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Pattern Formation During Animal Development

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At the beginning of this century, embryologists defined the central problems of developmental biology that remain today. These questions include how differentiated cells arise and form tissues and organs and how pattern is generated. In short, how does an egg give rise to an adult? In recent years, the application of molecular biology to embryological problems has led to significant advances and recast old problems in molecular and cellular terms. Although not necessarily comprehensive, this idiosyncratic review is intended to highlight selected findings and indicate where there are important gaps in our knowledge for those less than familiar with developmental biology.

IT'S NOT THE INGREDIENTS, IT'S HOW THEY'RE MIXED AND matched. A surprising and encouraging finding in recent years has been the recurring "discovery" of certain gene products in various developmental systems. Open a journal and one is likely to read that any particular gene involved in directing a given mouse cell to adopt a particular fate, after being cloned and sequenced, turns out to be related to a gene performing a similar, but not identical, function in worms, flies, or even yeast. For example, peptide growth factors, first studied for their ability to regulate cell division, now turn up in innumerable instances as signals that can tell a cell whether to become a muscle or skin cell (*1*). Similarly, fly genes involved in neurogenesis and worm genes involved in inducing vulval and gonadal lineages have both been found to encode a membrane protein with a region of amino acids related to epidermal growth factor, a gene product previously identified for its role in vertebrate epidermal differentiation (*2, 3*). Other examples include structural motifs, such as homeobox, zinc finger, helix-loop-helix, or pou domains, found in common among proteins coordinating the transcription of a set of genes and thereby directing a developmental program (*4*). These observations suggest that a few types of genes are used similarly by diverse organisms to specify cell fates during development. While differences in developmental programs between species obviously exist, these are most apparent when a developmental process is examined at the level of tissues and organs and not at the molecular level. It is perhaps self-evident that a present-day challenge for developmental biologists is to explain how relatively few types of genes (transcription factors, peptide growth factors, extracellular matrix components, cytoskeletal proteins, and so on) and mechanisms for specifying cell fates (cell-cell interactions or cytoplasmic localization among others) are used to produce such different animals. With this problem in mind, let us examine some recent findings in studies on pattern formation.

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Regulative or Mosaic Embryos

Observations on marine invertebrates and results of experiments in which parts of embryos were cut out and studied in isolation first led embryologists to divide organisms into two classes: regulative and mosaic. In regulative embryos, parts of the embryo can be removed and the remaining cells compensate for the loss to form a normally patterned animal. Two telling examples are presented by sea urchins and salamanders. Each blastomere of a cleaving sea

urchin embryo forms a normal, albeit small, larva after separation from one another at the four-cell stage (*5*). Similarly, if half of the prospective limb tissue from a salamander is removed, the remaining half is reorganized and a normal limb results (*6*). Thus, instead of forming just an eighth of a sea urchin or half of a limb, the remaining cells in both cases compensate for the missing parts and complete a normal pattern. In contrast, separating all eight blastomeres of an ascidian embryo does not give rise to eight miniature larvae, but rather to deformed partial embryos each resembling the portion each blastomere would have contributed to in the normal embryo.

The distinction between regulative and mosaic embryos could not be maintained in the light of further investigations and so many exceptions were found that the classification lost its utility. Nonetheless, in a historical sense, the distinction is significant because it focused attention on how the fates of cells are specified in embryogenesis. The phenomenon of regulation drew attention to the fact that groups of cells communicate and thereby set or reset specific programs of gene expression. For example, there are now many well-documented examples of embryonic induction in which one cell or group of cells tells another what genes to turn on (or off). This mechanism for specifying cell fates is employed over and over again in vertebrate development and may be the principal mechanism by which cell fates are determined in higher vertebrates. There is also increasing evidence that cell-cell interactions are used to specify fates in organisms with invariant cleavage patterns, many of which, such as worms and ascidians, were formerly considered to be classically mosaic embryos (*3, 7*).

The phenomena of mosaic development drew attention to localization of cytoplasmic "determinants" as a mechanism for specifying cell fates. In this case, the fate of a cell is thought to be determined by the cytoplasmic information (determinant) it inherits from the egg. Thus, in contrast to an inductive interaction, the fate of a cell is specified by internal rather than external information. In either case, there must be an initial difference in developmental information, a polarity to the system. Either one cell tells another what to do, or one region of the egg's cytoplasm contains information absent elsewhere. Thus, an understanding of how polarity is established is of primary importance for explaining how axes and tissue patterns arise during embryogenesis. In recent years, studies on the position of specific gene products in early embryos have given us a glimpse of how developmental polarity is established.

Polarity

In the case of the frog *Xenopus* an unfertilized egg has only one axis of developmental polarity (Fig. 1), the animal-vegetal (A-V) axis. Additional axes of polarity are added as development proceeds. The developmental polarity of the A-V axis is evidenced by the fact that animal and vegetal pole cells have completely different fates. The animal pole gives rise to the skin and nervous system, the vegetal pole gives rise to the gut, and the equatorial portion or marginal zone forms mesodermal tissues (notochord, muscle, blood, and other tissues). The isolation and recombination of parts of developing *Xenopus* embryos has led to the suggestion that animal pole cytoplasm contains localized determinants able to turn on genes that lead to ectodermal differentiation, whereas the vegetal pole cytoplasm directs the formation of endoderm. In addition, the vegetal pole appears to contain signaling molecules that induce mesoderm in overlying cells (*8*). The molecules responsible for specification of animal and vegetal cell fates are not known, but it has been demonstrated that a rare class of maternal mRNAs exists that are localized to one or other pole of the egg (*9, 10*). One of these mRNAs, called Vg1, is encoded by a gene that may participate in mesoderm induction, as Vg1 mRNA is localized to the vegetal pole and encodes a peptide growth factor that is a member of the transforming growth factor β (TGFβ) family (see below). With respect to the question of how developmental polarity is established, interest in Vg1 centers on the localization of its mRNA.

Early in oogenesis, Vg1 mRNA is initially distributed throughout the oocyte cytoplasm, but is translocated and anchored near the

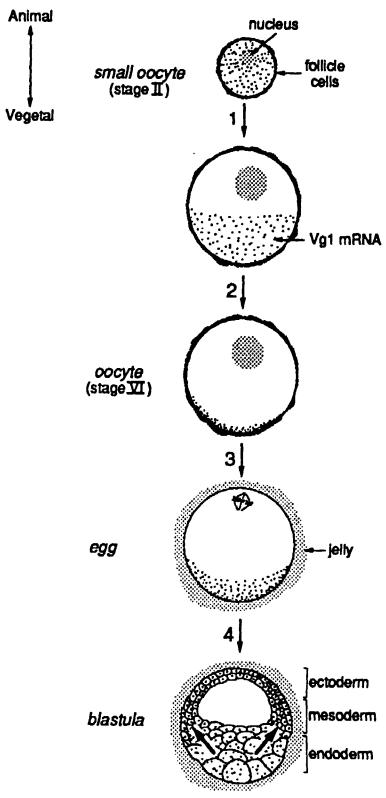

Fig. 1. Axis formation in *Xenopus* eggs. The primary axis of the egg, the animal-vegetal axis, is formed early in oogenesis. Vg1 mRNA, which encodes a TGFβ-like peptide, is synthesized early in oogenesis and is uniformly distributed in small (StII) oocytes. Vg1 mRNA is localized in two steps: (1) translocation that is dependent on intact microtubules and (2) anchoring of the mRNA to a subcortical region of the vegetal cytoplasm, dependent on intact microfilaments. After maturation (3), Vg1 mRNA is released and distributed to presumptive endoderm during cleavage divisions (4). At the early blastula stage, vegetal endoderm induces mesoderm in overlying cells (arrows). The cells that will give rise to ectoderm, mesoderm, and endoderm are roughly positioned in three layers, top to bottom.

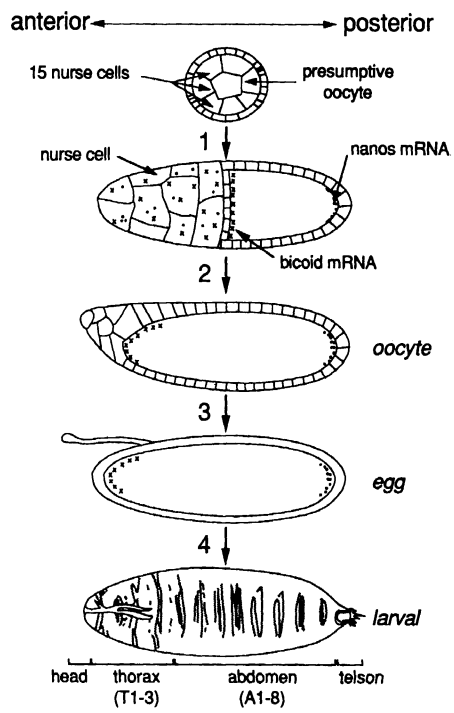

Fig. 2. Anterior-posterior axis formation in *Drosophila* eggs. An egg chamber is composed of 15 nurse cells and one oocyte, all surrounded by follicle cells. The oocyte grows (1) as nurse cells synthesize various maternal components and deposit them in the oocyte through cytoplasmic bridges. Among the various maternal components provided by transport across these bridges are factors involved in anterior and posterior specification of the embryo, the *bicoid* and *nanos* gene products, respectively. These and other maternal components initiate a cascade of regulatory events that affect the expression of the gap and pair rule genes, which leads in turn to a distinctive larval pattern. Gene products involved in specifying the termini and the dorsal-ventral axis are also active during the periods shown, but are not depicted [adapted from (*13*)].

vegetal pole as oogenesis proceeds. The Vg1 mRNA moves toward the vegetal pole before there is any cytological indication of an A-V axis, that is before yolk platelets or the nucleus show signs of A-V polarity. The translocation depends on intact microtubules, and the tight subcortical anchoring of Vg1 mRNA is sensitive to microfilament inhibitors. Biochemical fractionation suggests that cytokeratins may also participate in anchoring (*11*). While the components and mechanism of the translocation and anchoring machinery are not known, microinjection of synthetic Vg1 mRNAs shows that the signal in the RNA that interacts with this machinery is present in the 3′ untranslated region (*12*).

In some ways, the localization of information for pattern in the anterior-posterior (A-P) axis of *Drosophila* is similarly accomplished (*13*) (Fig. 2). The *bicoid* protein, which is a primary determinant of anterior development, is encoded by an mRNA that is localized to the anterior end of the egg. *Nanos*, a posterior determinant, is encoded by an mRNA localized at the posterior pole (*14*). Of the various gene products required for the correct localization of the *bicoid* and *nanos* mRNAs, one (*BicD*) encodes a cytoskeletal (coiled coil) protein (*15*). It is believed that *bicoid* mRNA is trapped at the anterior end of egg (perhaps by the *exu* and *swallow* gene products) (*13*), whereas *nanos* must be translocated to the posterior pole in a process dependent on *BicD* (*15*). Thus, at the molecular level, the translocation of *nanos* and Vg1 mRNAs may be similar processes in the sense that both are informational molecules localized by cytoskeletal elements. The same mechanism for distinguishing two ends of a cell could be used later in development, for example when a stem cell divides to produce another stem cell and its differentiated sister.

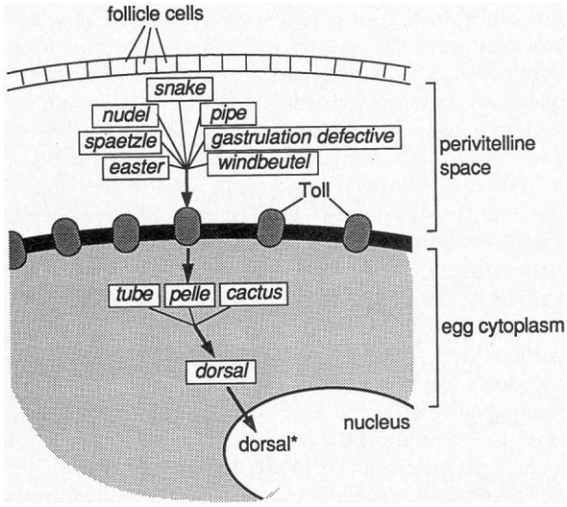

Fig. 3. Dorsal-ventral polarization of the *Drosophila* egg. A portion of the egg is shown with the perivitelline space between the egg and the follicle cells enlarged. The position of the seven genes outside the egg is not meant to imply that their products are necessarily found in the perivitelline space, but rather to indicate that these gene products are thought to signal the egg through a membrane receptor (the *Toll* gene product). Transduction of the signal in the cytoplasm leads to the migration of the *dorsal* gene product into nuclei on the ventral side of the egg. The *dorsal* protein and perhaps the other gene products shown, are uniformly distributed in the egg and are activated, in a graded fashion, only on the ventral side. Thus, it is a gradient of nuclear localization of the *dorsal* protein, and not a gradient of the protein as such, that is involved in polarizing the egg in the dorsal-ventral axis. The nuclear form of the *dorsal* protein (*) may be post-translationally modified (*17*).

Gene products that specify the axial polarity of the fly embryo, including *bicoid* and *nanos* mRNAs, are synthesized by associated nurse cells and transported in at the anterior end of the oocyte. Thus, the A-P axis in flies is established by the position of its associated nurse cells, which is the same for each oocyte in the ovariole and is coincident with the whole body axis of the fly. The situation in frogs is a bit different. Maternal mRNAs are synthesized by the egg itself, not by associated germline or somatic cells, and the A-V polarity of each egg is established without apparent relation to the site of follicle attachment or to the maternal body axis. The original orientation of the oocyte to its oogonial stem cell may set up the A-V axis (in which case there is some analogy with *Drosophila*), or alternatively the axis may be established randomly, perhaps by the relative positions of the nucleus and mitochondrial cloud (*16*). Thus, the primary developmental axis in frogs appears to principally depend on components synthesized and organized by the egg itself, whereas fruit flies have a type of template mechanism with the eggs all lined up, nurse cells at one end, in an assembly line. It is not yet known what signals establish the initial polarization of the entire "egg case" containing both oocyte and nurse cells such that the oocyte is the most posterior cell (*13*).

The fly egg also has another axis, the dorsal-ventral (D-V) axis, set by associated cells, this time follicle cells, but here the molecular mechanism does not appear to depend on the localization of mRNAs. A set of at least 12 genes has been identified as involved in establishing the D-V axis, the consequence of their sequential action being the nuclear localization of a protein (the *dorsal* gene product) in nuclei at the ventral side (*17*) (Fig. 3). The dorsal protein shares homology with a mammalian transcription factor, NF-κB, and controls cell fates by regulating gene transcription (*17*). In this case, proteins that are uniformly distributed in the D-V axis, such as a membrane receptor (*Toll*), are thought to receive and transmit a

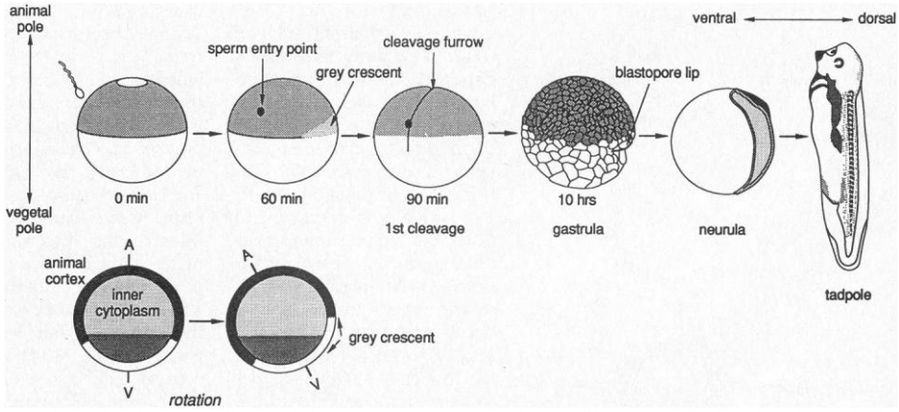

Fig. 4. Dorsal-ventral axis formation in amphibian eggs. The stages of early amphibian development are shown to illustrate the relationship between the animal-vegetal axis of the egg and the axes of the tadpole. Within the first hour after fertilization, the inner cytoplasm and outer cortex of the egg rotate 30° relative to one another. This rotation changes the relative positions of the inner cytoplasm and cortex and sets the dorsal-ventral axis. In some amphibia, the rotation creates an externally visible gray crescent on the future dorsal side, opposite to the site of sperm entry. Rapid cleavage divisions ensue, forming a blastula within 7 hours, after which the cell cycle slows and transcription of the embryonic genome first begins. At the beginning of gastrulation (10 hours), a blastopore lip forms on the prospective dorsal midline, marking the site at which cells invaginate. By the end of gastrulation, a neural plate is centered on the dorsal midline and the anterior-posterior axis is easily discerned. A swimming tadpole develops about a day later. For details see (*20, 21*).

signal that emanates from only some of the follicle cells. As yet, the nature of the signal from the follicle is unknown. It is believed therefore that the D-V axis, like the A-P axis, forms as a consequence of a polarity or difference in the surrounding follicle. A related mechanism for establishing polarity may occur for specification of the termini of the *Drosophila* egg. The *torso* gene product, a maternal membrane receptor, is uniformly distributed in the egg even though its function is only required in the terminal regions (*18*). Thus, the signaling apparatus is available throughout the egg but is activated in discrete locations.

Unlike fly eggs, the eggs of many other organisms do not obtain a second axis of developmental polarity until fertilization or shortly thereafter. Examples can be found among mollusks, echinoderms, ascidians, frogs, chickens, and perhaps worms in which the second axis of polarity forms relative to the site of sperm entry or the plane of first cleavage (*19*). One of the best studied examples of secondary axis determination is the amphibian, *Xenopus* (*20*), which reveals both an interesting story in cell biology and molecular puzzle.

An unfertilized amphibian egg is radially symmetric around its A-V axis (Fig. 4). The dorsal side of an amphibian egg forms opposite the site of sperm entry and is first indicated by a gray crescent visible on the surface in some species. The sperm can enter at any meridian around the egg, which means that any position around the equator can become "dorsalized." The dorsalization of the egg depends on and can be experimentally mimicked by a rotation of the subcortical cytoplasm relative to the outer surface. This rotation, dependent on and perhaps driven by microtubules (*21*), is oriented with respect to the site of sperm entry. How then is dorsalization initiated on one side? It is possible that there are two necessary molecular components, one in the vegetal cytoplasm and another in the animal hemisphere, and that the rotation mixes these two components to initiate a process leading to a dorsal center. For example, a maternal mRNA could be activated on one side by association with a translation initiation or elongation factor. Alternatively, an inactive protein (such as a peptide growth factor, see below) could be activated by cleavage, phosphorylation, or secretion. The molecular nature of the activation caused by a cytoplasmic rotation is an intriguing puzzle and its solution may be relevant to the establishment of polarity in mice, fish, chickens, and other animals.

While there has been progress in understanding how some axes are formed, major challenges remain. Rather little is known, for example, about how right-left asymmetry is established (*22*). This problem is highlighted in studies on the handedness of the twist of snail shells. For the snail *Lymnaea*, the dextral or sinistral pattern for its shell is determined by a maternally inherited gene product, identified genetically as the *D* locus (*23*). Cytoplasmic transfer experiments show that the dextral gene product can transform a sinistral into a dextral animal, if the dextral cytoplasm is injected before the second cleavage division. There are strong indications that the orientation of the third cleavage plane sets the spirality of the whole organism, but the mechanism by which the *D* gene product orients the mitotic spindle remains unknown.

Specification of Cell Fates

Related to, but independent of, the question of how polarity and axes are established is the question of how cell fates are specified. What tells a cell to become nerve or muscle? This question has been studied from so many angles and in so many organisms that only a few examples can be considered and these have been chosen to illustrate the distinction between intercellular communication and an inheritance of localized information from the egg cytoplasm.

As noted above, the idea that eggs have determinants localized in one region of the egg can be traced to observations on marine organisms with colored cytoplasm. The segregation of a green cytoplasm exclusively to blastomeres that will form ciliated cells in the ctenophore *Beroe* is a typical example (*24*). The close correlation between the presence of the green cytoplasm and the formation of specialized cilia, even in isolated blastomeres, strongly suggests that cell fates are determined by the inheritance of special cytoplasm (determinants) during cleavage. In sea urchins, also, the cells that form the larval skeleton are instructed to do so by a special region of vegetal cytoplasm (*25*). While examples of this type of correlation abound (*26*), the molecular nature of these cytoplasmic determinants long eluded embryologists. Recent studies in *Drosophila* have led to a clear picture of how one such determinant, the protein encoded by the *bicoid* gene, may work.

Bicoid mRNA is localized at the anterior end of the egg and consequently the protein is found in a steep concentration gradient with its maximum at the anterior pole (*27*). The mRNA is not translated until egg deposition, and the protein, a transcription factor containing a homeobox, is present for only the first 4 hours of embryogenesis, until gastrulation is under way. The protein determines the fates of cells in the anterior end in a concentration-dependent matter (*28*). A minimum threshold level of *bicoid* protein required for the correct development of the head and acron is satisfied only at the anterior end. Further posterior, *bicoid* protein acts as a positive transcription factor to activate the gap gene, *hunchback* (*29*), and as a negative regulator of a different gap gene, *Kruppel* (*30*), although the effects on *Kruppel* may be indirect. Thus, the same protein starts one cascade of gene expression in the anterior region of the embryo, leading to head and thorax development, and a different set of gene activations posteriorly. Evidently, this control is accomplished through the capacity of the *bicoid* protein to bind DNA and regulate the expression of other genes, including the *tailless, giant*, and *Deformed*, which are required for head and acron development (*13*).

There are still several aspects of *bicoid* function to be explained. As noted above, the mechanisms by which the mRNA is localized and its translation controlled are not yet fully understood. The interaction of *bicoid* protein with other maternal mRNAs, such as *caudal* mRNA, may reveal that the protein has the ability to control translation as well as transcription of other genes (*31*). Most interesting may be the question of how high concentrations of a single protein lead to one result, middle concentrations a second result, and so on. In this regard the *bicoid* protein, as well as the *dorsal* gene product, serve as examples for what is apparently a commonly used mechanism in development, namely the specification of different cell fates in response to different concentrations of a signaling molecule or morphogen. The molecular explanation for this phenomenon may prove to be analogous to the cooperative interactions so well described for λ repressor and which produce an on-off transcriptional switch (*32*) or reveal yet new twists on mechanisms of gene control.

One example of cell interactions is the induction of mesoderm in amphibians. Culturing pieces of blastula embryos in isolation and in various combinations suggested years ago that the mesoderm forms in the middle of a blastula because of a signal that emanates from the vegetal cells and instructs equatorial cells to form the muscular, skeletal, circulatory, and excretory systems (the mesoderm) of the tadpole (*8*). In the absence of this signal, the cells at the equator will form skin (ectoderm) only. The observations that soluble proteins could mimic the effects of vegetal cells (*33*) and that mRNAs localized to the vegetal hemisphere encode a TGFβ-like molecule (*9, 34*) lent support to the hypothesis that peptide growth factors act as the agents of mesoderm induction in *Xenopus*. Equally important

was the observation that purified fibroblast growth factor (FGF) induces mesoderm in isolated animal cap cells (*35*).

The striking nature of the embryonic inductive activities of peptide growth factors, previously identified by and known for their mitogenic activity, is perhaps best highlighted by induction with activin, a TGFβ family member (Fig. 5). When animal pole cells are isolated from an early blastula stage embryo and allowed to develop in a simple salt buffer, they divide and differentiate into ciliated epidermis. This is consistent with their normal fate, though these cells can also have the potential to form the nervous system if induced by adjacent mesoderm later in development. If an explant of animal pole cells is treated with activin A or activin B, the fate of the cells is dramatically altered and the explant can form a miniature embryo complete with head and a rudimentary trunk. Thus, exposing presumptive ectodermal cells to a pure protein changes their fate such that they will form an array of mesodermal and neural tissues (*36, 37*).

Activin is not, however, the only peptide growth factor that can affect embryonic induction and patterning in *Xenopus*. FGF, as mentioned above (*35*), can induce some mesodermal cell types, notably muscle, though induction of animal caps with FGF does not lead to the formation of miniature embryos as seen with activin. Also, overexpression of members of the *wnt* gene family causes a bifurcation of the dorsal axis (*38*). Although it is a significant advance to have identified the type of molecules (peptide growth factors of the FGF, *wnt*, and TGFβ families) involved in the induction and patterning of the mesoderm, there are numerous questions raised by these results. Foremost among these is which peptide growth factors are present in the early embryo, where are they found, and how do they act?

Numerous members of the TGFβ family have been implicated in mesoderm induction either because of their presence in the early embryo (Vg1, TGFβ5, and activin) or their ability to induce mesoderm in animal cap explants (TGFβ2 and β3) (*39*). Of these, special attention has been given to activin B (β_B homodimer). Like other homologous and heterologous activins, the β_B homodimer alone is sufficient to initiate the formation of dorsal axial mesoderm as well as anterior tissues in isolated animal caps. Furthermore, activin B is the first activin transcribed during embryonic development (at the blastula stage), hours before the activin A gene. Activin β_B is therefore present before gastrulation and at a time when it could act as the inducer of Spemann's organizer (*37, 40–42*). Notably, parallel studies in the chick have shown that activins can induce the formation of organized axial structures from isolated epiblasts and that activin B is expressed in the hypoblast, which normally induces axial differentiation in the epiblast (*43*).

Several members of the *wnt* gene family are also expressed during *Xenopus* early development. *Xwnt8*, for example, is expressed in the ventral mesoderm before gastrulation, and its ability to duplicate a dorsal axis when ectopically expressed suggests some involvement for it (or another member of the *wnt* family) with Spemann's organizer (*38, 44*). As in studies with activins, it is not yet clear whether this type of overexpression experiment reveals the normal function of *Xwnt8* or a cross-reactivity with a receptor that normally receives a signal from a closely related ligand. Until more is known about the expression patterns and inductive properties of other members of the TGFβ and *wnt* families, it is premature to say precisely which gene products are responsible for mesoderm induction and patterning in *Xenopus*. Indeed, in recent studies additional members of both the TGFβ and *wnt* families that are expressed during frog embryogenesis have been identified, but their functions have yet to be tested (*45*).

How does exposure to a peptide growth factor such as activin give rise to a miniature embryo with A-P and D-V polarity? One possibility is that cells in an animal cap are homogeneous and are exposed to different amounts of activin or are exposed for different lengths of time during these experiments, which would produce different responses leading to axial differentiation. In fact, dispersed animal cap cells do respond differently to high and low concentrations of activin (*46*). Another possibility is that there is an underlying pattern in the animal cap cells, that is, the animal cap cells are not homogeneous. Recent studies with the use of lineage tracers suggest that *Xenopus* animal cap cells do indeed have an inherent "prepattern" that determines the polarity and tissue types formed after uniform exposure to activin. These studies also show that the prepattern in the responding animal cap cells is established very early, probably by the cytoplasmic rotation that initiates dorsalization (*47*). These results are consistent with previous studies in chicks that showed that the high degree of organization generated by activin treatment can be explained by a weak polarity stored in the epiblast (*48*). In both the chick and the frog, it may be the case that the embryo employs a "double assurance" for setting axial pattern: both a localized source of an inducer (activin) able to set pattern and a predisposition or prepattern in the responding cells. In experimental manipulations, the prepattern in the responding cells can evidently be overridden by high concentrations of an inducer.

These results point to our ignorance of the state of the responding cells. It has been known for years that competence to respond to inducers is transient (*49, 50*), but it is not known whether this corresponds to the presence of a receptor or some other part of the signal transduction system. As a first step it is essential to isolate the receptors for the various peptide growth factors so that their expression patterns and ligand affinities can be determined. That information may help to explain the competence of cells and their predisposition to form certain cell types such as notochord or muscle. For example, there may be high and low affinity receptors for activins and this could help explain how different concentrations

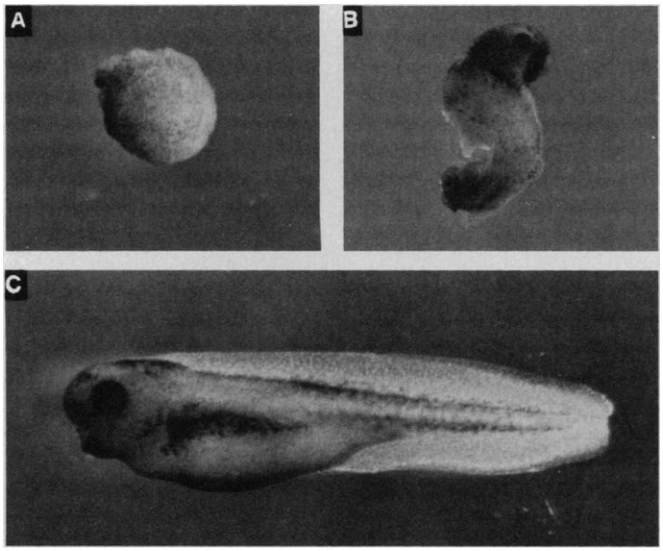

Fig. 5. Presumptive ectodermal cells were isolated from the top quarter (50 to 100 animal cap cells) of a frog blastula (see Fig. 1). A control of untreated animal cap cells (**A**) differentiates into a ball of ciliated epidermis, consistent with their normal fate as animal cap cells. Two days after a 2-hour incubation with *Xenopus* activin β_B protein, the animal cap cells form a tiny embryoid with a head, tail, and rudimentary body axis (**B**). Histological sections (not shown) reveal the presence of eyes, brain, notochord, muscle, and mesenchyme. The small tail-like structure twitches from muscular contractions. An unmanipulated blastula develops into a tadpole in the same time period (**C**). All photos shown at the same magnification. For experimental details, see (*36, 37*).

of one factor produce different cell types.

One clue about how peptide growth factors set pattern comes from studies showing that peptide growth factors turn on homeobox genes, such as *Mix1*, in responding cells (*41, 51*). Moreover, it has been shown in *Xenopus* that different peptide growth factors can set the amount of homeobox gene expression and that these levels correlate with A-P pattern. For example, low levels of the homeobox gene *Xhox3* expression are required for anterior development and high levels for posterior development. If these levels are artificially altered, headless and tailless embryos are produced (*42*). A similar connection between peptide growth factors and the activation of homeobox genes has been demonstrated in flies (*52*). This type of interaction establishes, in principle, how a signal from one cell can determine the fate of a responding cell, as homeobox genes encode transcription factors that can turn sets of genes on or off and thereby direct cell differentiation. This type of cascade from homeobox gene to peptide growth factor expression to another homeobox gene in a responding cell may also prove to be an explanation for how patterning information is transmitted from one germ layer to another (*53*).

The activation of homeobox genes like *Xhox3* and *Mix1* in response to peptide growth factors does not easily explain how different mesodermal cell types arise. Indeed, as far as is known the expression of these homeobox genes is pan-mesodermal; not restricted to one particular mesodermal lineage. How then is the mesoderm subdivided? In the case of muscle, there has been enormous progress in identifying genes that act early as regulators of a program for lineage-specific gene expression. A number of myogenic genes (*MyoD, myogenin*, and others) have been characterized in mammals, chicks, and frogs, and are certain to play key roles in committing cells to become muscle (*54*). For example, transient expression of one such gene, *MyoD*, can turn a fibroblast into a muscle cell (*55*). The early mesodermal expression of vertebrate homologs for the *Drosophila snail* and *twist* genes (*56*) may yet be connected in a pathway that includes myogenic genes and peptide growth factors that induce muscle. With respect to carving up the mesoderm anlage into more than just muscle, it is important to learn whether there are genes similar to *MyoD* operating in the respiratory, excretory, and skeletal lineages of the mesoderm.

Are there two ways to make muscle? Even though induction and cytoplasmic localizations are distinct as mechanisms for specifying cell fates, it is interesting that in at least three organisms there is evidence that both mechanisms are employed to produce muscle cells. In ascidians, the segregation of myoplasm into presumptive muscle blastomeres is a classic example of how a cytoplasmic localization may determine cell fates. Recent studies show, however, that some of the ascidian tadpole muscles can also be formed by inductive interactions (*57*). Similarly, in the nematode, pharyngeal and body wall muscle are formed as the result of inductive interactions as well as segregation of a cytoplasmic factor (*3,* 7). And finally, although there is abundant evidence that frog skeletal muscle is formed by induction, there is also support for the segregation of a muscle-forming determinant in the early embryo (*50, 58*). Once the early steps in the myogenic pathway are more fully elucidated it should be possible to find out whether the inductive and localization mechanisms for determining muscle converge at the molecular level (*19*).

Induction and Patterning of the Central Nervous System

As described above, experimental results suggest that peptide growth factors are responsible for inducing mesoderm, and there is circumstantial evidence for the idea that homeobox genes play a role in its patterning, at least in the A-P axis (*42*). In amphibia, transplantation experiments have shown that the mesoderm is responsible for the formation of the central nervous system (CNS) in the sense that mesoderm induces adjacent ectodermal tissue to become the CNS and imposes axial pattern: that is, head mesoderm induces anterior neural structures and tail mesoderm induces posterior neural tissue (*59*). Numerous studies have shown that homeobox genes serve as excellent early markers for positional differences in the vertebrate CNS. In mice it has been demonstrated that these A-P patterns of expression correspond to the organization of the homeobox genes in the genome, reminiscent of the relationship first noted in fruit flies (*60, 61*). Here again it may be that the positional information encoded in the mesoderm, and revealed by differences in homeobox gene expression, is transferred to the overlying ectoderm through a molecular cascade involving yet unidentified intercellular signals (*53*). For example, the regional expression of a homeobox gene in the mesoderm might direct the expression of a peptide growth factor that is secreted and in turn regionally activates a homeobox gene in the neural ectoderm.

It is not definitively known whether neural induction requires contact between the inducing mesoderm tissue and the responding ectoderm, nor is it known what molecules act as inducers (*62*). One class of molecules worth considering again is peptide growth factors. The possible involvement of extracellular matrix components has also not been fully explored. Whereas much attention is given to the inducing tissue (mesoderm) in neural induction, some recent studies suggest that the responding tissue (ectoderm) may contain some elements of the final pattern. Dorsal ectoderm, which is eventually induced to form neural tissue, is biased toward a neural fate at the blastula stage, before the inducing mesoderm has involuted (*63*). Whether this bias reflects, at the molecular level, the expression of proneural genes like those observed in *Drosophila* (*64*) remains to be determined.

Role of Retinoic Acid

The patterning of the CNS can also be influenced by retinoic acid (RA). Treatment of developing amphibia with RA causes an anterior to posterior transformation in the CNS, and disruption of axis formation in developing chicks (*65*). In these cases, it is not yet known whether the effects of RA are mediated through the inducing or responding tissues or both. As a patterning agent or morphogen, the effects of RA have been most thoroughly described in developing limbs (*66*). RA is present in a shallow gradient in developing chick limb buds (*67*), and exogenous addition of RA can symmetrically duplicate the anterior-posterior pattern of the digits (*68*). In these limb experiments, RA mimics the endogenous zone of polarizing activity (ZPA) which had been previously shown to induce axial pattern in the limb bud (*69*). RA can also alter the pattern formation of regenerating amphibian limbs (*70*).

Unlike the role for peptide growth factor receptors in intercellular inductions alluded to above, the effects of RA are mediated through a nuclear RA receptor (RAR). At least two forms of the receptor exist and these have significantly different affinities for their ligand, RA (*71*). In addition, much of the RA in cells is bound to a cytoplasmic RA binding protein (CRABP) which drastically limits the amount of free (and perhaps therefore morphogenetically active) RA (*72*). It has been suggested that a combination of different binding affinities of the receptors, a gradient of CRABP, and regulation of receptor synthesis by RA could work to modulate the gradient of RA (*66*).

An important hint about how RA may work comes from the observation that in some cases an early response to RA can be the transcriptional activation of homeobox genes (*73*). Various homeobox genes are expressed in discrete but overlapping regions of developing limb buds (*74*). It is remarkable that the order and graded expression of different homeobox genes in the A-P axis of the limb is not unlike that observed in the A-P axis of the body trunk (*61, 75*). And, as noted above, the sequential activation of the homeobox genes in this case is such that the gene located most 5′ in the chromosomal array is expressed last. The ordered activation of the genes in time and space, as revealed by in situ hybridization, is consistent with the idea that the ZPA provides the activating signal (in the form of RA). One expects that a causal connection between RA treatment and differential homeobox gene expression will soon be established. The consequences of altered homeobox gene expression for limb development are likely to generate instructive insights for understanding this patterning system.

Conclusions

There have been substantial advances in recent years in our understanding of developmental mechanisms, only some of which have been highlighted here. Of the many remaining challenges for developmental biologists, only a few are noted. In several developmental systems, the balance of evidence suggests that one type of signal (a morphogen) is presented in a graded fashion and generates a discontinuous pattern, that is, several different cell types or body parts. *Bicoid*, RA, and most probably some of the peptide growth factors act in this way. Whereas a simple threshold model can be used to explain a simple on-off switch (such as when a certain concentration of the morphogen turns a gene on), it is not clear how a graded distribution of a morphogen can produce discontinuous patterns with several states (*76*). With the present rate of discovery and research activity in this aspect of developmental biology, one anticipates that the answers will soon include a discussion of multiple binding sites on DNA, receptors with different affinities for the same ligand, and controls exerted at the level of signal transduction.

Looking ahead, one expects unabated success in identifying genes that play important roles in development and in understanding how the expression of such genes is controlled. Yet a more difficult challenge may be to understand how these genes exert their developmental effects at the cellular and supracellular levels; for example, how do the gene products affect morphogenetic movements? In this regard, one anticipates increased attention will be given to the role of the cytoskeleton in developmental processes. And, as more examples of cell interactions or inductions are uncovered, the role of the extracellular matrix is likely to be considered more vigorously by developmental biologists.

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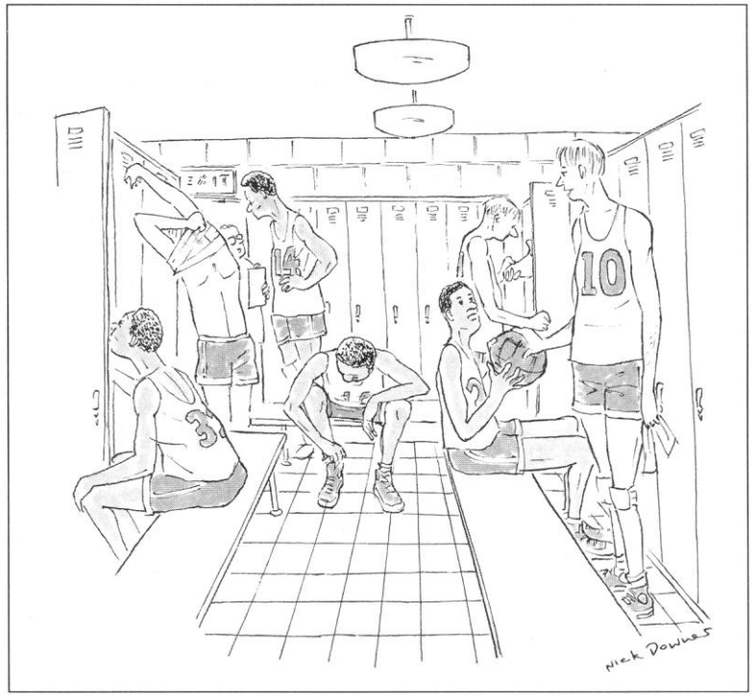

" So far, my endorsements have been a sneaker company, a line of sportswear and the makers of a recombinant human growth hormone."