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Commentary

Evolution of insect patterning

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Insect evolution, which spans several hundred million years, has generated the one million or so species of insects that inhabit the earth today, making the class Insecta the largest class in the animal kingdom (1). The insects display a staggering variety of adult morphologies and occupy an enormous range of ecological niches, but despite their diversity, they all share a remarkably well conserved stage of embryonic development. This constrained, or "phylotypic," point in insect development is known as the germ-band stage and is characterized by the overt segmentation of the body and the specification of defined head, thorax, and abdomen regions in the developing embryo (2).

From extensive genetic and molecular studies, we now have a detailed description of the events that lead up to the establishment of the germ-band stage embryo of the Dipteran *Drosophila melanogaster*, the basic outline of which is certainly familiar to most developmental biologists (for review, see refs. 3–5). Polarity is first established during oogenesis. After fertilization, maternally generated gradients emanating from the anterior and posterior poles of the egg initiate the regional expression of zygotic gap genes during the syncytial stages of embryogenesis. Just before cellularization, the gap genes establish the periodic patterns of pair-rule gene expression that are the first signs of metamerism in the embryo. As cellularization begins, the pair-rule genes initiate segment polarity gene expression patterns that in turn are responsible for maintaining segmentally repeated boundaries and generating even finer intrasegmental patterns after gastrulation is completed.

Thus, the *Drosophila* embryo is rapidly subdivided into progressively smaller units, leading to the nearly simultaneous establishment of segmental pattern throughout the embryo. In a largely concurrent process, the segmental units of the embryo are given regional identities by the homeotic genes, which are expressed in precise zones in response to gap and pair-rule gene inputs. The combined expression of the segment polarity and homeotic genes at the germ-band stage leads to the morphologically visible pattern of segments and regional specification characteristic of the germ-band stage of *Drosophila*.

Given the evolutionary conservation of the germ-band stage, one might expect that the homeotic and segment polarity gene expression patterns seen in *Drosophila* would be well conserved in all insect embryos, and studies over the last several years have generally borne out this prediction. For example, the segment polarity gene engrailed is expressed in the posterior portion of each segment at the germ-band stage in all insects examined (6–8). The conservation of this pattern is even seen in the crustacea, an arthropod group outside of the insects (6). Homeotic gene expression patterns are also generally well conserved at the germ-band stage, although some differences in later aspects of the homeotic patterns are seen that may be responsible for some of the variations seen in the morphology of structures that develop later in embryogenesis (see, for example, refs. 9 and 10).

It is also tempting to speculate that the genetic hierarchy of maternal, gap, and pair-rule genes that generates the homeotic and segment polarity expression patterns of *Drosophila* might also be at work in all other insects. However, there are reasons to believe that at least some aspects of the early *Drosophila* paradigm do not hold for all insects. Despite the conservation of the germ-band stage, differences in development leading up to this stage are seen in a variety of insects. Morphological differences are sometimes apparent, and even more striking are differences in the responses of different insect embryos to experimental perturbations. For example, ligation and cytoplasmic displacement experiments provide clear evidence for both posterior and anterior gradients in several Dipterans, including *Drosophila*. In damselflies and crickets, however, similar experiments have revealed a potential posterior gradient but provide no evidence for a gradient from the anterior pole of the egg (for review, see ref. 2).

In addition, UV-irradiation experiments have shown that the relative timing of segment specification along the axis of the developing embryo differs in various insects. Embryos such as those of *Drosophila*, which have established a complete body plan by the onset of gastrulation, are termed long-germ embryos. Embryos such as those of the grasshopper, which generate all body segments during a post-blastoderm growth phase, are termed short-germ embryos. Embryos whose segments are established as far posterior as the thorax or anterior abdomen at the blastoderm stage and that form the remaining more posterior segments after gastrulation are termed intermediate-germ embryos. In at least some short-germ embryos, the postblastoderm growth phase appears to involve a subterminal zone of proliferative cells, which would suggest that segmental pattern is being generated in a cellular environment rather than the syncytial environment that is typical of *Drosophila* (for review, see ref. 2).

These differences in germ type are mirrored by differences in the temporal generation of the engrailed stripes and the timing of homeotic gene expression (6–10). In *Drosophila*, all of the engrailed stripes appear more or less simultaneously at the onset of gastrulation (there is a slight anterior-to-posterior gradient). In grasshopper, in contrast, engrailed stripes appear well after gastrulation has started. When anterior stripes, such as those in the thorax, appear, the posterior region of the embryo is still forming via cell proliferation. When the more posterior stripes of the abdomen appear, extensive differentiation is already underway in the more anterior regions of the embryo.

Do these apparent differences in early development reflect real differences in the molecular mechanisms used by various insect embryos to reach the germ-band stage? One way to approach this question would be to carry out saturation mutagenesis screens for genes affecting embryonic pattern formation in a wide variety of insects. By comparing the phenotypes seen and the genes identified in such screens, it would be possible to make very detailed comparisons between pattern formation mechanisms in various insects. Mutational analysis, however, is technically practical in only a small number of insect species. An alternative approach, one that will yield less data but is much more rapid, is to isolate homologs of *Drosophila* segmentation genes in a variety of insects and to infer the potential developmental roles of these homologs by examining their expression patterns during embryogenesis. If early patterning mechanisms are conserved among all insects, then homologs of *Dro-*

sophila maternal, gap, and pair-rule genes should show similar patterns of expression in all insects. (The mere fact that homologs are present in all insects does not in and of itself argue that the early patterning mechanisms are conserved, because nearly all *Drosophila* segmentation genes serve multiple additional functions later in development.)

Over the past several years, this approach of examining the expression of segmentation gene homologs has shown that there may be variations in patterning mechanisms but that germ type designations do not necessarily predict the molecular mechanisms used to generate the segmental pattern at the germ-band stage. For example, homologs of the *Drosophila* pair-rule genes hairy and even-skipped are expressed in pair-rule patterns during *Tribolium* (flour beetle) development, although, consistent with the designation of *Tribolium* as a short-germ insect, these pair-rule stripes appear sequentially during development rather than simultaneously, as in *Drosophila* (11, 12). Homologs of the *Drosophila* pair-rule genes fushi tarazu and even-skipped, however, do not show any discernible pair-rule patterns of expression during the development of short-germ grasshopper embryos (13, 14). Thus, the process by which grasshopper embryos reach the conserved germ-band stage may be different from that used by *Drosophila* and *Tribolium*. *Drosophila* and *Tribolium*, however, may use the same mechanism despite their differences in germ type.

It therefore seems that, as Sander *et al.* (15) suggested, germ type designations, although they are a useful reminder of insect diversity, are not informative enough to predict the mechanistic and evolutionary origins of insect pattern formation. To understand the evolution of insect pattern formation, we need to gather extensive data on segmentation gene expression from a number of insect species and analyze the findings not just in the context of germ type designations but also in the context of the extensive information available about insect phylogeny (1). Thus, pair-rule patterns are observed in *Tribolium* and *Drosophila*, both of which are relatively phylogenetically advanced, but not in grasshoppers, which are phylogenetically primitive. Although the data are certainly still very limited, one possible interpretation of these results is that pair-rule prepatterning evolved during the appearance of phylogenetically advanced insects and might not have been present in the common ancestor to all insects. Further data from additional insects are needed to obtain a more complete and accurate picture of the evolution of insect pattern formation. In a recent paper, Kraft and Jäckle (16) provide such additional data and make an important contribution to our understanding of the evolution of pattern formation in insects by presenting an analysis of molecular markers of segmentation in a Lepidopteran, *Manduca sexta*.

The germ type classification of the Lepidoptera has been a matter of some debate (2, 9, 17). Perturbation experiments in both primitive and derived Lepidoptera suggest that they belong to the long-germ category (18, 19). Some workers, however, have placed Lepidoptera into the category of intermediate-germ insects based on morphological observations (17). Nevertheless, there is general agreement that Lepidoptera are quite closely related phylogenetically to Diptera, certainly more closely related to Diptera than to either Coleoptera or Orthoptera (the orders to which beetles and grasshoppers, respectively, belong).

Kraft and Jäckle (16) have cloned *Manduca* homologs of the *Drosophila* gap genes hunchback and Krüppel, the pair-rule gene runt, and the segment polarity gene wingless and examined the expression patterns of these genes during *Manduca* embryogenesis. The patterns they see are very similar to the corresponding expression patterns in *Drosophila* embryos. At the early blastoderm stage, *Manduca* hunchback is expressed in a broad anterior region and *Manduca* Krüppel is present in a band in the central region of the embryo. *Manduca* runt is expressed in a series of eight pair-rule stripes before the onset of gastrulation, and segmentally reiterated stripes of *Manduca* wingless appear in an anterior-to-posterior progression as gastrulation begins. Furthermore, the *Manduca* homolog of the *Drosophila* homeotic gene abdominal-A has been characterized and, like its *Drosophila* counterpart, is expressed in the presumptive abdominal region before the onset of gastrulation (9). In addition, a caudal homolog has been isolated from another Lepidopteran, *Bombyx mori*; caudal displays a similar concentration gradient from the posterior pole in both *Drosophila* and *Bombyx*, although the gradient is reported to form somewhat later in *Bombyx* than in *Drosophila* (20).

All these results suggest strong similarities in the genetic hierarchy that generates the segmental pattern in Lepidoptera and Diptera, a view consistent with their relatively close phylogenetic relationship. Furthermore, the observation that all eight pair-rule stripes of runt expression appear during the blastoderm stage suggests that the position of all the segmental units is defined in the initial germ anlage prior to gastrulation, supporting the designation of *Manduca* as a long-germ insect. Kraft and Jäckle (16) point out that although all of the segments are molecularly specified more or less simultaneously, morphologically visible segmentation later in development may appear in an anterior-to-posterior progression as a consequence of the anterior-to-posterior progression of gastrulation. In addition, the observation that the runt stripes are evenly spaced at the blastoderm stage argues against the existence of a proliferative growth zone for generating the abdominal regions of the embryo.

Kraft and Jäckle (16) conclude by suggesting that insects with similar germ type classifications do not necessarily use identical molecular patterning mechanisms. They emphasize the possibility that criteria not included in the germ type classification scheme, such as the mode of oogenesis, may be more informative in predicting variations in the mechanisms of segmentation (21). Support for this suggestion comes from recent studies on even-skipped expression in a variety of insect embryos (12). The data now available suggest that an understanding of the evolution of insect patterning mechanisms will come only from studying a variety of species spanning the entire range of insect phylogeny.

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