Pattern formation and developmental mechanisms
Unresolved issues of pattern formation
Editorial overview

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Introduction

To understand how animals are built requires understanding of the mechanisms that define: first, how anterior–posterior, dorsal–ventral and left–right axes of polarity are established, second, how this information is interpreted by cells to acquire specific fates, third, how cells translate this information to elicit particular cell behaviors (proliferation, shape change, migration) and fourth, how groups of cells coordinate their behaviors to form specific structures/tissues. Our current knowledge has arisen from work using a number of 'model' developmental systems. Most work is being conducted using *Caenorhabitis elegans*, *Drosophila*, *Xenopus*, zebrafish, chicks and mice. The challenge is not only to figure out for each system the mechanisms that govern these decisions, but also to elucidate the similarities and differences that have been selected by each organism during evolution.

In this issue of *Current Opinion in Genetics & Development*, we have selected a number of topics that illustrate some of the latest issues in pattern formation. We have combined a number of reviews on the various model systems in an attempt to illustrate the similarities, differences, approaches and unresolved issues. It is apparent that there are striking differences in our level of understanding of these processes between the different model systems. In *C. elegans* and *Drosophila*, many of the molecular players in development have been identified, as have some of the major relationships between them. In turn, the vertebrate systems fall into two categories: those like the frog, fish and mouse, where loss- and/or gain-of-function approaches can be followed (as in *Drosophila* and *C. elegans*), and those where cell lineage and transplantation are easier to study. In the former, it is also easier to discover pathways and molecular interactions, whereas the latter lend themselves better to an investigation of cellular movements, cell interactions ('inductions') and the dynamics of development. As a rule, these morphogenetic events are better understood in vertebrates than in invertebrates. It seems likely, therefore, that some of the differences in our understanding of the mechanisms underlying pattern formation between different species reflect the different approaches for which each organism is more suitable. A challenge for the future is to bridge this gap.

Determinants and establishment of polarity

The major event following fertilization is the establishment of polarity in the zygote. Whereas in most organisms such as *C. elegans* and vertebrates this occurs in a cellularized environment, in organisms such as *Drosophila* the first nuclear divisions occur in a syncytium. But animals may also be classified in another way. Some embryos, whose volume does not increase during early development (e.g. *Drosophila*, frog), establish their polarity by the localization of maternal messages. Other embryos (chick, mouse) increase in volume dramatically along with early cell divisions — these embryos are often characterized by an enormous power of regulation. The chick, for example, may be cut into many fragments at a stage when the embryo has more than 10^5 cells, and each fragment will spontaneously establish its own polarity and initiate axis development. In these animals, the localization of maternal determinants is unlikely to play a crucial, instructive role. Regardless of these initial differences, the first critical step in the development of all animals is the definition of polarity.

In the past few years, much knowledge has been obtained about the establishment of polarity in *C. elegans* and *Drosophila*. Bowerman and Shelton (pp 390–395) describe that begining with the first mitotic division in a *C. elegans* embryo, asymmetric cleavages establish much of the body plan. Further, they illustrate the role of both intrinsic and inductive signals in this process. In *Drosophila*, previous studies have illustrated how the two main body axes of the *Drosophila* embryo, antero-posterior and dorso-ventral polarity, are determined by a small number of asymetrically localized cues in the egg. The localization of these cues, which depends on earlier steps in oogenesis, is the focus of the review by van Eeden and St. Johnston (pp 396–404). They illustrate how the Cadherin-dependent positioning of the oocyte creates an anterior–posterior polarity that is transmitted to the embryo through the localized translation of *bicoid*, *oskar* and *nanos* mRNAs. Further, they review how dorsal–ventral polarity arises from the random segregation of the nucleus to the anterior of the oocyte, and how this ultimately effects the embryonic dorsal–ventral axis by restricting the expression of the *pipe* gene to ventral follicle cells.

In *Xenopus*, the localization of two maternal determinants in the cytoplasm of the fertilized egg establish initial dorso–ventral polarity in the embryo. One of these determinants is a member of the Vg1/activin (TGF-β) family, which becomes enriched in the vegetal pole. The other determinant, which is the focus of Sokol's contribution (pp 405–410), is the activation of the Wnt pathway. The polarity of its localization is achieved as a result of the cortical rotation that immediately follows fertilization, which causes β-catenin to

be placed at the dorsal side. These two determinants therefore form an orthogonal coordinate system, and the quadrant where they overlap becomes an important signaling region: the Nieuwkoop center — which subsequently emits signals that specify the organizer.

In the chick, as in amphibians, gravity plays some role in establishing a bias for axial polarity. But while the 'blastula' stage embryo does have visible polarity, this is not yet irreversibly established. Is there a region analogous to the Nieuwkoop center of the frog? Bachvarova (pp 411–416) reviews some substantial progress made recently, and reveals that one region, the posterior marginal zone, has very similar properties to those of the frog Nieuwkoop center. Neighboring regions (such as Koller's sickle), however, also appear to contribute to polarity — in ways that we do not yet understand fully.

It is rather remarkable that the rules underlying the establishment of embryonic polarity have not yet been answered definitively in mammals. The ease with which mouse chimeras can be constructed by random injection of dissociated cells, and the apparent lack of polarity of the early conceptus led to the traditional view that polarity and cell fates are labile until relatively late in development. Moreover, the polarity of the embryo is often related to the site of implantation in the maternal uterus. Gardner (pp 417–421) explains that recent research has uncovered a hitherto unknown polarity in the very early embryo, which includes both morphological and molecular asymmetries that relate to the future axis of the embryo. These new findings are starting to suggest that embryonic polarity is established prior to implantation, by signals emanating from future extraembryonic cells at one end of the conceptus. But both the nature of these signals, and the mechanisms that determine which become the signaling cells, remain unknown.

Until quite recently, most research effort on understanding the molecular determinants of embryonic polarity concentrated on two axes: head–tail and dorsal–ventral. The past few years have brought a major explosion of interest in the mechanisms that generate differences between left and right sides. Some of the molecular players have now been discovered and — surprisingly, as Yost (pp 422–426) discusses — some of these players, notably the secreted factor Nodal and the homeobox gene *Pitx2*, are common to all vertebrates, whereas events both up- and downstream of these appear to have diverged among the vertebrate classes.

Patterning fields of cells

Important advances have been made in understanding the mechanisms underlying patterning in tissues. Recent studies have started to pinpoint the signaling molecules underlying the activity of 'organizing centers' from which combinations of secreted factors have the ability to provide positional information to fields of cells. These include members of the Hedgehog, Wnts and TGF-β families. Capdevila and Izpisúa Belmonte (pp 427–433) describe the ability of these proteins to organize pattern and discuss various mechanisms by which these signaling molecules, in concert with their receptors and associated modulating molecules, operate to define the proper shape and size of the tissue. Although these secreted proteins contribute to generate global patterns, more local signaling mechanisms also exist. Irvine (pp 434–441) describes the role of Fringe proteins in positioning and restricting dorsal–ventral border cells in the *Drosophila* wing and eye through modulation of the Notch pathway. Perhaps surprisingly, this same pathway is also involved in patterning a more multicellular system: vertebrate somitogenesis. Here, temporal oscillations in the activity of a homolog of Fringe appears to modulate Notch function which is required for generating the spatially periodic pattern of somites in the vertebrate trunk. The function of Fringe and Notch proteins in establishing the dorsal–ventral boundary in the compound eye of the fly is described by Strutt and Strutt (pp 442–446), who also discuss in detail the contribution of other pathways (Wingless, JAK/STAT and JNK) involved in ommatidial polarity and in setting up long-range gradients of positional information that determine the mirror-image symmetry of photoreceptor clusters about the dorso-ventral midline of the eye. (The role of the JNK pathway in the establishment of planar polarity in *Drosophila* — possibly downstream of Frizzled receptors — is also reviewed by Noselli and Agnès [pp 466–472].)

Much progress has been made in the past few years on the identification of the signaling pathways that control pattern. Although we are beginning to have a good understanding of what these pathways can do and of their molecular architecture, we are still far from understanding why they have been selected for specific decisions. A striking finding is that only a rather limited numbers of signaling sytems have been selected during evolution to pattern animals (i.e. Wnts, TGF-β, Hedgehog, and TGF-α). As these pathways are used in multiple and intricate ways during development, it may not be too surprising that an increasing number of regulatory molecules are being identified. The complexity of these signaling pathways is illustrated by the review of Martinez Arias, Brown and Brennan (pp 447–454) on Wnt signaling. They describe how Wnt signaling in various contexts specifies different developmental decisions. They illustrate the similarities and differences found in signaling by Wnts in *Drosophila*, *C. elegans* and frogs, making a good case for the need to study these pathways both in multiple tissues and different organisms.

Co-ordinated movement of epithelial sheets

Patterning in multicellular assemblies cannot be explained fully by the localization of determinants and by inductive interactions generating local gene expression domains. Development is much more dynamic than this, and it is important to remember that the cell interactions are taking place among cells that constantly change position in the embryo. Much of what we know about cell movements during development comes from labeling and time-lapse studies during early development in chick and frog embryos. More

recently, several technical advances have made it possible to obtain information about the movements of epithelial sheets in other systems, as well as on the role of different cellular and extracellular components in morphogenetic events.

The chick limb has long been a favored model for studying pattern formation – that is, mechanisms that generate form within a field of cells that appears to be initially uniform and somewhat solid. By contrast, early embryos have traditionally offered a system for studying morphogenesis. Although the literal translation of this word from Greek is similar to 'pattern formation', it is usually employed to refer to situations when structures are generated primarily by cell rearrangement. Recent advances reviewed by Tickle and Altabef (pp 455–460) are starting to change this prejudice, and show that the study of development cannot overlook the contribution of cell lineages, movements and cellular rearrangements, even in a system like the limb.

It is also surprising how little we know about the movements of epithelial sheets even during early development. In the sea urchin — reviewed here by Ettensohn (pp 461–465) — sophisticated combinations of embryological manipulation and filming have revealed that the process of involution of the outer epithelial sheet begins much earlier than previously thought, and that bottle cells (which ingress individually) and the involution of epithelial sheets play sequential events in gastrulation. At the same time, *Drosophila* continues to be an excellent system for linking developmental events with their molecular effectors. Dorsal closure of the *Drosophila* embryo has emerged as an excellent system for identifying molecules involved in the coordinated movement of an epithelial sheet. Noselli and Agnès (pp 466–472) describe how lateral epithelial cells elongate dorsally and move in concert toward the dorsal midline where they ultimately fuse. They review a large amount of information that has been obtained in the past few years on the molecules involved in this process, in particular the role of the JNK and TGF-β pathway.

Cell migration of small groups of cells

In contrast to morphogenetic movements that depend on the coordinated movement of entire epithelial sheets, more subtle migration of groups of cells also occur in development. Such cells include neurons, neural crest cells, blood cells, cells of the immune system and primordial germ cells. Forbes and Lehmann (pp 473–478) describe three paradigms in *Drosophila* that permit a genetic and molecular dissection of the pathways underlying cell motility: tracheal cells which are under the control of a FGF receptor signaling pathway; primordial germ cell migration which depends on an attractant signal dependent on HMG CoA reductase, and a repellent which involves the phosphohydrolase, PAP-2; and migration of border cells during oogenesis which is under the control of E cadherin. Similarly, Branda and Stern (pp 479–484) discuss the roles of Netrin in dorsally and ventrally directed cell migrations and axon guidance in *C. elegans* and recent progress in identifying from genetic screens of intracellular proteins that transmit the extracellular signals that direct cell and axon migrations to the cytoskeleton.

Conclusions

Now that the complete sequence of several animal genomes are near completion, one may well ask the question 'if we knew the entire sequence of an animals DNA, would we be able to predict what it looked like?'. The answer, in all likelihood, is 'no'. As we approach the post-genome era, we still have a lot to discover about some of the most basic developmental mechanisms that contribute to generate pattern in both vertebrates and invertebrates, and some of the most fundamental questions are still unanswered. Each model system has its own flavor and can make an important contribution, and there is still considerable room for advances using both the bottom-up (gene to function) and the top-down (from developmental event to molecular players) approaches. Excitements to come in the next decade are likely not to include the discovery of new genes but understanding of increasingly sophisticated biological strategies for development.