Chick Embryo: Early Development

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CLEAVAGE, BLASTODERM FORMATION, AXIS FORMATION, AND HYPOBLAST FORMATION IN THE CHICK EMBRYO

Development of the bird egg begins with fertilization of the ovum in the mouth of the oviduct. Enveloping layers form around the zygote as it passes down the oviduct and enters the uterus. In the uterus, the zygote, which is a disc of cytoplasm atop a large yolk mass, cleaves into a thick disc of cells, the blastoderm, with a cavity beneath (Fig. 9A,B). The future anterior-posterior axis is determined while the blastoderm sheds many cells into the cavity beneath, and while other cells intercalate from the lower layers into the uppermost layer, forming a single-layered, more translucent region, in the central region of the blastoderm, called the area pellucida, from which the embryo will later arise (Figs. 9C, 10). The egg is laid in this condition and further development requires incubation. In

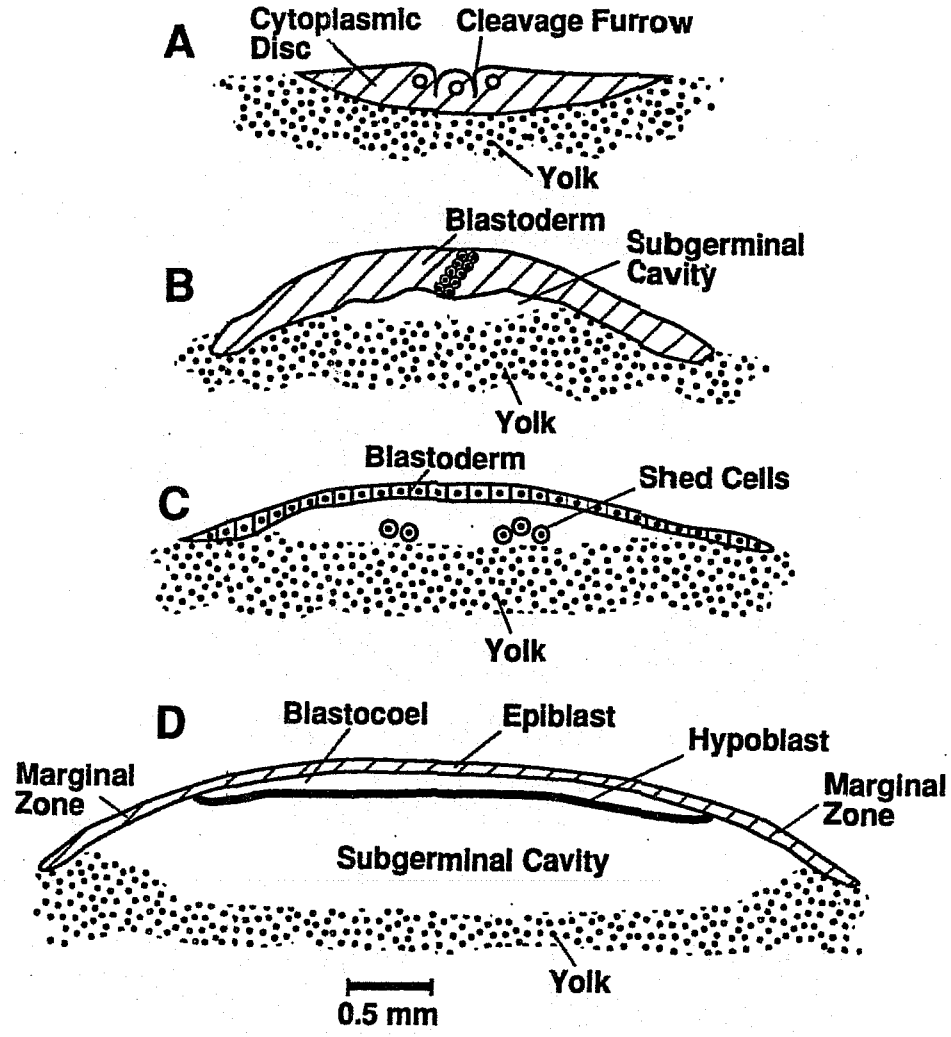

Fig. 9. Sections through the future midline of chick embryos during cleavage, axis formation, and hypoblast formation. Posterior is to the left and anterior to the right, using Von Baer's rule. **A.** Early cleavage. Eyal-Giladi and Kochav stage I. **B.** Cleavage has produced a blastoderm five to six cells thick. Stage VI. **C.** Blastoderm cells of the lower layers have shed into the subgerminal cavity, and others have intercalated into the upper layer which is now but one cell thick. Stage X, as the egg is laid. **D.** After the egg is laid and incubated, the hypoblast and blastocoel have formed. Stage XIII. All figures are drawn to the scale indicated. (Drawn from photos in Kochav and Eyal-Giladi, 1980.)

the first few hours of incubation, some cells ingress beneath the area pellucida and form a lower layer called the hypoblast (Figs. 9D, 10). We now describe these events in more detail.

The ovum of the chick, surrounded by its vitelline membrane, is shed into the infundibulum of the oviduct where it is fertilized. During the approximately five and a half hours that it takes the zygote to traverse the oviduct, layers of albumen and two shell membranes are enwrapped about the egg. The chick zygote consists of an island disc of cytoplasm atop the massive sphere of yolk. Cleavage begins after the zygote enters the uterus. The embryo takes about 20 hours to pass the length of the uterus and be laid. While in the uterus, the egg is rotated around its long axis by uterine muscles at 10 to 15 rounds per hour, and this rotation has a role in axis formation. The calcareous shell is secreted by the uterus.

To study development of the embryo in the uterus, the hen is sacrificed and the enclosed eggs examined, as done by Patterson (1910) and others, or the eggs may be aborted manually for study (Kochav and Eyal-Giladi, 1971). The considerable development of the egg that takes place during the 20 hours spent in the uterus and the first few hours of incubation after the egg is laid has been described and staged (roman numerals) by Eyal-Giladi and Kochav (1976) and Kochav, *et al.* (1980). The description that follows of this period of development is derived from those papers, and from the reviews by Eyal-Giladi (1984) and Vakaet (1984a).

Cleavage is superficial and is initially incomplete. Cells are open to the yolk underneath and peripherally (Fig. 9A). The earliest cleavage furrows are all vertical, but eventually furrows spread beneath some of the central cells to form closed cells. The pattern of cleavage is variable. Stages I to VI are cleavage stages and occur during the the first eleven hours that the egg is in the uterus. The disc of cytoplasm of the zygote is about 2 millimeters in diameter. During cleavage the diameter of the disc decreases somewhat while the thickness increases. By the end of the cleavage period (stage VI), the entire disc of cytoplasm has been cleaved into complete cells (Fig. 9B). The central area is five to six cells thick. Beneath the disc a subgerminal fluid-filled cavity has formed that separates the blastodisc from the yolk. The disc can now be considered a blastoderm. From this flat blastoderm, all of the parts of the embryo and the extraembryonic regions will

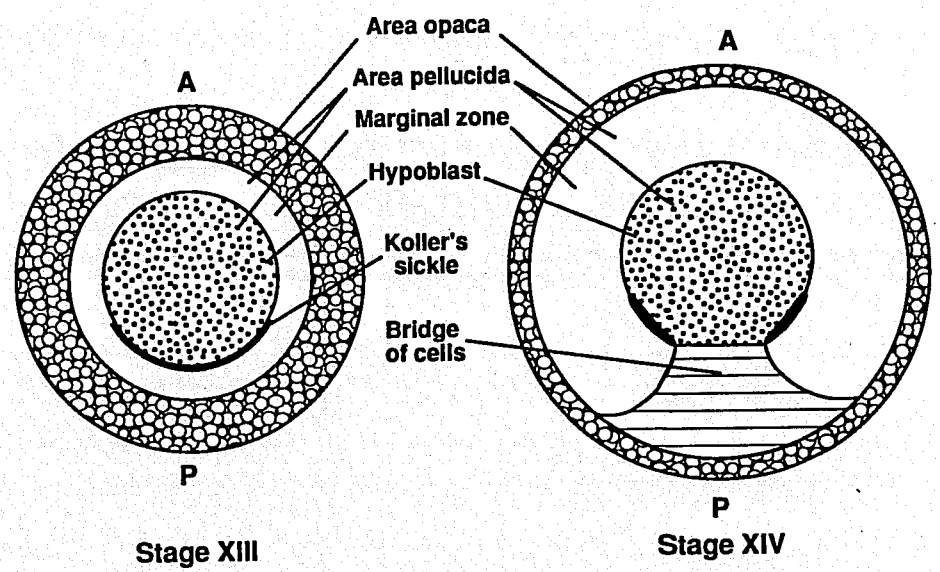

Fig. 10. Views of the ventral surfaces of chick embryos after hypoblast formation (Stage XIII), and later, after accumulation of a bridge of cells that will initiate the primitive streak (Stage XIV). Koller's sickle has been the source of the posterior hypoblast cells. Anterior (A) is at the top, and posterior (P) is at the bottom. (Drawn from photos in Eyal-Giladi and Kochav, 1975).

become segregated.

During cleavage stages, there is no basement membrane (no detectable fibronectin or laminin, Mitrani, 1982). During early cleavage, the nuclei lack nucleoli, but nucleoli form and begin to function by the end of cleavage stages (Eyal-Giladi, 1984).

Following cleavage stages, and during the rest of the period in the uterus (stages VII to X, 12 to 20 hours in the uterus), the area pellucida forms. Eyal-Giladi and Kochav (1976), Kochav *et al.* (1980), and Fabian and Eyal-Giladi (1981) describe the shedding of cells from the deeper layers of the blastoderm into the subgerminal cavity during formation of the area pellucida. The shed cells degenerate in the subgerminal cavity. Up to stage VI, the cells of the lower layer are interconnected by filopodia, but at stage VI some of these cells withdraw their filopodia and begin to round up (Fabian and Eyal-Giladi, 1981). The cells begin

to shed at stage VII. Shedding begins in the future posterior end of the embryo and proceeds anteriorly. By the time the egg is laid at stage X, the formation of the area pellucida is complete and the border with the thicker area opaca (the outer annulus of blastoderm still attached to the yolk) is sharply defined (Fig. 9C). During area pellucida formation, the thickness of the blastoderm is reduced from five to six cells to a layer just one cell thick.

Beginning at stage VII, after the cleavage stages, the diameter of the blastodisc begins to increase. Cell division, of course, continues during this period. The literature is not clear as to when cell division leads to growth, but Downie (1976) has observed that cell volumes in the extraembryonic epiblast are halved with each division during the first day of incubation after the egg is laid, but thereafter cell division is followed by a growth period that restores the size of the daughter cell to that of the mother cell. It would seem likely, therefore, that cell proliferation does not produce growth during uterine stages. This leaves open the question of how the blastoderm expands during these stages. One possibility is that expansion results when cells of the deeper layers intercalate with cells of the surface layer. Vakaet (1984a) favors the view that deeper cells intercalate with cells of the surface layer during area pellucida formation, leading to the expansion of the surface layer. Both Vakaet and Eyal-Giladi are likely correct, that is, both intercalation and cell shedding may be occurring during area pellucida formation. The newly-laid hen's egg has a blastoderm that is 4mm in diameter, compared to about 2mm in diameter in the zygote. Thus about a doubling of diameter of the blastoderm and a quadrupling of its surface area occurs during later development in the uterus.

The critical period for axis formation is from the end of stage VII to the middle of stage VIII (14 to 16 hours in the uterus) (Vintemberger and Clavert, 1960). This period coincides with area pellucida formation and cell shedding from the lower surface in the posterior regions of the blastoderm. The anterior-posterior axis of the embryo can be predicted with the well-known rule of Von Baer (1828). Holding the egg with the sharp end of the shell to your right, the anterior-posterior axis will be at right angles to the long axis of the shell, and with the posterior end toward you. Normally the egg is laid pointed end first.

Vintemberger and Clavert correlated the direction of rotation of the egg in the

uterus with the polarity of the axis. The future anterior end forms in the direction of rotation. In an egg at rest, the circular blastoderm sits atop the attached massive yolk. When the egg is rotated, the heavy yolk remains nearly stationary and the albumin, shell membranes and shell rotate about it. Kochav and Eyal-Giladi (1971) have demonstrated that gravity imposes the direction of the axis. They removed uterine eggs and rotated them, observing the position of the blastoderm. The blastoderm was tilted somewhat in the direction of rotation, and the posterior end always formed on the uphill side. They also showed the axis could be imposed by gravity without rotation by hanging the egg in a beaker of normal saline by one chalaza (the spiral of thick albumen at each end of the yolk), or by incubating whole eggs in vertical positions, either blunt end up or sharp end up. In these cases, the yolk is unable to rotate and the blastoderm is on one side rather than on top and the uppermost side of the blastoderm always formed the posterior end of the embryo. If the blastoderm is kept in a horizontal position with relation to gravity, then no axis is formed (Olszanska *et al.*, 1984).

When the egg is laid at stage X, after about 20 hours of development in the uterus, the area pellucida has formed and the underside of the blastoderm in the area pellucida has begun the next phase of development, the formation of the hypoblast. Polyinvagination (ingression) of small cells from the upper epithelial layer results in patches of epithelium on the lower surface. The basal surface of this new epithelium is upward toward the basal surface of the epiblast. The numerous investigators that have contributed to this understanding are cited by Eyal-Giladi (1984, p. 249). A second source of hypoblast cells, the prospective posterior portion of the hypoblast, is first seen as a concentration of cells in a crescent form (Koller's sickle, Fig. 10) near the posterior end of the area pellucida. A shelf of hypoblast epithelium emerges from this sickle of cells, which have emerged from what will be the posterior marginal zone (Spratt and Haas, 1965; Azar and Eyal-Giladi, 1979), and extends anteriorly, becoming confluent with the patches of ingressed cells encountered along the way until the hypoblast becomes complete. The overlying upper layer of blastoderm then can be termed the epiblast. The hypoblast does not extend peripherally to underlie the entire area pellucida. The border of the area pellucida that is not underlain by hypoblast is termed the

marginal zone. The important conclusion is that some hypoblast cells segregate themselves from the epiblast by ingression at multiple sites, and a shelf of confluent hypoblast cells emerging from the future marginal zone at the posterior border extends anteriorly, joining with the patches of ingressed hypoblast encountered along the way. By stage XIII the hypoblast is complete (Figs. 9D, 10).

The hypoblast becomes separated from the epiblast by a closed cavity, the blastocoel. Probably this cavity begins to form as soon as any portion of the hypoblast becomes complete, so it should be expected to appear first in the posterior end where the hypoblast is first a complete layer. The literature is not clear on this point. Sodium pumping might be involved in blastocoel formation, as well as in creating the ion currents that flow out of the primitive streak to be discussed below.

Hamburger and Hamilton (1951) have staged chick embryos commencing with incubation after the egg is laid. Reference to their stages is made in roman numerals. Stages X to XIV of Eyal-Giladi and Kochav (1976) fall within stage 1 of Hamburger and Hamilton.

EPIBOLY

Epiboly in the chick embryo is a monumental task, and is not completed until after four days of incubation. Eventually the blastoderm spreads to encompass the entire massive sphere of yolk. Epiboly does not begin until about eight to twelve hours of incubation.(Downie, 1974). Cells of the blastoderm do not attach to the vitelline membrane until five to six hours of incubation (Vakaet, 1962). Prior to ten hours of incubation, cultured epiblast cells stick to one another only briefly, but after ten hours of incubation, these cells stick to one another permanently (Downie, 1974). The active cells in the expansion of the epiblast are those that occupy the perimeter. These cells have localized adhesive sites on their outermost upper edges that adhere to the inner surface of the overlying vitelline membrane, and this adhesion appears to be necessary for the migratory behavior of this

marginal band of cells that expands the blastoderm, creating considerable tension on the rest of the epithelium of the epiblast. New (1959) found that blastoderms only expand when their margins are spreading, and a marginal band of cells isolated from the rest of the blastoderm will expand by itself on the vitelline membrane. New (1959) also demonstrated the localization of the adhesive region of the marginal cells by placing a blastoderm upside down on the undersurface of the vitelline membrane, after which the blastoderm edge turned under itself as the adhesive upper edge attached to and migrated on the membrane.

At the start of epiboly, the epiblast cells compose a low columnar epithelium, but soon the epiblast cells become flattened (squamous) and remain that way during the period of epiboly (Downie, 1976). This flattening is likely due to the tension in the blastoderm produced by the centrifugal expansion of the blastoderm caused by the edge cells migrating against the vitelline membrane. The amount of tension would be affected by the balance between proliferation of cells within the blastoderm and the pulling of the edge cells (Downie, 1976). The active edge cells are a band about three cells wide and layered two to three cells deep (Downie and Pegrum, 1971). These cells underlap one another so that the outermost perimeter consists of just the leading lamellipodia of a single set of outermost cells. These lamellipodia pull on the underside of the vitelline membrane. Trinkaus (1984b) critically reviews chick epiboly and points out the problem of whether or not the underlapping cells advance to take a turn at pulling, and whether there is cell intercalation and de-intercalation among the edge cells. Since the diameter of the ring of edge cells changes dramatically during epiboly, some mechanism is needed for increasing the ring size until the blastoderm reaches the equator of the yolk, then decreasing the ring size thereafter. Individual cell behavior will have to be examined to settle these questions. Proliferation of cells occurs in the blastoderm during epiboly, but the edge cells are not dividing. Since these cells do not divide, it is necessary to assume that intercalation of cells occurs as the ring of edge cells increases diameter. The lack of proliferation in the edge cells is consistent with the observation that actively migrating cells do not divide.

INDUCTION OF THE PRIMITIVE STREAK

Gastrulation of the flat blastoderms of bird embryos is accomplished through the activities of a morphological entity called the primitive streak. In this section we will examine how the primitive streak becomes induced in the epiblast by interactions with the underlying hypoblast.

Waddington (1932,1933) concluded on the basis of rotation experiments that the hypoblast induces the tissue movements in the epiblast that lead to formation of the primitive streak. Spratt and Haas (1960) suggested that the movements within the hypoblast influenced the formation of the primitive streak. In contrast, Eyal-Giladi and Wolk (1970) found primitive streak induction occurred after they had interposed a millipore filter between the hypoblast and the epiblast, and they concluded that primitive streak formation is not dependent upon movements within the hypoblast.

By rotation of hypoblast in relationship to the epiblast in precisely staged blastoderms, and by heterochronic recombinations of hypoblast and epiblast, Azar and Eyal-Giladi (1981) assessed the temporal changes in the inductive ability of hypoblast and in the response capability of the epiblast to form a primitive streak. They concluded that the hypoblast reaches a peak of ability to induce primitive streak at stage XIII (within stage 1). Within the hypoblast, ability to induce primitive streak is distributed in a gradient fashion with a maximum at the posterior end and declining laterally and anteriorly. The epiblast is also maximally competent to respond to primitive streak induction at stage XIII, with a similar maximum at the posterior end and a gradient of declining response laterally and anteriorly. When these two fields (the hypoblast and the epiblast) are rotated in relationship to one another at stage XIII, the inductive hypoblast dominates in fixing the site of primitive streak formation. Following stage XIII, competence of the epiblast to respond declines more rapidly than does the ability of hypoblast to induce primitive streak formation. Competence to form a primitive streak is limited to the posterior half of the epiblast at stage 2, and is gone at stage 3. The ability of hypoblast to induce a primitive streak (in younger epiblast) is still present at stage 3.

The ability to induce a primitive streak is restricted to that portion of the hypoblast that emerges from the posterior marginal zone. Azar and Eyal-Giladi (1979) removed the hypoblast from stage XIII embryos. New hypoblasts regenerated and primitive streaks were induced. However, if both the hypoblast and the marginal zone were removed, then the hypoblast that regenerated could not induce a primitive streak. They concluded that only that portion of the hypoblast that arises from the marginal zone can induce primitive streak.

Khaner and Eyal-Giladi (1986) rotated the hypoblast and epiblast in relationship to the marginal zone, or relocated pieces of the marginal zone from posterior to lateral regions and vice versa in embryos at stages X to XII. From these experiments, they concluded that the ability to induce primitive streak resides in the marginal zone at stage X, with a maximum at the posterior end. At stage XI, the posterior marginal zone is contributing cells to the inductive part of the hypoblast and the ability to induce the primitive streak is shifting from the marginal zone into the hypoblast. At stage XIII the hypoblast is maximally able to induce and the epiblast is maximally able to respond. Nevertheless, removal of the hypoblast at this stage is followed by regeneration of an inductive hypoblast if the posterior marginal zone is still present.

SUMMARY OF PREGASTRULAR EVENTS

Cleavage divides a disc of cytoplasm atop the yolk into a disc of cells. The cellular disc initially becomes several cells thick, but many cells beneath the surface layer are shed in a polar way into the subgerminal cavity (between the cellular disc and the yolk) where they appear to die. Other cells of the lower layers apparently intercalate into the surface layer as the area of this upper layer expands. These events coincide with axis determination and occur while the egg is being rotated within the uterus. The yolk and attached cellular disc are tilted with respect to gravity during rotation, and the axis is fixed so that the posterior end is on the highest side of the cellular disc. After shedding and spreading, the blastodisc is just one cell thick in the central region (area pellucida), but thicker peripherally

vhere it attaches to the yolk (area opaca).

The next event is the formation of the hypoblast, which occurs when the egg is ncubated after being laid. Cell ingress from the surface layer at many sites "polyinvagination") and form patches of prospective hypoblast. Other cells ngress from the posterior marginal zone of the blastodisc to form a shelf of cells nat then extends anteriorly, becoming confluent with the patches of hypoblast vhen encountered. The end result is a flat blastoderm composed of epiblast (upper ayer) and hypoblast (lower layer) with a fluid-filled cavity between (the lastocoel). These are the conditions when gastrulation begins. The posterior ypoblast induces the primitive streak which initiates gastrulation.

GASTRULATION

At the beginning of gastrulation, all three prospective germ layers of the mbryo are located in the epiblast. The hypoblast is not a part of the embryo, and t most is involved in gastrulation only passively. The main role of the hypoblast s to induce the primitive streak.

How a flat blastoderm gastrulates is best understood in the chick embryo. Rather than a whole sheet of cells involuting into the interior through a blastopore, s in amphibian and other embryos, chick gastrulation is a more subtle onvergence of epiblast cells, and ingression of endodermal and mesodermal cells rom the epiblast through a primitive streak. In this section we will describe the rganization of morphogenetic events that accomplish gastrulation in this form.

Mesoderm induction and the earliest phases of initiation of the primitive streak ccur during stages X to XIV of Eyal-Giladi and Kochav (1976). As noted above, nese stages are early phases of incubation following the laying of the egg, and oincide with Hamburger and Hamilton (1951) stage 1.

Gastrulation begins after the embryo has formed an epiblast and hypoblast, nd during most of gastrulation, epiboly exerts a centrifugal tension in the epiblast. At stage XIII there is a marginal zone free of hypoblast between the hypoblast and ne area opaca. At the end of stage 1 (at stage XIV), a bridge of cells accumulates

spanning the posterior marginal zone (Fig.10). These accumulating cells are the beginning of the primitive streak (Eyal-Giladi and Kochav, 1976).

After first appearing as a massing of cells at the posterior end of the epiblast, the primitive streak extends anteriorly to its fullest length at stage 4 (Fig. 11). During the extension of the primitive streak, the entire blastoderm is distorted from a disc into a pear shape (Fig. 11). The new forms probably involve distortions of the epiblast in response to the centrifugal force of epiboly and the polar convergence occurring in the streak, and rearrangements of the cells in respect to their neighbors since a permanent record of tensions is not reflected in the shapes of the cells, most of which become isodiametric after brief distortions.

The exact mechanisms of streak formation are not known, but the movements of the cells have been deduced from fate mapping, using markers of various sorts, and from time-lapse cinematography (see Bellairs, 1971 for references). As indicated in the drawings (Fig. 11), the streak is initiated as cells converge to the posterior midline, and as convergence continues, the streak elongates along the anterior-posterior axis. This convergence-extension is much like that seen in the amphibian embryo and may be due to similar intercalation behavior of the cells. However, a distinct difference in the avian embryo is the massive ingression of cells that occurs through the streak region even while the streak is elongating. As cells enter the streak, they deform, becoming bottle-shaped in the streak, and carrying the deformation to the extreme of reducing apical surface to a point, then detaching as they ingress beneath the streak. Because the cells all change to a bottle shape, the cell density is considerably greater in the streak than in the rest of the epiblast.

As the primitive streak is forming, individual streak cells ingress through a basal lamina (Mitrani, 1982; Vakaet, 1984b). Streak cells begin blebbing basally as they converge toward the midline of the streak (Vakaet, 1984b). As the streak forms, the basal lamina is lost beneath it (Sanders, 1984). When streak cells ingress, they detach from the epithelial epiblast and begin to migrate as mesenchyme cells. Considerable behavioral differences result from the conversion from epithelial cell to mesenchymal cell.

Epithelial cells have an apical-basal polarity and they attach laterally one to

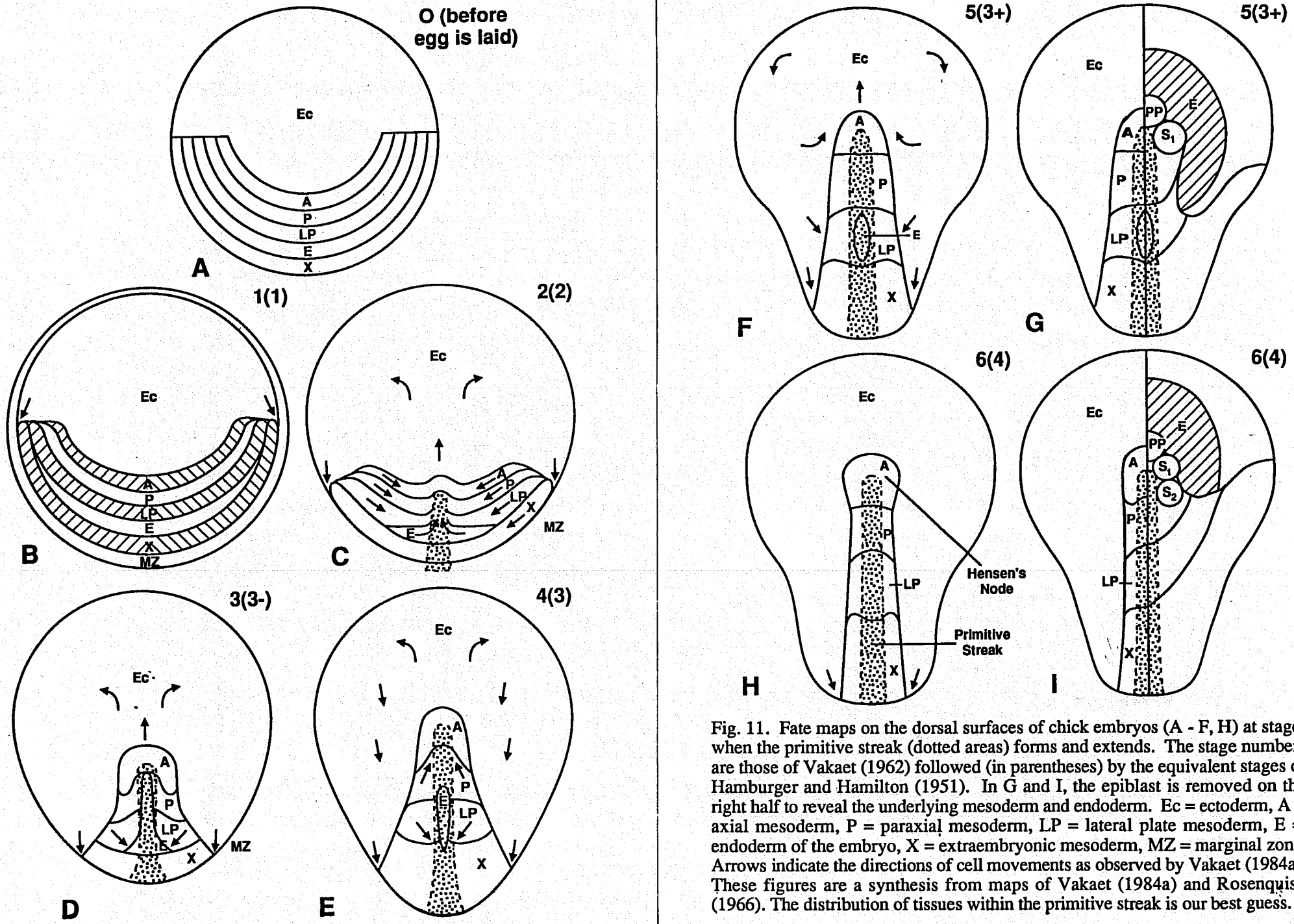

Fig. 11. Fate maps on the dorsal surfaces of chick embryos (A - F, H) at stages when the primitive streak (dotted areas) forms and extends. The stage numbers are those of Vakaet (1962) followed (in parentheses) by the equivalent stages of Hamburger and Hamilton (1951). In G and I, the epiblast is removed on the right half to reveal the underlying mesoderm and endoderm. Ec = ectoderm, A = axial mesoderm, P = paraxial mesoderm, LP = lateral plate mesoderm, E = endoderm of the embryo, X = extraembryonic mesoderm, MZ = marginal zone. Arrows indicate the directions of cell movements as observed by Vakaet (1984a). These figures are a synthesis from maps of Vakaet (1984a) and Rosenquist (1966). The distribution of tissues within the primitive streak is our best guess.

another by specialized junctions and by cell-cell adhesion. Cytoskeletal elements, including intermediate filaments and actin filaments, attach within the cells to the junctions so that a structural integrity exists across the whole epithelium. Mesenchymal cells migrate individually or in bunches and in general are surrounded by considerable extracellular matrix. Mesenchymal cells have no fixed apical-basal polarity. Contacts between cells are not extensive and are made and broken repeatedly. Nevertheless, cells that ingress through the primitive streak to emerge as mesenchymal cells that disperse beneath the epiblast are organized immediately into patterns.

The mechanical properties of mesenchyme and epithelium are quite different. Cells in an epithelium have extensive interfaces and adhesive contacts between the lateral surfaces of the cells, and are tightly bound to one another by apical junctional complexes. Epithelial cells transmit forces to one another directly, via intercellular junctions, and sustain these forces in the continuous epithelial sheet by way of the filaments attached to the junctions within the cells. Extracellular matrix associated with epithelia is mostly located as basal lamina at the basal surfaces of the cells. Mesenchymal cells are largely surrounded by extracellular matrix, and the cells make relatively few contacts with one another, and such contacts are made and broken easily. Much of the mechanics of mesenchyme depends on interactions between cells and extracellular matrix. Traction by mesenchymal cells may distort the extracellular matrix, and reciprocally, the cells may respond to forces exerted upon them by the extracellular matrix.

While the streak is still forming and elongating (stages 2 to 4), cells are ingressing. A population of the first cells to ingress intercalate into the hypoblast and give rise to the definitive endoderm. The original hypoblast cells are displaced peripherally during this process (Bellairs, 1953; Vakaet, 1962; Modak, 1966; Rosenquist, 1971; and others). As endoderm cells from the epiblast are intercalated into the hypoblast, a mosaic of the two cell types is formed and this mosaic persists for the period of streak formation (Azar and Eyal-Giladi, 1983). One consequence of this is that inductive hypoblast cells persist beneath the streak during its formation. Eventually, after stage 4, these two populations sort out so that the hypoblast cells are peripheral. Such sorting out has been observed in

explant combinations of the two cell types (Sanders, Bellairs, and Portch, 1978). The displaced hypoblast becomes the endoderm of the yolk sac stalk (Rosenquist, 1972).

By the middle of stage 3, the prechordal plate (the most anterior axial mesoderm) has formed as a round patch of cells in the midline anterior to the streak, and to each side of the prechordal plate, the paraxial mesoderm has organized into circular domains that are the first pair of somitomeres (Triplett and Meier, 1982)(Fig. 12). The cells of these structures have just ingressed through the streak. By stage 4, a second pair of somitomeres is organized lateral to Hensen's node, and at stage 5, as the streak begins regression (regression is a posterior displacement and shortening of the streak), additional somitomeres form in the paraxial mesoderm just lateral to the regressing node, and the incipient notochord at the midline is laid down in segmental patches (Triplett and Meier, 1982) (Fig. 12). How the loose and wandering mesenchyme cells that compose these patterns manage to do so is still unknown, but similar patterns are found in gastrulating embryos of six other species of amniotes, amphibia and teleosts (Jacobson, 1988).

The primitive streak is used up as it regresses and lays down the axial and more lateral mesoderm. As the streak disappears, the node moves to the caudal end and its remnants are eventually incorporated into the tail bud (see Bellairs, 1986).

MODELS OF CHICK GASTRULATION

Ingression of the hypoblast could be the result of adhesive differences between epiblast and hypoblast cells, but since many hypoblast cells seemingly ingress randomly, it is difficult to suggest how such adhesive differences arise. Alternatively, adhesive differences could emerge between epiblast and hypoblast cells after ingression of the hypoblast cells, and the ingression could be the result of other factors.

Once the hypoblast is segregated and formed into a complete layer, a closed compartment is created between epiblast and hypoblast that could facilitate the

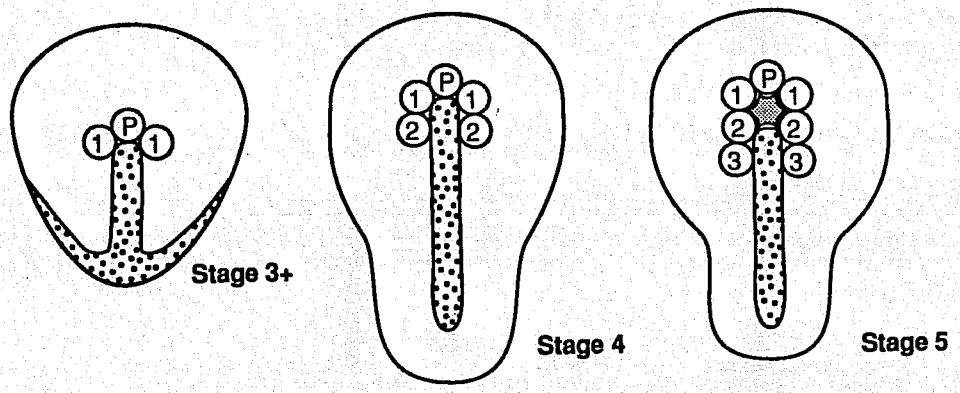

Fig. 12. Dorsal views of chick embryos with the epiblast removed to show the distribution of axial and paraxial mesoderm at three Hamburger and Hamilton stages. The primitive streak is indicated by the dotted areas. Stage 3+, extending streak. Stage 4, maximal extension of the streak. Stage 5, after some streak regression. P = prechordal plate (the most anterior axial mesoderm). The circles indicate somitomeres, and the numbers within the circles the order of their appearance. At stage 5, notochord (shaded) lies between prechordal plate and streak. (Modified from Jacobson, 1988).

organization of electrical currents that arise during primitive streak formation. Primitive streak formation and stabilization may rely on these ion currents. The primitive streak is the site of exit of strong electrical currents that re-enter through the epiblast (Jaffe and Stern, 1982). These currents were measured with a vibrating probe. Stern (1982) has found that the epiblast has a trans-epithelial potential such that the basal surface is more positive than the apical surface. Sodium ions enter the apical surface passively and are pumped out at the basolateral surface by a sodium-potassium ATPase. Water follows the osmotic gradient that the pump generates. Sodium and water accumulate in the cavity between the epiblast and the hypoblast.

The ATPase has been localized by autoradiography of epiblast after exposure to tritiated ouabain (Stern and Mackenzie, 1983). In the epiblast, the oubain binds to the basal surface, but binds basolaterally in the forming streak, and binds on the upper surface in the formed primitive streak. The polarity is thus reversed within the streak. Experimental reversal of polarity of electric currents through the epiblast results in positioning of extracellular matrix of basal lamina on the upper

rather than the lower surface of the epiblast (Stern, 1982). These observations may account for the ingression of cells through the streak.

Stern (1984) proposes that the epiblast transports sodium and water into the blastocoel between its basal surface and the hypoblast. When the basal concentration of sodium or the hydrostatic pressure exceed a threshold, the sodium-potassium ATPase stops pumping. This likely starts beneath the forming streak where the hypoblast is first continuous. As cells accumulate sodium, their junctions with adjacent cells open and they detach from the epiblast. Calcium is released from intracellular pools and activates contraction of apical microfilament bundles that then deform the cells to a flask shape and aids their detachment. The calcium also activates migratory activity and the cells ingress into the cavity below the epiblast. The opening of the junctions allows sodium and water to flow to the upper surface, a reversal of polarity in the cells, transport of sodium up through the primitive streak, and a circulating current that stabilizes and localizes the streak. Transport of sodium by the epiblast could thus lead to formation and stabilization of the primitive streak and ingression of cells through it.

Patterns of Adhesion and Fate Maps in a Chick Embryo

During gastrulation, there is considerable distortion of the epithelial epiblast. These distortions are seen in fate maps in Fig. 11. These maps, modified from Vakaet (1984a) and Rosenquist (1966), are a synthesis from previous maps made by following marked cells using vital staining, carbon marks, transplantation of cells treated with tritiated thymidine to non-treated hosts, or transplantations of cells between chick and quail embryos. Vakaet also followed movements with time-lapse video cinematography. These changes in the fate maps during gastrulation might be partly accounted for by adhesive differences that lead to sorting out in the plane of the epiblast. Sorting out of cells in a two-dimensional tissue has been demonstrated by Garrod and Steinberg (1973).

We propose a model invoking adhesive differences among the domains of prospective tissues seen on the surface of the epiblast in the fate maps. The model

is generalized and modified from the differential adhesion hypothesis developed by Steinberg (1963; 1970). Many of the movements seen could be explained if the following hypotheses were true:

1) The epiblast consists of different domains of cells. The adhesive affinity between cells varies among the domains in the following hierarchy: Marginal zone (MZ) > Ectoderm (Ec) > Apical mesoderm (A) >Paraxial mesoderm (P) > Lateral Plate mesoderm (LP) > Extraembryonic mesoderm (X) > Endoderm (E). This proposed order is similar to that which has been demonstrated for affinities among frog deep cells, that is, ectoderm > mesoderm > endoderm, as discussed above.

2) The cells at a particular level have a greater affinity for cells higher in the order than among themselves, and less for cells lower in the order.

3) The cells of the epiblast migrate within the plane of the epithelium and change contact relations with neighboring cells in ways that will increase the strengths of adhesion of their contacts.

4) When the axis is determined, the prospective marginal zone becomes most adhesive at its posterior edge.

Given the proposed adhesion hierarchy, the preincubation pattern is unstable and neighbor exchanges may occur that will reorder the pattern and produce greater stability. The pattern that can emerge is constrained by the original ordering to those new sorts of contacts that cells can make, since cells can interact adhesively only with other cells that they contact. For example, the highly adhesive marginal zone originally surrounds all the other tissues, and there is no way that the expected engulfment of the marginal zone by any of the other tissues it encounters can occur.

Extraembryonic mesoderm and ectoderm both abut the marginal zone, but ectoderm is much higher in the adhesive hierarchy than this mesoderm, so as cells move randomly about at the common boundary of ectoderm, extraembryonic mesoderm and marginal zone, ectodermal will replace mesodermal contacts with the marginal zone and the ectoderm-marginal zone boundary will increase in length. This would produce a convergence of ectodermal cells toward the posterior midline (Fig. 11C), and concomitant rearrangements of cells within the mesodermal regions as they become driven toward the posterior midline.

The cells within the semi-annulus that represents the region of prospective endoderm (Fig. 11A) are out of their hierarchical adhesive order in relation both to the lateral plate cells to one side and the extraembryonic mesoderm cells to the other. Lateral plate and extraembryonic mesoderm are higher in the adhesive hierarchy than is endoderm, so they will adhere more with themselves and with one another than either will with endoderm. Similarly, the mesoderms should adhere more strongly with ectoderm than would endoderm. As cells move about, probably randomly, mesoderm cells will replace endoderm cells at the ectoderm boundary, then the two mesoderms will stay abutted whenever they contact at the expense of contacts with endoderm. The endodermal cells are inevitably driven into the configuration that provides the least boundary with surrounding mesoderm, that is, a circle. The beginning of this process is seen in Fig. 11B. This set of cellular rearrangements amounts to a convergence toward the posterior midline, augmented by the effects of the ectoderm rearranging along the marginal zone boundary (Fig. 11C).

As the lateral-posterior ectoderm converges toward the posterior midline, a tension would be exerted on the remaining ectoderm that would draw the ectoderm in a wheeling motion away from the anterior midline and also pull on the midline of the mesoderm, stretching it toward the anterior pole (arrows, Fig.11C, D).

As cells converge toward the posterior midline, some cells pile up at the midline, forming the incipient primitive streak (Fig. 11C). The arrival of excess cells at the midline may relax locally some of the constant centrifugal tension produced by epiboly, allowing the posterior edge of the blastoderm to bulge. The stretching of the forming primitive streak in the anterior-posterior axis may be partly due to relaxation of some of the tensions of epiboly, and partly due to pulling anteriorly by the ectoderm in the midline there (but this topic is discussed further below).

The process of convergence produces a rounding up of the mesodermal and endodermal areas that would reduce the lengths of some boundaries between domains of cells of different adhesiveness, as should be expected. The rounding up is distorted from circles by stretching in the anterior-posterior axis as the primitive streak elongates (Fig. 11D, H). This stretching in the anterior-posterior

direction is not accounted for by the adhesion model and will be discussed further below.

Some distortion from a circle of the endodermal area is possible without increasing its boundary with surrounding tissues because ingression of endodermal cells through the streak, starting at least by Vakaet stage 4 (Hamburger and Hamilton stage 3) (Fig. 11D), reduces the area of endoderm that remains on the surface. Likewise, some reduction of the area composed of endoderm in the streak occurs when these endodermal streak cells elongate and reduce their apical areas. Extraembryonic mesoderm ingresses through the streak possibly beginning even earlier than the endoderm.

Paraxial mesoderm and axial mesoderm begin ingressing through the streak between the stages shown in Figs.11E and 11H. The prechordal plate (axial) and the first pair of somitomeres (paraxial) are established during the stage shown in Fig. 11E, and a second pair of somitomeres is established by the stage shown in Fig. 11H (Triplett and Meier, 1982).

Maximum length of the primitive streak is reached at the stage shown in Fig. 11H. The streak then regresses posteriorly while ingression reduces it volume and length. Notochord is laid down in segmental units in the axis by the regressing streak (Triplett and Meier, 1982), and additional paraxial somitomeres and lateral plate are laid down laterally. During streak regression, as ingression of cells reduces its volume, the boundary of mesoderm with the more adhesive ectoderm is reduced as should be expected. Eventually the ectoderm converges posterior to the regressing streak so the whole streak is surrounded by ectoderm.

The hypothesis that the axis is defined by an adhesion gradient highest in the most posterior marginal zone is one simple way of relating the site of primitive streak formation to the determinative events of axis formation that occurred in the uterus. The bridge of cells that gather at the posterior marginal zone (Fig. 10) as the forerunners of the primitive streak could gather there by following an adhesion gradient. As the ectoderm converges toward the posterior midline, its cells would be running up an adhesion gradient. Until replacement by the ectoderm, the prospective extraembryonic mesoderm should also converge along the adhesion gradient of the posterior marginal zone. This hypothesis is not essential to the

general argument of this model, but it adds some greater efficiency to it.

Events That May Contribute to the Elongation of the Primitive Streak

As noted above, the adhesion model does not account for the distortion of the endodermal region into a rod extending between lateral plate and paraxial mesoderm in the anterior streak (Fig. 11D). Vakaet (1984a) states that endoderm ingresses through the anterior streak beginning at his stage 4 (Fig. 11E). He says that the anterior streak is a mixture of endoderm and mesoderm with endoderm dominating at his stage 4, and mesoderm dominating at his stage 6. We have drawn the tissues within the streak at these stages (Figs.11C-H) as we interpret they may be. There is no firm data on their actual distribution. Since Triplett and Meier (1982) have shown that some axial and paraxial mesoderm has already ingressed by Vakaet stage 5 (Fig. 11F), there must be at least some of these tissues in the anterior streak at the previous stage (Vakaet stage 4).

In any event, the endoderm protrudes cranially between lateral plate and paraxial mesoderm at some stages, and this is contrary to expectations from the adhesion model. Other events are occurring that may contribute to this arrangement.

As the primitive streak forms, the cells that occupy the streak become densely packed because they assume bottle shapes with greatly reduced apical areas. It is in these cells that the ion current is the reverse of that in the rest of the epiblast (discussed above). This reverse ion current, besides perhaps influencing the cells to ingress, might at least temporarily moderate the adhesion characteristics of the streak cells. The streak cells lack the basal lamina found under the rest of the epiblast, so they can adhere only laterally, while the rest of the epiblast cells adhere basally to a basal lamina as well as to neighboring cells.

Another factor affecting epiblast cells as they enter the streak is a possible response to chemical signalling. A microelectrode loaded with cAMP (cyclic adenosine monophosphate) placed on the ventral surface of an explanted chick embryo causes the elongating primitive streak to divert toward the source

(Robertson and Gingle, 1977). Robertson, Grutsch and Gingle (1978) also found that chick embryo cells release cAMP when stimulated with cAMP within a limited physiological range of concentration. Chemical signalling may thus have a role in cell behavior during primitive streak formation, and this could over-ride adhesive characteristics. If so, one would expect that the normal signalling source would be Hensen's node.

The directions of movements that would result from the processes proposed here and illustrated in Fig. 11 are those that are actually seen in the epiblast during gastrulation (Vakaet, 1984a, p. 78).

Cell Migration Following Ingression, and Regression of the Primitive Streak

After cells ingress through the primitive streak, they then encounter a complex environment that includes variations in extracellular matrix and in adhesive properties of matrix and neighboring cells. The means by which the mesoderm cells become properly distributed beneath the ectoderm (Fig. 11G, I) are still unresolved (see review by Bellairs, 1986), but many studies have begun to define the nature of the extracellular matrix at these stages. Certainly one or more components of the extracellular matrix, if properly distributed, could help guide the migrating mesodermal cells to their proper positions. The cells could, for example, be following an adhesion gradient. The mesodermal cells could also be sorting out amongst themselves, or they could be moving laterally due to population pressure, and their fates be set by their order of ingression and/or by interactions with the local environment. These possibilities are not mutually exclusive, so any combination of them, and possibly of others as well, could be in operation.

The primitive streak reaches its maximum length at Vakaet stage 6, or Hamburger and Hamilton stage 4 (Fig. 11H). This stage is followed by a regression of the primitive streak posterally and a laying down of the body axis anterior to the streak as it regresses. Regression of the primitive streak is a posterior displacement of the entire streak, and a simultaneous decrease in streak

length and volume. Streak regression probably begins when the amount of ingression of cells begins to exceed the adding of new cells to the streak from convergence of lateral epiblast.

As axial mesoderm is put into place anterior to the streak, it begins or continues its typical convergent extension movements and this may help drive the streak caudad. As explained below, the notoplate in the midline of the emerging neural plate also does convergent extension, helping to elongate the axis. The streak may also be pulled caudad by the centrifugal tensions of epiboly.

COMPARISON OF CLEAVAGE THROUGH GASTRULATION IN THE AMPHIBIAN AND THE CHICK

Axis determination begins earlier in the frog than in the chick. In both, an essential radial symmetry is converted to bilateral symmetry by processes that normally involve gravity.

The entire egg cleaves ("complete cleavage") in many amphibian embryos, but cleavage in the larger chick egg is limited to the patch of segregated cytoplasm on the upper surface of the massive yolk, and cleavage of the patch is superficial and incomplete. In the amphibian with complete cleavage, a spherical blastula with an internal cavity, the blastocoel, results. The end result of cleavage in the chick egg is a flat disc of cellular blastoderm over a subgerminal cavity. In the chick, the blastocoel is formed later between a later-forming bottom layer, the hypoblast, and the overlying epiblast.

Sodium pumping by the prospective blastocoel roof may have a role in blastocoel formation in the frog embryo, and possibly also in the chick embryo. Once the chick forms a closed blastocoel, sodium pumping creates ion currents that could help form and stabilize the primitive streak. There are ion currents in amphibian gastrulae that may also affect blastopore formation and gastrulation. Nieuwkoop (1969) points out that the timely appearance of the blastocoel of the amphibian embryo prevents the entire ectoderm from being induced to form mesoderm by induction from the vegetal endoderm. Once the blastocoel is in

existence, only the ectoderm that abuts the vegetal endoderm around the equator is induced to form mesoderm, and one special region of that endoderm (Nieuwkoop's organizer) induces the axial area.

In the chick embryo, the mesoderm is induced by hypoblast that emerges as a sheet from the posterior prospective marginal zone (from the region of Koller's sickle, see Fig. 10). The leading edge of the posterior hypoblast (extraembryonic endoderm) is comparable in the chick to Nieuwkoop's organizer in the amphibian. This leading edge induces axial mesoderm from areas closest to the center of the blastoderm, and paraxial mesoderm, lateral plate, embryonic endoderm, and extraembryonic mesoderm are induced from regions successively more peripheral. Spemann's organizer, located on the dorsal lip of the blastopore in the amphibian early gastrula, is located in Hensen's node at the anterior end of the primitive streak in the chick embryo. In both cases, transplantation of the organizer to another site in the early embryo may induce a second axis.

The amphibian embryo has bottle cells that initiate blastopore formation. The comparable cells in the chick embryo would be the anterior endoderm cells. These cells ingress rather than invaginate, except they may, as they shrink their apical surfaces before ingressing, help form the pit and furrow of the streak. Whether cells that becomes bottle shaped ingress or cause the epithelium to invaginate is likely due to slight differences in the adhesive relationships with their neighbors.

During gastrulation in both amphibia and chicks, rearrangements of the prospective ectoderm, mesoderm and endoderm may be guided by adhesive differences among the different domains of cells. Once the mesoderm and endoderm are internal, by invagination and involution of cells through the blastopore in the amphibian, or by convergence and ingression of cells through the primitive streak in the chick, the mesoderm migrates extensively under the blastocoel roof or the epiblast. Migration in each case is on an extracellular interface, the basal lamina of the amphibian blastocoel roof or the basal lamina of the chick epiblast. The basal lamina contains, among other things, fibronectin and laminin, though the laminin is mostly on the side toward the overlying cells (Meier and Drake, 1984). Some evidence suggests that the extracellular matrix may have some role in the migration of the gastrulated mesoderm.

Literature Cited

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J D (1989) *Molecular Biology of the Cell*; second edition. Garland, New York

Armstrong PB (1989) Cell sorting out: The self-assembly of tissues in vitro. CRC Critical Reviews in Biochemistry and Molecular Biology 24:119-149

Azar Y, Eyal-Giladi H (1979) Marginal zone cells - the primitive streak inducing component of the primary hypoblast in the chick. J Embryol Exp Morph 52:79-88

Azar Y, Eyal-Giladi H (1981) Interaction of epiblast and hypoblast in the formation of the primitive streak and the embryonic axis in chick, as revealed by hypoblast rotation experiments. J Embryol Exp Morph 61:133-144

Azar Y, Eyal-Giladi H (1983) The retention of primary hypoblast cells underneath the developing primitive streak allows for their prolonged inductive influence. J Embryol Exp Morph 77:143-151

Baker P (1965) Fine structure and morphogenetic movements in the gastrula of the treefrog, *Hyla regilla*. J Cell Biol 24:95-116

Bellairs R (1953) Studies on the development of the foregut in the chick blastoderm I. The presumptive foregut area. J Embryol Exp Morph 1:115-127

Bellairs R (1971) *Developmental processes in higher vertebrates*. Logos Press, London

Bellairs R (1986) The primitive streak. A review. Anat Embryol 174:1-14

Bellairs R (1987) The primitive streak and the neural crest. Comparable regions of cell migration? In: Maderson P (ed) *Developmental and Evolutionary Aspects of the Neural Crest*. John Wiley & Sons, New York

Bellairs R, Ede D A, Lash J W (eds) (1986) *Somites in Developing Embryos*. Plenum, New York, London

Belousov L V (1988) Contact polarization of embryonic cells of *Xenopus laevis* during gastrulation. I. Contact polarization as a response to mechanical relaxation. Ontogenez 19:48-54

Berne R M, Levy M N (1972) *Cardiovascular Physiology*, second edition. C V Mosby Co., St. Louis

Black SD, Vincent J-P (1988) The first cleavage plane and the embryonic axis are determined by separate mechanisms in *Xenopus laevis*. II. Experimental dissociation by lateral compression of the egg. Dev Biol 128:65-71

Boucaut J-C, Darribere T (1983) Fibronectin in early amphibian embryos. Migrating mesodermal cells contact fibronectin established prior to gastrulation. Cell Tiss Res 234:135-145

Bray D, White J G (1988) Cortical flow in animal cells. Science 239:883-888

Brick I, Weinberger C (1984) Electrophoretic properties, cell surface morphology and calcium in amphibian gastrulation. Amer. Zool. 24:629-647. (discussion of unpublished work by Twersky and Brick.)

Burnside B (1971) Microtubules and microfilaments in newt neurulation. Dev Biol 26:416-441

Burnside B (1973) Microtubules and microfilaments in amphibian neurulation. Am Zool 13:989-1006
Burnside B, Jacobson A G (1968) Analysis of morphogenetic movements in the neural plate of the newt *Taricha torosa*. Dev Biol 18:537-552
Caplan AI (1986) Bone development and repair. BioEssays 6:171- 175
Carlson BM (1975) The effects of rotation and positional change of stump tissues upon morphogenesis of the regenerating axolotl limb. Dev Biol 47:269-291
Cheng LY (1987a) Deformation analysis in cell and developmental biology. Part I: Formal methodology. J Biomech 109:10-17
Cheng LY (1987b) Deformation analysis in cell and developmental biology. Part II: Mechanical experiments on cells. J Biomech 109:18-24
Cheng L Y, Murray J D, Odell G M, Oster G F (1987) The cortical tractor model: A new model for epithelial morphogenesis. In Teramoto E, Yamaguti M (eds), *Lecture Notes in Biomathematics: Mathematical Topics in Population Biology, Morphogenesis and Neurosciences*. Springer-Verlag, Berlin. pp. 209-216
Cooke J (1972) Properties of the primary organization field in the embryo of *Xenopus laevis*. III. Retention of polarity in cell groups excised from the region of the early organizer. J Embryol Exp Morph 28:47-56
Cooke J (1983) Evidence for specific feedback signals underlying pattern control during vertebrate embryogenesis. J Embryol Exp Morph 76:95-114
Cooke J, Summerbell D (1980) Cell cycle and experimental pattern duplication in the chick wing during embryonic development. Nature 287:697-701
Crawford K, Stocum D L (1988a) Retinoic acid coordinately proximalizes regenerate pattern and blastema differential affinity in axolotl limbs. Development 102:687-698
Crawford K, Stocum D L (1988b) Retinoic acid proximalizes level-specific properties responsible for intercalary regeneration in axolotl limbs. Development 104:703-712
Davis G S (1984) Migration-directing liquid properties of embryonic amphibian tissues. Amer. Zool. 24:649-655
del Pino E M (1989) Marsupial frogs. Sci Amer 260(5):110-118
Downie J R (1974) Behavioural transformation in chick yolk-sac cells. J Embryol Exp Morph 31:599-610
Downie J R (1976) The mechanism of chick blastoderm expansion. J Embryol Exp Morph 35:559-575
Downie J R, Pegrum S M (1971) Organization of the chick blastoderm edge. J Embryol Exp Morph 26:623-635
Edelman G M (1988) *Topobiology: An Introduction to Molecular Embryology*. Basic Books, New York
Elinson R P, Rowning B (1988) A transient array of parallel microtubules in frog eggs: Potential tracks for a cytoplasmic rotation that specifies the dorso-ventral axis. Dev Biol 128:185-197
Etheridge A L (1968) Determination of the mesonephric kidney. J Exp Zool 169:357-369

Ettensohn C A (1985) Gastrulation in the sea urchin is accompanied by the rearrangement of invaginating epithelial cells. Dev Biol 112:383-390
Eyal-Giladi H (1984) The gradual establishment of cell commitments during the early stages of chick development. Cell Differentiation 14:245-255
Eyal-Giladi H, Kochav S (1976) From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. Dev Biol 49:321-337
Eyal-Giladi H, Wolk M (1970) The inducing capacities of the primary hypoblast as revealed by transfilter induction studies. W Roux' Arch Entwicklungsmech. Org 165:226-241
Fabian B, Eyal-Giladi H (1981) A SEM study of cell shedding during the formation of the area pellucida in the chick embryo. J Embryol Exp Morph 64:11-22
Feinberg R N, Beebe D C (1983) Hyaluronate in vasculogenesis. Science 220:1177-1179
Fristrom D (1988) The cellular basis of epithelial morphogenesis. A review. Tiss & Cell 20:645-690
Fristrom D, Fristrom J W (1975) The mechanism of evagination of imaginal discs of *Drosophila melanogaster*. I. General considerations. Dev Biol 43:1-23
Garrod D R, Steinberg M S (1973) Tissue-specific sorting-out in two dimensions in relation to contact inhibition of overlapping. Nature 244:568-569
Geduspan J S, MacCabe J A (1989) Transfer of dorsoventral information from mesoderm to ectoderm at the onset of limb development. Anat Rec 224:79-87
Gerhart JC (1980) Mechanisms regulating pattern formation in the amphibian egg and early embryo. In: Goldberger R F, (ed) *Biological Regulation and Development*. Vol. 2: Molecular Organization and Cell Function . Plenum, New York. pp. 133-316
Gerhart J C (1987) Determinants of early amphibian development. Amer Zool 27:593-605
Gerhart J C, Keller R (1986) Region-specific cell activities in amphibian gastrulation. Ann Rev Cell Biol 2:201-229
Gierer A (1977) Physical aspects of tissue evagination and biological form. Quart Rev Biophys 10:529-593
Gimlich R L, Gerhart J C (1984) Early cellular interactions promote embryonic axis formation in *Xenopus laevis*. Dev Biol 104:117-130
Goodwin B C (1988) The evolution of generic forms. In: Maynard Smith J, Vida G, (eds) *Organizational Constraints on the Dynamics of Evolution*. Manchester University Press (in press)
Goodwin B C, Trainor L E H (1983) The ontogeny and phylogeny of the pentadactyl limb. In: Goodwin B C, Holder N, Wylie C C (eds) *Development and Evolution*. Cambridge University Press, New York. pp. 5-98

Gordon R (1985) A review of the theories of vertebrate neurulation and their relationship to the mechanics of neural tube birth defects. J Embryol Exp Morph 89(Supplement):229-255
Goss R J (1969) *Principles of Regeneration.* Academic: New York
Greenspan H P (1977) On the dynamics of cell cleavage. J Theor Biol 65:79-99
Hamburger V, Hamilton H L (1951) A series of normal stages in the development of the chick embryo. J Morphol 88:49-92.
Hardin J, Keller R (1988) The behaviour and function of bottle cells during gastrulation of *Xenopus laevis.* Development 103: 211-230.
Harris A K (1976) Is cell sorting caused by differences in the work of intercellular adhesion? A critique of the Steinberg hypothesis. J Theor Biol 61:267-285.
Harrison L G (1987) What is the status of reaction-diffusion heory thirty-four years after Turing? J Theor Biol 125:369- 384.
Harrison R G (1921) On relations of symmetry in transplanted limbs. J Exp Zool 32:1-136.
Heintzelman K F, Phillips H M, Davis G S (1978) Liquid-tissue behavior and differential cohesiveness during chick limb budding. J Embryol Exp Morph 47:1-15.
Hinchliffe J R (1977) The chondrogenic pattern in chick limb morphogenesis: a problem of development and evolution. In: Ede D A, Hinchliffe J R, Balls M (eds) *Vertebrate Limb and Somite Morphogenesis.* Cambridge University Press, New York
Hinchliffe J R, Johnson D R (1980) *The Development of the Vertebrate Limb.* Clarendon, Oxford
His W (1874) *Unsere Körperform und das physiologische Problem ihrer Enstehung, Briefe an einen befreundeten Naturforscher.* Vogel, Leipzig
Holtfreter J (1944) A study of the mechanics of gastrulation. J Exp Zool 95:171-212
Holtfreter J (1968) Mesenchyme and epithelia in inductive and morphogenetic processes. In: Fleischmajer R, Billingham R E (eds) *Epithelial-Mesenchymal Interactions.* Williams & Wilkins, Baltimore
Honda H (1983) Geometrical models for cells in tissues. Int Rev Cytol 81:191-248
Jacobson A G (1978) Some forces that shape the nervous system. Zoon 6:13-21
Jacobson A G (1981) Morphogenesis of the neural plate and tube. In: Connelly T G, Brinkley L L, Carlson B M (eds) *Morphogenesis and Pattern Formation.* Raven Press, New York. pp. 233-263
Jacobson A G (1984) Further evidence that formation of the neural tube requires elongation of the nervous system. J Exp Zool 230:23-28
Jacobson A G (1985) Adhesion and movement of cells may be coupled to produce neurulation. In: Edelman G M, Thiery J P (eds) *The Cell in Contact: Adhesions and Junctions as Morphogenetic Determinants,* John Wiley & Sons, New York. pp 49-65
Jacobson A G (1988) Somitomeres: mesodermal segments of vertebrate embryos. Development 104 (Supplement):209-220

Jacobson A G, Gordon R (1976) Changes in the shape of the developing vertebrate nervous system analysed experimentally, mathematically, and by computer simulation. J Exp Zool. 197:191-246
Jacobson A G, Odell G M, Oster G F (1985) The cortical tractor model for epithelial folding: Application to the neural plate. In: Edelman G M (ed) *Molecular Determinants of Animal Form, UCLA Symposia on Molecular and Cellular Biology* (new series), Vol 31. Alan R Liss, New York. pp 143-167
Jacobson A G, Oster G F, Odell, G M, Cheng L Y (1986) Neurulation and the cortical tractor model for epithelial folding. J Embryol Exp Morph 96:19-49
Jacobson A G, Sater, A K (1988) Features of embryonic induction. Development 104:341-359
Jacobson A G, Tam P P L (1982) Cephalic neurulation in the mouse embryo analysed by SEM and morphometry. Anat Rec 203:375-396
Jacobson C O (1962) Cell migration in the neural plate and the process of neurulation in the Axolotl larva. Zool Bidr Uppsala 35:433-449
Jaffe L F, Stern C D (1982) Strong electrical currents leave the primitive streak of chick embryos. Science 206:569-571
Jarzem J, Meier S P (1987) A scanning electron microscope survey of the origin of the primordial pronephric duct cells in the avian embryo. Anat Rec 218:175-181
Javois L C (1984) Pattern specification in the developing chick limb. In: *Pattern Formation.* Malacinski G M, Bryant S V (eds), Macmillan, New York pp. 557-579
Kaneda T, Hama T (1979) Studies on the formation and state of determination of the trunk organizer in the newt, *Cynops pyrrhogaster.* W Roux's Arch Dev Biol 187:25-34
Keller R E (1975) Vital dye mapping of the gastrula and neurula of *Xenopus laevis.* I. Prospective areas and morphogenetic movements of the superficial layer. Dev Biol 42:222-241
Keller R E (1978) Time-lapse cinematographic analysis of superficial cell behavior during and prior to gastrulation in *Xenopus laevis.* J Morphol 157:223-248
Keller R E (1980) The cellular basis of epiboly: An SEM study of deep-cell rearrangement during gastrulation in *Xenopus laevis.* J Embryol Exp Morph 60:201-234
Keller R E (1981) An experimental analysis of the role of bottle cells and the deep marginal zone in gastrulation of Xenopus laevis. J Exp Zool 216:81-101
Keller R E (1984) The cellular basis of gastrulation in *Xenopus laevis:* Active, postinvolution convergence and extension by mediolateral interdigitation Amer Zool 24:589-603
Keller R E (1986) The cellular basis of amphibian gastrulation. In: Browder L (ed) *Developmental Biology: A Comprehensive Synthesis.* Plenum, New York. pp. 241-327
Keller R E (1987) Cell rearrangement in morphogenesis. Zool Sci 4:763-779

Keller R E, Danilchik M (1988) Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. Development 103: 193-209

Keller R E, Danilchik M, Gimlich R, Shih J (1985) The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*.. J Embryol Exp Morph (Supplement) 89:185-209

Keller R E, Hardin J (1987) Cell behavior during active cell rearrangement: Evidence and speculation. J Cell Sci (Supplement) 8:369-393

Keller R E, Trinkaus J P (1987) Rearrangement of the enveloping layer cells without disruption of the epithelial permeability barrier as a factor in *Fundulus* epiboly. Dev Biol 120:12-24

Khaner O, Eyal-Giladi H (1986) The embryo forming potency of the posterior marginal zone in stages X through XII of the chick. Dev Biol 115:275-281

Kitchin J C (1949) The effects of notochordectomy in *Ambystoma mexicanum*. J Exp Zool 122:393-415

Kochav S, Eyal-Giladi H (1971) Bilateral symmetry in chick embryo, determination by gravity. Science 171:1027-1029

Kochav S, Ginsburg M, Eyal-Giladi H (1980) From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. II. Microscopic anatomy and cell population dynamics. Dev Biol 79:296-308

Kolega J (1986) The cellular basis of epithelial morphogenesis. In: Browder L (ed) *Developmental Biology: A Comprehensive Synthesis*. Plenum, New York. pp. 103-143

Kubota H Y, Durston A J (1978) Cinematographical study of cell migration in the opened gastrula of *Ambystoma mexicanum*. J Embryol Exp Morph 44:71-80

MacCabe A B, Gasseling M T, Saunders J W Jr. (1973) Spatiotemporal distribution of mechanisms that control outgrowth and antero-posterior polarization of the limb bud in the chick embryo. Mechanisms of Ageing and Development 2:1-12

MacCabe J A, Saunders J W Jr., Pickett M (1973) The control of the anteroposterior and dorsoventral axes in embryonic chick limbs constructed of dissociated and reaggregated limb-bud mesoderm. Dev Biol 31:323-335

McClay D R, Ettensohn C A (1987) Cell adhesion in morphogenesis. Ann Rev Cell Biol 3:319-345

Malicinski G M, Youn B W (1981) Neural plate morphogenesis and axial stretching in "notochord-defective" *Xenopus laevis* embryos. Dev Biol 88:352-357

Meier S P (1979) Development of the chick mesoblast. Formation of the embryonic axis and establishment of the metameric pattern. Dev Biol 73:25-45

Meier S, Drake C (1984) SEM localization of laminin in the basement membrane of the chick corneal epithelium with immunolatex microspheres. Dev Biol 106:83-88

Mitrani E (1982) Primitive streak-forming cells of the chick invaginate through a basement membrane. W Roux's Arch 191:320-324

Mittenthal J E (1981) The rule of normal neighbors: A hypothesis fo morphogenetic pattern regulation. Dev Biol 88:15-26

Mittenthal J E (1985) Morphogenetic fields and the control of form in the limbs o decapods. In: Wenner A M (ed) *Crustacean Growth: Factors in Adul Growth*. Balkema, Rotterdam. pp. 47-71

Mittenthal J E (1986) A model for the role of interfacial tensions within cell sheet in the early morphogenetic events of amphibian development. In: Slavkin H (ed) *Progress in Developmental Biology*. Part A. Liss, New York

Mittenthal J E (1987) The shaping of cell sheets: An application of mechanics i developmental biology. In: Akkas N (ed) *Biomechanics of Cell Division* Plenum, New York. pp. 327-346

Mittenthal J E (1989) Physical aspects of the organization of development. In Stein D (ed) *Complex Systems, SFI Studies in the Sciences of Complexity* Addison-Wesley Longman, Reading, Masachusetts

Mittenthal JE, Mazo RM (1983) A model for shape generation by strain an cell-cell adhesion in the epithelium of an arthropod leg segment. J Theor Bic 100:443-483

Mittenthal J E, Nuelle J R (1988) Discontinuities of pattern and rules fo regeneration in limbs of crayfish. Dev Biol 126:315-326

Modak S P (1966) Analyse expérimentale de l'origine de l'entoblast embryonnaire chez les oiseaux. Rev Suisse Zool 73:877-908

Mookerjee S, Deuchar E M, Waddington C H (1953) The morphogenesis o the notochord in amphibia. J Embryol Exp Morph 1:399-409

Moury J D, Jacobson A G (1989) Neural fold formation at newly create boundaries between neural plate and epidermis in the axolotl. Dev Biol 133 44-57

Muneoka K, Bryant SV (1982) Evidence that patterning mechanisms developin and regenerating limbs are the same. Nature (Lond) 298:369-371

Nakatsuji N (1986) Presumptive mesoderm cells from *Xenopus laevis* gastrula attach to and migrate on substrata coated with fibronectin or laminin. J Cel Sci 86:109-118

Nakatsuji N, Johnson K E (1983) Conditioning of a culture substratum by th ectodermal layer promotes attachment and oriented locomotion by amphibia gastrula mesodermal cells. J Cell Sci 59:43-60

Nardi J B (1981) Induction of invagination in insect epithelium: Paradigm fo embryonic invagination. Science 214:564-566

Nardi J B, Stocum D L (1983) Surface properties of regenerating limb cells evidence for gradation along the proximodistal axis. Differentiation 25:27-31

New D A T (1959) The adhesive properties and expansion of the chicl blastoderm. J Embryol Exp Morph 7:146-164

Newman S A (1988) Lineage and pattern in the developing vertebrate limb Trends in Genetics 4:329-332

Newman S A, Frisch H L (1979) Dynamics of skeletal pattern formation i developing chick limb. Science 205:662-668

vman S A, Frisch H L, Percus J K (1988) On the stationary state analysis of reaction-diffusion mechanisms for biological pattern formation. J Theor Biol 134:183-197
vport J, Kirschner M (1982) A major developmental transition in early *Xenopus* embryos: I. Characterization and timing of cellular changes at the midblastula stage. Cell 30:675-686
uwkoop P D (1969) The formation of the mesoderm in urodelean amphibians. I. The induction by the endoderm. Roux' Arch Entwmech Org 162:341-373
bler-Jung K (1977) Pattern stability in the insect segment. I. Pattern reconstitution by intercalary regeneration and cell sorting in *Dysdercus intermedius* Dist. W Roux Arch Dev Biol 183:17-40
ell G M, Oster G, Alberch P, Burnside B (1981) The mechanical basis of morphogenesis. I. Epithelial folding and invagination. Dev Biol 85:446-462
zanska B, Szolajska E, Lassota Z (1984) Effect of spatial pattern of uterine quail blastoderms cultured *in vitro* on bilateral symmetry formation. Wilhelm Roux Arch 193:108-110
no Y, Shinozaki A (1989) Cell dynamical systems. Forma (in press)
er G F (1984) On the crawling of cells. J Embryol Exp Morph 83(Supplement):329-364
er G F, Murray J D, Harris A K (1983) Mechanical aspects of mesenchymal morphogenesis. J Embryol Exp Morph 78:83-125
er G F, Murray J D, Maini P K (1985) A model for chondrogenic condensations in the developing limb: the role of extracellular matrix and cell tractions. J Embryol Exp Morph 89:93-112
er G F, Shubin N, Murray J D, Alberch P (1988) Evolution and morphogenetic rules: The shape of the vertebrate limb in ontogeny and phylogeny. Evolution 42:862-884
terson J T (1910) Studies on the early development of the hen's egg. I. History of the early cleavage and the accessory cleavage. J Morph 21:101-134
ry M, Waddington C H (1966) Ultrastructure of the blastoporal cells in the newt. J Embryol Exp Morph 15:317-330
llips H M, Davis, G S (1978) Liquid-tissue mechanics in amphibian gastrulation: Germ-layer assembly in *Rana pipiens*. Amer Zool 18:81-93.
ple T J, Steinberg M S (1981) Amphibian pronephric duct morphogenesis: Segregation, cell rearrangement, and directed migration of the *Ambystoma* duct rudiment. J Embryol Exp Morph 63:1-16
chman D P, Stewart R M, Hutchinson J W, Caviness V S, Jr. (1975) Mechanical model of brain convolutional development. Science 189:18-21
bertson A, Gingle A R (1977) Axial bending in the early chick embryo by a cyclic adenosine monophosphate source. Science 197:1078-1079
bertson A, Grutsch J F, Gingle A R (1978) cAMP production by embryonic chick cells. Science 199:990-991
binson K R, Stump R F (1984) Self-generated electrical currents through *Xenopus* neurulae. J Physiol (Lond.) 352:339-352

Rooney P, Archer C, Wolpert L (1984) Morphogenesis of cartilaginous long bone rudiments. Symp Soc Develop Biol 42:305-322
Rosenquist G C (1966) A radioautographic study of labelled grafts in the chick blastoderm: development from primitive streak to stage 12. Contr Embryol Carnegie Inst 38:71-110
Rosenquist G C (1971) The location of the pregut endoderm in the chick embryo at the primitive streak stage as determined by radioautographic mapping. Dev Biol 26:323-335
Rosenquist G C (1972) Endoderm movements in the chick embryo between the early short streak and head process stage. J Exp Zool 180:95-104
Sanders E J (1984) Labelling of basement membrane constituents in the living chick embryo during gastrulation. J Embryol Exp Morph 79:113-123
Sanders E J, Bellairs R, Portch P A (1978) *In vivo* and *in vitro* studies on the hypoblast and definitive endoblast of avian embryos. J Embryol Exp Morph 46:187-205
Saunders J W Jr. (1948) The proximo-distal sequence of the origin of the parts of the chick wing and the role of the ectoderm. J Exp Zool 108:363-404
Schaeffer B E, Schaeffer H E, Brick I (1973) Cell electrophoresis of amphibian blastula and gastrula cells: The relationship of surface charge and morphogenetic movement. Dev Biol 34:66-76
Scharf S R, Gerhart J C (1983) Axis determination in eggs of *Xenopus laevis*: A critical period before first cleavage, identified by the common effects of cold, pressure and ultraviolet irradiation. Dev Biol 99:75-87
Scharf S R, Rowning B, Wu M, Gerhart J C (1989) Hyperdorsoanterior embryos from *Xenopus* eggs treated with D_2O. Dev Biol 134:175-188
Schechtman A M (1942) The mechanism of amphibian gastrulation. I. Gastrulation-promoting interactions between various regions of an anuran egg (*Hyla regilla*). Univ Calif Publ Zool 51:1-39
Schoenwolf G C (1985) Shaping and bending of the avian neuroepithelium: Morphometric analysis Dev Biol 109:127-139
Schoenwolf G C, Alvarez I S (1989) Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. Development 106:427-439
Schroeder TE (1970) Neurulation in *Xenopus laevis*. An analysis and model based upon light and electron microscopy. J Embryol Exp Morph 23:427-462
Slack C, Warner A E (1983) Intracellular and intercellular potentials in the early amphibian embryo. J Physiol (London) 232:313-330
Slack J M W (1984) The early amphibian embryo -- a hierarchy of developmental decisions. In: Malacinski G M, SV Bryant S V (eds) *Pattern Formation.* Macmillan, New York. pp 457-480
Slack J (ed) (1985) *Early Amphibian Development.* J Embryol Exp Morph 89 (Supplement)
Smith D W (1981) Mechanics in morphogenesis: Principles and response of particular tissues. In: *Recognizable Patterns of Human Deformation.* Saunders, Philadelphia. pp 110-144

lursh M (1984) Ectoderm as a determinant of early tissue pattern in the limb bud. Cell Differentiation 15:17-24

emann H (1938) *Embryonic Development and Induction.* Hafner, New York.

ratt N T, Haas H (1960) Integrative mechanisms in development of the early chick blastoderm. I. Regulated potentiality of separate parts. J Exp Zool 145:97-138

ratt N T, Haas H (1965) Germ layer formation and the role of the primitive streeak in the chick. J Exp Zool 158:9-38

einberg M S (1963) Reconstruction of tissues by dissociated cells. Science 141:401-408

einberg M S (1970) Does differential adhesion govern self-assembly processes in histogenesis? Equilbrium configurations and the emergence of a hierarchy among populations of embryonic cells. J Exp Zool 173:395-434

einberg M S, Poole T J (1982) Cellular adhesive differentials as determinants of morphogenetic movements and organ segregation. Symp Soc Develop Biol 40:351-378

ern C D (1982) Experimental reversal of polarity in chick embryo epiblast sheets *in vitro.* Exp Cell Res 221:395-404

tern C (1984) A simple model for early morphogenesis. J Theor Biol 107:229-242

tern C D, Goodwin B C (1977) Waves and periodic events during primitive streak formation in the chick. J Embryol Exp Morph 41:15-22

tern C D, MacKenzie D O (1983) Sodium transport and the control of epiblast polarity in the early chick embryo. J Embryol Exp Morph 77:73-98

tocum D L (1984) The urodele limb regeneration blastema. Determination and organization of the morphogenetic field. Differentiation 27:13-28

tocum D L, Fallon J F (1984) Mechanisms of polarization and pattern formation in urodele limb ontogeny: A polarizing zone model. In: Malacinski G M, Bryant S V (eds) *Pattern Formation.* Macmillan, New York. pp 507-520

tock G B, Krasner G N, Holder N, Bryant S V (1980) Frequency of supernumerary limbs following blastemal rotations in the newt. J Exp Zool 214:123-126

rinkaus J P (1984a) Mechanism of Fundulus epiboly -- a current view. Amer Zool 24:673-688

rinkaus J P (1984b) *Cells into Organs. The Forces that Shape the Embryo.* Prentice-Hall, Englewood Cliffs, New Jersey

riplett R L, Meier S (1982) Morphological analysis of the development of the primary organizer in avian embryos. J Exp Zool 220:191-206

akaet L (1962) Some new data concerning the formation of the definitive endoblast in the chick embryo. J Embryol Exp Morph 10:38-57

akaet L (1984a) Early development of birds. In: Le Douarin N M, McLaren A (eds) *Chimeras in Developmental Biology,* Academic Press, London, pp. 71-88

akaet L (1984b) The initiation of gastrular ingression in the chick blastoderm. Amer Zool 24:555-562

Vintemberger P, Clavert J (1960) Sur le déterminisme de la symétrie bilatérale chez les oiseaux. XIII. Les facteurs de l'orientation de l'embryon par rapport à l'axe de l'oeuf et la régle de Von Baer, à la lumiére de nos expériences d'orientation dirigée sur l'oeuf de poule extrait de l'uterus. C R Soc Biol 154:1072-1076

Von Baer K E (1828) *Entwicklungsgeschichte des Hühnchens im Ei.* Bonntragen, Königsberg, p. 315

Waddington C H (1932) Experiments on the development of chick and duck embryos, cultivated *in vitro.* Phil Trans R Soc London B 211:179-230

Waddington C H (1933) Induction by the endoderm in birds. W Roux' Arch Entwicklungsmech Org 128:502-521

Wilby O K, Ede D A (1976) Computer simulation of vertebrate limb development. In: Lindenmayer A, Rozenberg G (eds) *Automata, Languages, Development.* North-Holland, Amsterdam. pp. 143- 161

Wilson P A, Oster G, Keller R (1989) Cell rearrangement and segmentation in Xenopus: Direct observation of cultured explants. Development 105:155-166

Winfree A T (1984) A continuity principle for regeneration. In: Malacinski G M, Bryant S V (eds) *Pattern Formation.* Macmillan, New York. pp 103-124

Wolpert L (1971) Positional information and pattern formation. Curr Top Devel Biol 6:183-224

Wolpert L, Stein W D (1984) Positional information and pattern formation. In: Malacinski G M, Bryant S V (eds) *Pattern Formation.* Macmillan, New York. pp 3-21