Gastrulation in birds: a model system for the study of animal morphogenesis

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Lewis Wolpert is alleged to have stated that *'it is not birth, marriage or death, but gastrulation which is truly the most important time in your life'* (quoted in Slack [30]). 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.

Bird embryos have been for some time the 'classical' vertebrate in which gastrulation has been studied. More recently, however, progress in avian gastrulation has lagged behind that made using other vertebrates, particularly because of two disadvantages: first, unlike amphibian embryos, the cells of bird embryos are small, which makes the study of cell lineage by injection of markers into individual cells very difficult. Second, unlike the mouse, very little is known about the genetics of birds. Nevertheless, avian embryos offer some attractive advantages for the study of gastrulation: unlike most other vertebrate embryos, they are flat, fairly transparent and considerably larger than either amphibian or mammalian embryos, they lend themselves to sophisticated microsurgery and can be obtained cheaply. The lack of genetic markers is overcome, to some extent, by the availability of the chick-quail chimaera technique introduced by Le Douarin [22], which has been used extensively for fate-mapping.

The process of gastrulation, like the rest of embryonic development, consists of a series of processes that fall into two major categories: cytodifferentiation and morphogenesis. The first concerns the divergence of cell fates from pluripotent progenitor cells to give rise to different cell types. The second refers to the processes that ensure that these different cell types are distributed correctly within the embryo and that the correct pattern is generated. 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 believed to be required to bring certain tissues together in the embryo, and the interactions (*'induction'*) between these tissues will influence the fate of the 'responding' or 'competent' cells. The mesoderm of amphibian embryos is assumed to arise in this way, as the result of the influence of the primitive endoderm on the ectoderm, as indeed is the mesoderm of the chick and other vertebrate embryos [30]. The nervous system also arises from an inductive interaction, between the mesoderm and the ectoderm, a discovery for which Hans Spemann received the Nobel prize for Physiology and Medicine in 1935 [33].

The process of gastrulation therefore represents a good model system in which the relationships between morphogenesis and cell diversification can be studied. In this paper we will survey the classical views of chick gastrulation, and we will present some new data which may throw some light on these complex processes. We will argue that the evidence in favour 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 organised prior to the onset of major morphogenetic movements. Just prior to gastrulation, the chick embryo is a flat disc about 2 mm in diameter, consisting of two regions, each of which is two-layered. The epiblast, a pseudostratified epithelium, is continuous over both regions of the embryonic disc. The ventral aspect of the outer area opaca is composed of large (up to 150–200 μm in diameter), yolky cells, while the ventral aspect of the central area pellucida is composed of a loose layer of yolky cells (about 80–120 μm in diameter), 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.

Dorso-ventral polarity of the epiblast

The cells of the epiblast are polarised along their apical-basal (= dorso-ventral) axis. Sodium [42] and water [43] are transported from apical to basal aspects; this unidirectional transport generates a trans-epithelial potential of some 25 mV (basal side positive [15, 42]). Like all transporting epithelia, the epiblast is also polarised morphologically. The polar features include apical intercellular junctions and some apical microvilli, basal nuclei and a hyaluronate-rich basal lamina secreted continuously by the epiblast but receiving a contribution from the hypoblast as well [50]. The apical-basal polarity of the epiblast is not fixed but labile; it can be reversed quickly by applying a trans-epithelial potential of opposite polarity to that measured across it (35 mV, apical side positive) [42]. It can also be reversed by placing it in a pH

gradient so that the apical side is about 3 pH units more acid than the basal side (unpublished observations).

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 sub-blastodermic fluid is slightly acid (pH 6.5)[43]. Since a pH gradient of 3 units is sufficient to reverse the polarity of the epiblast experimentally, it is probable that this asymmetry, set up by the mother, plays a role in determining the polarity of the epiblast. As the epiblast develops, the trans-epithelial potential generated could also serve to maintain its polarity. 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[15,42,43]. A simple model has been proposed to explain this local reversal of polarity[37].

Cranio-caudal polarity of the embryo: breaking radial symmetry

How is bilateral symmetry set up in the embryonic disc? Kochav and Eyal-Giladi[18] have suggested that cranio-caudal polarity is established during the descent of the egg in the oviduct, under the influence of gravity. 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 cranio-caudal midline of the hypoblast sheet marks the future midline of the embryo. Waddington[52] suggested 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 cranio-caudal axis of the embryo. Mitrani and Eyal-Giladi[24], however, suggested that both the epiblast and the hypoblast have a polarity, 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 cranio-caudal axis of the embryo.

What determines the origin and the shape of the primitive streak? Spratt and Haas[34,35] suggested that the primitive streak arises preferentially at the margin between the area pellucida and area opaca (the region called 'marginal zone' by Eyal-Giladi and colleagues[4,17]); this region is undoubtedly special in some way. The reason for the rod-like 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 proliferation of the cells in the epiblast portion of the primitive streak[5,36,37,40].

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 cinephotomicrography.

Morphogenesis of the lower layer

The earliest tissue to cover the lower layer is the hypoblast; its origin has been the subject of some controversy. Eyal-Giladi and her colleagues[10] argue that it arises by ingression of cells from the epiblast at many different sites. Others[41,48,49] have suggested that it derives mainly from the caudal margin of the germ wall (area opaca endoderm). The latter suggestion receives support from the following arguments: (i) the epiblast cells at the centre of the area pellucida are small and non-yolky, while the germ wall cells resemble the hypoblast more closely in size and amount of intracellular yolk particles; (ii) grafting of a quail marginal zone into a chick host results in quail cells being found in the host lower layer. On the other hand, the proposal that the hypoblast is derived from the central area pellucida epiblast is more consistent with the appearance of the hypoblast precursor cells in 'clumps' all over the ventral aspect of the epiblast prior to their coalescence into a sheet of cells. Time-lapse analysis of the formation of the hypoblast should resolve this question; preliminary films (unpublished observations) have shown that both views may be correct: the islands of hypoblast precursors appear to arise in situ, but the coalescence of the hypoblast into a sheet of cells takes place by two mechanisms: the addition of cells derived from the caudal germ wall, and the spreading and joining of cells in the primitive islands. Thus, the primitive lower layer may be of mixed origin. Vakaet[48,49], among others, has advanced the view that the islands seen in young stages constitute a primitive, or primary hypoblast, and that the secondary hypoblast that completes the primitive lower layer is derived from the germ wall margin, particularly at the caudal margin of the blastodisc. Mitrani, Shimoni and Eyal-Giladi[25] have suggested that it is this secondary hypoblast that is responsible for inducing the primitive streak.

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, which give rise to the 'junctional endoderm'. The definitive endoderm inserts into the centre of the original sheet of hypoblast[5,29,41], while the junctional endoderm arises at its periphery. As a consequence of these contributions and of complex *'Polonaise'* movements of the hypoblast sheet[48], the original hypoblast becomes confined to a region close to the cranial area pellucida/area opaca margin by the end of gastrulation (full primitive streak stage, Hamburger and Hamilton[12] stage 4), forming a region known as the 'germinal crescent' because the primordial germ cells are associated with it. The forces that cause the hypoblast to migrate *en*

Polonaise are not known, nor is the role played by this migration.

Morphogenesis of the upper layer and formation of the primitive streak

The upper layer (epiblast) also goes through complex movements[48]. Some of these movements are due to the expansion of the blastoderm on the vitelline membrane. 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 towards the midline. High-power time-lapse observations (unpublished) of the epiblast at stages X–XIV show that there is considerable mixing of cells in all regions of the epiblast, which is perhaps surprising in a polarised epithelium containing intercellular tight junctions. The movement of the cells towards the axis of the primitive streak, however, does not appear to be related to the appearance of the mesoderm. Neither Vakaet's nor our own time-lapse observations (both unpublished, but see Vakaet[49]) lend any support to the textbook view of gastrulation as being a convergence of cells to the primitive streak accompanied by a sheet-like involution of the epiblast into it to form the mesoderm. Moreover, 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 towards 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 Hamburger and Hamilton[12] stage 2 embryo, and in time-lapse films the formation of the primitive streak can be seen only 'in retrospect', by projecting the film backwards. This suggests that formation of the primitive streak is not a massive ingression of presumptive mesoderm cells all at once, but rather represents the coalescence of cells that were already under the surface of the epiblast[49].

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[12] stage 3$^+$, at about the same time as 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 paraxial segmental plates, from which the somites will arise, represent the most medial, and therefore the most recently formed mesoderm. The left and right halves of the mesoderm later become separated from each other by the regression (shortening) of the primitive streak that occurs after the end of gastrulation[40]. 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 activity of hyaluronidase[38]. Since the basal lamina of the overlying epiblast consists primarily of hyaluronate, it is possible that this high level of activity is concerned with degrading the overlying basal lamina to encourage more mesoderm cells to ingress.

Induction and cytodifferentiation

The appearance of cell diversity

By the time of laying (about stage X of Eyal-Giladi and Kochav[9]), several distinct cell types are already recognizable by morphological criteria. The epiblast consists mostly of small, columnar, polarised epithelial cells, the ventral surface of the embryo displays islands of hypoblast cells, which are larger and more yolky, and the ventral surface of the area opaca has even larger and more yolky cells, the germ wall. 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 than those of the latter. At the extreme margin of the area opaca, the epiblast cells possess wide lamellae which attach to the vitelline membrane. These cells are responsible for the expansion of the blastoderm on this membrane.

During gastrulation, other cell types appear. The mesoderm of the primitive streak is a mesenchymal tissue, with small, fibroblastic, stellate, non-yolky cells. Initially these cells are packed tightly at the primitive streak, but later they migrate out to give rise to the lateral and segmental plates and to the notochord. The cells of the notochord later become very vacuolated. During gastrulation, the definitive (gut) endoderm also makes its appearance; this consists of flat cells that are more tightly adherent to one another than those of the hypoblast. The junctional endoderm also forms at this time, but its cells are not easily distinguished from 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, the lack of morphological differences between any other potential cell types does not necessarily indicate that the cells of tissues that look uniform are the same as one another[39]. It is somewhat disconcerting that although some workers have described the existence of antigenic differences between different tissues of the early chick embryo a decade ago[54], these have never been followed up or used to resolve questions about the mechanisms of cell diversification in the chick embryo. The solution to these problems is of importance if we are to understand the process of induction.

Mesoderm induction

According to several authors[26,30] there are at least two distinct inductions that occur during the early develop-

ment 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 and the epiblast (competent ectoderm). The second is neural induction, which was first described in the amphibian embryo by Spemann and Mangold in 1924[33]. 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. The cells of the responding epiblast become columnar and later fold to give rise to the neural tube. It was C. H. Waddington who first extended Spemann and Mangold's findings to bird embryos, by grafting Hensen's nodes between a donor and a host embryo[53]. He found that a secondary axis was produced at the site of the graft. Later it was confirmed using the quail/chick chimaera technique that this result was indeed an induction[13, 26, 30]. In the rest of this discussion, we shall concern ourselves with induction of the mesoderm.

In amphibians, the mesoderm is said to form as a result of an inductive interaction between endoderm and ectoderm. In *Xenopus laevis,* the cells that give rise to the mesoderm are already in a deep layer associated with the superficial ectoderm cells[16, 30]. This interaction will also 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[11, 30]. Slack[30] has argued that this interaction is 'instructive' rather than 'permissive' because: (a) there is no increase in volume in the explants or in the embryo during the relevant stages of development, (b) there is no visible cell death in the cultures, and (c) in confrontation cultures several markers characteristic of mesodermal derivatives are expressed (e.g. muscle myosin). He states (p. 26): 'It is not conceivable... that the 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 cell ...'. Nevertheless, there is as yet no evidence for induction at the single cell level (see Gurdon[11], p. 294 for a lucid, albeit brief, discussion of this problem). Recently, Slack and Smith and their collaborators have demonstrated that several substances are capable of inducing the expression of mesodermal markers in cultures of *Xenopus* ectoderm. They include fibroblast growth factor[32], an extract from chick embryos and a protein secreted by an amphibian cell line, XTC[31, 32]. All of these have relative molecular weights of the order of 16,000 daltons.

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; Waddington[51, 52] rotated the hypoblast of young chick embryos through 180° cranio-caudally and provided evidence that the hypoblast contributed in determining the orientation of the primitive streak. Subsequently, Azar and Eyal-Giladi[3] showed that the result of this interaction depends critically upon the stage of the operation. 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.

Of course, virtually nothing is known about either the nature of the inductive signal or the nature of the response in bird embryos. Unlike the case in amphibians, the mesoderm cannot be induced easily by heterologous stimuli in birds[26]. Zagris and Matthopoulos[55] investigated the pattern of ^{35}S-methionine incorporation into proteins resolved by two-dimensional SDS-polyacrylamide gel electrophoresis. They found a single 30 Kilodalton (kD) protein unique to the epiblast and two proteins (22 kD and 37 kD) unique to the hypoblast. The hypoblast and epiblast are such disparate tissues that it would be surprising if these were the only differences between them in protein synthetic activity. For the study of mesoderm induction, it would be more appropriate to examine the differences in synthetic activity between the epiblast before induction and the primitive streak. To our knowledge, this has not yet been done.

A factor capable of inducing secondary axes

Based on the rather disjointed information available in 1983, Stern[37] proposed a simple model to account for the formation and maintenance of the primitive streak. The model makes a specific prediction: that virtually anything capable of reducing the resistance of the epiblast to mechanical tension or ionic flow in a local way will result in the formation of a primitive streak at that location. Recently, Stoker and his colleagues[44, 45] have described a factor secreted by a human embryo cell line, MRC5, which is capable of 'scattering' cultured epithelial cells such as the MDCK cell line. They interpreted the scattering activity as reflecting the breakdown of intercellular junctions between the responding epithelial cells. The factor is an acidic protein of apparent molecular mass 57 kD, secreted into the medium by producing cells, and which appears to be lost after transformation. More recently, Ireland, Stern and Stoker[14] have investigated the possibility that scatter factor-like activity might induce secondary primitive streaks to form from the epiblast by disrupting intercellular junctions: we grafted small pellets of MRC5 cells (up to 1000 cells each) into chick embryos of appropriate stages of development and found that in some cases secondary axes were formed, while grafts of cell lines that do not produce scatter factor had no effect. Primitive streak-like structures were formed in some of the embryos grafted with MRC-5 cells, and it was striking to find that supernumerary neural plates were found in about 80% of the grafted embryos (as opposed to some 50–60% after grafting a Hensen's node). 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 and that responding tissues such as epiblast can respond to purified scatter factor.

Unlike the case in amphibians, the ectoderm of amniote embryos cannot easily be induced to form mesoderm by heterologous factors. The finding that cells that produce scatter factor activity can induce secondary axes in the chick could provide a useful tool for the study of mesoderm induction in these embryos. Nevertheless, 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 able to distinguish between different cell populations in the embryo at 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 in the expression of macromolecules or enzyme activities were to be found, it would still have to be shown that these differences reflect 'real' differences between cell types with different developmental potential and are not merely a reflection of momentary cell behaviour within a diverse cell population. Nevertheless, in our present state of ignorance, any regional differences at or before gastrulation will be helpful in the search for real markers. In the last few years, several such differences have been identified. They are summarised in the table. In the following discussion we shall concentrate on the expression of one of these regional markers, and consider how it may help us to understand the relationships between cell diversification and morphogenesis.

Antibodies recognising a complex sulphated carbohydrate epitope known as L2 (such as monoclonal antibody HNK-1, originally raised by Abo and Balch [1]) show characteristic patterns of binding in early chick embryos. HNK-1 is known to recognise chick trunk neural crest cells, notochord and neuronal processes, and cross-reacts with other cell adhesion-related glycoproteins such as myelin-associated glycoprotein, myelin basic protein. J1/cytotactin, F11 and N-CAM [19, 20, 28, 47]. In stage 2–3 chick blastoderms, the L2 epitope is present in the primitive streak mesoderm and in the hypoblast as well as in a few cells in the posterior (caudal) margin of the germ wall (area opaca endoderm) (Canning and Stern, in preparation). Western blot and immunohistochemical studies showed that these regional differences in chick blastoderms are not due to differences in N-CAM. At stage XIII and earlier, long before the primitive streak makes its appearance, the hypoblast already contains L2-bearing molecules. We were surprised, however, to find that some cells in the epiblast are also labelled by these antibodies. These cells are distributed in an apparently random way in the epiblast, giving a 'pepper and salt' appearance. The staining pattern obtained with certain tetrazolium blue dyes sensitive to intracellular redox potential, also reveal a 'pepper and salt' appearance in the epiblast at this stage as well as the mesoderm of the primitive streak but not the hypoblast. We do not yet known whether the L2-positive cells and/or the redox dye-positive cells of the epiblast are mesodermal precursors and are investigating these possibilities. If they were, the notion that the mesoderm of amniote embryos results from an instructive induction will have to be revised.

Even before gastrulation, therefore, there may be different populations of cells which can be characterised biochemically and immunologically. The differences include several enzyme activites, cell adhesion molecules, a cellular oncogene product, cell surface glycoconjugates and intracellular redox potential. Their existence may reflect the presence of differences in various properties of the cells that may affect their behaviour in a developmentally significant way. The discovery of such regional differences offers new and exciting possibilities to help develop an understanding of the cellular processes that control all aspects of gastrulation. If it can be shown, for example, that L2-positive cells in the epiblast of the early embryo are indeed mesoderm precursors, new light would be thrown to our understanding of mesoderm induction, be-

Summary of regional differences found in the chick embryo at gastrulation

Marker and reference	Hypoblast	Msoderm P.S.	Msoderm Lat.pl.	Epiblast	Germ wall	Endoderm
L2/HNH-1*	+++	+++	–	+/–	+ (post.)	–
N- & L-CAM [6, 8, 46]	–	–	–	+/–	–	–
G4*	++	–	–	–	+ (edge)	–
AChE*, [7, 21, 27]	+++	+++	–	+/–	+	–
BChE*	+/–	(+)	–	+/–	+/–	–
hsc24, 70 & 89*	–	–	–	–	+++	–
P. lipaseD-lipid*	+	–	–	–	+/–	+/–
Cholera toxin*	+	–	–	–	+ (edge)	–
αD-glucosidase*	?	+++	–	–	–	–
pp60^{src} [2, 23]	–	–?	–?	+/–	–?	–
Hyaluronidase [38]	–	+++	+	–	–	–
Redox dyes*	?	+	?	+/–	+	–

* Canning and Stern (in preparation); * Buckingham, Canning, Carlson, MacKay, Maughan and Stern (in preparation).

cause this finding implies that the mesoderm cells are determined as such prior to the formation of the primitive streak, and that they simply sort out from the non-mesodermal cells of the epiblast. Most importantly, they may, at last, allow us to understand the relationships between the processes of cell diversification and morphogenesis.

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Ionic currents in morphogenesis

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Summary. Morphogenetic fields must be generated by mechanisms based on known physical forces which include gravitational forces, mechanical forces, electrical forces, or some combination of these. While it is unrealistic to expect a single force, such as a voltage gradient, to be the sole cause of a morphogenetic event, spatial and temporal information about the electrical fields and ion concentration gradients in and around a cell or embryo undergoing morphogenesis can take us one step further toward understanding the entire morphogenetic mechanism. This is especially true because one of the handful of identified morphogens is Ca^{2+}, an ion that will not only generate a current as it moves, but which is known to directly influence the plasma membrane's permeability to other ions, leading to other transcellular currents. It would be expected that movements of this morphogen across the plasma membrane might generate ionic currents and gradients of both electrical potential and intracellular concentration. Such ionic currents have been found to be integral components of the morphogenetic mechanism in some cases and only secondary components in other cases. My goal in this review is to discuss examples of both of these levels of involvement that have resulted from investigations conducted during the past several years, and to point to areas that are ripe for future investigation. This will include the history and theory of ionic current measurements, and a discussion of examples in both plant and animal systems in which ionic currents and intracellular concentration gradients are integral components of morphogenesis as well as cases in which they play only a secondary role. By far the strongest cases for a direct role of ionic currents in morphogenesis is the polarizing fucoid egg where the current is carried in part by Ca^{2+} and generates an intracellular concentration gradient of this ion that orients the outgrowth, and the insect follicle in which an intracellular voltage gradient is responsible for the polarized transport from nurse cell to oocyte. However, in most of the systems studied, the experiments to determine if the observed ionic currents are directly involved in the morphogenetic mechanism are yet to be done. Our experience with the fucoid egg and the fungal hypha of *Achlya* suggest that it is the change in the intracellular ion concentration resulting from the ionic current that is critical for morphogenesis.
Key words. Ionic currents; vibrating probe; membrane potential; fucoid egg polarization; animal-vegetal polarity; polarization; voltage gradients; calcium; vesicle secretion; *Achlya;* oocytes; insect follicle; insect ovariole; polarized transport; egg activation; mouse blastomere; epithelial morphogenesis; limb bud.

Introduction

The mechanisms underlying morphogenesis must have the capability of generating pattern over relatively long distances of 10–100 µm, based on the dimensions of many well-studied examples of morphogenesis, including ooplasmic segregation, segmentation in *Drosophila,* somite formation in vertebrates, and tentacle formation in *Hydra.* Such morphogenetic fields must be generated by mechanisms based on known physical forces which include gravitational forces, mechanical forces, electrical forces, or some combination of these. These fields have been referred to as 'electro-mechanico-chemical fields'[39] because they generally involve interactions of these physical forces and morphogens such as Ca^{2+} with specific cellular targets such as the cytoskeleton or a population of membrane proteins. Because of these complex interactions, it is unrealistic to expect a single force, such as a voltage gradient, to be the sole cause of a morphogenetic event. However, spatial and temporal information about the electrical fields and ion concentration gradients in and around a cell or embryo undergoing morphogenesis can take us one step further toward understanding the entire morphogenetic mechanism. This is especially true because one of the handful of identified morphogens is