Developmental Evolution: Insights from Studies of Insect Segmentation

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Rapid advances have been made in the understanding of the genetic basis of development and pattern formation in a variety of model systems. By examining the extent to which these developmental systems are conserved or altered between different organisms, insight can be gained into the evolutionary events that have generated the diversity of organisms around us. The molecular and genetic basis of early pattern formation in *Drosophila melanogaster* has been particularly well studied, and comparisons to other insects have revealed conservation of some aspects of development, as well as differences that may explain variations in early patterning events.

Even before the concept of evolution was expressed, naturalists had debated the interpretation of parallels between development and the grouping and classification of organisms. With the emergence of evolutionary theory, many focused on the relationship between the development of an organism and its evolutionary history (*1*). In *The Origin of Species*, Darwin referred to development and embryology as "one of the most important subjects in the whole round of history" (*2*). With the combination of ideas from development, evolution, and genetics, it was possible to conceptualize how the selection of heritable genetic changes in developmental programs could have generated much of the diversity of life. But now, as we begin to understand the specific genes and molecular genetic systems that control the development of organisms, we can actually examine the evolution of particular developmental processes at the level of the specific genes that control them.

Geneticists and developmental biologists have, on occasion, considered the evolutionary implications of their findings. For example, when Lewis described his analysis of the bithorax complex, a group of genes that control regional specification of the *Drosophila* body plan, he pointed out the potential importance of these findings for the understanding of the evolution of the arthropod body plan (*3*). Although some of Lewis' ideas have required modification as additional data have been collected, the seminal nature of his line of thought is clear.

Interest in the links between development and evolution have been heightened recently by the discovery that developmentally interesting genes identified in one organism often have homologs (based on sequence similarity) in a range of distantly related creatures. In many instances, this sequence similarity reflects a conservation of biochemical function. In several cases, these homologous genes serve similar developmental functions in a number of diverse organisms. Probably the best known example of this evolutionary conservation of a developmental mechanism comes from the comparison of the HOM/Hox genes, which include the bithorax complex genes described by Lewis (*4*). Originally discovered by genetic analysis of pattern formation in *Drosophila*, these genes encode a closely related family of homeodomain-containing transcription factors that determine regional identity during *Drosophila* development. Because of the conservation of the homeobox sequences of these genes and the preservation of the genomic structure of the gene complex, multiple homologs were identified in a number of organisms and subsequently shown to serve roughly similar functions during development. Thus, this set of evolutionarily conserved genes shows similarity at four levels: sequence, genomic organization, biochemical function, and developmental context. In other instances, genes with developmentally related functions have been independently identified in two widely separated model systems, and only after they were both cloned was it realized that similar genes had been characterized. For example, nematode *unc-6* and the vertebrate netrins were both characterized for their roles in the circumferential growth of axons and were only subsequently recognized to be closely related genes (*5*).

The list of apparently homologous genes that serve developmentally conserved functions in distantly related organisms grows daily. For example, the highly related *Pax-6* (*small eyes*) gene of mice and the *eyeless* gene of *Drosophila* are both required for normal eye development (*6*); the genes of the myo-D family are involved in muscle development in nematodes, *Drosophila*, and mice (*7*). These and similar findings represent a triumph of developmental biology, but to understand the evolution of animal diversity, we need to learn about the genetic basis of the variations (not just the similarities) in developmental programs. Diversity in the molecular genetic level of developmental pathways has been documented in several instances, even in cases in which superficial similarities exist. For example, genetic studies have identified the steps involved in sex determination in both *Drosophila* and nematodes. Although the general organization of the pathway is similar in these two animals, the molecular machinery that underlies the process appears quite different (*8*).

In some cases, closely related gene products in two organisms share biochemical functions but act in different developmental contexts. For example, the signal transduction system involved in *Drosophila* dorsal-ventral patterning and mammalian immune system activation rely on a similar set of proteins and biochemical pathways [Toll/IL-1 receptor, Dorsal/NF-κB, Cactus/IκB (*9*)]. Furthermore, single genes often serve multiple developmental functions in an organism. The *runt* gene of *Drosophila*, for example, functions in segmentation, neural specification, and sex determination (*10*). Assuming that these three functions did not arise simultaneously during evolution, it is possible that distantly related organisms that branched off before all three functions evolved in the lineage leading to *Drosophila* will use *runt* homologs for only one or two of these processes. Of course, independent loss of function or adoption of novel functions in other lineages is also possible.

Changes in the pattern of expression of the HOM/Hox genes may help us understand some of the changes in morphology that have occurred during animal evolution, but overall, extensive conservation is seen in the deployment of HOM/Hox genes. It has been suggested that the stage at which HOM/Hox genes establish body pattern represents a zootypic stage for animals of many different phyla (*11*). That is, whereas animal embryos from two different phyla may appear morphologically quite different at this stage, at the molecular level they have established a common ground plan, the zootype. The notion of the zootype also fits with the older idea that there is a phylotypic stage for all

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embryos within each phylum. The phylotypic stage is defined as the stage at which embryos within a phylum show the greatest level of morphological similarity (*12*). For example, chordate embryos pass through a step in development at which they have a similar arrangement of neural tube, notochord, and somites. Because the phylotypic point of development is also the stage at which HOM/Hox genes are establishing regional identities, there is significant overlap between the zootypic stage between phyla and the phylotypic stage within each phylum.

In spite of the conservation of the phylotypic and zootypic stages, developmental biologists have long recognized that the initial stages of embryonic development within each phylum are characterized by a great deal of diversity. For example, whereas human, chicken, and zebrafish embryos look similar at the phylotypic stage, their earlier development appears morphologically quite different. An important question is how this earlier diversity manifests itself at the molecular level. Given that all embryos within a phylum reach a similar phylotypic stage, it may be that all the molecular genetic steps that control pattern formation up to this conserved stage are also identical, with the differences in early morphology representing only differences in topology or in the relative timing of various developmental events. Alternatively, fundamental differences in the molecular machinery and developmental programs may parallel the diversity seen in the stages that precede the phylotypic point.

Insect Segmentation as a Model for Evolutionary Change

Several attributes make the arthropod phylum, and insects in particular, well suited for the investigation of the molecular nature of variation in early development. First, many years of investigation have yielded a detailed picture of the steps that generate anterior-posterior segmental pattern during the early embryonic development of the model insect, *Drosophila melanogaster* (*13*). Discussion here will be limited to four basic classes of mutations that affect the process of anterior-posterior segmentation (Fig. 1, A through E). The first category is maternal effect mutations that result in the deletion of large regions of the body plan; examples include *bicoid*, *nanos*, *staufen*, and *oskar*. The second is zygotic gap mutations that create gaps in the pattern of embryonic segments; examples include *Krüppel*, *hunchback*, *knirps*, and *giant*. Third is zygotic pair-rule mutations that cause the deletion of every other segmental unit; examples include *even-skipped*, *fushi tarazu*, *runt*, *hairy*, and *odd-Oz*. Fourth is zygotic and maternal segment polarity mutations that affect patterning within each segmental unit; examples include *engrailed*, *wingless*, *armadillo*, and *hedgehog*.

An analysis of these mutations, and the normal functions of the genes altered by these mutations, provides a detailed picture of the molecular basis of *Drosophila* segmen-

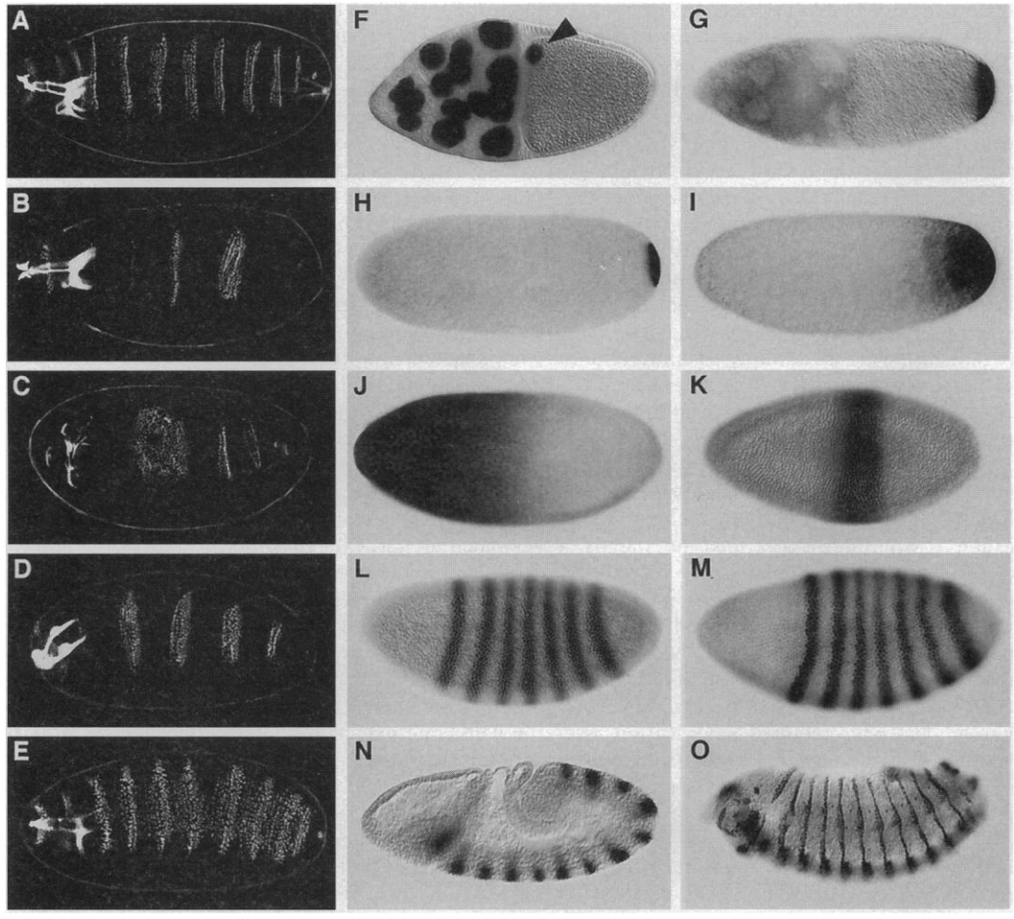

Fig. 1. *Drosophila* anterior posterior pattern formation. (A through E) Cuticle preparations illustrating different classes of mutations. (**A**) Wild-type. (**B**) Maternal class mutation *oskar* deletes most of the abdomen. (**C**) Gap class mutation *Krüppel* deletes the thorax and anterior two-thirds of the abdomen. (**D**) Pair-rule class mutation *fushi tarazu* deletes every other segment unit. (**E**) Segment polarity mutation *gooseberry* deletes the naked cuticle portion of each segment and replaces it with a mirror image duplication of the adjacent denticle belt pattern. (F and G) Steps in the generation of pattern during oogenesis and embryogenesis. (**F**) During oogenesis, incomplete cytokinesis generates a cyst of sixteen cells; fifteen form nurse cells and one becomes the oocyte (arrow points to oocyte nucleus; other stained nuclei are nurse cell nuclei). Asymmetry is already quite apparent at the morphological level in that the anterior end of the oocyte is closest to the attachment to the nurse cells and the oocyte nucleus is in an anterior dorsal position within the oocyte. (**G**) During oogenesis, several maternal transcripts become spatially localized in the oocyte, such as *oskar*, which is localized to the posterior pole. (**H**) Localization of *nanos* mRNA at the posterior end of the egg. (**I**) Diffusion of the *nanos* protein product forms a gradient from the posterior pole. (**J**) Maternal *hunchback* protein gradient forms in a syncytial blastoderm embryo as translation of uniformly distributed *hunchback* mRNA is repressed by the posterior gradient of *nanos* product. (**K**) Zygotic gap gene *Krüppel* protein expression in the blastoderm embryo. Seven-stripe protein patterns of pair-rule genes *even-skipped* (**L**) and *fushi tarazu* (**M**) in cellular blastoderm embryos. (**N**) Transcripts of the segment polarity gene *gooseberry* appear in segmental stripes by the onset of gastrulation. This embryo is beginning a morphogenetic movement known as germband extension, which will transiently cause the posterior end of the embryo to curl around toward the head. (**O**) Protein distribution of segment polarity gene *engrailed* at mid-embryogenesis. Each stripe marks the posterior portion of a segment.

tation (Fig. 1, F through O). Briefly, maternally supplied *bicoid* and *nanos* messenger RNA (mRNA) are localized at the anterior and posterior ends of the embryo, respectively, before fertilization. The protein products produced from these two mRNAs diffuse through the syncytial embryo to generate protein gradients that act to establish the large domains of gap gene expression. Interactions in the syncytial embryo among these gap genes, which encode a variety of classes of transcription factors, then generate the patterned expression of specific pair-rule genes. The expression of the pair-rule genes, whose products are almost exclusively transcription factors, is the first sign of periodic patterning in the embryo; most are expressed in patterns of seven stripes with two-segment periodicity in the cellularized blastoderm embryo. Interactions among the pair-rule genes establish the so-called parasegment domains. The parasegments have segmental periodicity but are slightly out of phase with the morphologically visible segments; these domains, rather than the morphologically obvious segmental domains, are the relevant genetic and molecular units used during early *Drosophila* pattern formation. The pair-rule genes also regulate the expression or activity of the final tier in the segmentation hierarchy, the segment polarity genes. These genes, most of which are expressed in segmental periodicity, act to maintain and refine the borders established by the pair-rule genes. Some of the segment polarity genes encode transcription factors, but the remainder are involved in intracellular communication and intercellular signal transduction.

In a process concurrent with segmentation, the expression of homeotic genes gives unique identities to the segments established by the segmentation hierarchy. Expression of homeotic genes is initiated by the gap genes, and their precise boundaries are further refined by the pair-rule and segment polarity genes. Thus, the *Drosophila* embryo is progressively subdivided into smaller and smaller units by the action of the segmentation gene hierarchy, and identities are assigned to the individual segments by the homeotic genes. This entire process occurs rapidly: Within 3 hours after fertilization, the homogeneous-looking blastoderm stage *Drosophila* embryo contains an accurate representation of the larval body plan.

After gastrulation, the *Drosophila* embryo enters the germband stage. By this time, the earlier expression of maternal, gap, and pair-rule genes has decayed. Morphological segmentation and the regional specializations of the body plan are clearly visible. Segment polarity gene expression is maintained, and the cellular signaling systems mediated by these genes continue to refine the patterns within each segment. Homeotic genes are expressed in their characteristic regional domains but their expression is also constantly refined and they continue to influence the development of region- and segment-specific structures (Fig. 2A). It is this germband stage that represents the phylotypic point for arthropods. All arthropod embryos look very similar at the morphological level during this stage, and a comparison of only insects reveals even more extensive similarities. At the germband stage, all insect embryos possess a head composed of a procephalic region plus three gnathal segments that form the mouth parts, three thoracic segments, and eight to eleven abdominal segments.

The second reason that insects are ideal for studies of the evolution of patterning mechanisms is that developmental diversity is quite obvious within this group of organisms. Differences in development after the phylotypic point lead to variations in larval and adult morphologies, but there is also diversity in the steps leading up to the phylotypic point. Some evidence for diversity in early development comes from perturbation experiments, which have been performed on a variety of insect embryos (*14*). As discussed above, genetic and molecular analyses illustrate that an essentially complete pattern of body segments is established by the end of the blastoderm stage in *Drosophila*. However, perturbations such as ligations and localized cell ablation had already indicated that this was the case for a number of insect embryos, including those of flies and bees. These embryos, in which the entire body plan is already established by the blastoderm stage, are called long-germ embryos. This mode of development, however, is not typical of all insects. In embryos such as those of grasshopper, a syncytial and cellular blastoderm is formed just as in *Drosophila*, but only a small fraction of the blastoderm, called the germ anlage, contributes to the embryo. The remaining blastoderm regions give rise to extraembryonic membranes. Experimental perturbations indicate that the germ anlage does not contain a complete representation of the body plan. Only the head region appears to be specified initially. All of the more posterior regions of the embryo are generated from a growth zone that produces the material for the rest of the embryo by cell proliferation after gastrulation. Embryos like these, in which all segments posterior to the head appear to be specified after gastrulation, are termed short germ. Between the short- and long-germ extremes are a range of intermediate-germ embryos in which the head and thorax appear to be established in the germ anlage, with the remaining abdominal regions generated later.

These differences in the timing of segment formation relative to the stage of embryonic development can be thought of as examples of heterochrony. The entire body plan of long-germ embryos is specified in the blastoderm before the start of gastrulation. By contrast, short-germ embryos complete the majority of their body plan after gastrulation. It is possible that the molecular machinery of segmentation is identical in long- and short-germ embryos but that the timing of the process is shifted relative to other morphological processes in the embryo. However, one argument against this possibility is that the *Drosophila* long-germ system as we understand it requires a syncytium for the diffusion of the maternal and gap gene products. Delay of some of these steps until after cellularization and gastrulation would seem to present obvious difficulties to the conservation of this patterning system.

An additional advantage to the use of insects for an analysis of the evolution of developmental systems is that the evolutionary relationships of most insects are well understood (*15*) (Fig. 3A). Because the fossil record is unlikely to reveal many details about insect embryonic pattern formation mechanisms, our analysis is dependent

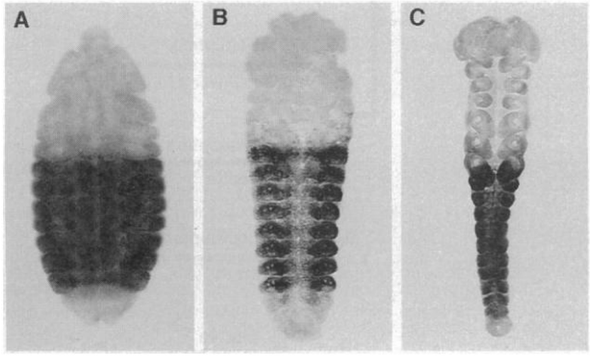

Fig. 2. Similarities in the overall body plan of insects as revealed by homeotic gene expression. In *Drosophila* (**A**), *Tribolium* (**B**), and *Schistocerca* (**C**) the Ubx/Abd-A protein expression domain [detected with an antibody that recognizes both gene products (*57*)] extends through most of the abdomen. There are differences in the position of the posterior boundary at different stages which may account for changes in the later delineation of posterior abdominal segments (*18*).

on the analysis of extant insects. Evolutionary analyses based on extant organisms are often hindered by evolutionary gaps, but the relatively low rate of extinction of insect orders suggests that these gaps will be less problematic for insects than for most other animal groups (*16*). Thus, the insects constitute a system in which an outline of evolutionary relationships is available and all embryos establish a morphologically similar body plan but in which there is reason to believe that there is diversity in the steps that lead up to this conserved stage of development.

Drosophila belongs to one of the most phylogenetically derived insect orders (Diptera); thus, although it is the most well-studied insect, it may not be representative of other insect groups, especially those that belong to more phylogenetically primitive orders. During the course of the subsequent discussion, it is important to bear in mind that the short-, intermediate-, and long-germ classification scheme is a useful reminder of the diversity of insect pattern formation mechanisms, but by itself is not sufficient to describe the evolution of insect patterning. Whereas long-germ embryos are found only in the most phylogenetically derived insects, intermediate- and short-germ embryos are scattered throughout insect phylogeny, and single-insect orders, such as Coleoptera, sometimes contain representatives of all three germ types.

To analyze the evolution of pattern formation, comparative molecular data are needed from a variety of insect embryos that span not only a range of germ types, but, more importantly, from a wide range of insect phylogeny. Because normal pattern formation in *Drosophila* requires the expression of most segmentation genes in their wild-type spatial patterns, and because the consequences of both lack of expression as well as misexpression of these segmentation genes are known, it is possible to make some predictions about the potential conservation of developmental function or lack thereof by examining the expression of particular segmentation gene homologs in other insect embryos. Comparative molecular studies have been undertaken by a number of laboratories, and extensive descriptions of the expression of multiple homologs of *Drosophila* homeotic and segmentation genes are now available for a number of insects. Below I summarize some of the data from insects outside of the Diptera to illustrate the kind of information that has been obtained from these studies. I focus particularly on those findings that may help us understand the basis for the developmental differences seen prior to the conserved germband stage.

Comparative Molecular Studies

Homeotic genes. The earliest results from comparative studies provided molecular support for the conservation of the germband stage of insect development. The constraint of the germband stage of development had led to the prediction that those genes active in the *Drosophila* germband, that is, homeotic and segment polarity genes, would also be well conserved in the germband stage of other insect embryos. This prediction has been borne out by a number of studies. The expression of several homeotic genes has been analyzed in a range of insect embryos (*17*). Just as the overall morphological body organization of all insect embryos is well conserved, the expression pattern of homeotic genes is generally well conserved in a variety of insect embryos (Fig. 2). Whereas the broad domains of homeotic gene expression are the same in various insects, the precise boundaries vary somewhat. This is especially apparent in a comparison of the domain of *abdominal-A* expression in the posterior regions of the abdomen in *Drosophila* and *Schistocerca* (Orthoptera; grasshopper) (*18*). These changes may account for the extent to which abdominal segments become overtly differentiated and visible during the development of these insects. Although no data are yet available, the variation in the

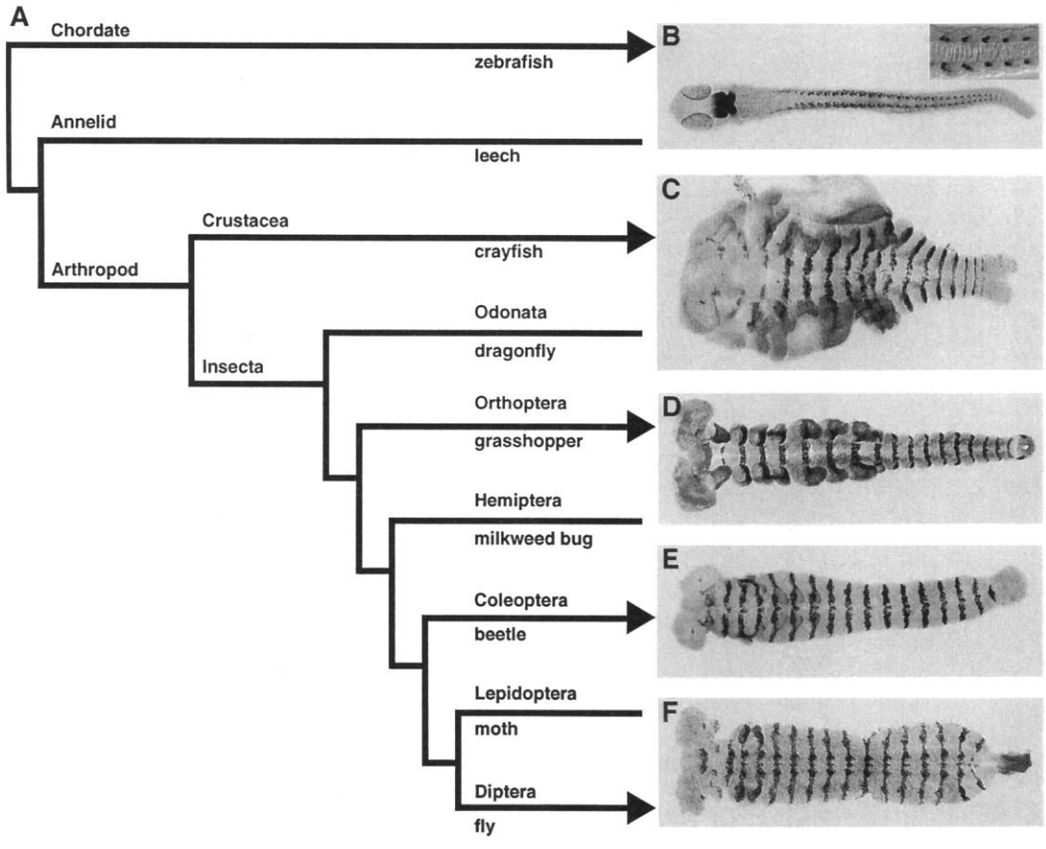

Fig. 3. (**A**) Phylogenetic tree of several insect orders and their relationship to another member of the arthropod phylum, crustacea, and the relationship of the arthropods to the chordate and annelid phyla. (**B** through **F**) The *engrailed* expression during embryogenesis. A well-conserved pattern of *engrailed* stripe expression is seen at the germband stage in (C) *Procambarus* (crayfish), (D) *Schistocerca*, (E) *Tribolium*, and (F) *Drosophila*. In the zebrafish (B), *engrailed* homologs are expressed in the midbrain-hindbrain region and in a segmentally repeated pattern in the somites (shown at higher magnification in the inset). As described in the text, the segmentally repeated *engrailed* patterns in vertebrates occur only after morphologically visible segmentation is already present.

placement of flight appendages between the Diptera and its sister order, Strepsiptera, may also be the result of modifications in the precise boundaries and levels of homeotic gene expression (*19*).

Segment polarity genes. The expression of the segment polarity gene *engrailed* has been examined in many insects (*20*) (Fig. 3). In *Drosophila*, *engrailed* is expressed in the posterior portion of each segment during the germband stage. Later, *engrailed* is still expressed in ectodermal stripes, but additional expression is found in a specific subset of neurons (*21*). These expression patterns are conserved in all of the insects examined so far and have also been seen in another group of arthropods, the crustacea (*20*) (Fig. 3C). The conservation of the precise boundaries of *engrailed* expression, as well as the conservation of the expression boundaries of several of the homeotic genes, also indicates that the parasegmental units, rather than the morphologically visible segmental units, are the fundamental units of molecular pattern formation, not only for *Drosophila*, but also for other insects and even crustaceans (*22*). In addition, homologs of the segment polarity gene *wingless* are expressed in *Tribolium* (Coleoptera; flour beetle) and *Manduca* (Lepidoptera; tobacco hawkmoth) in the same pattern as in *Drosophila* (*23*, *24*).

Whereas the expression patterns of the homeotic and segment polarity genes are well conserved at the germband stage, the initiation of gene expression differs between *Drosophila* and several other insects. In long-germ *Drosophila*, homeotic gene expression is initiated prior to the onset of gastrulation (*13*). Segment polarity genes such as *engrailed* and *wingless* show a slight anterior-to-posterior gradient of expression even though there is no strict gradient for pair-rule gene expression. All the body stripes are, nevertheless, initiated by the onset of gastrulation (*13*). In long-germ *Apis* (Hymenoptera; honeybee), the anterior-to-posterior gradient of *engrailed* expression is more obvious, but as in *Drosophila*, all of the body stripes appear by the onset of gastrulation (*25*). In long-germ *Manduca*, *wingless* expression shows a pronounced anterior-to-posterior temporal gradient, which may be linked to an anterior-to-posterior gradient in the gastrulation process in this insect (*24*).

In short-germ *Schistocerca*, however, neither *engrailed* nor any of the characterized homeotic genes is expressed before the onset of gastrulation (*20*, *17*). Instead, these genes are expressed in a distinct temporal and spatial sequence after gastrulation. For example, *Schistocerca engrailed* stripes appear first in the thoracic region and then subsequently in more anterior and posterior regions. In the posterior regions, a rapid phase of cell proliferation first generates an abdominal region and *engrailed* stripes then appear one at a time in an anterior-to-posterior progression. Similarly, *engrailed* stripes in short-germ *Tribolium* embryos also appear sequentially as the embryo elongates, although in this case the stripes begin in the gnathal region (*26*). Thus, the sequential appearance of morphologically visible segments in short-germ embryos is preceded by the sequential appearance of molecular markers of the segmentation process; compressed patterns of gene expression are not seen in the proliferative zone of the germ anlage.

Pair-rule genes. In long-germ embryos of *Manduca*, as in *Drosophila*, all of the stripes of *runt*, a pair-rule gene, appear at the blastoderm stage and are equally spaced over the body region of the embryo (*24*). In short-germ *Tribolium*, gene expression in pair-rule patterns has been seen for homologs of *Drosophila even-skipped* (*eve*), *fushi tarazu* (*ftz*), and *hairy* (*27–29*) (Fig. 4, A through D). The pair-rule patterns in *Tribolium* do not, however, appear in their entirety at the blastoderm stage. Instead, only about two pair-rule stripes of any of these genes are seen in the *Tribolium* germ anlage before gastrulation; the remaining stripes appear as the embryo elongates. In the intermediate-germ beetle *Dermestes*, four *even-skipped* pair-rule stripes appear before gastrulation, and in the long-germ beetle *Callosobruchus*, six pair-rule stripes appear before gastrulation. In both beetles, the remaining *eve* pair-rule stripes appear after gastrulation (*27*).

Although *eve* and *ftz* display pair-rule periodicities in *Tribolium*, the actual patterns are not identical to the corresponding *Drosophila* patterns (*27*, *28*). In *Tribolium*, as in *Drosophila*, the anterior margin of the peaks of *ftz* expression correspond to the position in which even-numbered *engrailed* stripes will form, but unlike *Drosophila*, *ftz* expression is also seen in the areas between forming *engrailed* stripes. This may not be a trivial difference, because when *Drosophila ftz* is forced into a *Tribolium*-like expression pattern (for example, by misexpression with a heat shock promoter) defects occur in segmentation (*30*). Furthermore, deletion of *Tribolium ftz* does not appear to cause a pair-rule defect (although the defect may be masked by the deletion of adjacent homeotic genes) (*28*). Thus, although *Tribolium ftz* may be expressed in a roughly pair-rule pattern, it may lack pair-rule function (see below).

The relationship between *eve* and *engrailed* expression starts out the same in *Drosophila* and all three beetles; the anterior margin of each *eve* pair-rule stripe predicts the location of each odd-numbered *engrailed* stripe (which corresponds to the anterior margin of each odd-numbered parasegments), and the *eve* pair-rule stripes narrow just before *engrailed* expression begins. In both *Drosophila* and *Tribolium*, *eve* expression also undergoes a transition to form segmental stripes. In *Drosophila*, weak *eve* stripes form de novo in even-numbered parasegments. In all three beetles, *eve* stripes also appear in even-numbered parasegments, but they form from the posterior edge of the initial pair-rule stripes, and the level of *eve* expression in these stripes is equal to that of *eve* stripes in odd-numbered parasegments (*27*, *28*). Again, this difference may seem trivial, but experiments in *Drosophila* suggest that variation in the levels of *eve* expression is responsible for the different regulatory roles of *eve* in even- versus odd-numbered parasegments (*31*). Thus, like the results obtained from the analysis of *Tribolium ftz*, the observations on *Tribolium eve* suggest that there are differences between the pair-rule gene networks in *Drosophila* and *Tribolium*.

Homologs of *eve* and *ftz* have also been characterized from *Schistocerca* (*32*, *33*) (Fig. 4E). Both genes are expressed in conserved patterns in the nervous systems of *Schistocerca* and *Drosophila* (*32*, *33*) (Fig. 4, F through H), but no pair-rule expression patterns have been detected in *Schistocerca*, which suggests that neither gene serves a pair-rule function in this insect. Both genes show a posterior domain of expression prior to gastrulation and during the period in which the embryo is elongating by cell proliferation. A variety of observations suggest that both genes may have had ancestral HOM/Hox functions before they adopted pair-rule functions. In the case of *ftz*, additional sequence motifs, its sharply demarcated expression boundaries in the nervous system, and its position within the homeotic clusters of *Drosophila* and *Tribolium* suggest that it could have arisen from the duplication of an adjacent Antp class homeotic gene (*28*, *33*). The finding that *eve* homologs are part of the vertebrate (and possibly even the coral) Hox complexes suggests that *eve* had an ancestral function as part of the HOM/Hox cluster before taking on pair-rule functions (see below for further discussion) (*34*, *35*).

Gap genes. Homologs of the gap genes *hunchback* and *Krüppel* have been characterized from *Manduca*, and regional patterns of expression at the blastoderm stage appear to be roughly equivalent in *Manduca* and *Drosophila* (*24*). *Krüppel* expression has also been studied in *Tribolium* (*29*). In *Drosophila*, the *Krüppel* expression domain is positioned in about the middle of the blastoderm embryo (thorax plus most anterior parts of the abdomen). In short-germ *Tribolium*, the thorax and abdomen both appear to arise from the most

posterior parts of the germ anlage; consistent with this, the *Krüppel* domain initially forms a cap at the posterior end of the *Tribolium* egg (*29*). The segment polarity gene *wingless* also has a terminal domain of expression in early *Drosophila* development, and this terminal pattern also appears to be conserved in *Tribolium* (*23*). In *Drosophila*, the *Krüppel* domain and terminal *wingless* domains do not overlap. By contrast, although the appropriate double-labeling experiments remain to be done in *Tribolium*, the available descriptions indicate that the two domains overlap in the posterior end of the blastoderm *Tribolium* embryo. Later in *Tribolium* development, however, after the embryo has begun to elongate, the *Krüppel* expression domain is restricted to the thorax and anterior abdomen (*29*). Thus, with the data currently available, it is not clear whether the posterior boundary of *Krüppel* expression in the *Tribolium* blastoderm corresponds to the same posterior boundary seen once the embryo begins to elongate. Data on gap genes expressed in more posterior regions of the abdomen would be useful in determining how complete gap gene patterning actually is at the blastoderm stage of *Tribolium* (that is, would more posterior gap genes also be expressed in the *Tribolium* blastoderm, or would their expression begin only once the embryo had started elongating).

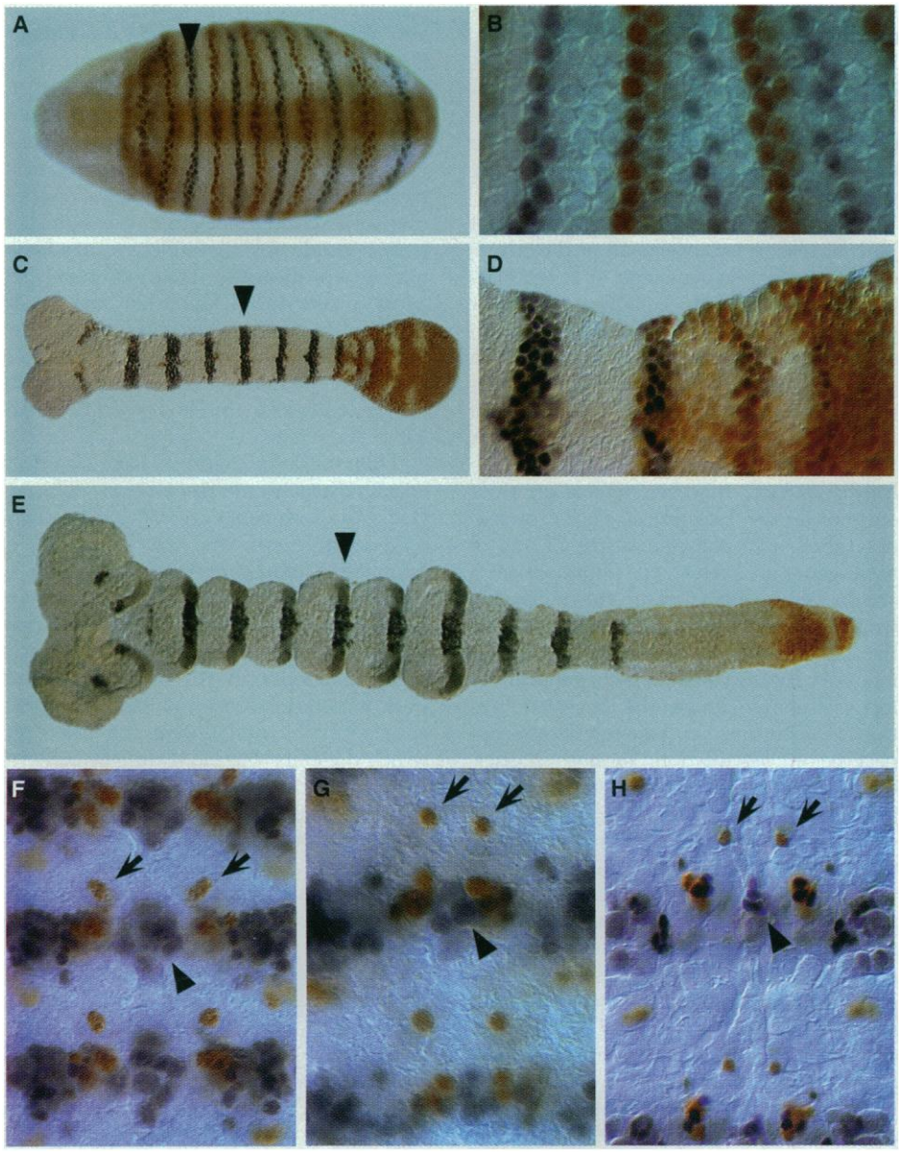

Fig. 4. Comparisons of *eve* (brown) and *engrailed* (gray) expression in various insects. In *Drosophila*, *engrailed* appears in a 14-stripe segmental pattern and *eve* is in a 7-stripe pair-rule pattern. (**A**) is the ventral view, with a higher magnification lateral view in (**B**). Odd-numbered *engrailed* stripes appear at the anterior margin of each *even-skipped* pair-rule stripe. Weak *eve* stripes at the position of even-numbered *engrailed* stripes are not visible in this preparation. In *Tribolium*, *engrailed* and *eve* stripes form sequentially, in an anterior-posterior progression, as the embryo elongates. (**C**) is the ventral view, with a higher magnification view in (**D**). The anterior margin of *eve* pair-rule stripes corresponds to the position at which odd-numbered *engrailed* stripes will appear. Secondary segmental *eve* stripes also form that mark the location at which even-numbered *engrailed* stripes will form. In *Schistocerca* (**E**) there does not appear to be any relationship between the expression of *eve* and the formation of *engrailed* stripes. As the abdominal *engrailed* stripes appear, *eve* is not expressed in any sort of stripe pattern, but is instead seen in a region at the posterior end of the embryo (the staining at the very posterior tip is the anal pad expression which is seen in all insects). Arrowheads in (A), (B), and (C) indicate the *engrailed* stripe of the first thoracic segment (stripe number four). Expression patterns of *eve* and *engrailed* are well conserved in the central nervous system of *Drosophila* (**F**), *Tribolium* (**G**), and *Schistocerca* (**H**). Arrows point to the *eve*-expressing RP2 neurons and arrowheads indicate the *engrailed*-expressing progeny of the median neuroblast.

Maternal genes. Homologs of the maternal class genes *bicoid*, *oskar*, and *nanos* have been characterized in a number of Diptera (*36*), but in no other insect orders. Data from nematodes and *Xenopus*, however, suggests that *nanos* homologs might have an evolutionarily ancient role in the establishment of axes in animal embryos (see below for further discussion) (*37*, *38*). In *Drosophila*, *caudal* is expressed both maternally and zygotically; the initially uniform maternal mRNA forms a posterior gradient prior to the blastoderm stage (*13*). A *caudal* homolog has been characterized in *Bombyx mori* (Lepidoptera; silkworm moth) and this mRNA is found in a gradient that extends from the posterior end, although the gradient is reported to form somewhat later in *Bombyx* than in *Drosophila* and it has not been determined if this mRNA is produced by maternal or zygotic transcription (*39*).

Whereas preblastoderm gradients have not been observed outside of the Diptera, the results of experimental manipulations strongly suggest that morphogenetic gradients exist early in development in many insect orders (*14*). Particularly compelling is the evidence for a gradient from the posterior pole of the egg. It remains to be seen how these gradients will be related to the Bicoid and Nanos protein gradients seen in Diptera.

Data from Groups Outside the Insects

In presenting an evolutionary picture of insect segmentation, it is also useful to present some relevant data from groups outside the insects. As discussed above, homeotic gene involvement in regional patterning seems to be present in all animals (*4*). In all crustaceans examined, the

segment polarity gene *engrailed* is expressed in segmentally repeated ectodermal stripes and in nervous system patterns very similar to those seen in *Drosophila* (*20*) (Fig. 3C). In the leech, an annelid, *engrailed* also shows metamarically repeating patterns of expression in several lineages, and it has been argued that this pattern is reminiscent of the early segmental patterns seen in *Drosophila* (*40*). Furthermore, leech *engrailed* is also expressed in a subset of neurons later in development. Vertebrate homologs of *Drosophila* segment polarity genes do not appear to play a role in the overall generation of metameric patterns, although in some instances they are involved in the differentiation of structures that are themselves segmentally reiterated. For example, *engrailed* homologs in vertebrates are expressed in the midbrain-hindbrain regions and some also show segmentally repeated patterns in the somites and spinal cord, but these repeated patterns appear well after morphologically visible segmentation (*41*) (Fig. 3B). Mutations of *engrailed* homologs in mice show various defects in neural development but not in body segmentation (*42*). The expression of pair-rule and gap gene homologs has not been examined in crustaceans or annelids. Although vertebrate homologs of the *Drosophila* pair-rule genes *runt* and *eve* have been characterized, data do not suggest that these genes have a pair-rule type patterning function in vertebrates (*43*, *44*).

Evolutionary Picture of Insect Segmentation

The challenge now is to extract an evolutionary picture from the comparative information that has been, and continues to be, collected from various insects. First, however, it is essential to appreciate the restrictions on any analysis of the data. The comparative studies summarized above are still limited to a relatively small number of genes, are biased toward insect orders relatively closely related to Diptera, and are usually based on data from only one or two species per order. Moreover, in most cases, we are attempting to assess the function of genes from just their expression patterns and have yet to directly test the developmental functions of most of these genes outside of *Drosophila*. Nevertheless, it is possible to envision some of the similarities and differences that might exist in the overall segmentation hierarchy in extant insects and to use the information to develop a picture of the evolution of the segmentation process seen in *Drosophila*. We can specifically use the data to address (i) the possible molecular basis for differences in germ type, (ii) the overall evolution of the genetic segmentation hierarchy, and (iii) the evolution of specific segmentation genes.

Molecular Basis for Variations in Germ Type

The data from comparisons of long-germ *Drosophila*, short-germ *Tribolium*, and short-germ *Schistocerca* illustrate the molecular distinctions between short- and long-germ insects. In *Drosophila*, all *engrailed* stripes form by the onset of gastrulation (*13*, *21*). In short-germ *Schistocerca* and *Tribolium*, all the stripes form more or less sequentially after the embryo has gastrulated (*20*, *26*). At the level of the pair-rule genes, however, we see that there may be significant differences in the ways that short-germ *Tribolium* and *Schistocerca* generate *engrailed* stripes. In *Tribolium*, sequentially appearing pair-rule stripes appear to establish segment polarity stripes (*27*–*29*). The intermediate-germ beetle, *Dermestes*, and long-germ beetle, *Callosobruchus*, appear to use pair-rule patterning to establish segment polarity gene expression like *Tribolium* does, but they differ in the extent to which patterning has proceeded down the length of their bodies by the start of gastrulation (*27*). In *Schistocerca*, however, no evidence for pair-rule patterning has been seen (*27*, *32*). In addition, the specific relationship between *eve* and *engrailed* stripes in long-germ *Callosobruchus* is more like that found in short-germ *Tribolium* than in long-germ *Drosophila* (*27*).

Thus, these results suggest that the germ type designation system accurately reflects the relative timing of segmentation, but not necessarily the variations that are present in the hierarchy of segmentation genes. The molecular control of segmentation in short-germ *Tribolium* is probably more closely related to that of long-germ *Drosophila* than that of short-germ *Schistocerca*; segmentation in long-germ *Callosobruchus* is probably more closely related to that in short-germ *Tribolium* than to that in long-germ *Drosophila*.

Variations in germ type may, therefore, have several possible molecular explanations depending on the insect species being examined. All beetles probably use nearly identical molecular systems for segmentation, but simple shifts in the relative timing of this molecular process and the morphological process of cellularization and gastrulation may allow beetles to span a continuum of germ types. On the other hand, short-germ *Schistocerca* may use a more divergent patterning system than that found in either Diptera or Coleoptera, and this system may simply be one that functions only after the completion of gastrulation. Whereas germ type comparisons are invaluable, a more useful way to look at potential variations in molecular mechanisms is to place the various results into a phylogenetic framework (Fig. 3).

Overall Evolution of the Segmentation Hierarchy

Evidence for gap, pair-rule, segment polarity, and homeotic gene patterning is seen in extant Diptera, Lepidoptera, and Coleoptera. Thus, it is reasonable to suggest that the common ancestor to these three orders already had evolved the basic genetic hierarchy that we know from *Drosophila*. One might say that development is essentially *Drosophila*-like in all three orders, but it is important to remember that there are significant differences. For example, the segmental *eve* pattern seen in Coleoptera is quite distinct from that seen in *Drosophila*, suggesting that the specific interactions between pair-rule genes in *Drosophila* and Coleoptera may be different (*27*, *28*).

The observations from short-germ *Tribolium* also suggest that the *Drosophila* gap patterning system can function in a cellular, as opposed to a syncytial environment. This might be possible if, as has been suggested, the cells of *Tribolium* are connected by junctions that allow the diffusion of various segmentation gene products (*29*). An alternative explanation is suggested by the structure of several of the gap genes themselves. Both *tailless* and *knirps* belong to a steroid receptor superfamily that contains additional members that are dependent on small ligand molecules (*45*). Some of the products of gap genes in *Tribolium* may act as receptors for small ligands that diffuse between cells, obviating any need for gap gene products themselves to diffuse between cells (*45*). Finally, gradients could also be established by dilution of gene products as cells proliferate and the embryo elongates.

Evidence for homeotic and segment polarity patterning steps have been found in the Orthopteran, *Schistocerca* (*20*, *17*). No evidence for pair-rule patterning has yet been found in *Schistocerca*; neither *eve* nor *ftz* homologs appear in pair-rule stripes during development (*32*, *33*). If Orthoptera does not use pair-rule patterning, then one interpretation would be that the common ancestor to Orthoptera and Diptera did not utilize pair-rule patterning and that this step in the segmentation hierarchy evolved sometime during the evolution of the more phylogenetically advanced insect orders.

There are, however, several reasons to question this interpretation. First, pair-rule prepatterning has not been completely ruled out in *Schistocerca*. Homologs of other pair-rule genes, such as *hairy* and *runt*, may be expressed in pair-rule patterns in *Schistocerca*. Recently, a novel pair-rule gene,

called *odd-Oz*, was identified from *Drosophila* (*46*). This pair-rule gene is unique because it encodes a cell surface molecule. It is intriguing to speculate that such a pair-rule gene might function in the cellular environment of *Schistocerca*. Moreover, even if pair-rule patterning is not used by *Schistocerca*, this does not necessarily mean that pair-rule patterning was not an ancestral trait; it may simply have been lost in the lineage leading to *Schistocerca*. Data from additional phylogenetically primitive insects as well as from other arthropods, such as crustacea, may help resolve this question.

If *Schistocerca* turns out not to utilize pair-rule patterning, other mechanisms might establish segment polarity stripes in this insect. Whereas some of the *Drosophila* segment polarity genes encode transcription factors, many others encode secreted molecules or cell surface receptors that function in cell-cell signaling pathways that act to maintain and refine segment polarity expression patterns. Although segment polarity gene expression is initiated by pair-rule genes in *Drosophila*, it is possible that in *Schistocerca* the segment polarity genes propagate a repeating pattern of their own expression starting from a single point of discontinuity (*20*, *47*). It should be kept in mind, however, that the expression patterns of *eve* and *Abd-B* in *Schistocerca* provide evidence that boundaries are established well ahead of *engrailed* stripe formation (*18*, *27*). In the case of *eve*, for example, a precise anterior boundary of expression is seen within the abdomen just after the onset of abdomen elongation, but well before the appearance of the first abdominal *engrailed* stripe (*27*).

Two other observations may also be relevant to thinking about how pattern formation may occur in the cellular environment of *Schistocerca*. First, the introduction of excess copies of *bicoid* into *Drosophila* results in embryos with noticeably altered patterns of segmentation gene expression (all patterns are shifted toward the posterior). The larvae that hatch out, however, are normal, suggesting that there is some later compensatory mechanism at work (*48*). The nature of this compensation is still unknown, but its mode of action may shed some light on the generation of pattern in more phylogenetically primitive insects. A second result concerns the generation of pattern in the nematode. The *glp-1* gene, which encodes a cell surface receptor closely related to Lin-12 and Notch, plays an important role in the initial generation of asymmetry, and the Glp-1 protein itself is asymmetrically distributed in 2- to 28-cell embryos (*38*). The *glp-1* mRNA, however, is initially distributed uniformly. The asymmetric protein distribution is due to sequences in the untranslated part of the mRNA that prevent translation in the more posterior cells of the embryo. The sequences in *glp-1* that are responsible for this translational control are reminiscent of the *nanos*-responsive elements present in the *Drosophila hunchback* mRNA that prevent its translation in the posterior part of the *Drosophila* embryo (*38*). Although no *nanos* homolog has yet been identified in the nematode, an intriguing possibility is that similar molecules could generate the asymmetric distribution of a transcription factor (Hunchback) in one context, and the asymmetric distribution of a cell surface receptor (Glp-1) in another context. Given that pattern formation in *Schistocerca* occurs in a largely cellular environment, it is possible that the posterior morphogenetic gradient in this insect also ultimately sets up a gradient of some cell surface receptor.

Outside the insects there is evidence for segment polarity patterning in other arthropods (crustacea) and possibly in another phylum (annelids). So far, chordates do not appear to use homologs of *Drosophila* gap, pair-rule, or segment polarity genes for generating metameric pattern (although it may turn out that they use a logically similar scheme of subdividing a region into smaller and smaller units). Taken together, these results argue that homeotic gene patterning is shared by all animal phyla but that the *Drosophila* segmentation hierarchy (at least the segment polarity part of the system) first appeared in the common ancestor to annelids and arthropods. It still cannot be ruled out, however, that parts of the *Drosophila* system of generating metameric patterning are ancestral but were lost in the lineage leading to chordates.

Evolution of Specific Genes

Although our picture of the evolution of the overall segmentation hierarchy is incomplete, we can make some observations regarding specific genes within the hierarchy. Two genes in particular, *engrailed* and *eve*, can be traced through a number of phyla. As discussed already, *engrailed* has a highly conserved expression pattern during segmentation and neurogenesis in insects and crustaceans (*20*) (Fig. 3). Both expression patterns are also found in annelids (*40*). In chordates, however, *engrailed* expression is seen during neurogenesis, but not during the initial generation of metameric pattern (*44*). Thus, although neural development in arthropods and chordates has diverged so greatly that it is not obvious how to homologize the expression patterns seen in the two phyla, a role in neurogenesis may be an ancestral function of *engrailed*. This hypothesis can be tested by examining the expression of *engrailed* homologs in other phyla such as echinoderms, molluscs, and nematodes.

In *Drosophila*, *eve* is expressed in pair-rule and segmental stripes, in a subset of neurons, in the anal pad, and in the dorsal mesoderm, which contributes to the heart plus dorsalmost muscle fibers (*49*). In *Schistocerca*, expression is seen in the same subset of neurons, in the anal pad, and in the dorsal mesoderm, but there are no pair-rule or segmental stripes (*32*) (Fig. 4). There is, however, a posterior domain of expression that moves posteriorly as the embryo elongates (*32*). In vertebrates, *eve* homologs are expressed by a subset of spinal cord neurons and also in the more posterior regions of the embryo during gastrulation (*44*). The vertebrate *eve* genes are located at the end of two of the four Hox complexes (the relative position of *Schistocerca eve* and the homeotic complex is not known) (*35*). The available data suggests that the common ancestor to both arthropods and chordates may have used *eve* for neural specification, for axial patterning, or for both. The investigation of *eve* homologs in a variety of additional phyla should help test this idea and may also reveal which of the two expression patterns is evolutionarily older.

The evolution of *ftz* homologs is also revealing. In *Drosophila*, *ftz* is involved in both pair-rule patterning and neurogenesis (*13*). Expression during neural development is well conserved in *Schistocerca* and *Tribolium* (*28*, *33*). No pair-rule patterns are seen in *Schistocerca*, but an imprecise pair-rule pattern is seen in *Tribolium* (*28*, *33*). An analysis of a deletion mutant of *Tribolium*, however, suggests that its *ftz* gene does not serve a pair-rule patterning function (*28*). Thus, *ftz* may have adopted a pair-rule pattern, but no pair-rule function, in the common ancestor to *Drosophila* and *Tribolium*. This pair-rule pattern without pair-rule function has been conserved into present-day *Tribolium*. Thus, we may actually be observing an example of a gene adopting a new function during development. Analysis of additional insect species will help test this idea.

An intriguing question is, Why are so many of the segmentation genes used in both neurogenesis and segmentation? Even in *Drosophila*, a number of genes involved in neurogenesis show pair-rule or segmental stripe expression, but analysis of mutants does not indicate a pair-rule or segment polarity patterning function (*50*). One explanation is that the nervous system provides a large reservoir of useful transcription factors already established in interactive networks. When one gene is co-opted into a new function, such as in segmentation, it pulls the expression of several other genes along with it. As long as there are no harmful results, these other genes might maintain this new expression pattern for some

time before being utilized for new developmental roles.

Looking at the evolution of individual genes also provides insight into the formation of a complex developmental event whose step-by-step evolution is difficult to imagine. For example, in *Drosophila* there are at least nine different pair-rule genes (*13*, *46*). The elimination of any one causes disastrous consequences for the embryo, yet it is hard to imagine that all nine genes came to have a pair-rule patterning function simultaneously during evolution. By studying homologs of each pair-rule gene in various insects, we may be able to reconstruct the order in which the pair-rule system was constructed. Again, understanding how *Tribolium* accomplishes pair-rule patterning without *ftz* may be particularly revealing.

General Conclusions and Future Directions

The available data suggest that variations in early development sometimes disguise well-conserved molecular systems and at other times are indicative of more fundamental changes in developmental mechanisms. Contrary to some initial assumptions, it appears that the basic *Drosophila* segmentation paradigm can function in a cellular environment such as that found in *Tribolium*. Future studies may reveal what is responsible and required for the heterochronic shift between these two particular insects. In some insect embryos, more fundamental changes in the patterning mechanism may have occurred. For example, *Schistocerca* may not employ a pair-rule prepatterning step, or at least it does not appear to use *eve* and *ftz* for this step. Further analysis of *Schistocerca* development may soon explain how segment polarity gene expression patterns are established under these situations.

It is clear that more comparative data from a variety of organisms will help answer many questions that have been raised. In addition, techniques for the disruption of gene function in organisms not easily subjected to standard genetic manipulation are needed to test many hypotheses that are currently based solely on comparisons of expression patterns. Finally, these comparative studies are unlikely to identify novel patterning mechanisms that might be at work in various insects and thus new model species must be developed that are amenable to the genetic analysis of pattern formation. Already, preliminary screens are being carried out to identify pattern formation mutants of *Tribolium* (*51*) and *Nasonia* (wasp) (*52*).

The basic approach described here to study the evolution of insect development is being applied to many other systems. As we learn more about the genetic basis of vertebrate development, for example, comparative studies may reveal many of the molecular and genetic underpinnings for the diversification of vertebrate body forms and the evolution of variation in early vertebrate development. Specific evolutionary studies are already being undertaken to analyze a wide variety of developmental processes ranging from nematode vulva formation (*53*), to ascidian larval development (*54*) to sea urchin cell lineage control (*55*). All of these approaches will provide valuable insights into how developmental processes evolve and lead to a deeper understanding of the commonalities that link all embryos as well as of the differences that account for the diversity of embryonic development (*56*).

REFERENCES AND NOTES

1. S. J. Gould, *Ontogeny and Phylogeny* (Belknap, Cambridge, 1977), pp. 69–114.
2. C. Darwin, *The Origin of Species* (John Murray, London, 1859), (Random House, New York, 1993), p. 586.
3. E. Lewis, *Nature* **276**, 565 (1978).
4. W. McGinnis and R. Krumlauf, *Cell* **68**, 283 (1992); R. Krumlauf, *ibid.* **78**, 191 (1994); C. Kenyon, *ibid.*, p. 175.
5. E. M. Hedgecock, J. G. Culotti, D. H. Hall, *Neuron* **2**, 61 (1990); T. Serafini *et al.*, *Cell* **78**, 409 (1994); C. S. Goodman, *ibid.*, p. 353.
6. R. Quiring, U. Waldorf, U. Kloter, W. J. Gehring, *Science* **265**, 785 (1994); R. E. Hill *et al.*, *Nature* **354**, 522 (1991).
7. L. Chen, M. Krause, M. Sepanski, A. Fire, *Development* **120**, 1631 (1994); A. M. Michaelson, S. M. Abmayr, M. Bate, A. Martinez Arias, T. Maniatis, *Genes Dev.* **4**, 2086 (1990); B. M. Patterson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3782 (1991); M. A. Rudniki *et al.*, *Cell* **75**, 1351 (1993).
8. J. Hodgkin, *Nature* **344**, 721 (1990).
9. R. Chasan and K. V. Anderson, in *The Development of* Drosophila melanogaster, M. Bate and A. Martinez-Arias, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1993), pp. 387–424. It should also be noted that there is evidence that at least part of this pathway may be involved in the *Drosophila* immune response [Y. T. Ip *et al.*, *Cell* **75**, 753 (1993)] and plant viral resistance [S. Whitman *et al.*, *Cell* **78**, 1101 (1994)].
10. J. B. Duffy, M. A. Kania, J. P. Gergen, *Development* **113**, 1223 (1991); J. B. Duffy and J. P. Gergen, *Genes Dev.* **5**, 2176 (1991).
11. J. M. Slack, P. W. H. Holland, C. F. Graham, *Nature* **361**, 490 (1993).
12. K. Sander, in *Development and Evolution*, B. C. Goodwin, N. Holder, C. C. Wylie, Eds. (Cambridge University Press, Cambridge, 1983), pp. 137–159.
13. D. St. Johnston and C. Nüsslein-Volhard, *Cell* **68**, 201 (1992); P. Ingham, *Nature* **335**, 25 (1988); M. Akam, *Development* **101**, 1 (1987); C. Nüsslein-Volhard and E. Wieschaus, *Nature* **287**, 795 (1980); C. Nüsslein-Volhard, E. Wieschaus, H. Kluding, *Roux's Arch. Dev. Biol.* **193**, 267 (1984); G. Jürgens, E. Wieschaus, C. Nüsslein-Volhard, H. Kluding, *ibid.*, p. 283; E. Wieschaus, C. Nüsslein-Volhard, G. Jürgens, *ibid.*, p. 296; T. Schüpbach and E. Wieschaus, *ibid.* **195**, 302 (1986).
14. K. Sander, *Adv. Insect. Physiol.* **12**, 125 (1976).
15. F. E. Schwalm, *Insect Morphogenesis* (Karger, Basel, 1988), pp. 1–5; N. P. Kristensen, *Annu. Rev. Ent.* **26**, 135 (1981).
16. C. C. Labandeira and J. J. Sepkoski Jr., *Science* **261**, 310 (1993).
17. M. Akam *et al.*, *Development* (suppl.), in press.
18. G. Tear, M. Akam, A. Martinez Arias, *Development* **110**, 915 (1990); R. Kelsh, I. A. Dawson, M. Akam, *ibid.* **117**, 293 (1993).
19. M. F. Whiting and W. C. Wheeler, *Nature* **368**, 696 (1994).
20. N. H. Patel *et al.*, *Cell* **58**, 955 (1989); N. H. Patel, *Development* (suppl.), in press.
21. S. DiNardo, J. M. Kuner, J. Theis, P. H. O'Farrel, *Cell* **43**, 59 (1985); T. L. Karr, M. P. Weir, Z. Ali, T. Kornberg, *Development* **105**, 605 (1989).
22. A. Martinez Arias and P. Lawrence, *Nature* **313**, 639 (1985); P. Lawrence, *Development* (suppl.) **104**, 61 (1988).
23. L. M. Nagy and S. Carroll, *Nature* **367**, 460 (1994).
24. R. Kraft and H. Jäckle, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6634 (1994).
25. R. Fleig, *Roux's Arch. Devl. Biol.* **198**, 467 (1990).
26. S. J. Brown, N. H. Patel, R. E. Denell, *Dev. Genet.* **15**, 7 (1994).
27. N. H. Patel, B. G. Condron, K. Zinn, *Nature* **367**, 429 (1994).
28. S. J. Brown, R. B. Hilgenfeld, R. E. Denell, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
29. R. J. Sommer and D. Tautz, *Nature* **361**, 448 (1993).
30. G. Struhl, *Nature* **318**, 677 (1985); D. Ish-Horowicz and H. Gyurkovics, *Development* (suppl.) **104**, 67 (1988).
31. A. S. Manoukian and H. M. Krause, *Genes Dev.* **6**, 1740 (1992).
32. N. H. Patel, E. B. Ball, C. S. Goodman, *Nature* **357**, 339 (1992).
33. R. Dawes, I. Dawson, F. Falciani, G. Tear, M. Akam, *Development* **120**, 1561 (1994).
34. A. Miles and D. J. Miller, *Proc. Roy. Soc. B* **248**, 159 (1992); D. J. Miller and A. Miles, *Nature* **365**, 216 (1993).
35. E. Boncinelli, A. Simeone, D. Acampora, F. Mivilio, *Trends Genet.* **7**, 329 (1991).
36. P. J. Webster, J. Suen, P. M. Macdonald, *Development* **120**, 2027 (1994); R. Schröder and K. Sander, *Roux's Arch. Dev. Biol.* **203**, 34 (1993); R. Sommer and D. Tautz, *Development* **113**, 419 (1991); M. A. Seeger and T. C. Kaufman, *EMBO J.* **9**, 2977 (1990); P. M. Macdonald, *Development* **110**, 161 (1990); D. Curtis and R. Lehmann, personal communication.
37. L. Mosquera, C. Forristall, Y. Zhou, M. L. King, *ibid.* **117**, 377 (1993).
38. T. C. Evans, S. L. Crittenden, V. Kodoyianni, J. Kimble, *Cell* **77**, 183 (1994).
39. X. Xu, P.-X. Xu, S. Yoshiaki, *Development* **120**, 277 (1994).
40. C. J. Wedeen and D. A. Weisblat, *ibid.* **113**, 805 (1991).
41. C. A. Davis, D. P. Holmyard, K. J. Millen, A. L. Joyner, *ibid.* **111**, 287 (1991).
42. W. Wurst, A. B. Auerbach, A. L. Joyner, *ibid.* **120**, 2065 (1994); A. L. Joyner, K. Herrup, A. Auerbach, C. A. Davis, J. Rossant, *Science* **251**, 1239 (1991).
43. E. Ogawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6859 (1993).
44. A. Ruiz i Altaba and D. A. Melton, *Development* **106**, 173 (1989); *Cell* **57**, 317 (1989); H. Bastian and P. Gruss, *EMBO J.* **9**, 1839 (1990); M. K. Dush and G. R. Martin, *Dev. Biol.* **151**, 273 (1992).
45. M. J. Pankratz and H. Jäckle, in *The Development of* Drosophila melanogaster, M. Bate and A. Martinez-Arias, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1993), pp. 467–516.
46. A. Levine *et al.*, *Cell* **77**, 587 (1994).
47. N. H. Patel, T. B. Kornberg, C. S. Goodman, *Development* **107**, 201 (1989); M. Klingler, *BioEssays* **16**, 391 (1994).
48. W. Driever and C. Nüsslein-Volhard, *Cell* **54**, 95 (1988).
49. P. M. MacDonald, P. Ingham, G. Struhl, *ibid.* **47**, 721 (1986); M. Frasch, T. Hoey, C. Rushlow, H. Doyle, M. Levine, *EMBO J.* **6**, 749 (1987); N. H. Patel, in *Evolutionary Conservation of Developmental Mechanisms*, A. C. Spradling, Ed. (Wiley-Liss, New York, 1993), pp. 85–110.
50. E. Bier, H. Vaessin, S. Younger-Shepherd, L. Y. Jan, Y. N. Jan, *Genes Dev.* **6**, 2137 (1992); C. Q. Doe, Q. Chu-LaGraff, D. M. Wright, M. P. Scott, *Cell* **65**, 451 (1991).
51. I. Sulston and K. Anderson, personal communication.
52. M. A. Pultz, personal communication.
53. R. J. Sommer and P. W. Sternberg, *Science* **265**, 114 (1994).

54. W. R. Jeffrey and B. J. Swalla, *BioEssays* **14**, 219 (1992).
55. G. A. Wray and R. A. Raff, *Dev. Biol.* **132**, 458 (1989).
56. E. H. Davidson, *Development* **108**, 365 (1990).
57. R. Kelsh, R. O. J. Weinzierl, R. A. H. White, M. Akam, *Dev. Genet.* **15**, 19 (1994).

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Cytoskeletal Functions During *Drosophila* Oogenesis

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Organismal morphogenesis is driven by a complex series of developmentally coordinated changes in cell shape, size, and number. These changes in cell morphology are in turn dependent on alterations in basic cytoarchitecture. Elucidating the mechanisms of development thus requires an understanding of the cytoskeletal elements that organize the cytoplasm of differentiating cells. *Drosophila* oogenesis has emerged as a versatile system for the study of cytoskeletal function during development. A series of highly coordinated changes in cytoskeletal organization are required to produce a mature *Drosophila* oocyte, and these cytoskeletal transformations are amenable to a variety of experimental approaches. Genetic, molecular, and cytological studies have shed light on the specific functions of the cytoskeleton during oogenesis. The results of these studies are reviewed here, and their mechanistic implications are considered.

Drosophila ovaries are composed of parallel bundles of developmentally ordered egg chambers, each of which supports the development of a single oocyte. These bundles, called ovarioles, are divided into anterior and posterior compartments [Fig. 1A; for a comprehensive review of *Drosophila* oogenesis, see (*1*)]. Oogenesis is initiated in the anterior compartment of the ovariole, or germarium (Fig. 1B), by a stem cell division that produces a cystoblast and regenerates a stem cell (Fig. 1C). The cystoblast proceeds through four mitotic divisions to produce a cyst of 16 germline cells that will differentiate to form the single oocyte and 15 nurse cells found in each egg chamber. During oogenesis, the nurse cells synthesize maternal components for transport to the oocyte (Fig. 1D). Cytokinesis is incomplete at each of the cystoblast divisions, which leaves the 16 germline cells interconnected by large cytoplasmic bridges called ring canals, which are maintained through the completion of oogenesis.

Germariums are divided into four cytologically distinct regions that contain developmentally arrayed germline cysts (Fig. 1B). The stem cells and the mitotically dividing cystoblasts lie within germarial region 1, whereas newly formed 16-cell cysts are located in region 2a. When cysts progress into region 2b, they become lens-shaped and span the width of the germarium. The future oocyte is positioned at the center of the lens-shaped cysts from region 2b. By the time the 16-cell cyst occupies region 3 of the germarium, the oocyte is located at the posterior pole. The oocyte remains at the posterior of the germline cell cluster through the completion of oogenesis. In region 2a, somatic follicle cells begin to migrate between the 16-cell germline cysts. When they reside in region 3, the cysts are surrounded by a monolayer of follicle cells and are referred to as stage 1 egg chambers.

Stage 2 egg chambers bud from the germarium and enter the posterior compartment of the ovariole, or vitellarium (Fig. 1A). During stages 2 through 6, the egg chambers increase in size while remaining roughly spherical. The oocyte grows at approximately the same rate as a single nurse cell. Oocyte growth during stages 2 through 6 is the result of the transport of nutrients into the oocyte from the nurse cells.

During stages 7 through 10a, the oocyte endocytoses yolk proteins synthesized by fat bodies and follicle cells. Consequently, oocyte growth is more rapid than nurse cell growth, and by stage 10 the oocyte occupies the entire posterior half of the egg chamber (Fig. 1D). The morphogenetic molecules that specify the embryonic axes are asymmetrically positioned within the oocyte during these stages. Messenger RNA (mRNA) of *bicoid*, the primary anterior morphogen, is localized to the anterior cortex (*2*); the Vasa (*3*, *4*) and Staufen proteins and *oskar* mRNA (*5*), which are required for pole cell formation and posterior patterning, are positioned at the posterior pole; and *gurken* mRNA, which plays a key role in dorsoventral axis specification, accumulates between the dorsally located oocyte nucleus and the cortex (*6*).

During stages 10b through 12, the remaining nurse cell cytoplasm is transferred to the oocyte. As the nurse cells shrink, the oocyte expands (*7*). Nurse cell cytoplasm enters the oocyte and is mixed with the existing ooplasm by rapid ooplasmic movements. During stages 13 and 14, these ooplasmic movements stop and the meiosis I spindle assembles. The oocyte remains in the metaphase of the first meiotic division until it enters the oviduct and egg activation and fertilization initiate embryonic development.

Oocyte Specification

As outlined above, oogenesis in *Drosophila* begins with the formation of a cyst of 16 cells. Although the 16 sibling cells are interconnected by cytoplasmic bridges, only a single oocyte is produced. Oocyte differentiation thus reflects the establishment of a specialized region of cytoplasm within a syncytium.

The pattern of the four incomplete cystoblast divisions is precisely controlled and leads to the production of a cyst containing two cells with four ring canals, two cells with three ring canals, four cells with two ring canals, and eight cells with a single ring canal (Fig. 1C). One of the two cells with four ring canals invariably forms the oocyte, indicating that specification of the cytoplasmic compartment that will ultimately form the oocyte is linked to this cystoblast division pattern. The geometry of the cystoblast divisions, in turn, appears to depend on a structure called the fusome (*8*). The fusome is a region of cytoplasm that is rich in vesicles and membrane-associated cytoskeletal proteins that forms along mitotic spindle remnants during the cystoblast divisions (*9*). At the completion of each division, newly formed segments of fusome merge with material from previous mitoses. As a result, the fusome becomes a continuous branched structure that extends through the intercellular bridges that connect all of the germline cells (Fig. 2D). One spindle pole in each mitotic cystoblast is always anchored in the fusome (Fig. 2C). Because the orientation of the spindle determines the mitotic cleavage plane, the fusome has a direct effect on the geometry of the cystoblast divisions. Once all four mitotic cell cycles are complete, the fusome disappears.

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