 1500 cells. (l) Same pluteus larva, seen from left side. [S. Hörstadius, in (*5*); courtesy of *Biological Reviews of the Cambridge Philosophical Society*]

the case for any of the remaining tiers of blastomeres.

Hörstadius (*5*) has shown that the fates of all the other blastomeres in the 16-cell embryo can be altered by placing them in chimeric combinations with other blastomeres. For example, the behavior of the animal pole cap of cells depends completely on which other blastomere tiers are included in the chimera. If the tiers labeled veg_1 and veg_2 in Fig. 1 are removed, but the micromeres are included, they induce the formation of a gut from the animal pole blastomeres. Normally (Fig. 1) the gut forms exclusively from veg_2 cells, and the animal blastomere tiers give rise only to larval ectoderm and mouth. Similarly, in the absence of veg_2 cells, the gut is induced to form from veg_1 cells, which in normal embryos give rise to the larval ectoderm on the anal side (Fig. 1), while in the absence of micromeres, skeleton is formed from veg_2 descendants, which of course also contain vegetal pole cytoplasm. Many additional constructions and chimeric recombinations (*5*) provide the general conclusion that the fate of the *an* and *veg* blastomere tiers is affected by the apposition of different neighboring cells that adjoin them in the normal embryo. Decisive inductive interactions thus must occur between the adjacent tiers. The inductive potency of isolated micromeres has been demonstrated directly by implanting them into the other blastomere tiers, and into meridional half or quarter embryos (*5*). Micromere implantation inhibits apical tuft formation by animal pole isolates, turns presumptive ectoderm into endoderm, and in some cases creates new embryonic axes.

A view of the normal process of developmental determination along the animal-vegetal axis that is implied by these classical results on experimentally perturbed embryos is the following. Localized maternal cytoplasmic determinants specify certain cells in the normal embryo, in particular (although probably not exclusively) the micromeres and the archenteron precursors near the vegetal pole. These cells then determine inductively the fates of neighboring blastomeres, which interact in turn with their neighbors. This mechanism requires plasticity in many of the mid-cleavage stage blastomeres until they have engaged in the appropriate intercellular interactions, although not in the cells initially specified by inherited cytoplasmic determinants, such as the micromeres. The chimeric recombination experiments display directly both the plasticity and the inductive capabilities of the 4th to 6th cleavage blastomere tiers.

The mechanism by which the oral-aboral axis is determined remains obscure. In the overall structure, this axis of bilateral symmetry becomes evident only at the gastrula stage, when the embryo flattens on the future oral side, the oral skeletal rods appear, and the secondary mesenchyme cells contact the ectodermal wall, followed by the tip of the archenteron, thus inducing the formation of the mouth (*11*) (see Fig. 1). The earliest morphological manifestation is the formation of bilateral aggregations of primary mesenchyme cells at the base of the oral side, where they begin the secretion of the triradiate spicules (*11*). The results of chimeric recombination studies and of various vital staining experiments (*12*) suggest, but do not demonstrate, that the oral-aboral axis was already specified by early cleavage. For example, Czihak showed that staining for the mitochondrial enzyme cytochrome oxidase is localized as early as the eight-cell stage in the blastomeres of the future oral side. Whenever the initial specification might occur, the fates of presumptive oral and aboral ectoderm cells remain plastic far into cleavage, and probably are determined inductively. Thus meridional half embryos and embryos in which the animal half has been rotationally reoriented with respect to the vegetal half are all able to establish normal oral-aboral axes (*5*).

Expression of Specific Markers in the Aboral Ectoderm Lineage

In the pluteus stage embryo, there are about 350 to 400 aboral ectoderm cells, by which is denoted the single layer of cells forming the body wall of the pluteus stage embryo in the region away from the mouth and arms, from about the juncture of the oral and anal skeletal rods to the vertex of the larva. These cells descend mainly from the an_1 and an_2 blastomere tiers, with some contribution from veg_1 on the anal side (Fig. 1). The aboral ectoderm lineage is constructed during late cleavage and early blastulation, when most of the cells in the embryo are multiplying at a logarithmic rate (except for the micromere lineage; see below). Autoradiographic data of Cohen (*13*) show that after the 9th or 10th cleavage, the rate at which these cells divide falls sharply, and remains low for the remainder of embryogenesis. Thus, from 18 to 19 hours after fertilization incorporation of ^{3}H-labeled thymidine is confined to a progressively smaller fraction of these cells (*13*), and in contrast to dividing cells elsewhere in the embryo, their content of histone messenger RNA (mRNA) is greatly diminished, as shown by in situ hybridization procedures (*14*). By gastrula stage, cell division appears to have ceased in most of the cells of this lineage. The aboral ectoderm cells of the late pluteus are therefore exactly the same cells as were present on the prospective aboral side in the late blastula. They do not migrate or otherwise alter their relative positions, although they spread and flatten at the pluteus stage. During the postembryonic growth period, dividing cells are very ocassionally observed in the aboral ectoderm of the feeding larva (*13*).

Genes for two sets of proteins that could be functionally related are expressed specifically in the aboral ectoderm cells. These are the cytoskeletal *CyIII* actin genes (*15*) and the *Spec* (*Strongylocentrotus purpuratus* ectoderm) genes, which code for a family of calcium-binding proteins related in sequence to the vertebrate protein superfamily that includes troponin C and calmodulin (*16, 17*). Two-dimensional gels reveal about 10 to 12 *Spec* proteins which, on the basis of sequence homology, fall into two classes called *Spec1* and *Spec2*. The major species of the *Spec* proteins are produced by the *Spec1* genes, while the *Spec2* genes code for a number of related minor species. *Spec1* mRNA's can first be detected 20 hours after fertilization, at the hatched blastula stage, and about 10 hours later, at the early gastrula stage, *Spec2* transcripts begin to accumulate (*17*). The cytoskeletal actin genes *CyIIIa* and *CyIIIb* are utilized only in the embryonic and larval periods of the life cycle, and their transcripts are totally absent from the post-metamorphosis sea urchin (*15*). About 95 percent of these transcripts derive from gene *CyIIIa*. *CyIII* actin mRNA's apparently begin to accumulate slightly earlier than do *Spec1* transcripts, at about 14 hours of development. The levels of both the *Spec* and *CyIII* transcripts attain peak values by late in gastrulation, except for the relatively minor *CyIIIb* message, which continues to increase during the pluteus stage.

The in situ hybridizations such as those shown in Fig. 2, a and c, demonstrate that *Spec1* mRNA's and *CyIII* actin mRNA's are confined to the aboral ectoderm cells of the pluteus stage embryo. All the cells of this region of the ectoderm appear to express these particular genes, although if there were scattered individual cells in which these genes are quiescent, they might not have been detected. However, immunostaining reactions (*16*) show the *Spec1* pro-

teins in virtually all the dorsal ectoderm cells in each section. Figure 2, b and d, shows hybridizations of the *Spec1* and *CyIII* actin probes to mRNA's in blastula stage embryos, long before even the earliest morphological distinction is evident between the future oral and aboral sides of the embryo. It is clear that each probe reacts with transcripts present in a contiguous patch of blastula cells, namely, those constituting the prospective aboral ectoderm. Observations made on intermediate-stage embryos confirm this identification (*18, 19*). The data suggest that the *CyIII* actin genes and the *Spec1* genes are activated about the time that the cells of this lineage decrease their rate of division (*13*), followed later by the *Spec2* genes. Absolute synthesis rate and turnover measurements for the *Spec1* mRNA's (*20*), combined with mRNA accumulation data (*17, 18*), show that the appearance of these mRNA's is indeed due to transcriptional activation, although this has not yet been substantiated by direct transcription rate measurements.

The physiological function of the *Spec1* and the *CyIII* cytoskeletal actin gene products in the cells of the aboral ectoderm is not known. The aboral ectoderm may perform an active role at metamorphosis, by contracting to provide an exit for the emergence of the juvenile sea urchin. Carpenter *et al.* (*16*) have suggested that this event might be mediated by alterations in intracellular calcium ion concentrations controlled by the *Spec* proteins. These proteins, and the *CyIII* actins, might also constitute functional components of the ectodermal cytoskeleton that are required to establish and maintain the form of the body wall during the larval growth phase. Whatever their actual role, the kinetics of transcript accumulation for both sets of genes suggests that the respective proteins are required exclusively by the aboral ectoderm cells late in embryonic life or in the following larval period.

A contrasting pattern of expression is shown (Fig. 3a) (*19*), by an in situ hybridization with a probe specific for transcripts of a different cytoskeletal actin gene, *CyI* (*15, 21*). This cytoskeletal actin gene is also activated early in development when its transcripts are present ubiquitously (unpublished data). Later they become confined to certain embryonic lineages, including the oral ectoderm, but they are absent from the aboral ectoderm. Accumulation of *CyI* mRNA in the oral ectoderm provides an additional example of specific expression imposed long before morphological distinctions are evident.

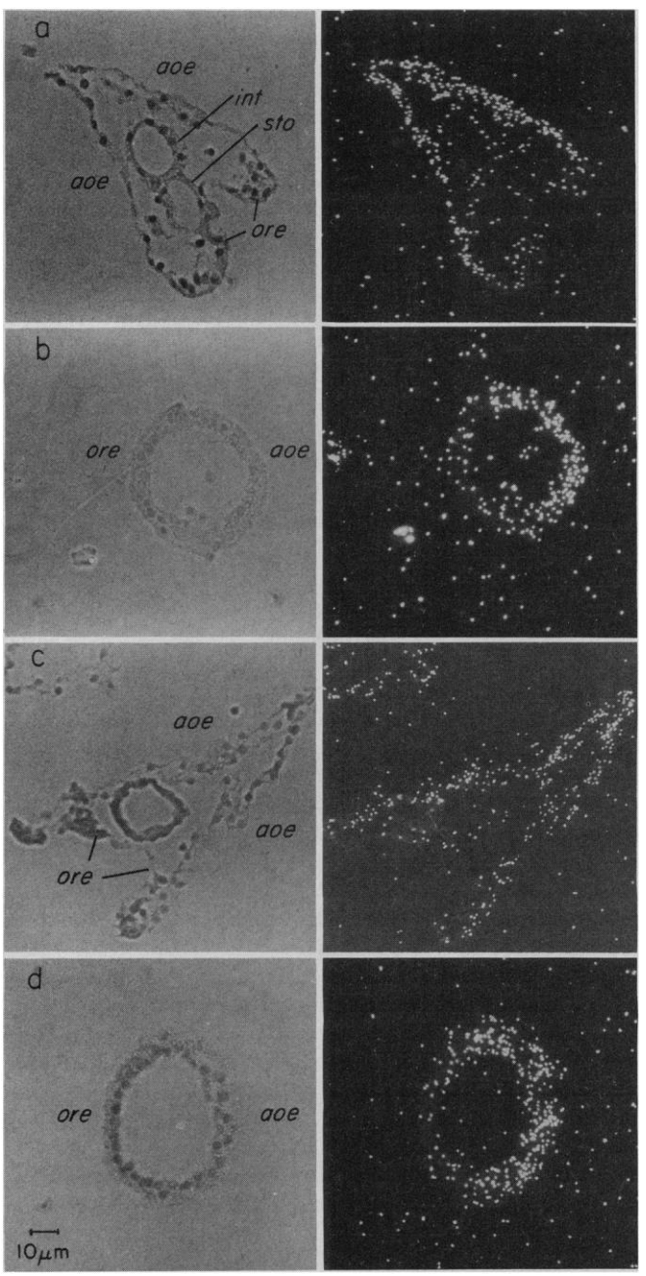

Fig. 2. Expression of cloned genes in the aboral ectoderm. In situ hybridization of 5 μm embryonic sections of *S. purpuratus* embryos photographed with phase-contrast optics (left), and in dark field (right). The hybridizations were carried out as described by Cox *et al.* (*14*) with single-stranded RNA probes transcribed in vitro from recombinant templates carrying Sp6 bacteriophage promoter (*46*). Autoradiographic exposure times were 40 to 70 days. Nonspecific background was estimated from sections hybridized with the transcripts of the vector (not shown). Grain density over lineages other than aboral ectoderm are not dectectably different from this nonspecific background. (a and b) *Spec1* probe, reacted with a section of 82-hour pluteus (a) and 23-hour blastula (b). Distribution of *Spec1* sequences has been described (*18*). (c and d) *CyIIIb* cytoskeletal actin probe, reacted with sections of 82-hour pluteus (c) and 29-hour blastula (d). This and other actin transcript hybridizations in this article are from the study of Cox *et al.* (*19*). The *CyIIIb* probe utilized cross reacts with *CyIIIa* transcripts (*15*). Abbreviations: aoe, aboral ectoderm, which at the pluteus stage forms a V shape with the vertex to the upper left in (a) and the upper right in (c) (Fig. 1, k and l); int, intestine; sto, stomach; ore, oral ectoderm.

The Primary Mesenchyme Cell Lineage

The development of the primary mesenchyme cell lineage may be conveniently divided into three phases. The first begins with the formation of the micromeres at 4th cleavage, and lasts until their descendants enter the blastocoel as free wandering mesenchyme cells. At 5th cleavage the micromeres undergo an unequal division, which lags slightly behind other mitoses in the embryo (*22*) and gives rise to four small and four larger cells (*8*). The four small cells divide once more, and the ring of eight vegetal pole cells thus formed appears much later in the pharyngeal region of the larval digestive tract (*8, 23*). The mesenchyme cell lineage is the only product of the four larger cells. In the sea urchin *Mespilia globulus*, where their lineage is exactly known, the presumptive mesenchyme cells undergo just three further divisions, resulting in two rings of 16 cells each that are embedded in the vegetal region of the blastula wall, while the cells elsewhere in the embryo are carrying out their 6th, 7th, 8th, and 9th cleavages. The presumptive mesenchyme cells then cease to divide.

For many hours thereafter, that is, until ingression, the presumptive mesenchyme cells display a polarized ultrastructure identical to that of the neighboring blastomeres (*24*). The nuclei are located toward the inner surface, which is covered with a basal lamina, and the mitochondria are concentrated toward the external or apical surface, which bears a single cilium, and displays a specific affinity for hyalin protein (*25*) that coats the whole outer surface of the embryo. When ingression begins all of these structural properties change. In *Lytechinus pictus* (*24*), the presumptive mesenchyme cells become teardrop-shaped, the basal lamina disappears locally, and the nucleated basal ends are protruded into the blastocoel. The cilia disappear from the apical ends, where the membrane apparently loses its affinity for hyalin (*25*), and numerous other cytological changes occur, including cytoskeletal realignments and disappearance of the lateral desmosomes originally joining the adjacent columnar cells. The cells become motile and by means of pulsatile movements they escape into the blastocoel (*26*). There they round up and begin the second phase of their existence as ameboid mesenchyme cells. This phase corresponds to the late blastula and early gastrula period of embryogenesis, a period of many hours during which the primary mesenchyme cells can be found wandering about the blastocoel. Mitoses are reported to be very rare amongst them at this stage (*22*). The third phase is defined by skeleton deposition. This begins with the bilateral aggregation of the mesenchyme cells on the future oral side of the invaginating archenteron, and the secretion of triradiate spicules. The skeletal rods are then deposited within linear syncytial aggregates of mesenchyme cells formed by fusion of their pseudopodial extensions. These multicellular structures are oriented with respect to the embryo by cytoplasmic strands that connect the cell bodies to the inner surface of the ectodermal wall. The rods brachiate in a species-specific way, and the ability to construct a given pattern is partly an inherent property of the mesenchyme cells. However, it is clear that the final organization of the skeletal rods is controlled by the spatial pattern of interactions between the mesenchyme cells and the inner wall of the blastocoel (*26–28*).

As shown by Okazaki for several Japanese sea urchin genera (*29*) and for *S. purpuratus* (*28*), isolated micromeres in culture exhibit the same three phases of differentiation, on about the same temporal schedule, as they display in vivo. Thus they first aggregate and undergo several divisions, then they develop pseudopodia and become singly motile, and finally they reaggregate, and form multicellular cables within which brachiated skeletal rods are secreted. The form of the skeletal structures secreted in mesenchyme cell cultures is abnormal, but can be improved in the direction of the normal pattern by contact with reaggregated gastrula ectoderm cells (*28*).

From the moment of their formation micromeres differ in their molecular and cellular properties from the other blastomeres of the 16-cell embryo (*30–32*). However, not until later in the development of this cell lineage can most of its specific and unique biosynthetic products be detected. Because of their small size, the ratio of nucleus to cytoplasm is, from the beginning, several times greater in micromeres than in the other blastomeres, and consequently the ratio of newly synthesized message to maternal message may be much higher than elsewhere in the 4th or 5th cleavage stage embryo. This could be the cause of the significantly greater ratio of histone to nonhistone protein synthesis that has been reported for micromeres (*33*). Synthesis of high complexity nuclear RNA is delayed in micromeres until well after 5th cleavage, while it is active in other

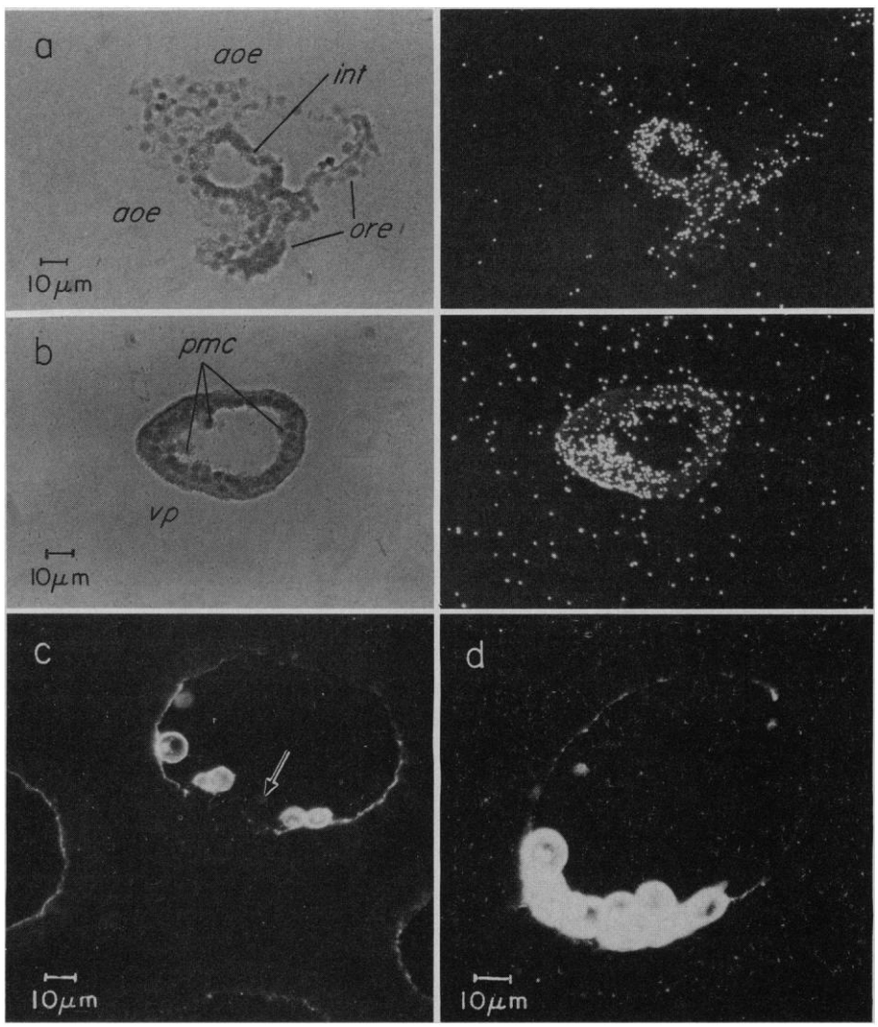

Fig. 3. Genes active in primary mesenchyme cells. (a and b) Hybridizations in situ of sections of *S. purpuratus* embryos with a *CyI* cytoskeletal actin probe. (a) An 82-hour pluteus. The oral ectoderm and the intestinal region of the gut are heavily labeled, while there is no labeling over the aboral ectoderm. Although it is difficult to distinguish in this section, mesenchyme cells are also labeled by this probe (*19*). (b) A 23-hour blastula. Primary mesenchyme cells in the blastocoel are labeled, as are the presumptive primary mesenchyme and other cells of the vegetal region of the blastocoel wall (*19*). (c and d) Reactions of a monoclonal antibody designated Ig8 with sections of *Lytechinus pictus* embryos, visualized by immunofluorescence, reproduced from a study of McClay *et al.* (*39*). In (c) the primary mesenchyme cells that have completed ingression stain, while those still embedded in the blastocoel wall (arrow) do not. (d) Primary mesenchyme cells after ingression. The secondary mesenchyme cells that appear at the late gastrula stage do not react with this antibody. Abbreviations: pmc, primary mesenchyme cells; vp, vegetal plate; other labels as in Fig. 2.

embryo nuclei before this (*31*). There are also differences in the core nucleosomal histones of the micromere chromatin (*34*). Ernst *et al.* (*31*) also found that micromeres lack a set of nonpolysomal maternal cytoplasmic RNA sequences that is present in the other blastomeres. The implication of the mode of micromere formation is thus that their nuclei reside in a distinct cytoplasmic domain. Such a domain could include the specific conditions, or factors, required for the determination of the micromere cell lineage.

There is still no evidence that mRNA sequences are present in micromeres that are absent elsewhere in the 16- to 32-cell embryo. Thus within the limits of resolution of the RNA excess hybridization method, the same set of polysomal mRNA sequences appear to be present in micromeres as in the other embryo blastomeres (*31*). Similarly, Tufaro and Brandhorst (*35*) and Harkey and Whiteley (*36*) found all of the clearly discernible protein species that can be resolved in two-dimensional gels are synthesized alike by micromeres, mesomeres, and macromeres.

The significance of these negative results is limited, in that the two-dimensional protein synthesis analyses concern only those proteins produced by the moderately prevalent and abundant messages, while the mRNA complexity comparison is incapable of resolving small differences, for example, anything less than about 10 percent of the 10^4 or so diverse transcripts present in the embryo polysomal RNA (*1*). Later in phase I of their development, the presumptive mesenchyme cells may undergo some characteristic changes in their patterns of protein synthesis, according to a two-dimensional analysis carried out on proteins labeled during the equivalent stage of an in vitro micromere culture (*36*). Synthesis of several of the prominent proteins initially made is sharply reduced, and synthesis of a few protein species, including an actin, is initiated. However, in vitro cultivation can sharply affect protein synthesis patterns, including the synthesis of cytoskeletal actin (*37*), and it is difficult to authenticate these changes in the presumptive mesenchyme cells of undisturbed embryos while they are still embedded in the blastocoel wall. In Fig. 3b is reproduced an in situ hybridization that shows an early biosynthetic activity of phase I mesenchyme cells. Here cells at the vegetal pole of the blastula, presumably including the presumptive mesenchyme cells, express the *CyI* actin gene prior to ingression (Fig. 3a).

Significant changes in the cell surface macromolecules would be expected to occur at ingression. For example, immunofluorescent staining (*38*) indicates that fibronectin appears on the mesenchyme cells after ingression. A dramatic visualization of another mesenchyme-specific surface macromolecule is illustrated in Fig. 3, c and d, from the work of McClay *et al.* (*39*). Here a monoclonal antibody is demonstrated by fluorescent staining to react strongly with primary mesenchyme cells just after ingression, but (Fig. 3c) not until they have become free of the blastocoel wall does this antigen appear. A major set of changes occurs in the pattern of protein synthesis carried out by developing micromere cultures, just at the time when ingression occurs in normal embryos (*36*). Most of these newly synthesized proteins are also being translated actively in primary mesenchyme cells freshly isolated at the gastrula stage. Of the 17 most prominent newly synthesized mesenchyme cell proteins 13 are not found elsewhere in the embryo. These proteins are thereafter synthesized continuously by the primary mesenchyme cells throughout embryogenesis, with a few additional new species appearing at the stage when skeletal spicules begin to be deposited. Initial results (*40*) confirm the presence of cloned mesenchyme-specific mRNA's beginning at phase II of mesenchyme cell differentiation. None of the cloned sequences so far examined is expressed in micromeres or in cultured phase I presumptive mesenchyme cells (*40*). The only identified newly appearing mRNA in phase I mesenchyme cells is the *CyI* actin message (Fig. 3b).

Among the most interesting lineage-specific proteins are those constituting the organic matrix of the skeletal rods. These have been partially characterized (*41*), and genes that encode them are being isolated (*42*). These genes are evidently activated autonomously in culture as well. The impressive repertoire of biosynthetic functions exhibited by cultured micromeres and their descendants shows that this cell lineage is endogenously determined to utilize on schedule the sets of genes that endow it with the complex of functional properties reviewed: in phase I the expression of division functions for several further mitotic cycles, and later the activation of genes responsible for the cytological changes that in situ lead to ingression; in phase II the synthesis of cytoskeletal and cell surface proteins associated with motile behavior; and in phase III the synthesis of proteins required for aggregation and spicule deposition. The events that will result in the activation of the many individual genes that must be required for all these functions are evidently set in train when the lineage is established at 4th cleavage, by the sequestration of nuclei in the vegetal cytoplasm of the egg.

Markers for the Early Differentiation of the Gut Cell Lineage

The gut arises from a thickened vegetal plate, composed of cells descendant from the veg_2 tier of blastomeres (Fig. 1). At gastrulation these cells develop the capacity to invaginate, and their descendants give rise to all parts of the digestive tract, except the mouth, which derives from the an_1 blastomere tier. The mechanical basis of the invagination is somewhat similar to that responsible for mesenchyme cell ingression (*43*). Thus the initially polar cells of the vegetal plate display pulsatile, pseudopodial movements on their basal surface, accompanied by reduction in intercellular contact. These contacts are maintained, however, on the apical surface, and a contiguous wall of cells is thus carried inward by the invagination. The secondary mesenchyme cells are budded off after the archenteron has extended part way across the blastocoel, and the remainder of the invagination is apparently pulled by contractions of pseudopodia that emerge from cells at the tip of the archenteron and make contact at the opposite wall of the blastocoel (*43*). Unlike the primary mesenchyme and the aboral ectoderm, the cells of the vegetal plate remain mitotically active into the gastrula stage (*13*). Division continues among the secondary mesenchyme cells and also in the archenteron tip in late gastrulation, and thereafter as new cell lineages develop in the coelomic rudiments, the oral region, and the oral ectoderm surrounding the larval arms (*13*). We are here concerned only with the original gut cell lineage that participates in the initial invagination of the archenteron.

The distribution of an archenteron-specific cell surface antigen is displayed by a monoclonal antibody isolated by McClay *et al.* (*39*) (Fig. 4, a and b). This antibody reacts with the cell surface antigens of the stomach and intestine regions of the differentiated archenteron of the pluteus (Fig. 4a), but not with those of the esophageal region. A similar pattern is displayed by the distribution of the *CyIIa* cytoskeletal actin transcripts in the pluteus (Fig. 4c), in that the probe labels portions of the stomach and intestine, but not the esophagus. The *CyIIa*

probe reacts as well with mesenchyme cells, while the antigen displayed by the fluorescent antibody is absent from these cells. The most interesting result (Fig. 4) is that the *CyIIa* probe and the gut-specific monoclonal antibody both react with some cells of the archenteron secondary mesenchyme lineage in the vegetal plate prior to invagination (Fig. 4, b and d). Neither marker is expressed until just before this event. Thus, expression of a set of lineage-specific sequences precedes visible morphological differentiation and can be used, in this case, to identify the patch of cells that are the archenteron progenitors. The subsequent process of archenteron morphogenesis involves additional forms of differentiation that probably require the activation of additional sets of genes. Thus McClay *et al.* (*39*) have reported monoclonal antibodies that in later embryos react only with the cells of the forming mouth, or with hindgut cells.

Discussion and Conclusions: Gene Regulation in Embryonic Cell Lineages

Obvious parallels exist in the processes by which each of the three lineages we have discussed approaches the differentiated state. Specification of the micromere lineage and of the gut cell lineage deriving from the veg_2 blastomere tier clearly occurs when there are only a very small number of progenitor cells. This could be true of the aboral ectoderm lineage as well. Although the developmental stage at which the oral-aboral axis is established is unknown, chimeric recombination experiments show that polarization along this axis begins by the very early blastula stage (*5*), which is in any case significantly before the final number of cells in the embryonic aboral ectoderm is attained. We ignore in this argument the differences between the irreversible determination, or commitment, imposed locally on the micromeres, and the more leisurely determination that occurs in aboral ectoderm and gut progenitor cells by intercellular induction. In either case the result is evidently the imposition of a heritable change in the progenitor cell nuclei, which potentiates the future expression of certain genes in each lineage. This change is then propagated by mitosis to the much larger number of cells ultimately required by the functional, differentiated lineage. In the embryogenesis of animals such as the sea urchin the propagation phase cannot be regarded as growth, since there is no increase in cytoplasmic mass. Its major results are rather the appearance of a large number of nuclei committed to the functional biosynthetic activities to be carried out by each lineage when the time comes, and the spatial disposition of the lineage at the appropriate staging locations in the wall of spherical blastula. In sum, for specification only a few target nuclei are required, whereas for function, a large number of nuclear factories are required.

Differentiation, defined narrowly as the biosynthesis of lineage-specific macromolecules, occurs at different stages of embryogenesis in the three examples considered. The aboral ectoderm cells are the earliest to display known, lineage-specific differentiated functions, in *S. purpuratus* 20 hours after fertilization, and is followed closely by the primary mesenchyme cells after their ingression at about 22 to 24 hours, and a few hours thereafter by the presumptive archenteron cells of the vegetal plate. Thus the embryo as a whole may not undergo differentiation at any particular stage, nor behave overall as a regulatory field, except for those defined in terms of its constituent cell lineages. Each lineage conducts its own, temporally organized regulatory processes, which may, or may not, require signals from adjacent lineages. Ultimately some of the lineages considered brachiate further, giving rise to new lineages that no doubt express additional sets of genes.

There are relatively few experimental systems in which cloned probes have been used to define the onset of lineage-specific differentiation in early embryonic development. The identification of lineage-specific mRNA's and proteins provides a precise set of molecular indices, with which to approach the central problem of the mechanism by which specific patterns of gene activity are imposed in given cell lineages. It is interesting that multiple examples of lineage-specific genes have already been uncovered in each of the three cases considered. The gut and aboral ectoderm markers were discovered in studies not focused initially on these particular cell lineages (*18, 19, 39*). The implication is of course that the known lineage-specific markers are initial samples of much larger sets of genes. The more extensive observations

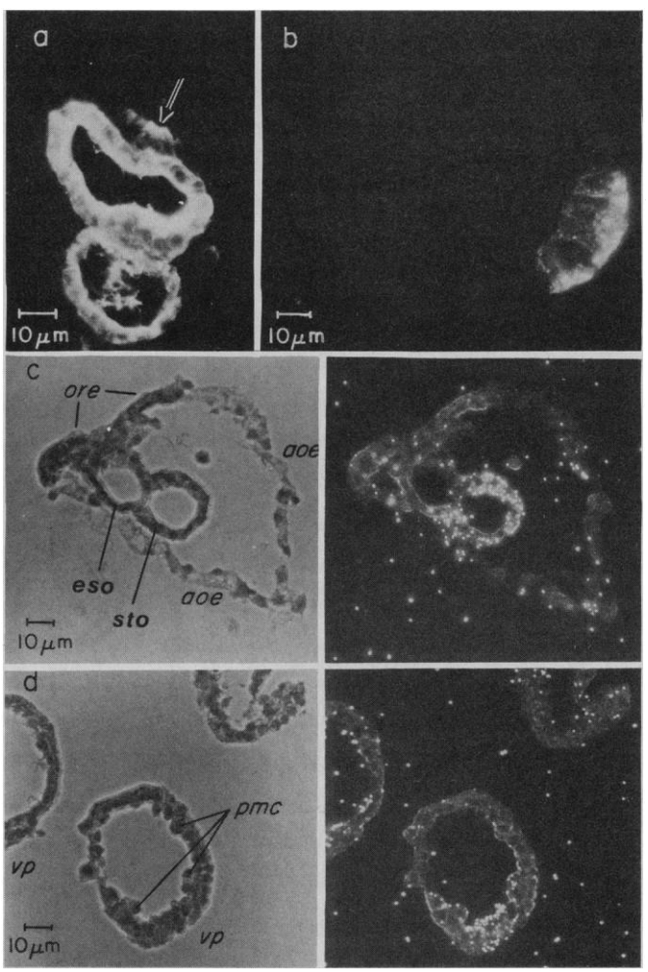

Fig. 4. Genes active in embryonic gut. (a and b) Reactions of the gut-specific monoclonal antibody designated 5c7, from (*39*). (a) Advanced embryo, photographed in the fluorescence microscope. Only the intestine and stomach areas of the archenteron stain; the esophageal area does not, except for a few cells indicated by the arrow. (b) Mesenchyme blastula. The primary mesenchyme cells, which are not visualized by this antibody, have already been released into the blastocoel, and the stained cells of the vegetal plate are presumptive gut cells, seen just as invagination begins. The apical surfaces of the presumptive gut cells stain most intensely. (c and d) In situ hybridization of *CyIIa* cytoskeletal actin probe (*19*). (c) A 68-hour pluteus stage embryo, showing reaction with stomach region of the embryonic gut. The *CyIIa* probe labels the mesenchyme cells as well. (d) A 29-hour mesenchyme blastula, vegetal plate at bottom of figure, showing labeling specifically in the presumptive archenteron-secondary mesenchyme cells.

available for the mesenchyme cell lineage indeed demonstrate a much larger complex of specific gene products (*36*). The differentiation of embryonic cell lineages is a pleiotropic event, clearly involving multiple loci. The early cell lineages, which carry out distinct functions, can thus be regarded as the first biological units of differential gene regulation in the embryo.

The following generalizations relate what is so far known of lineage-specific gene sets to the overall patterns of mRNA representation in the sea urchin embryo. (i) Transcripts of lineage-specific genes begin to accumulate only at given stages of development and are not significantly represented in the maternal RNA. Moreover, there is as yet no evidence in the sea urchin embryo for sequestration of specific maternal messages to given progenitor blastomeres (*34–36*). Thus it is possible that all lineage-specific mRNA's are transcribed de novo in the embryonic cell nuclei. (ii) Not all late-appearing mRNA transcripts are lineage-specific. For instance the *CyI* actin gene is evidently activated during development (*15, 19, 21*), although its messages are originally also present at low concentration in the egg. However, this gene is expressed in several different cell lineages (Fig. 3). Similarly, of the 17 major late mesenchyme cell proteins identified by Harkey and Whiteley (*36*), four are also present elsewhere in the embryo. (iii) No more than about 10 percent of the sequence diversity of cytoplasmic embryo transcripts of all prevalence classes (≥one molecule per average cell up) can be accounted for as nonmaternal, late-appearing sequences (*1*). There are about 10^4 different transcription units represented in the embryo polysomes (*1*). Thus, unless there exist many heretofore undetected lineage-specific maternal mRNA's, there are unlikely to be more than about 10^3 lineage-specific structural genes used throughout embryogenesis, including those producing rare messages. Lineage-specific transcripts thus do not appear to account for a major fraction of the high complexity observed for embryonic mRNA populations overall (*1*). The functional significance of most of these early mRNA's, which are actively transcribed both during oogenesis and in the embryo (*1, 7, 20*), requires further exploration.

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