CHAPTER 5

The Roles of Cell Lineage and Cell Interactions in the Determination of Cellular Fates in Vertebrate Embryos

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Introduction

During the development of any multicellular animal or plant, two types of tasks must be accomplished: the production of the correct cell types and their appropriate assembly in space. An organism or organ system can adopt one of two strategies to carry out these tasks (Fig. 1): (1) cells can become committed to their various fates irrespective of their position within the embryo (for example, by their previous lineage history), and then migrate to their correct sites, die, or sort out (Fig. 1a) or (2) cells may become determined at their final sites by local interactions, that is, as a result of their position within the embryo (Fig. 1b). There are advantages and disadvantages associated with both methods of generating cellular diversity. For example, while the second method requires less cell movement, no cell death, and little or no cell sorting, it is more difficult to produce the correct proportions of two or more different cell types in this manner.

If cell diversity is set up prior to and independently of cell interactions (in other words, if development is mosaic), there must be some extranuclear organization in the cytoplasm, cortex, or cell membrane that creates diversity upon cell division. It is this organization that constitutes the theme of this book.

To understand the relationship between cell diversity and pattern, it is important to recognize the difference between fate (the cell types normally
Cell diversity and pattern formation: two different strategies

Cells determined randomly, ... then sort out

Cells determined at their correct positions, by interactions

FIGURE 1. Two strategies by which cell diversity can be generated in relation to the final pattern. (a) Cells are assigned different fates regardless of their position within the embryo, for example by their lineage history. The correct pattern is then generated by cell movements, cell sorting (differential adhesion?), or by differential survival of the two cell types at different locations. (b) All cells have the potential to become determined as either of the two cell types. Fates are allocated as a result of interactions of the cells with their microenvironment. The second strategy can be described as an “instructive induction,” while the first may be viewed as a “permissive induction.”

generated by a particular progenitor cell) and developmental potential (the ability of a progenitor cell to give rise to any given set of cellular phenotypes). The difference is illustrated by considering an experimental example: The fate of each of the two blastomeres of a 2-cell-stage amphibian embryo is to give rise to one-half of the adult body. However, each has the potential to give rise to a whole embryo. Each cell is able to realize its potential if the two blastomeres are separated carefully (see Refs. 28, 30). In fact, all four blastomeres generated by the second cleavage division are totipotent in terms of the cell types that they are able to generate. After the third cleavage, however, this is no longer the case: the developmental potential of the four animal blastomeres is no longer the same as that of the vegetal four. Commitment to a particular fate (or determination) occurs when the developmental potential of a cell no longer differs from its fate.

In this chapter I shall review the relationships between the generation of cell diversity and the formation of pattern during two important steps in the building of the vertebrate body plan: (1) gastrulation and (2) the laying down of segmental organization. Using these examples, I will argue that both the
position-independent and local-interaction strategies for cell diversity and pattern formation are used, even in the same species and during the development of the same organ system.

Gastrulation

Lewis Wolpert (30) has stated the importance of gastrulation very succinctly: “it is not birth, marriage or death, but gastrulation which is truly the most important time in your life.” Indeed, gastrulation is the period of early embryonic development in which the third germ layer, or mesoderm, arises as a distinct tissue. From the mesoderm will arise the skeleton, the muscle, and many of the internal organs of the adult organism. The segmental pattern of somites that develops in the mesoderm also dictates the pattern of some structures that do not derive from the mesoderm, such as the peripheral nervous system.

Like the rest of embryonic development, gastrulation consists of a series of processes that fall into two major categories: cytodifferentiation and morphogenesis. It is commonly assumed that morphogenesis precedes and is required for the allocation of cell fates (30). Thus, cell diversity is thought to result from geometry: the morphogenetic movements of gastrulation are required to bring certain tissues together in the embryo, and the interactions (“induction”) between these tissues are believed to influence the fate of the “responding” or “competent” cells.

The process of gastrulation therefore represents an excellent model system in which the relationships between morphogenesis and cell diversification can be studied. In the first part of this chapter I will survey the classical views of chick gastrulation and present some data which may question the accepted beliefs. I will argue that the evidence in favor of geometry as the causative force for cell diversification is not as strong as is generally assumed, at least in birds.

Polarity and Symmetry-Breaking

In order for morphogenesis to take place in an orderly fashion, the geometry of the embryo must be organized prior to the onset of major morphogenetic movements. Just prior to gastrulation, the chick embryo is a flat disc, about 2 mm in diameter, with two concentric regions: a central area pellucida, and a peripheral area opaca, each of which is two-layered. The epiblast, a pseudo-stratified epithelium, is continuous over both regions of the embryonic disc. The ventral layer of both regions consists of large, yolky cells. In the area pellucida this layer is known as the hypoblast. In addition to generating a third germ layer (mesoderm), the process of gastrulation must break the initial radial symmetry of the disc and generate a bilaterally symmetrical embryo.
DOOR-VENTRAL POLARITY OF THE EPIBLAST

The cells of the epiblast are polarized along their apical-basal (i.e., dorsoventral) axis. Sodium (43) and water (44) are transported from apical to basal aspects, and this unidirectional transport generates a transepithelial potential of some 25 mV (basal side positive; Refs. 14, 43). Like all transporting epithelia, the epiblast is also polarized morphologically. The polar features include apical intercellular junctions and some apical microvilli, basal nuclei, and a hyaluronate-rich basal lamina. The apical-basal polarity of the epiblast is labile; it can be reversed quickly by applying a transepithelial potential of opposite polarity to that measured across it (35 mV, apical side positive; 43). It can also be reversed by placing the epiblast in a pH gradient so that the apical side is about 3 pH units more acid than the basal side (unpublished observations).

3. How is the apical-basal polarity of the epiblast set up and maintained? In freshly laid eggs, the albumen that bathes the apical aspect of the epiblast is strongly alkaline (pH 9.5), while the subblastodermic fluid is slightly acidic (pH 6.5). Since a pH gradient of 3 units is sufficient to reverse the polarity of the epiblast experimentally, it seems likely that this asymmetry, set up by the mother, plays a role in determining the polarity of the epiblast. As the epiblast develops, the transepithelial potential generated could also serve to maintain the polarity of the epiblast. The primitive streak region appears to be a zone where the apical-basal polarity of the epiblast is reversed, or at least disturbed: ionic currents from the interior of the embryo escape through it (14, 43, 44).

CRANIOCAUDAL POLARITY OF THE EMBRYO: BREAKING RADIAL SYMMETRY

How is bilateral symmetry set up in the embryonic disc? It has been suggested that craniocaudal polarity is established during the descent of the egg in the oviduct, under the influence of gravity (21). The earliest manifestation of this polarity can be observed during the formation of the hypoblast, which coalesces into a continuous sheet of cells starting at the caudal end; the craniocaudal midline of the hypoblast sheet marks the future midline of the embryo. Waddington (49) was the first to suggest that it is the hypoblast that induces the formation of the primitive streak, since rotation of the hypoblast through 180° at the appropriate stage of development results in 180° reversal of the craniocaudal axis of the embryo. Other evidence (26), however, suggests that both the epiblast and the hypoblast have their own craniocaudal polarity. This view is based on the results of experiments of reaggregation of dissociated hypoblast and epiblast: When the hypoblast is dissociated and combined with an intact epiblast, the polarity of the epiblast dictates the orientation of the future craniocaudal axis of the embryo, and vice versa (26).

What determines the origin and the shape of the primitive streak? It has been suggested (1, 35) that the primitive streak arises preferentially at the margin between the area pellucida and area opaca (the region called the “marginal zone”; (1, 19); this region is undoubtedly special in some way. The rea-
son for the rodlike appearance of the primitive streak is unclear, but it is likely that two major forces contribute to determine its shape: the tension generated by the expansion of the blastoderm on the vitelline membrane, and some change in the shape, arrangement, and rate of proliferation of the cells in the epiblast portion of the primitive streak (4, 36, 38).

**Morphogenesis**

To date, the information available about the origin, movements, and subsequent development of each of the three germ layers is based entirely on direct observation of embryos at the appropriate stages of development, a few simple grafting experiments, and a few observations using time-lapse cinemicrography.

Briefly, the stages in the development of the chick embryo in the 36 h after the egg is laid are as follows (Fig. 2): At stage X, which corresponds to the time of laying, the embryo is a flat disc, where the central area pellucida is essentially single-layered. The early hypoblast at this stage is no more than a series of separate islands of a few cells each. By stage XII, about 4 h later, the hypoblast is starting to form a sheet from the future caudal end of the embryo (bottom of the diagram). At stage XIV, about 7 h after laying, the hypoblast sheet is complete. Shortly afterward (stage 2) the primitive streak

![Diagram illustrating the stages of development of the chick embryo in the 36 h after the egg is laid. Stages denoted by Roman numerals (I–XIV) apply to the earlier stages of development, and follow the staging system described by Eyal-Giladi and Kochav (7), while those marked by Arabic numerals (2–45) correspond to the later stages of development and follow the system of Hamburger and Hamilton (11).](image-url)

Morphogenesis of the Lower Layer

The earliest tissue to cover the lower layer is the hypoblast (Fig. 2). Its origin has been the subject of some controversy: one group of workers (8) argues that it arises by ingression of cells from the epiblast at many different sites, while others (40, 48) have suggested that it derives mainly from the caudal margin of the germ wall (lower layer of the area opaca). However, both views may be correct (see Ref. 28). The islands of hypoblast seen at very early stages (Fig. 2, stage X) appear to arise in situ, but the coalescence of the hypoblast into a sheet of cells takes place by two mechanisms: (1) the addition of cells derived from the caudal germ wall and (2) the spreading and joining of cells in the primitive islands. Thus, the primitive lower layer may be of mixed origin. The islands seen in young stages may constitute a primary hypoblast, while the secondary hypoblast that completes the primitive lower layer may be derived from the caudal margin of the germ wall (19, 48). This secondary hypoblast is said to be responsible for inducing the primitive streak (27).

During gastrulation, the hypoblast is gradually displaced by the appearance of the "definitive," or "gut," endoderm, which is derived from the cranial portion of the primitive streak, and by cells continuing to migrate centrally from the marginal germ wall (4, 40). The original hypoblast therefore becomes confined to a region close to the cranial area pellucida–area opaca margin by the end of gastrulation (full primitive streak stage; Fig. 2, stage 4; Ref. 11), forming a region known as the "germinal crescent" because the primordial germ cells are associated with it. The hypoblast itself does not contribute to the embryo proper; it gives rise only to the endoderm of the yolk sac stalk.

Morphogenesis of the Upper Layer and Formation of the Primitive Streak

The upper layer (epiblast) undergoes complex cellular movements (see Refs. 28, 48 for reviews). Some of these movements are due to the expansion of the blastoderm on the vitelline membrane, while others are related to the change in shape that the blastoderm undergoes during gastrulation, from a circle to a pear shape. Around the periphery of the area pellucida, the epiblast
cells move centrifugally, while near the primitive streak they move toward the midline. High-power time-lapse observations (unpublished) of the epiblast at stages X to XIV (7) (Fig. 2) show that there is considerable mixing of cells in all regions of the epiblast, which is perhaps surprising in a polarized epithelium containing intercellular tight junctions. The movement of the cells toward the axis of the primitive streak, however, does not appear to be related to the appearance of the mesoderm. Neither Vakaet’s nor my own time-lapse observations (both unpublished, but see Ref. 48) lend any support to the widely accepted view that gastrulation consists of a convergence of cells to the primitive streak accompanied by a sheetlike involution of the epiblast into it to form the mesoderm. When a visible “groove” forms in the primitive streak, the cells lining it are elongated cranio-caudally and do not move. Close to the end of gastrulation, while cells continue to converge toward the midline, no movement of cells into the primitive streak region can be seen.

**Morphogenesis of the Middle Layer**

The appearance of the primitive streak is a remarkably rapid process. It is unusual to find a true stage 2 (11) embryo (Fig. 2), and in time-lapse films the formation of the primitive streak can be seen only “in retrospect,” by projecting the film backward. This suggests that formation of the primitive streak is not a massive migration of presumptive mesoderm cells but rather represents the coalescence of cells that were already under the surface of the epiblast (48).

The middle layer arises from the caudal portion of the primitive streak. After the primitive streak has formed, middle-layer cells migrate out of it to give rise to the lateral plate. Time-lapse films show that this happens at Hamburger and Hamilton’s stage 3 (Fig. 2) at about the same time that the groove appears in the primitive streak. Before formation of the lateral plate, the mesoderm is packed densely at the primitive streak; as the lateral plate forms, it migrates massively away from the axis of the streak. The left and right halves of the lateral plate later become separated from each other by the regression (shortening) of the primitive streak that occurs after the end of gastrulation (38). The notochord is laid down as a rod of mesoderm by the cranial tip of the primitive streak (Hensen’s node) and elongates as the primitive streak regresses. The mesoderm at the primitive streak displays an elevated level of hyaluronidase activity (37), and this enzyme may degrade the overlying basal lamina and, thereby, encourage more mesoderm cells to ingress (36).

**Induction and Cytdifferentiation**

From the foregoing discussion, it can be seen that interactions between cells and their environment play an important role in establishing the overall pattern of the early embryo. We must now consider the mechanisms that lead to
the production of cell diversity. It has long been assumed that the mesoderm arises as the result of cell interactions. In the discussion that follows, I shall question whether current knowledge about gastrulation and induction supports this assumption.

**The Appearance of Cell Diversity**

By the time of laying (about stage X of Eyal-Giladi and Kochav, Ref. 7; Fig. 2), several distinct cell types are already recognizable by morphological criteria in the chick embryo. The epiblast consists mostly of small, columnar, polarized epithelial cells; the ventral surface of the embryo displays islands of hypoblast cells, which are larger and more yolky; and the germ wall has even larger and more yolky cells. In addition, the epiblast of the *area opaca* differs from that of the *area pellucida* in that the cells of the former are smaller and more cuboidal. During gastrulation, other cell types appear. The mesoderm of the primitive streak is a mesenchymal tissue, with small, fibroblastic, non-yolky cells. Initially these cells are packed tightly at the primitive streak, but later they migrate to give rise to the lateral and segmental plates and to the notochord. The notochord cells later become very vacuolated. The definitive (gut) endoderm also makes its appearance during gastrulation; it consists of flat cells that are more tightly adherent to one another than are those of the hypoblast.

Although these morphological differences help somewhat in understanding the origin and relations between the discernible cell types, they are not sufficiently well defined to represent good markers for these cell types. Moreover, lack of morphological differences does not necessarily indicate that the cells of tissues that look uniform are the same as one another.

**Mesoderm Induction**

According to several authors (28, 30) there are at least two distinct inductions that occur during the early development of amniote embryos. The first is induction of the mesoderm, which, in birds, is claimed to be the result of an interaction between the hypoblast (inducer) and the epiblast (competent ectoderm). The second is neural induction, which was first described in the amphibian embryo and for which Hans Spemann received the Nobel prize for Physiology and Medicine in 1935 (33). Here, the mesoderm is the inducer and the ectoderm the responding tissue. In birds, neural induction results from the interaction between the mesoderm of Hensen's node and notochord with the overlying epiblast to form the neural plate. In the rest of this discussion, we shall concern ourselves with induction of the mesoderm.

In *Xenopus laevis*, the cells that give rise to the mesoderm are already in a deep layer associated with the ectoderm (15), but the mesoderm is said to become determined as a result of an inductive interaction between the endoderm and ectoderm. This interaction can be made to occur in culture if an
explant of ectoderm (containing both superficial and deep layers) is confronted with cells from the appropriate region of the endoderm (10, 30). Slack (30) has argued that this interaction is “instructive” rather than “permissive” because (1) there is no increase in volume in the explants or in the embryo during the relevant stages of development, (2) there is no visible cell death in the cultures, and (3) in confrontation cultures several markers characteristic of mesodermal derivatives are expressed (e.g., muscle actin). He states (p. 26): “It is not conceivable…that these interactions…are permissive in character since they are clearly the foundation of the progressive regional subdivision and consequent increase in complexity of the body plan. If they are permissive then it means that some completely unknown process is responsible for generating the different types of cells…” Nevertheless, there is as yet no evidence for induction at the single cell level (see Ref. 10, p. 294 for a lucid, albeit brief, discussion of this problem). Recently, Slack and Smith and their collaborators (31, 32) have demonstrated that several substances are capable of inducing the expression of mesodermal markers in cultures of Xenopus ectoderm. They include fibroblast growth factor, an extract from chick embryos, and a protein secreted by an amphibian cell line, XTC, which may be identical to the transforming growth factor TGFβ2. All of these have relative molecular weights on the order of 16,000.

In the chick, our knowledge about mesodermal induction is even more limited. The avian equivalent of the inducing endoderm of the amphibian is the hypoblast. Since craniocaudal reversal of the chick hypoblast leads to reversal of the craniocaudal axis of the embryo (49), the hypoblast clearly plays a role in controlling the orientation of the primitive streak. Subsequently, it was shown that the result of this interaction depends critically upon the stage of the operation (2). It has never been shown, however, that the interaction between hypoblast and epiblast is truly an instructive induction in that it changes the fate of the cells of the epiblast. An alternative would be that it is only permissive, allowing the expression of cellular fates that otherwise do not become overt (see Fig. 1). Of course, virtually nothing is known about either the nature of the inductive signal or the nature of the response in bird embryos, and, unlike the case in amphibians, the mesoderm in birds cannot be induced easily by heterologous factors (28).

A FACTOR CAPABLE OF INDUCING SECONDARY AXES

Recently, a 50-kilodalton factor has been discovered (46) which is secreted by a human embryo cell line, MRC-5, and which is capable of “scattering” cultured epithelial cells. We (12) found that scatter factor can induce a secondary primitive streak to form in the epiblast: We grafted small pellets of MRC-5 cells into chick embryos of appropriate stages of development and found that secondary axes were formed, while grafts of cell lines that do not produce scatter factor had no effect. Primitive-streak-like structures formed in embryos grafted with MRC-5 cells, and supernumerary neural plates were found in about 80 percent of the grafted embryos (compared to some 50 to 60 percent
after grafting the “natural” inducer, Hensen’s node). Purified scatter factor applied locally is also effective (unpublished observations). We are investigating the possibility that chick cells with inducing ability, such as Hensen’s node, primitive streak mesoderm, and hypoblast, produce scatter factor–like activity themselves.

The finding that cells that produce scatter factor can induce secondary axes in the chick could provide a useful tool for the study of mesoderm induction in these embryos. However, we still do not know whether mesoderm induction in amniotes is permissive or instructive. In order to address this question, it would be of great interest to identify stable cell-type-specific markers that could distinguish between different cell populations in the embryo during and prior to gastrulation.

Regional Markers

Embarking on a hunt for cell-type-specific markers is obviously a difficult task without some direction to guide the search. If regional differences were found, it would still have to be shown that these differences identify cell types with different fates and are not merely a reflection of momentary cell behavior within a diverse cell population. In our present state of ignorance, however, any regional differences at or before gastrulation will be helpful in the search for real markers. Recently, several such differences have been identified (Stern et al., in preparation). In the remainder of this section I shall discuss how one of these markers may help us to understand the relationships between cell diversification and morphogenesis.

Antibodies recognizing a complex sulfated carbohydrate epitope known as “L2,” which is present on certain adhesion-related glycoproteins (N-CAM) show characteristic patterns of binding in early chick embryos (5). In stage 2 to 3 chick blastoderms, the L2 epitope identifies all of the primitive streak mesoderm, the hypoblast, and a few cells in the posterior (caudal) margin of the germ wall (area opaca endoderm) (Fig. 3). Western blot and immunohistochemical studies have shown that these regional differences are not due to differences in the distribution of N-CAM.

At stage XIII and earlier, long before the primitive streak makes its appearance, the hypoblast cells already bear the L2 epitope on their surface. We were surprised, however, to find that some cells in the epiblast are also labeled. These cells are distributed in an apparently random way in the epiblast, giving a “pepper and salt” appearance (Fig. 3). We do not yet know whether the L2-positive cells of the epiblast are mesodermal precursors, and we are investigating this possibility. If they were, the widely accepted notion that the mesoderm of amniote embryos results from an instructive induction will have to be revised. If it could be shown that L2-positive cells in the epiblast of the early embryo are indeed mesoderm precursors, this would imply that the mesoderm cells are determined as such prior to the formation of the primitive streak, and that they sort out from the nonmesodermal cells of the epiblast.
FIGURE 3. Diagram illustrating the distribution of the carbohydrate epitope L2 at two different stages of development in the early chick embryo. The upper diagrams show a general view of the embryo, while the lower diagrams show the distribution of L2 in a cross-section through the region marked by a dotted line in the corresponding upper diagram. At stage XIII, the hypoblast cells express L2 (heavy outline and hatching), while the epiblast contains a mixture of labeled and unlabeled cells. At stage 3, about the middle of gastrulation, the hypoblast is still positive, although it has become displaced to the periphery of the area pellucida. The cells of the forming primitive streak are also positive, but the rest of the epiblast no longer displays the epitope. Cartoon based on the results of Canning and Stern (5).

Summary

In summary, therefore, it seems that the dorsoventral and craniocaudal polarity of the early embryo are controlled as a result of dynamic interactions between cell populations and their environment. The production of the mesoderm as a new cell type during gastrulation has long been assumed also to be the result of cell interactions. However, careful consideration of the evidence in favor of this notion reveals that it is not sufficiently well founded to be accepted without further investigation, and it may yet turn out that the mesoderm and other cell types are allocated as a result of the lineage history of the progenitor cells. Cell lineage mapping, combined with transplantation and an analysis of new cell-type-specific markers as they appear should resolve this issue.

Setting up Segmental Organization

The segmental organization of vertebrate embryos is most obvious in the pattern of somites from which derive the vertebrae and ribs of the axial skeleton,

the dermis of the trunk, and all the voluntary musculature of the adult are derived. The metameric pattern of somites determines the segmental arrangement of other structures in the embryo, such as the peripheral nervous system (16, 17, 41, 42, 45).

In the chick embryo, some 55 pairs of somites form, each somite being, at first, an epithelial sphere that buds off the rostral (anterior) end of each of the paired segmental plates of paraxial mesoderm that appear about 1.5 days after the egg is laid. Each pair of somites takes about 1.5 h to form. Some 6 to 8 h after its initial appearance, each somite splits up into two further components: (1) the dermomyotome dorsally, which retains some epithelial characteristics that give rise to the dermis of the trunk and to skeletal muscle and (2) the sclerotome ventromedially, which is a loose mesenchyme that gives rise, along with the notochord, to the axial skeleton. Each sclerotome is subdivided into a rostral (anterior) and a caudal (posterior) half (16, 17). Differences between the cells of the two halves determine the segmental organization of the peripheral nervous system: motor nerves and neural crest cells (16, 17, 29) are restricted to the rostral half of each sclerotome and are unable to colonize the caudal half.

During the formation of a somite, its progenitor cells have to make several decisions in order to realize their morphogenetic potential. Among them, we can distinguish: (1) When does a cell become committed to be part of a somite rather than part of any other mesodermal derivative? (2) When do sclerotome cells decide to become rostral or caudal? (3) When do cells choose to become dermomyotome as opposed to sclerotome? (4) When, if at all, are regional differences between somites determined?

**Determination of Somitogenic Potential**

Since the cranial portion of isolated segmental plates can form somites, while the caudal portion cannot (3, 34), the somitogenic potential of a cell may be determined during its sojourn in the plate.

A recent, albeit preliminary, experiment using lineage labels injected into single cells (39) may help us determine more precisely the time at which cells become restricted to a somitic fate. The results of this experiment, summarized in Fig. 4, show that as late as two cell divisions prior to segmentation, somite progenitor cells also contribute to other mesodermal tissues such as lateral plate and mesonephric kidney. A single injected cell at the caudal end of the segmental plate gives rise, 2 days later, to some 24 to 26 cells in a single somite and to some 28 cells scattered among other mesodermal tissues. Since the somite progenitor cells at this time appear to be scattered over a broad region at the caudal end of the segmental plate and primitive streak of the chick embryo, it seems unlikely that the decision between somite and nonsomite is made as a result of position. It is more likely that an unequal division of each progenitor cell gives rise to one daughter with somitogenic

FIGURE 4. Summary of results from single-cell lineage experiments. The diagram on the left illustrates the embryo at the time of injection of rhodamine-lysine-dextran into a single cell, while the drawings in the center and right summarize the distribution of the progeny of the injected cell two days later in the whole embryo (center) and in transverse section (right). A cell injected anywhere within the cranial half of the segmental plate gives rise to a clone of about 16 to 64 cells which is restricted to one somite, but not to any particular portion of it. A cell injected in the caudal one-third of the segmental plate also gives rise to about the same cell number of labeled cells that are restricted to one somite, but a large number of labeled cells are also seen in the intermediate and lateral plate mesoderm, in the endothelium of the floor of the aorta, and in circulating blood. Based on the results of Stern et al. (39).

potential and a slow rate of cell division (9 to 10 h) and one with nonsomatic fate that divides faster (5 h) (Fig. 4). Thus, the commitment to a somitic fate is probably a consequence of cell lineage history, rather than of position (Fig. 5).

Rostral-Caudal Determination

When do sclerotome cells become committed to being rostral or caudal? If either half of a newly formed somite is excised and transplanted into any other site in the embryo, it always gives rise to sclerotome with the properties of the half of origin, irrespective of the position into which it is grafted (42). This rules out the possibility that the rostrocaudal fate of somite cells is determined after the time of overt somite formation.

**FIGURE 5.** Diagram illustrating the positions at which the various cellular commitments involved in somite formation might take place. Since somite formation is a progression in time and space, such that “younger” cells are more caudally placed than more mature ones, the more caudal positions (lower portion of diagram) correspond to earlier points in the developmental sequence.
If rostrocaudal commitment is made before segmentation, there must be some mechanism to ensure that the correct cells end up in the correct half of the somite. There are two possibilities: (1) cells are fixed in position after determination or (2) cells are able to move in the segmental plate but can sort out according to their rostrocaudal nature (Fig. 1). There are arguments against both of these alternatives. An experiment by Menkes and Sandor (25) rules out the first possibility. They dissociated the segmental plate and found that it still produced normal somites. We have found that the rostrocaudal composition of the resulting sclerotomes is also normal. If cells are fixed in position in the segmental plate mesoderm, a restriction consistent with the existence of somitomeres (morphological presomite condensations in the segmental plate; Ref. 24), dissociation of the segmental plate should result in a loss of the rostrocaudal pattern. Moreover, cell movement has been reported to occur in the segmental plate mesoderm (41, 47, unpublished observations). If, on the other hand, cells move within the segmental plate, it would be difficult to maintain a fixed pattern of somitomeres. It would also be difficult to explain why rotation of the segmental plate results in reversal of the pattern (16), since cells should sort out to restore the original arrangement. Similarly, the experiment of Menkes and Sandor (25) should lead not to a normal repeated rostrocaudal sequence but rather to one large rostral and one large caudal half-sclerotome. Finally, in order for rostral and caudal cells to recognize each other for the purpose of sorting out, they would have to express some rostrocaudal differences in the segmental plate. There is no evidence for this at present.

Therefore, it seems likely that the rostrocaudal decision is made during somite formation, perhaps in relation to the length of time each cell spends adjacent to the developing segment border (41). One interesting consequence of this is that, since the determination of the rostral half of a newly forming somite as rostral would coincide with the determination of the caudal half of the preceding somite as caudal, rostrocaudal determination would be parasegmental, as has been suggested for the epidermal segments of Drosophila (22). If this is the case, rostrocaudal determination would be an example of a developmental decision made in relation to position, rather than lineage history of the cells (Fig. 5).

Specification of Sclerotome and Dermomyotome

The dorsoventral polarity of the somites forming from the cranial tip of the segmental plate can still be reversed by inverting the plate (9, 13). This finding indicates that somite cells are specified as dermomyotome or sclerotome close to the time of somite formation, but before subdivision, some 6 to 8 h later. The commitment as dermomyotome or sclerotome therefore depends upon position, by interactions with the adjacent epiblast and endoderm (Fig. 5).
Regional Specification

Vertebrae in different regions of the spinal column are morphologically different from one another, suggesting that individual somites have defined regional identities. At what stage are somite cells determined to form particular skeletal elements? When thoracic segmental plate mesoderm is grafted into the cervical region, ribs develop in the neck (20). The same is true for the plumage pattern, which is derived from the dermatomes (23). The muscle pattern, on the other hand, does not behave in this way: when nonwing level somites are transplanted to the wing region, they give rise to normal wing muscles (6) and are innervated appropriately for their new position (18). These results could be interpreted to mean that skeletal and dermal derivatives of the somite are regionally determined in the segmental plate or earlier, while the voluntary muscles become determined much later. However, it is possible that regional specification for dermis and sclerotome does not take place until later; this alternative explanation requires that cells behave autonomously within the plate, being unable to take positional cues from other regions. Clearly, these transplantation experiments do not help us to determine when regional specification occurs.

Because somite pairs form sequentially, specification as cervical, thoracic, etc., could be linked to the time of formation of each somite pair. Heat-shock and other experiments (reviewed in Ref. 17) suggest that somite progenitor cells have an internal “clock” that makes them competent to segment at a particular time. This mechanism could control the size of somites by regulating the number of cells that segment at any one time. The experiments suggest that this clock is linked directly to the cell division cycle, and that this clock is already operating four division cycles before segmentation, that is, about two divisions before cells decide to become somitic (see above, “Determination of Somitogenic Potential”). It is possible that regional specification for sclerotome and dermal derivatives of the somite could also be linked to this clock. If this is the case, this would be another example of a lineage-related decision (Fig. 5). On the other hand, the myogenic cells, at least at limb levels, can become any muscle and be innervated by any motor nerve until they enter the limb, 24 h or more after the corresponding dermomyotomes form. The commitment to form a particular muscle, therefore, is an example of a position-related decision, as myoblasts would need to interpret positional cues from the rest of the limb (Fig. 5).

Summary

The preceding discussion illustrates that even within what appears to be a single process, that of somite formation, six of the developmental decisions that can be recognized are evenly divided in their use of the two strategies for creating cell diversity in relation to pattern: somitogenic potential and the regional properties of the dermis and axial skeleton all appear to be determined by lineage history (Fig. 1a), while it is likely that the decisions between rostral
and caudal sclerotome, between sclerotome and dermomyotome, and between
different skeletal muscles all depend upon cell interactions with neighboring
tissues (Fig. 1b). In the case of the decisions we can identify during somite
formation, therefore, it appears that those decisions that are made early (two
cell cycles or more before overt segmentation) depend upon the lineage history
of the cells, while those that are made late (during segmentation or later) de-
pend upon cell interactions (Fig. 5).

Conclusions

During the development of vertebrate embryos, therefore, the fates of cells di-
verge both as a result of cell lineage history and as a result of interactions
with their environment. Although it is easy to list advantages and disadvan-
tages to each of these strategies, the mechanisms that cause cells to use one or
the other method to generate diversity in relation to their final spatial ar-
rangement are not always immediately obvious.

It is worth considering the possibility that different organisms use different
strategies to generate similar structures. Because of the importance of cell
diversity and pattern, it is possible that form is controlled by evolution more
directly than the mechanisms used to produce it.

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General References


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cell lineage determines fate) tends to be favored by molecular biologists. Unlike those who study them, embryos seem to use all the mechanisms available to them to give rise to organized diversity.

2. What are the prospects for superimposing a genetic approach on the vertebrate somite development problem?

I am very optimistic. In the last 5 years, we have learned a huge amount about somite formation and about the subsequent development of vertebrate segments at the cellular level. This knowledge should help to bridge the gap between the pattern and the genetic approach. Many mouse mutations displaying abnormalities in various aspects of somite development have been known for many years (see Ref. 17 for review and references), but only a few have been studied in detail. This was probably due to a lack of understanding of the cellular processes involved in somite development. As this situation is changing, many more investigators are turning their attention to the study of these mutations. Another major advance will derive from vertebrates that have been introduced recently as developmental model systems. The most interesting recent addition is the zebrafish embryo, which allows a study of development at the molecular, genetic, and cellular levels.

To give a full answer to the editor’s question, however, requires an understanding of what “the vertebrate somite problem” is. I see it as comprising many separate but related aspects. My aim in this chapter was to attempt to separate out some aspects of somite development that will probably have to be studied as independent issues. In order to confront the challenge of seeking the molecular bases of cellular decisions, we have to know the time and place at which these decisions are made.

But will meaningful results come from those approaching the problem from the genetic level or from those approaching it from the cellular-tissue level? Both are systematic, but while the latter is more elegant because experiments can be designed to follow each other logically, the former has proved to be more efficient. If asked for my own preference, I would choose logical elegance rather than efficiency. The indiscriminate molecular approach feels too much like solving a crossword puzzle by searching through the dictionary from “A” to “Z,” looking for words that fit each clue.