REVIEW ARTICLE

Segmentation: Painting Stripes From Flies to Vertebrates

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INTRODUCTION

This issue of Developmental Genetics focuses on the topic of segmentation. Included are discussions of this process in a wide range of organisms, spanning the evolutionary ladder from insects to fish, birds, and mammals. A definition of segmentation is worth considering. Bateson (1984) defined segmentation as a repetition of pattern elements along the major body axis. In insects, this segmental pattern is distinct and clearly visible in the external structures of both the larva and adult. The role of segmentation in establishing the vertebrate body plan, which has been more controversial, is discussed here and in three other articles in this issue.

How are segments established in Drosophila?

The process of segmentation is best understood for the fruit fly, Drosophila melanogaster, largely as a result of the work of Christiane Nüsslein-Volhard, Eric Weischaus, and co-workers [1980, 1984, 1985, 1994] as well as Lewis [1978, 1994], who pioneered studies of Drosophila homeotic genes. The basic segmented body plan of Drosophila is specified by positional information laid down in the early embryo by an interacting group of regulatory genes [for review, Akam, 1987; Gergen et al., 1986; Ingham and Martinez-Arias, 1992; Lawrence, 1992; Scott and O’Farrell, 1986; St. Johnston and Nüsslein-Volhard, 1992]. Many of these genes were identified in massive genetic screens designed to identify lethal mutations that alter the pattern of the larval cuticle [Nüsslein-Volhard et al., 1985; Nüsslein-Volhard and Wieschaus, 1980]. The results of these screens suggested a model whereby the embryo is subdivided into increasingly specified body regions by the sequential action of regulatory genes. Maternally active coordinate genes specify the polarity of the egg, whereas zygotically active gap, pair-rule, and segment polarity genes are required for the development of the segmented body plan of the animal [Nüsslein-Volhard and Wieschaus, 1980]. The homeotic genes convert equivalent segmental units into ones having unique identities [Lewis, 1978, 1994]. These regulatory genes chart out pathways of embryonic development, but are not themselves directly involved in differentiation [García-Bellido, 1975].

In Drosophila, the division of the anterior-posterior axis into repeated metameric units is controlled by the pair-rule genes [urgens et al., 1984; Nüsslein-Volhard et al., 1984, 1985; Nüsslein-Volhard and Wieschaus, 1980; Wieschaus et al., 1984]. Mutations in the pair-rule genes result in lethality accompanied by deletions of alternating regions of the body. The periodic deletions caused by the eight different pair-rule genes are out of frame with each other. Together, these genes act to direct the development and differentiation of segmental units (parasegments) [Martinez-Arias and Lawrence, 1985] of the larva and adult. How do these genes specify embryonic pattern? One due to their function comes from the fact that these genes are expressed in spatially and temporally restricted patterns in the embryo. For the most part, these restricted expression patterns correlate well with the domains of action of these genes as reflected by their mutant phenotypes. For example, the pair-rule gene fushi tarazu (ftz) is expressed in a striped pattern in the primordia of the even-numbered parasegments that are missing in ftz mutant embryos (Fig. 1) [Hafen et al., 1984]. In the absence of ftz function, these regions of the embryo fail to develop, whereas the remaining regions develop autonomously, resulting in approximately half-size embryos that fail to hatch as larvae (see Fig. 2). Mis-expression studies have highlighted the importance of the restricted, striped expression domains of the pair-rule genes. Expression outside of their striped domains results in lethality, accompanied by distinct and specific cuticular defects [Struhl, 1985]. Thus two broad questions must be addressed to understand the function of the pair-rule genes: (1) how are their periodic striped patterns established, and (2) once correctly positioned, how do these gene products provide the information to direct the growth and differentiation of body segments that form the basis of the three-dimensional body plan of the fully developed organism?

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PAIR-RULE STRIPES ARE FIRST INDICATION OF PERIODICITY IN THE EMBRYO

Genetic studies demonstrated that embryonic regulatory genes act hierarchically to establish the Drosophila body plan. The wide-type function of genes acting earlier in the hierarchy is necessary for the correct expression of downstream genes. That is, maternal coordinate gene function is necessary for correct expression of the gap genes, which are in turn required for correct pair-rule expression, and so on [see e.g., (Carroll and Scott, 1986; Frasch et al., 1988; Gutjahr et al., 1993; Harding et al., 1989; Howard and Ingham, 1986; Ingham et al., 1988; Ingham and Martinez-Arias, 1986; Martinez-Arias et al., 1987)]. Since the pair-rule genes are the first genes to be expressed in a repeated pattern, it is thought that they have a mechanism to "interpret" nonperiodic information laid down by maternal and gap genes, which they "convert" into periodic information provided by their striped patterns. For some pair-rule genes, this "interpretation" is done by cis-acting regulatory elements that integrate information provided by graded expression patterns of maternal and/or gap gene products to generate a single stripe [Hartman et al., 1994; Howard et al., 1988; Howard and Struhl, 1990; Langeland et al., 1994; LaRosee et al., 1997; Pankratz and J. ackle, 1990; Pankratz et al., 1990; Riddihough and Ish-Horowicz, 1991; Small et al., 1991, 1992, 1993]. Detailed analysis of one of these regulatory elements—the eve stripe 2 element—showed that this stripe is formed by input from maternal and gap genes: transcription of stripe 2 is activated by bicoid and hunchback in the anterior region of the embryo and repressed by the gap gene giant and Kruppel at the anterior and posterior borders to form a sharp stripe [Small et al., 1992]. DNA binding, transcription studies, and mutagenesis experiments in vivo have demonstrated that these effects are direct: the eve stripe 2 element contains binding sites for each of these gene products that mediate regulation. These studies set a precedent by demonstrating that aperiodic information from maternal and gap genes can be interpreted directly by a pair-rule regulatory element to form a stripe in the embryo. However, even for eve, and also the pair-rule gene hairy, which has modular, single stripe regulatory elements, the situation is not so simple. Although an eve stripe 3 element has been identified [Small et al., 1993, 1996], single stripe elements have not been found for all of the eve stripes. Therefore, it is not clear yet whether the elegant eve stripe 2 story is the rule and has taught us how generic stripes are made in the embryo, or whether this story is the exception among more complex regulatory modes.

The situation for other pair-rule genes such as ftz and paired (prd) is more complicated [Gutjahr et al., 1993,
Other pair-rule genes affect striped patterns at the level of refinement and maintenance only [Ingham and Gergen, 1988; Gutjahr et al., 1993; Klingler and Gergen, 1993; Yu and Pick, 1995]. The situation for the pair-rule gene runt appears to be a hybrid between the two scenarios described above: both seven stripe elements and single stripe elements are necessary to reconstruct wild-type levels of correctly positioned runt stripes [Butler et al., 1992; Klingler et al., 1996]. Together, studies of these regulatory elements suggest that the seven stripe elements that establish ftz, prd, and runt stripes use a novel mechanism for generating periodicity that remains to be elucidated.

In sum, a general concept has emerged to explain how periodicity can be established in the embryo through the refinement of hierarchical information provided by a network of regulatory genes. However, despite intensive investigation in a number of laboratories, it is not yet possible to provide a mechanistic explanation of a complete striped pattern for any single pair-rule gene. Clearly, much remains to be learned about how periodicity is generated, even in Drosophila.

Reinitz and Sharp [1995] and Mjolsness et al. [1991] are taking a novel approach to address the issue of stripe formation, using a combination of computer modeling and “bench” experiments. This approach attempts both to quantitate and integrate the huge body of experimental data obtained from analyzing the evolution of gene expression patterns in wild-type and mutant embryos. Here, Reinitz et al. [1998] present a provocative paper which suggests that combinations of gap gene input can direct the formation of only one set of stripes in the embryo, and these stripes correspond to the domains of expression of even-skipped. This conclusion is intriguing in light of the open questions about various modes of stripe formation for different pair-rule genes, described above. The future challenge for the “gene circuit model” will be to form testable predictions about how these other sets of stripes are established in the embryo.

DOWNSTREAM FUNCTIONS OF PAIR-RULE GENES

Getting the pair-rule genes expressed in stripes is not enough: Once expressed in their proper position, the products of these genes must do something to promote the development and differentiation of segments. We know surprisingly little about this aspect of pair-rule gene function and even less about the cell biology required for segment differentiation and assembly. When the pair-rule genes were cloned in the late 1980s, they were found to encode DNA binding transcription
factors, as were many of the other genes isolated in the "Nüsslein-Volhard screen." It seemed straightforward, therefore, to presume that these genes would directly regulate the expression of downstream target genes—the "realizator genes" of García-Bellido [1975]—that do the job of making segments. However, most of the target genes identified thus far are other regulatory genes in the hierarchy and often the clearest targets are autoregulatory. Clearly, a cascade of transcription factors is not enough to build an embryo.

The identification of target genes for many of these proteins has been hampered by the fact that they encode DNA binding proteins that are members of larger families and that significant DNA binding often requires unknown protein cofactors. A clear example of this are homeodomain proteins: DNA binding specificity is relatively low and the binding sites for different family members are overlapping [reviewed in Mann, 1995]. For proteins such as Runt, bHLH proteins such as Hairy, and even homeodomain proteins such as Ftz, physiologically significant DNA binding seems to require partner proteins that are only now being identified [Fisher et al., 1996; Florence et al., 1997; Golling et al., 1996; Guichet et al., 1997; Ohsako et al., 1994; Yu et al., 1997]. This has precluded the use of standard in vitro DNA binding assays for identifying targets, even for these well-characterized pair-rule gene products. Thus the identification of direct target genes has required more labor-intensive combinations of molecular, biochemical, and genetic approaches [see, e.g., Schier and Gehring, 1992].

What is the general wild-type function of the pair-rule genes? Two views have been proposed that are best summarized by the title of a review by Peter Lawrence [1987]: "Pair-rule genes: Do they paint stripes or draw lines?" The "painting stripes" view is a combinatorial one: the interdigitating/overlapping expression patterns of different pair-rule genes generates a repeated code along the anterior-posterior axis. Combinations of different pair-rule products in different cells within this repeated unit result in assignment of particular cell fates [Gergen et al., 1986; Scott and O'Farrell, 1986]. The "painting stripes" view proposes that the function of at least two pair-rule genes (eve and ftz) is to draw a line that marks the anterior parasegment border. This border would form a boundary between neighboring developmental fields, and each parasegment would be defined from this border, possibly by a gradient of a diffusible morphogen within each parasegmental zone [Lawrence, 1987]. Support for the latter view comes from the finding that Ftz and Eve stripes have sharp anterior borders that overlap precisely with the anterior borders of engrailed expression [Lawrence et al., 1987]. Engrailed (en) is a segment polarity gene expressed in 14 stripes in the embryo that define the posterior compartment of each segment [DiNardo et al., 1985; Fjose et al., 1985; Kornberg, 1981; Kornberg et al., 1985; Lawrence and Morata, 1976]. Ftz and Eve are each responsible for the establishment of alternating en stripes [see Fig. 3; DiNardo and O'Farrell, 1987; Howard and Ingham, 1986], and, for Ftz, there is strong evidence that this regulation is direct [Florence et al., 1997].

A short communication in this issue [Lawrence and Pick, 1998] tests the hypothesis that graded expression of Ftz itself within a parasegment defines the parasegment border. According to this model, en is expressed only in response to high concentrations of Ftz and, therefore, only at the anterior border of the Ftz stripe. Two experimental observations presented here clearly rule out this model. First, the authors show that Ftz itself is not expressed in a gradient within each stripe. Earlier presumptions that this is the case were based upon analysis of β-galactosidase protein expressed from ftz/ lacZ constructs. In this case, it is the perdurance of the protein in late embryos that creates the appearance of a gradient. Second, varying the dosage of Ftz in the embryo did not affect en expression, as would have been expected for the gradient model. This rules out the possibility that a gradient of Ftz defines the en border, but leaves open the question of what the wildtypefunction of Ftz is.

One extreme view emerging from studies of pair-rule regulation of en, described above, is that the sole function of Ftz and Eve in the embryo is to regulate en. The "business end" of making segments would then be in the hands of the segment polarity genes. This view ignores other obvious candidate direct targets of these genes such as the homeotic genes [Ingham and Martinez-Arias, 1986]. However, the possibility that the pair-rule genes regulate only other regulatory genes must still be considered.

At the other end of the spectrum is the more recent proposal that Ftz and Eve regulate virtually every gene that is expressed in the blastoderm embryo (Z. Liang and M.D. Biggin, unpub. data). Support for this view is based in part upon in vivo UV crosslinking experiments that suggest that Ftz and Eve bind DNA as promiscuously in embryos as they do in the test tube [Walter et al., 1994]. Distinguishing between these views, or finding a compromise between them, will require a better understanding of how pair-rule proteins regulate transcription in the embryo.

Two papers in this issue address this question by examining regulation of target genes by the pair-rule proteins Runt and Prd. Tsai and Gergen [1994] showed previously that ectopic expression of Runt protein leads to repression of the head gap gene orthodenticle (otd). Here, they show that otd is also a target of Runt in wild-type embryos since otd expression is ectopically activated in the posterior of runt mutants [Tsai et al., 1998]. Whether or not otd is a direct target of Runt remains to be determined; however, Tsai et al. [1998] show here that repression of otd by Runt does not require the conserved VWRPY motif necessary for interaction with the co-repressor Groucho [Paroush et al., 1994]. Interaction with Groucho was previously shown to be necessary for Runt repression of specific eve
and hairy stripes, but not for repression of en stripes [Aronson et al., 1997]. Thus, Runt appears to have at least two independent molecular modes of repression in the embryo by which it regulates genes at three levels of the hierarchy: gap, pair-rule, and segment polarity segmentation genes.

Unlike other pair-rule genes, the Prd protein contains two DNA binding domains—the Pdr domain and the homeodomain [Frigerio et al., 1986; Kilcherr et al., 1986; Treisman et al., 1991; Wilson et al., 1995; Xu et al., 1995]. Both of these binding domains are essential for Pdr function in vivo and are necessary for activation of gooseberry (gsb, a segment polarity gene that also contains a Pdr domain) [Baumgartner et al., 1987; Gutjahr et al., 1993; en and eve, three candidate direct targets of Pdr [Bertuccioli et al., 1996; Li et al., 1993; Li and Noll, 1996; Miskiewicz et al., 1996]. The fact that Pdr protein itself contains two DNA binding domains suggested that unlike other homeodomain proteins discussed above, Pdr may have intrinsic DNA binding specificity and selectivity that override the requirement for interaction with other cofactors. This idea was supported by the finding that the two domains bind cooperatively to an optimized Pdr binding site in vitro [Jun and Desplan, 1996]. These observations raised the question of whether all Pdr targets contain binding sites for both of these domains and whether cooperative binding to these sites is what allows for selective regulation by Pdr of downstream genes in the embryo [Miskiewicz et al., 1996]. Direct activation of eve by Pdr through a target element that contains both Pdr domain and homeodomain binding sites [Fujikura et al., 1996] has provided a model for Lan et al. [1998] to study these questions in the embryo. Using a combination of in vivo assays in transgenic embryos and transcription assays in cell culture, Lan et al. [1998] have designed a system to study the relationship between the two Pdr DNA binding domains and their relative roles in regulating expression of downstream genes. Alterations in binding specificity as well as in spacing abrogated eve activation in the embryo, whereas other targets were less severely affected. Interestingly, even for eve regulation, in vitro optimized binding sites for a single binding domain could replace the composite site. These results suggest that high affinity binding of Pdr to DNA determines target site selection in vivo and that different Pdr-responsive regulatory elements may utilize each DNA binding domain to different extents. Thus, Pdr may indeed require additional cofactors for selection of some downstream targets.

SEGMENTATION IN OTHER INSECTS

Drosophila is a long germ band insect: the entire germ band and segmental pattern of the body plan is established simultaneously [Sander, 1976]. Segmentation is established more or less synchronously along the anterior-posterior body axis by the time cellularization is completed in the blastoderm embryo [Chan and Gehring, 1971]. Thus the pair-rule genes, which establish segments, are expressed in their characteristic seven stripe patterns at this time and the 14 stripes of En arise more or less en masse in response to this uniform expression of pair-rule genes. In contrast, segments arise sequentially in the short and intermediate germ band insects, as development proceeds. Studies of the molecular basis of segmentation in these insects has, therefore, been of interest, since morphology would suggest mechanisms that are principally different from those operating in Drosophila.

Homologues of Drosophila segmentation genes have been studied in the extreme short germ band grasshopper, Schistocerca americana [Dawes et al., 1994; Patel et al., 1992] and in short, intermediate, and long germ band beetles [Patel et al., 1994]. Gap gene homologues also have been identified [Sommer and Tautz, 1993]. Tribolium homologues of Drosophila hairy [Sommer and Tautz, 1993], ftz [Brown et al., 1994], and eve [Brown et al., 1997; Patel et al., 1994], and runt [cited in Brown and Denell, 1996] have been identified and all are expressed in striped patterns in this short germ band insect. In keeping with the sequential addition of segments to the body plan, stripes for these pair-rule genes arise one-by-one during the growth phase, suggesting that segments are successively specified as development proceeds [reviewed in Patel, 1994]. Furthermore, engrailed homologues are expressed in stripes that mark the posterior segment compartment in a wide range of insects [Patel et al., 1989], reviewed in Patel [1994]. These observations suggest that basic mechanisms directing segment establishment are conserved in different types of insects. However, it should be noted that the although the Tribolium homeotic complex (HOM-C) does contain a ftz homologue that is expressed in stripes, a deletion within the complex does not generate an obvious pair-rule phenotype [Brown et al., 1994; Stuart et al., 1991]. This may suggest different functions of the pair-rule genes in different insects, a higher degree of redundancy in Tribolium than in Drosophila, or a specific feature of this HOM-C deletion that obscures identification of pair-rule function.

Sulston and Anderson [1996] carried out a genetic screen in Tribolium to identify genes responsible for segmentation in this insect without the bias created from reliance upon Drosophila segmentation genes for their identification. From a screen for cuticular defects, five mutants were identified that caused defects in 3

3There has been some discussion of whether Tribolium should be classified as a short or intermediate germ band insect; see Patel et al. [1994]; Sommer and Tautz [1993]; Sulston and Anderson [1996] for various points of view.
segmentation reminiscent of what is seen in Drosophila. In this issue, these authors have analyzed the expression patterns of Tribolium segmentation genes eve and en in some of these mutants [Sulston and Anderson, 1998]. Both similarities and differences between Drosophila and Tribolium segmentation are highlighted here. Future analysis of these mutant phenotypes and molecular dissection of Tribolium segmentation genes will elucidate the molecular basis of segmentation in Tribolium and allow for further comparisons to the Drosophila system.

**SEGMENTATION IN VERTEBRATES**

The role of segmentation in vertebrate development has been much more controversial. The most obvious segmented structures in vertebrates are the somites [reviewed in Gossler and de Angelis, 1998], as well as the rhombomeres of the hindbrain. Somites are metameric divisions of the paraxial mesoderm that give rise to the major segmented structures of the vertebrate body: the axial skeleton, skeletal musculature, and dermis. Somites also impose segmentation on the peripheral nervous system, which is derived from neural crest [Christ and Ordahl, 1995; Keynes and Stern, 1988; Psychoyos and Stern, 1996]. Somites form in vertebrates from the presomitic mesoderm (PSM) in an anterior-posterior axis. The timing of each wave of somitogenesis occurs sequentially, only two to three her1 stripes are observed at any one point in time: her1 stripes arise in the emerging posterior PSM as more anterior stripes fade, before morphological evidence of somite formation is visible. This mode of expression is very similar to pair-rule striping in Tribolium.

Equally unexpected was the expression of the chick hairy homologue, c-hairy1 [Palmerin et al., 1997]. c-hairy1 is expressed in the PSM in cyclic waves along the posterior-anterior axis. The timing of each wave of c-hairy1 expression corresponds to the time required for formation of a single somite (~90 minutes in the chick). The wave emanates from the posterior tail bud region and progresses anteriorly to mark each somite before it has become morphologically distinct. Reiterated waves of expression emerge sequentially suggesting the existence of a “molecular clock” that times the periodic expression of this pair-rule gene. Thus the major control of the repeated c-hairy pattern would be temporal, in contrast to the pair-rule stripes in Drosophila, which are repeated spatially. These results suggest that pair-rule genes function to specify metameres in vertebrates, as they do in Drosophila. A further speculation is that pair-rule patterning was used in a common segmented ancestor [Kimmel, 1996]. Genetic analysis will be required to determine the function of these vertebrate genes and to identify other pair-rule-type genes that may not be structurally homologous to the known Drosophila pair-rule genes.

Large-scale genetic screens were carried out by Nusslein-Volhard’s group and others to identify mutations in the zebrafish that affect embryonic development [Driever et al., 1996; Haffter et al., 1996; Kimmel et al., 1989]. Among many mutations identified were two groups of genes involved in somite formation [van Eeden et al., 1996]. The first group (fused somites, fss-group) fails to establish anterior-posterior somite boundaries, whereas the second group (you-group mutants) establishes somites but has defects in somite patterning [van Eeden et al., 1996]. Interestingly, one of these, sonic you, encodes a homologue of Drosophila hedgehog, a segment polarity gene that is widely conserved [Schauerte et al., cited in van Eeden et al., this issue]. Here, van Eeden et al. [1998] further analyze the defects seen in these zebrafish segmentation mutants. They show that irregular somite boundaries that can form at later stages in fss mutants require the activity of the you-type genes that have apparent similarity to Drosophila segment polarity genes. They further use...
the her1 gene as a marker to analyze pair-rule patterning in these mutants: all fss-class mutants affect the expression of her1, although to varying extents. Creating double mutants for two different fss-type genes effectively abolished her1 expression. Surprisingly, no pair-rule phenotype (analogous to Drosophila) was found here. This intriguing result suggests that either pair-rule stripes play a different role in fish than in flies, or that redundancy in the vertebrate system (Nüsslein-Volhard, 1994) compensates for the loss of one set of pair-rule stripes.

Palmerin et al. (1998) examine, in this issue, the molecular basis for formation of rostro-caudal compartments in chick somites. Interestingly, genes implicated in this process in mouse and chick are homologues of the Drosophila Delta and Notch genes, which play quite different roles in establishing cell fate in the fly [reviewed in Campos-Ortega, 1995; Muskavitch, 1994]. In mouse, Delta is expressed in the presomitic mesoderm and in the posterior compartments of the somites [Bettenhausen et al., 1995]. In accord with this, loss-of-function Delta mutations cause loss of anterior-posterior polarity in the segments, similar to a Drosophila segment polarity phenotype [de Angelis et al., 1997]. Here, Palmerin et al. (1998) show that chick Delta and Notch are expressed in partially overlapping domains in the presomitic mesoderm and in the posterior compartment of the somites. In contrast to the chick hairy homologue, discussed above, these genes are not expressed in waves, consistent with different modes of regulation for genes acting at different levels of the hierarchy. In vitro culture experiments show that these expression patterns are maintained in the absence of overlying ectoderm. However, although repeated patterns of gene expression are still detectable, somite formation did not occur in the absence of ectoderm. As discussed above for Drosophila, these results highlight questions about what is required, once regulatory genes are correctly positioned in the embryo, in order to direct the differentiation and growth of three dimensionally patterned body structures in the animal.

The work by Zachgo et al. (1998) addresses later aspects of somite patterning in the mouse. The Danforth's short tail (Sd) mutation was first identified by Dunn et al. in 1940 and results in abnormalities of the axial skeleton [see also Gluecksohn-Schoenheimer, 1945; Gluecksohn-Schoenheimer, 1943]. Recent experiments have shown that Sd function is required in the notochord for maintenance of notochord integrity: breakdown of the notochord in these mutants appears to be responsible for the wide range of defects seen in the somite-derived vertebral column seen in Sd mutants [Maatman et al., 1997]. Here, Zachgo et al. (1998) show that an enhancer trap insertion that results in lac Z expression in the notochord, and a recessive lethal phenotype, has targeted the Sd locus. Their genetic analysis suggests that the original Sd mutation is a gain-of-function mutation. These observations light the importance of interactions in patterning the somites once metamization is established in vertebrates. With an enhancer trap line in hand, it will be possible to clone the Sd gene and begin to identify the molecular basis for patterning of the somites by the notochord in wild-type embryos.

CONCLUDING REMARKS

Tremendous progress has been made in recent years in understanding the process of segmentation. Most is known in Drosophila where saturation screens have identified most of the genes involved in this process. However, even in the fly, many basic mechanisms have still to be elucidated. Although it is clear that the pair-rule genes are responsible for generating periodicity in the Drosophila embryo, how they do so is not as clear as it may seem. Much remains to be learned about how repeated patterns of gene expression are established and about the downstream functions of these regulatory genes. Recent advances in studies of segmentation in other insects and in vertebrates have revealed a surprising conservation of the basic mechanisms used in the fly. Many of the genes involved are the same, and, most surprisingly, pair-rule stripes are found in higher vertebrates. Substantially more of the regulatory hierarchy appears to be conserved than was thought just a few years ago. How far will this functional conservation go? The stripes of pair-rule homologues suggest functional similarities across phyla, but this has yet to be tested. Given the conservation of genes at all levels of the embryonic regulatory hierarchy, a major goal in the coming years will be to describe the pathways that define evolutionary divergence.

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