Pattern formation and developmental mechanisms

Editorial overview

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Abbreviations

bHLH	basic helix-loop-helix
BMP	bone morphogenetic protein
EGF	epidermal growth factor
LCR	locus control region
MADS	MCM1, agamous, deficiens, serum response factor
msl	*male-specific lethal*
RTK	receptor tyrosine kinase
TGF	transforming growth factor
UTR	untranslated region

Developmental regulatory mechanisms, pattern formation, and evolution

This issue of *Current Opinion in Genetics & Development* examines mechanisms by which pattern is established during the development of a broad range of organisms and in a wide variety of tissues. Perhaps the most important message to emerge is that, on the whole, developmental mechanisms have been extraordinarily well conserved during evolution. Each embryo appears to have at its disposal a fundamental 'toolkit' of regulators and regulatory pathways with which to construct an organism. Most chapters in this issue discuss the tools; the last chapter, by contrast, addresses the evolutionary question of how different embryos give rise to distinct organisms with essentially the same 'tool-kit' of molecules during development.

The first and arguably most fundamental event in development is the establishment of asymmetry in the early embryo. The symmetry-breaking event is poorly understood in vertebrates (and particularly so in the mammalian embryo) but great advances have been made in the invertebrates *Drosophila melanogaster* and *Caenorhabditis elegans*. In these species, polarization of the embryo involves the localization of regulatory molecules either during oogenesis or in the newly fertilized zygote. The classic view in *Drosophila* has been that the antero-posterior and dorso-ventral axes are controlled independently but recent evidence suggests that the two are linked. As reviewed by Grünert and St Johnston (pp 395–402), RNA encoding a TGFα homologue, Gurken, is localized to the posterior of the oocyte, and secreted Gurken protein acts through a homologue of the EGF receptor, Torpedo, to specify adjacent follicle cells as posterior. The posterior follicle cells then establish, by unknown means, a polarized microtubule network, along which the oocyte nucleus migrates to occupy a corner of the oocyte's anterior cortex. Gurken RNA becomes localized to one side of the nucleus, and secreted Gurken again acts through Torpedo, this time to cause overlying follicle cells to adopt a dorsal, rather than a ventral, fate.

Not only can maternal mRNAs be regulated by localization but they can also be precisely controlled at the translational level. Macdonald and Smibert (pp 403–407) review evidence from both invertebrate and vertebrate embryos that the 3′ untranslated region (UTR) can control translation both in time and in space. One of the most surprising recent observations is that bicoid protein, well known as a transcriptional activator, binds to the 3′UTR of *caudal* RNA and represses its translation. The gradient of embryonic *bicoid* therefore transcriptionally activates certain genes (e.g. *hunchback*) and translationally represses at least one maternal mRNA, *caudal*, in the anterior part of the embryo. In this way, the anterior-to-posterior gradient of bicoid establishes an opposing posterior-to-anterior gradient of caudal.

In *Drosophila*, early patterning occurs in a syncytium, with multiple nuclei in a common cytoplasm. In this respect *Drosophila* differs from most embryos, which undergo cleavage divisions. Guo and Kemphues (pp 408–415) describe recent progress in understanding the establishment of polarity in one of these 'other' embryos — that of the nematode *C. elegans*. Here specification of the anterior–posterior axis relies on the position of sperm entry. Fertilization then sets in motion a cascade of cytoplasmic events that results in a precisely regulated series of asymmetric divisions. These asymmetric divisions require the products of the *par* genes, which are themselves asymmetrically localized. Interestingly, the polarity of the *Xenopus* embryo is also established at fertilization. In this case, the sperm entry point defines the axis of rotation of a microtubule-driven movement of cortical cytoplasm. This rotation, through an unknown mechanism, specifies the dorso-ventral axis of the embryo. Unfortunately, no unifying theme has yet emerged for the establishment of polarity in *Drosophila*, *C. elegans*, or *Xenopus*, although it is noteworthy that the cytoskeleton is important in all three.

Once the broad strokes of pattern have been established, what is the next step? How is this underlying information used to control the development of organs at the right time and in the right place? This question is first addressed by Rusch and Levine (pp 416–423) in *Drosophila*. A gradient

of the *dorsal* transcription factor leads to the establishment of three territories: dorsal ectoderm, neuroectoderm, and mesoderm. Analysis of the promoter regions of five different *dorsal* target genes has revealed that specific threshold responses to the *dorsal* gradient are generated by the differential occupancy of *dorsal* binding sites in the promoters of these genes.

The three territories thus defined are then further subdivided. Rusch and Levine (pp 416–423), and also Ferguson (pp 424–431), discuss the dorsal ectoderm, where a high effective concentration of decapentaplegic, a member of the TGF-β family, specifies differentiation as amnioserosa, whereas low levels cause the formation of epidermis. Ferguson emphasizes the remarkable similarities in molecular details for establishing dorso-ventral pattern in *Drosophila* and in *Xenopus* and also notes that the dorso-ventral axes of the two species appear to be inverted: the ventral side of *Drosophila* corresponds to the dorsal side of *Xenopus*. This suggests that an inversion of the dorso-ventral axis occurred between the arthropods and the vertebrates.

The similarities between *Drosophila* and *Xenopus* dorso-ventral patterning include the TGF-β family member (*decapentaplegic* or its vertebrate homologue *BMP4*) to specify position and a second secreted protein (*short gastrulation*, or its vertebrate homologue *chordin*) that modulates activity of the TGF-β family member. This theme is taken up by Hogan (pp 432–438), who also discusses the roles of other members of the TGF-β family such as BMP7 and the *nodal*-related genes. Hogan also describes gene targeting experiments in the mouse, which reveal BMP4 to be required for early postimplantation development.

In *Drosophila* and *C. elegans*, one can go from phenotype to gene, secure in the knowledge that the gene plays an important and defined role in development. Until recently, there was no such luxury in vertebrates, where the primary approach has been to guess which genes might be interesting and then knock them out. Such guesses have been inspired in a variety of ways: identification of key regulators in *Drosophila* or *C. elegans*, effects on tissue culture cells, expression patterns during development, or consequences of over-expression in *Xenopus*. Sometimes the guess has been good, as with BMP4, and other times bad. In their chapter, St-Jacques and McMahon (pp 439–444) describe recent mouse knock-outs affecting initial establishment of the body plan, skeletal development (concentrating on *Hox* genes), and limb development. The rate of progress in this area is nothing short of staggering and, as St-Jacques and McMahon emphasize, next year promises to be even more extraordinary. We can anticipate the generation of compound mutants, techniques that allow excision of the positive selection cassette (thus preventing interference with complex loci), tissue-specific knock-outs, generation of large chromosomal alterations, and the production of more 'knock-ins', in which the coding sequence of one gene is replaced by that of another.

Molkentin and Olson (pp 445–453) describe the use of knock-out technology combined with experimental embryology and molecular biology to unravel the cellular interactions directing formation of skeletal muscle. Inductive signals emitted from notochord and floorplate (probably Sonic hedgehog), and from dorsal neural tube (Wnts), function cooperatively to activate expression of myogenic basic helix-loop-helix (bHLH) factors such as MyoD. The bHLH proteins themselves interact in a cooperative fashion with MEF2 factors and together they induce muscle structural gene expression either by binding directly to muscle-specific control regions or through indirect interactions. In terms of identifying signals, target genes, and targets of the targets, this is one of the best understood of all vertebrate inductive interactions, though little is known about the signal transduction machinery by which inductive signals activate the bHLH genes.

Vertebrate heart development is not as well understood as the development of somitic muscle, but Lyons (pp 454–460) shows that some of the same principles are involved. Like somitic muscle, cardiac muscle is formed through an inductive interaction, in this case by a signal derived from anterior endoderm. MyoD and the other somitic muscle bHLH transcription factors do not regulate cardiac muscle development but two novel bHLH proteins are expressed and the MEF2 family of MADS-box containing proteins also appear to be involved. Finally, members of other families of transcription factors make important contributions to heart development. In particular, GATA proteins and a homeodomain protein called Nkx-2.5 direct various aspects of cardiac morphogenesis and gene expression.

Identifying potential vertebrate regulatory genes on the basis of homology with *Drosophila* or *C. elegans* has the disadvantage that developmental events specific to vertebrates will be overlooked. To overcome this problem, two laboratories have recently undertaken large-scale mutant screens in the zebrafish *Danio rerio*. These screens are discussed by Granato and Nüsslein-Volhard (pp 461–468), who refer to no fewer than 36 papers soon to appear in *Development*. The mutants fall into several classes, including those for dorso-ventral patterning, axis formation, organ formation and locomotion. Progress in positional cloning techniques should allow molecular identification of many of the mutant genes and it is encouraging that some genetically identified mutants, such as *floating head*, map to molecularly defined genes, such as the zebrafish homologue of the *Xenopus* gene *Xnot*.

The screens described by Granato and Nüsslein-Volhard have concentrated on phenotypes visible by dissecting microscope but they also mention more specialized screens in which, for example, mutations affecting retinotectal projections are isolated by anterograde labelling of axons with fluorescent dyes. Müller, Bonhoeffer and Drescher (pp 469–474) describe three families of molecules involved in this process: the netrins (the *C. elegans* homologue of which is UNC-6), the semaphorins/collapsins, and the Eph receptor tyrosine kinases (RTKs) together with their ligands. Within these families, there is great scope for diversity of effects and therefore for subtle modulations of function which are likely to be required for accurate wiring of the nervous system. For example, chemorepulsive and chemoattractive effects of UNC-6, the *C. elegans* homologue of netrin-1, are encoded by different domains of the protein, and in the Eph family 13 RTKs and 7 ligands have already been identified.

Tremendous progress has been made in plant development over the past few years. Best known is the stellar progress on how individual parts of a flower are specified. In this issue, we turn to two new topics. Meyerowitz (pp 475–479) focuses on how cleavage planes are regulated in plants and the importance of this regulation to pattern formation. Typically, cell divisions follow a tightly controlled pattern during plant development with respect to both orientation and number of cleavages. The control of cleavage plane has therefore been interpreted as a central mechanism for plant development. Consistent with this idea, various mutants that profoundly disrupt development of distinct tissues — for example *clavata1* and *shootmeristemless* — also interfere with the normal pattern of cell divisions in those tissues. Nonetheless other mutations or experimental manipulations drastically alter cell divisions with little effect on final structure. Meyerowitz concludes that communication between cells can, in some instances, override cell division defects. The situation bears some similarity to *C. elegans* development, where cleavage planes are dramatically orchestrated, a fact that drove the initial focus of research in nematode development. The past decade, however, has shown that cell interactions are instrumental to nearly every aspect of *C. elegans* development. Is *C. elegans* straddling the fence between plant and animal development? Or are plants more similar to animals than once thought? Stay tuned over the next few years — the rapid rate of progress in plants will soon answer these fundamental questions.

The developmental switch from vegetative growth to flowering can be considered the plant equivalent of puberty in animals. Amasino (pp 480–487) reviews evidence addressing mechanisms by which this profound change is controlled in plants, with an emphasis on genetic evidence from *Arabidopsis*. During normal development in many plants, flowers are induced by the combined effect of cold (vernalization) and day-length. Mutants have now been found that dramatically alter the time of flowering, with the most dramatic defects leading to flowers during embryogenesis. Such mutants can be likened to the heterochronic mutants of *C. elegans*. Regulatory genes and pathways are emerging and cloned genes are being expressed in various plant species to alter flowering drastically. Not only are the fundamental controls becoming understood but also the power of manipulating those controls for agricultural or horticultural purposes is becoming accessible.

Correct development depends on making sure that genes are expressed in the right cells, at the right time, and at the right level. Two articles address these problems and emphasize the role of chromatin structure in gene regulation. Martin, Fiering and Groudine (pp 488–495) consider regulation of β-globin gene expression, and conclude that the locus control region (LCR), located 6–22 kb upstream of the human globin gene cluster, functions predominantly to maintain the globin locus in a state permissive for transcription, whereas elements closer to the genes control developmental switching. Bashaw and Baker (pp 496–501) then discuss dosage compensation in *Drosophila*, the phenomenon by which the single X chromosome in the male is transcribed at an increased rate. Four genes (three *msl* genes and *mle*) are required for this hypertranscription. The MSL proteins bind, probably in a complex, to sites along the male X chromosome. These sites tend to be associated with a form of histone H4 that is acetylated at lysine 16, suggesting that MSL function and enhanced transcription is linked to regulation of chromatin structure. An important question, of course, is how MSL function is restricted to males and recent work indicates that this is under a post-transcriptional control by SXL. Although the mechanism is not yet understood, potential SXL binding sites in the 5′ and 3′ UTRs appear to be required for the sex-specific expression of MSL-2. An intriguing possibility is that SXL may regulate RNA at several levels, including the now classic regulation of alternative splicing as well as effects on RNA export or translation.

Finally, Palopoli and Patel (pp 502–508) review current thinking about how similar molecular components might have evolved to regulate the development of distinct structures. The theme that underlies their article — and indeed this entire issue of *Current Opinion in Genetics & Development* — is the similarity between the molecular mechanisms controlling pattern formation in invertebrates and vertebrates. The same regulatory proteins and regulatory pathways have been used repeatedly throughout evolution. Sometimes these proteins are used for essentially the same process (e.g. *short gastrulation* and *chordin* both control dorso-ventral patterning) and at other times they are used for distinct biological events (e.g. the *ras* pathway controls mesoderm formation in *Xenopus*, vulval development in *C. elegans*, and photoreceptor

differentiation in *Drosophila*). Palopoli and Patel argue that it will be difficult to understand this problem by thinking about large numbers of small changes in gene function and expression, each of which individually might have only a small effect on phenotype. Rather, it may be more fruitful to consider adaptation involving changes in a small number of genes which have a large effect on phenotype. Palopoli and Patel provide persuasive evidence for this view, a view that should encourage further investigation of the molecular details of the evolution of development.