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The evolution of arthropod segmentation: insights from comparisons of gene expression patterns

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SUMMARY

The comparison of gene expression patterns in a number of insect and crustacean species has led to some insight into the evolution of arthropod patterning mechanisms. These studies have revealed the fundamental nature of the parasegment in a number of organisms, shown that segments can be generated sequentially at the molecular level, and suggested that pair-rule pre-patterning might not be shared by all insects.

Key words: evolution, segmentation, *Drosophila*, *Manduca*, *Tribolium*, *Schistocerca*, Acheta, Crustacea, *engrailed*, *even-skipped*

INTRODUCTION

The 1988 Development Supplement entitled 'Mechanisms of Segmentation' contained twenty-three articles dealing with the establishment of metameric pattern in animals as diverse as *Drosophila*, mice and leeches. At that time – and at present, the situation remains largely unchanged – the process generating anterior-posterior segmental pattern during development was best understood in *Drosophila*, and this detailed knowledge was built on a foundation of intensive study of *Drosophila* development at the genetic level. At least six articles in that Development Supplement went on to pose specific questions regarding the extent to which the *Drosophila* paradigm could be applied to understanding segmentation in other arthropods. Several of these articles also suggested that studies of pattern formation in additional insects could help us understand the evolution of the developmental system seen in *Drosophila*.

Few other insects, however, are as amenable to genetic analysis as *Drosophila*, and several authors outlined an alternative method that might provide some initial information about segmentation in other insects and arthropods. This approach involved the isolation of homologs of *Drosophila* segmentation genes from additional arthropods and the subsequent comparison of the expression patterns of these genes in various arthropod embryos. Six years later, many of the questions raised in that 1988 Development Supplement are still with us, but we have obtained a number of answers and some significant insights by pursuing the comparative molecular approach. I will summarize a portion of the progress that has been made and describe answers to several of the questions that had been posed.

(1) ARE PARASEGMENTS UNIVERSAL IN INSECTS?

While segment boundaries are morphologically obvious and segments are historically the units used to describe the metameric properties of insects, genetic and molecular studies of *Drosophila* point to a more developmentally relevant unit - the parasegment. An individual parasegment includes the posterior portion of one segment plus the anterior portion of the next more posterior segment. Thus, parasegments span the same length as segments, but their boundaries lie between the segment boundaries. Molecularly, the parasegment boundaries lie at the interface of the *engrailed* and *wingless* expression domains (reviewed by Martinez Arias, 1993). In the 1988 Development Supplement, Lawrence (1988) provided a concise summary of the data supporting the hypothesis that parasegments are the fundamental units of design in the *Drosophila* embryo (Martinez Arias and Lawrence, 1985): (1) parasegments are the first metameres to be defined during development, (2) parasegment boundaries provide important lineage boundaries during development, and (3) parasegments are the domains in which key genes (such as homeotic genes) are expressed during development. Lawrence ended his discussion on parasegments by stating that ' ..... it would be astounding if other insects, and even annelids, were made of fundamentally different units.' A few pages later, Sander (1988) indicated that comparisons of gene expression patterns between *Drosophila* and other insects might be used to determine whether parasegment organization was a common feature of insect development and that this approach was being actively pursued by a number of laboratories.

Ultimately, these sorts of comparative molecular studies showed that parasegment metamery is found throughout the insects and crustaceans. Homologs of two *Drosophila* genes in particular, *engrailed* (a segment polarity gene) and *abdominal-A* (a homeotic gene), have been studied in a number of species, and an analysis of their expression patterns reveals the evolutionary conservation of parasegmental domains. In the case of *engrailed*, expression is seen in the

posterior portion of each segment in all insects and crustaceans examined so far (Fig. 1; for additional examples see Patel et al., 1989a, 1989b; Fleig, 1990, Sommer and Tautz, 1993; Scholtz et al., 1993; Manzanares et al., 1993; Brown et al., 1994). In these species, as in *Drosophila*, the anterior (parasegmental) border of each *engrailed* stripe rapidly resolves into a sharp boundary, whereas the posterior (segmental) border is not clearly demarcated until much later in development, thus illustrating that the parasegments are the first metameres to be resolved (Patel et al., 1989b). Furthermore, *wingless* expression has been characterized in the beetle, *Tribolium castaneum*, and is found to abut the anterior margin of the *engrailed* stripes, just as in *Drosophila* (Nagy and Carroll, 1994). Expression of *abdominal-A* has been studied in *Manduca* (tobacco hawkmoth), *Tribolium*, and *Schistocerca* (grasshopper), and in all three of these insects, as in *Drosophila*, the anterior boundary of expression coincides with the parasegment boundary within the first abdominal segment (Nagy et al., 1991; Staurt et al., 1993, Tear et al., 1990). In addition, mutations in the *Tribolium abdominal-A* homolog lead to defects that transform parasegmental domains (Stuart et al., 1993). Thus, at least some homeotic genes obey parasegmental boundaries in a variety of insects.

In *Drosophila*, careful lineage analysis reveals that the parasegmental boundary, as marked by the anterior margin of each *engrailed* stripe, defines a stable lineage boundary throughout much of development (Vincent and O'Farrell, 1992; Martinez Arias, 1993). Studies of the development of a number of crustacean species reveal even more striking ectodermal lineage units, which have been termed 'genealogical units' (see for example Dohle, 1976; Dohle and Scholtz, 1988; Scholtz, 1992). Each genealogical unit starts as a single row of cells (roman numeral row), which then undergoes two rounds of division to yield four orderly rows of cells (Fig. 2D). Dohle showed that these genealogical units do not correspond to segments since a segment groove lies within each genealogical unit and not between each genealogical unit. It was speculated that these geneological units might bear some relationship to *Drosophila* parasegments (Martinez Arias and Lawrence, 1985; Dohle and Scholtz, 1988). Indeed, the analysis of *engrailed* expression in the crayfish, *Procambarus clarki*, suggests that these lineage units show striking similarities to *Drosophila* parasegments as the anterior boundary of each of the crustacean geneological units is demarcated by the stable anterior margin of each *engrailed* stripe (Patel et al., 1989b), and similar results have been obtained in additional crustacean species (Fig. 2; Scholtz et al., 1993; N. Patel, unpublished data). Thus, parasegmental units are easily visualized in a wide range of insects and crustaceans. More importantly, these parasegment units appear to satisfy the criteria for fundamental units of design as stipulated by Lawrence (1988).

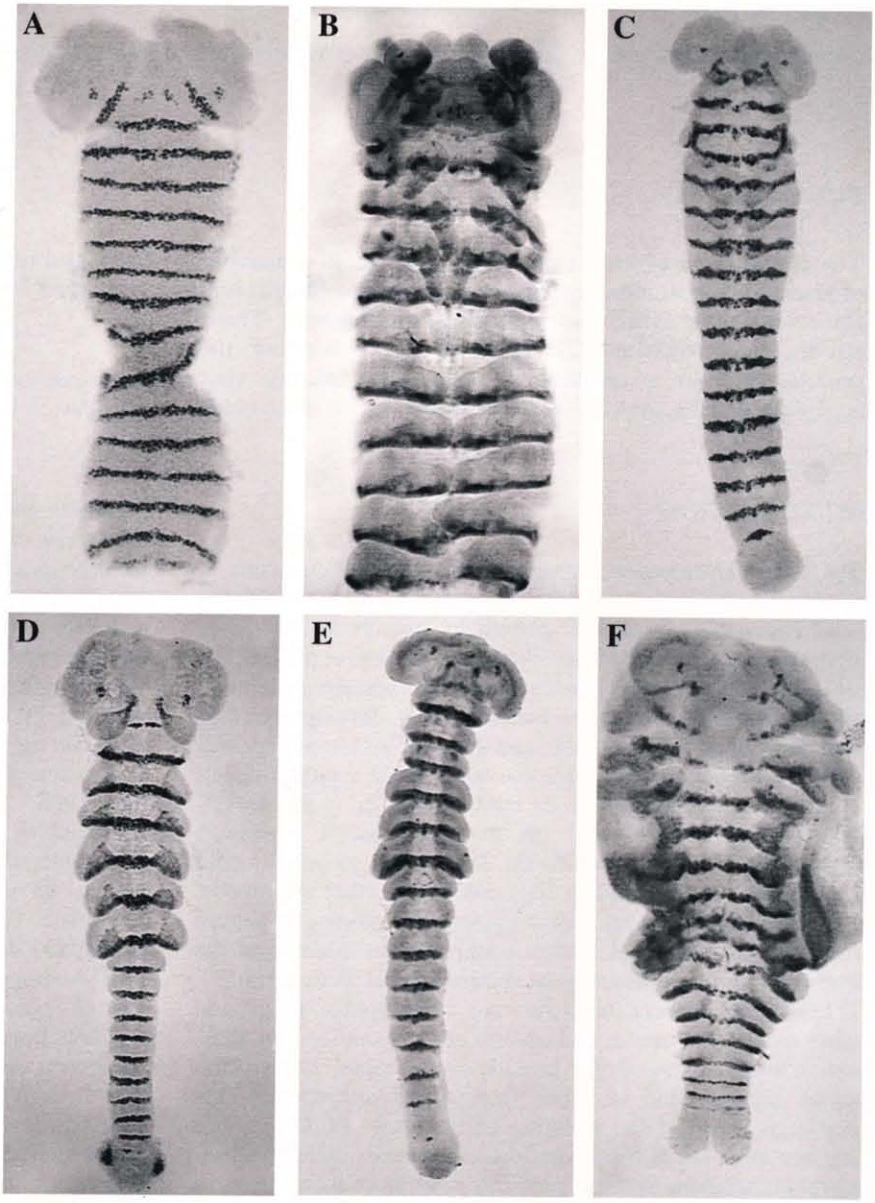

Fig. 1. Expression of *engrailed* in a variety of insects and a crustacean. (A) *Drosophila melanogaster* (fruit fly), (B) *Manduca sexta* (tobacco hawkmoth), (C) *Tribolium castaneum* (red flour beetle), (D) *Acheta domestica* (cricket), (E) *Schistocerca americana* (grasshopper), (F) *Procambarus clarki* (crayfish). *engrailed* is expressed in the posterior portion of each body segment. The stage 9 *Drosophila* embryo in A has been dissected flat and the proctodeum and posterior midgut have been removed (see Fig. 3A,B for undissected specimens). The faint *engrailed* stripe at the posterior end of this *Drosophila* embryo is part of the ninth abdominal segment. In B, all segments posterior to A7 have been removed. Embryos in C–F have not completed the formation of some of the most posterior *engrailed* stripes. All embryos are oriented anterior up and are viewed from the ventral side. mAb 4D9 was used to detect *engrailed* in all embryos except *Manduca*, where mAb 4F11 was used instead (Patel et al., 1989a).

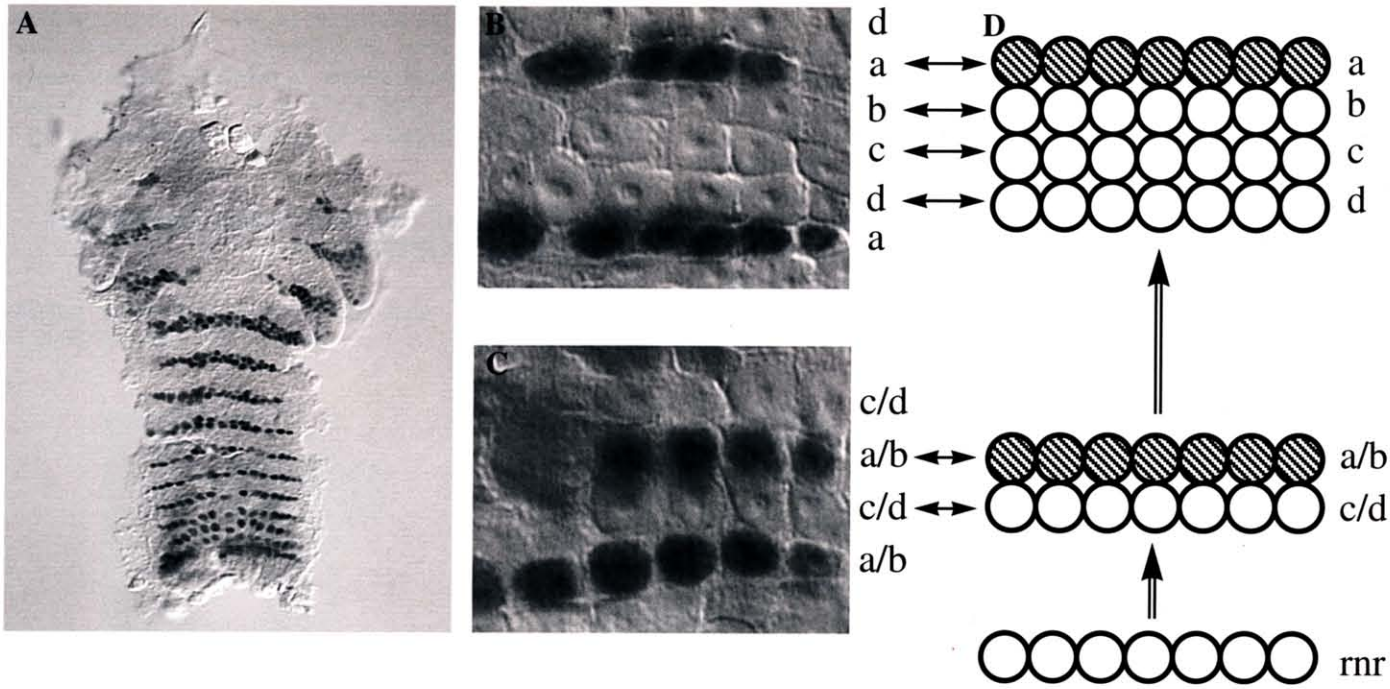

Fig. 2. Expression of *engrailed* during the formation of genealogical units in the crustacean, *Mysidium columbiae*. (A) *Mysidium* embryo stained with mAb 4D9 to visualize the expression of *engrailed*. (B,C) Higher magnification views of some of the rows of *engrailed*-expressing cells. (D) Schematic illustration of the generation of 'genealogical' units in *Mysidium*. In *Mysidium*, segments are generated sequentially from a posterior growth zone. The *Mysidium* growth zone consists of an organized row of cells called ectoteloblasts. The ectoteloblasts undergo a series of asymmetric divisions, each division generating a row of cells known as a 'roman numeral row' (rnr; Dohle, 1976; Dohle and Scholtz, 1988). Each roman numeral row divides symmetrically to generate a/b and c/d rows. Each of these two rows then divides symmetrically to yield a, b, c, and d rows. Since this division pattern provides an inherent temporal and spatial gradient, all steps are visible in a single embryo of the appropriate stage; ectoteloblasts at the very posterior, newly formed roman numeral rows just anterior to the ectoteloblasts, a/b and c/d rows slightly more anterior, and a, b, c, and d rows even further anterior. *engrailed* expression is not seen in the ectoteloblasts nor in roman numeral rows. C shows a level of the *Mysidium* embryo in which a/b and c/d rows have just formed and at this time *engrailed* is expressed in the a/b row (shaded in the corresponding section of D). After the a/b row divides, *engrailed* expression is lost from the b row cells and is maintained in the a row cells. B shows a region of the *Mysidium* embryo in which a, b, c, and d rows of cells are visible and *engrailed* protein is only in the a row cells (shaded in the corresponding section of D). Later, some b row cells will regain *engrailed* expression, and after the next round of division, *engrailed* will be maintained in all a row progeny, but in only the more anterior b row progeny. When the segmental groove forms, it will traverse between the progeny of the b row. For further details, see the description of *engrailed* expression in *Procambarus* (Patel et al., 1989b) and *Cherax* (Scholtz et al., 1993).

(2) ARE SEGMENTS ESTABLISHED SEQUENTIALLY AT THE MOLECULAR LEVEL IN SHORT GERM INSECTS?

The articles by French (1988), Tear et al. (1988), and Sander (1988) discussed an important classification system that has been used to divide insect embryos into three large developmental categories (reviewed by Sander, 1976). This classification scheme uses data from a variety of embryonic manipulations to determine the extent to which the body plan is established in the initial germ anlage. Embryos such as those of *Drosophila*, which have established a complete body plan by the onset of gastrulation, are termed long germ embryos. *Schistocerca* embryos, which belong to the short germ category, appear to consist of only a head region and a subterminal proliferative zone at the end of the blastoderm stage. All the body segments appear to be generated subsequently as the embryo elongates by cell proliferation (Mee and French, 1986). Finally, embryos whose segments are established as far posterior as the thorax or anterior abdomen at the blastoderm stage and that specify the remaining, more posterior segments after gastrulation are termed intermediate germ embryos.

In long germ *Drosophila*, the establishment of the entire segmental pattern of the body is revealed at the molecular level by the patterns of segmentation and homeotic genes during the blastoderm stage (reviewed byAkam, 1987; Ingham, 1988). For example, all fourteen *engrailed* stripes of the body are visible in the *Drosophila* embryo by the time gastrulation begins, and these stripes are more or less evenly spaced out over the body region of the germ anlage (Fig. 3A,B). A priori, there were two possibilities for molecular specification of segments in short germ embryos: all segments could be established at the molecular level within the prospective proliferative zone during the blastoderm stage and simply expand and differentiate sequentially during the growth phase, or the proliferative zone could generate a sheet of cells that would be sequentially segmented at the molecular level after the growth phase. Studies with both *engrailed* and a number of homeotic genes suggests that the latter is true. In short germ embryos of *Schistocerca*, the first *engrailed* stripes appear in the thorax

and at this time there is no compressed pattern of *engrailed* stripes in the region that will proliferate to provide the cells of the abdomen (Fig. 3E). Instead, abdominal *engrailed* stripes appear sequentially, later in development, in a region that has been newly generated by cell proliferation (Fig. 3F; Patel et al., 1989b). In *Tribolium*, the first *engrailed* stripe (mandibular segment) appears at the onset of gastrulation, and the remaining *engrailed* stripes of the body appear sequentially during development (Fig. 3C,D; Brown et al., 1994). Similar sequential appearance of body stripes has also been found for the pattern of *Tribolium wingless* (Nagy and Carroll, 1994). The analysis of homeotic gene expression patterns also supports the notion that segments are not specified simultaneously during short germ development – *Schistocerca Antp*, *Ubx*, *abd-A*, and *Abd-B* appear in sequence as the embryo develops (Tear et al., 1990; Kelsh et al., 1994, 1993; E. Ball, N. Patel, D. Hayward, and C. Goodman, unpublished results).

(3) WHAT ARE THE EVOLUTIONARY ORIGINS OF PAIR-RULE ORGANIZATION?

In *Drosophila*, the expression patterns of segment polarity genes are established by the actions of the pair-rule class of

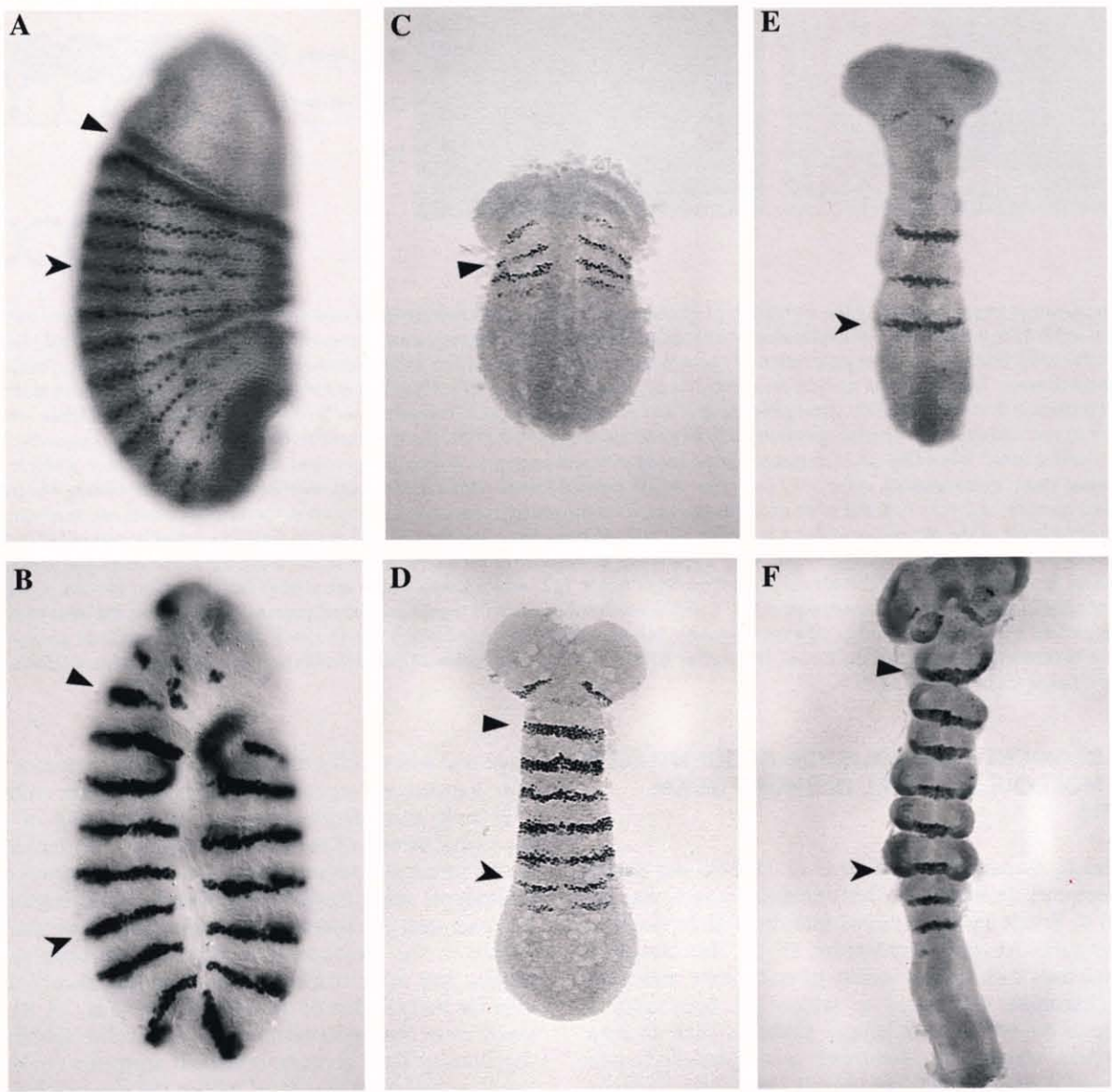

Fig. 3. Establishment of *engrailed* patterns in *Drosophila*, *Tribolium*, and *Schistocerca*. In all panels, the triangle marks the *engrailed* stripe of the mandibular segment and the arrowhead indicates the position of the *engrailed* stripe of the third thoracic segment. (A) At stage 6, the *Drosophila* embryo has just started gastrulation and germband extension and all fourteen *engrailed* stripes of the body are already visible. The mandibular stripe is slightly hidden as it is folded into the cephalic furrow. (B) By stage 11, germband formation is completed and additional patches of *engrailed* expression are now present in the terminal regions. In *Tribolium*, only the most anterior *engrailed* stripes are present shortly after the start of gastrulation and germband extension (C) and the remaining stripes appear as the germband elongates (D). In *Schistocerca*, *engrailed* stripes first appear in the thorax (E) and more posterior *engrailed* stripes appear only after the abdominal region has expanded by cell proliferation (F).

genes (Fig. 4A,B; reviewed by Martinez Arias, 1993). The maintenance and refinement of the segment polarity expression patterns, however, involves a system of cell-cell interactions mediated by the products of the segment polarity genes themselves (reviewed by Martinez Arias, 1993). Because *Schistocerca* pattern formation appears to occur in a cellular (as opposed to syncytial) environment, it was proposed that the generation of segment polarity expression patterns in short germ *Schistocerca* embryos might not involve a pair-rule pre-patterning system, but instead might rely on a system of cell-cell interactions like those used to maintain segment polarity patterns in *Drosophila* (Tear et al., 1988; Patel et al., 1989b). Furthermore, whereas most *Drosophila* segment polarity genes, including *engrailed*, show an initial 'pair-rule' pattern of intensity as their expression first begins, no such 'pair-rule' patterns are seen during the generation of *engrailed* stripes in *Schistocerca* (Patel et al., 1989b). Sander (1988), however, argued that the pair-rule pre-patterning system is of ancient

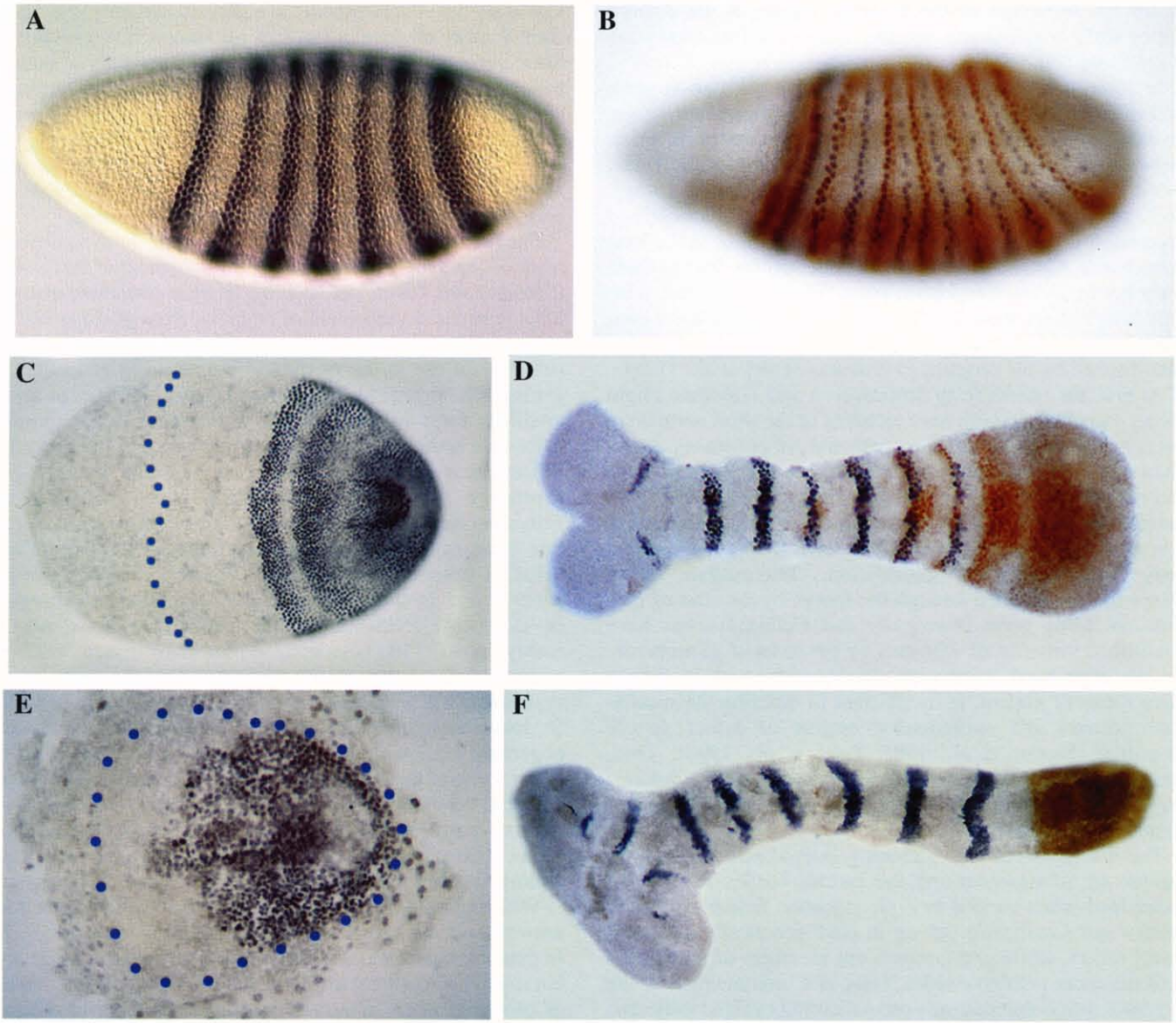

Fig. 4. Relationship of *even-skipped* and *engrailed* expression. *Drosophila* (A,B), *Tribolium* (C,D), and *Schistocerca* (E,F) embryos stained for *even-skipped* alone (A,C,E) or *even-skipped* and *engrailed* (B,D,F; *even-skipped* in brown, *engrailed* in black). In *Drosophila*, all seven *even-skipped* pair-rule stripes are present at the blastoderm stage (A). At the onset of gastrulation, odd numbered *engrailed* stripes appear at the anterior margin of each *even-skipped* pair-rule stripe (B). In *Tribolium*, *even-skipped* also displays a pair-rule expression pattern, but only the first two pair-rule stripes are present at the onset of gastrulation (C). The remaining *even-skipped* stripes appear as the embryo elongates (D). As in *Drosophila*, the anterior border of each *Tribolium even-skipped* pair-rule stripe marks the position of odd-numbered *engrailed* stripes (see Patel et al., 1994 for a more detailed discussion of *even-skipped* expression in *Tribolium*, particulary for details concerning the formation of segmental secondary stripes). In *Schistocerca*, *even-skipped* is expressed in a posterior domain at the onset of gastrulation with a crescent of unstained cells at the posterior end of the gastral furrow (E), and expression remains in a posterior domain as the embryo elongates (F). No pair-rule patterns have been observed for *Schistocerca even-skipped*. The blue dots in C and E indicate the boundary between embryonic and extra-embryonic cells. *Even-skipped* was detected using mAb 2B8 (Patel et al., 1994) and *engrailed* with mAb 4D9 (Patel et al., 1989a).

origin based on the morphologically visible patterns of two segment periodicity in the myriapods, although it should be noted that *Drosophila* does not itself display these sorts of morphological two segment periodicities.

Schistocerca homologs of the *Drosophila* pair-rule genes *fushi tarazu* and *even-skipped* have been characterized (Dawes et al, 1994; Patel et al., 1992, 1994). Neither is expressed in any discernable pair-rule pattern, although both show conserved expression patterns within the developing nervous system. Curiously, both *Schistocerca even-skipped* and *fushi tarazu* are expressed in the posterior region of the embryo during early development, but the function of this expression is unknown (Fig. 4E,F).

In contrast to *Schistocerca* embryos, however, short germ *Tribolium* embryos do display pair-rule pre-patterning, as revealed by the expression of *Tribolium* homologs of *hairy*, *fushi tarazu*, and *even-skipped* (Fig. 4C,D; Sommer and Tautz, 1993; Patel et al., 1994; S. Brown, J. Parrish, and R. Denell, personal communication). In addition, *even-skipped* expression patterns reveal pair-rule pre-patterning in the long germ beetle, *Callosobruchus maculatus*, and the intermediate germ beetle, *Dermestes frischi*, but consistent with their germ type designations, these beetles differ from *Tribolium* and each other in the relative number of *even-skipped* stripes that have been formed by the onset of gastrulation (Patel et al., 1994).

At first, the results from *Schistocerca* and *Tribolium* might seem contradictory: both have embryos of the short germ type, but only *Tribolium* seems to use pair-rule pre-patterning. A resolution to this apparent contradiction comes from a more precise consideration of germ type designations. Germ type designations simply reflect the timing of segment specification - both *Tribolium* and *Schistocerca* embryos establish most body segments after gastrulation. Intermediate germ *Dermestes* is patterned through the thorax by the start of gastrulation. Long germ *Drosophila* and *Callosobruchus* have established virtually all segments by the onset of gastrulation. On the other hand, germ type classification, with its simple three category system, is insufficient to describe the mechanistic details and evolutionary origins of insect pattern formation (Sander et al., 1985; Patel et al., 1994). Thus, embryos with shared germ type designations, such a *Schistocerca* and *Tribolium*, do not necessarily share identical pattern formation mechanisms.

The data obtained by examining *even-skipped* expression patterns in *Schistocerca* and the various beetles is easier to understand when viewed in a phylogenetic framework. Both beetles and *Drosophila* belong to phylogenetically advanced insect orders, while grasshoppers are members of a phylogenetically more primitive order. Thus, one interpretation of the available data is that pair-rule pre-patterning evolved sometime during the evolution of the phylogenetically more advanced insects. However, it is possible that evidence for pair-rule pre-patterning will emerge when additional *Schistocerca* homologs of *Drosophila* pair-rule genes are characterized. Certainly the results from *Tribolium* prove that development in a cellular environment and the sequential appearance of *engrailed* stripes do not necessarily rule out the presence of pair-rule pre-patterning. Alternatively, as Sander (1988) pointed out, pair-rule pre-patterning may be an ancient property of insects that has simply been lost in the evolution of the lineage leading to *Schistocerca*. The validity of each of these hypotheses can be tested by looking at the expression of additional pair-rule gene homologs in *Schistocerca* as well as by expanding these sorts of studies to additional phylogenetically primitive insects and to other arthropods outside of the insects.

CONCLUSIONS

The molecular comparisons made between various organisms have given us important insights into the evolution of insect segmentation. Parasegments are almost certainly the fundamental units of development in all insects and crustaceans. Short germ insects generate segments sequentially at both the molecular and morphological levels. Pair-rule patterning is evident in several orders of phylogenetically advanced insects but may be absent from phylogenetically primitive insect orders. Many questions that were posed in the 1988 Development Supplement remain unanswered, of course, and what new data we have also raises a number of new questions.

For example, work in *Tribolium* points to the clear involvement of gap gene patterning in the development of this embryo (Sommer and Tautz, 1993); will gap gene patterning also participate in the development of embryos from phylogenetically more primitive insects? In *Drosophila*, gap gene products presumably diffuse in the syncytial blastoderm to generate local gradients (reviewed by Pankratz and Jäckle, 1993). Can similar gradients form in the cellular environment of the growing *Tribolium* embryo? If pair-rule pre-patterning is not at work in *Schistocerca* development, how are segment polarity gene expression patterns initiated? While both *Drosophila* and *Tribolium* display pair-rule prepatterning, some details of pair-rule gene expression are different between the two insects. What is responsible for these slightly different pair-rule patterns and how do these differences influence subsequent development? Evidence for the maternal establishment of embryo polarity has been obtained for many insects, but is the maternal information always transmitted by the same genetic system that has been characterized in *Drosophila* (reviewed by St. Johnston and Nüsslein-Volhard, 1992)? Does the mode of oogenesis (i.e., with or without nurse cells attached to the anterior end of the developing oocyte) limit the extent to which maternal components can be used to establish the body plan of the embryo (French, 1988; Patel, 1993)? Most analyses have been confined to insects and crustaceans, but what sorts of patterning systems will be seen in myriapods and chelicerates?

While continued molecular comparisons will provide some answers to these questions, additional approaches are needed. In particular, gene expression patterns can be quite suggestive, but it will be important to devise ways to eliminate the function of particular genes in various arthropods in order to critically access their developmental roles. Moreover, although identifying homologs of *Drosophila* segmentation genes has been a useful approach, it will not identify novel patterning mechanisms that might be at work in other arthropods. Thus, additional organisms amenable to genetic analysis must be sought out. In another five or six years, significant progress will certainly be made in our continuing efforts to understand the processes that have guided the evolution of pattern formation in the arthropods. The results obtained will also help us to understand how complex developmental patterning systems in other phyla may have evolved.

REFERENCES

Akam, M. (1987). The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101**, 1-22.

Brown, S. J., Patel, N. H. and Denell, R. E. (1994). Embryonic expression of the single *Tribolium engrailed* homolog. *Dev Genet.* **15**, 7-18.

Dawes, R., Dawson, I., Falciani, F., Tear, G. and Akam, M. (1994). *Dax*, a locust Hox gene related to *fushi-tarazu* but showing no pair-rule expression. *Development* **120**, 1561-1572.

Dohle, W. (1976). Die Bildung und Differenzierung des postnauplialen Keimstreifs von *Diastylis rathkei* (Crustacea, Cumacea). II. Die Differenzierung und Musterbildung des Ektoderms. *Zoomorphologie* **84**, 235-277.

Dohle, W. and Scholtz, G. (1988). Clonal analysis of the crustacean segment: the discordance between genealogical and segmental borders. *Development* **104 Supplement,** 147-160.

Fleig, R. (1990). *Engrailed* expression and body segmentation in the honeybee *Apis millifera*. *Roux's Arch. Dev. Biol.* **198**, 467-473.

French, V. (1988). Gradients and insect segmentation. *Development* **104 Supplement**, 3-16.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**, 25-34.

Kelsh, R., Dawson, I. and Akam, M. (1993). An analysis of *Abdominal-B* expression in the locust *Schistocerca gregaria*. *Development* **117**, 293-305.

Kelsh, R., Weinzierl, R. O. J., White, R. A. H. and Akam, M. (1994). Homeotic gene expression in the locust *Schistocerca*: An antibody that detects conserved epitopes in Ultrabithorax and abdominal-A proteins. *Dev. Genet.* **15**, 19-31.

Lawrence, P. (1988). The present status of the parasegment. *Development* **104 Supplement**, 61-65.

Manzanares, M., Marco, R. and Garesse, R. (1993). Genomic organization and developmental pattern of expression of the *engrailed* gene from the brine shrimp *Artemia*. *Development* **118**, 1209-1219.

Martinez Arias, A. and Lawrence, P. A. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**, 639-642.

Martinez Arias, A. (1993). Development and patterning of the larval epidermis of *Drosophila*. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez Arias), pp. 517-608. New York: Cold Spring Harbor Laboratory Press.

Mee, J. and French, V. (1986). Disruption of segmentation in a short germ insect embryo I. The localization of segmental abnormalities induced by heat shock. *J. Embryol. exp. Morph.* **96**, 245-266.

Nagy, L. M., Booker, R. and Riddiford, L. M. (1991). Isolation and embryonic expression of a *abdominal-A*-like gene from the lepidopteran, *Manduca sexta*. *Development* **112**, 119-129.

Nagy, L. M. and Carroll, S. (1994). Conservation of wingless patterning functions in the short-germ embryos of *Tribolium castaneum*. *Nature* **367**, 460-463.

Pankratz, M. J. and Jäckle, H. (1993). Blastoderm segmentation. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez Arias), pp. 467-516. New York: Cold Spring Harbor Laboratory Press.

Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989a). Expression of *engrailed* proteins in arthropods, annelids, and chordates. *Cell* **58**, 955-968.

Patel, N. H., Kornberg, T. B. and Goodman, C. S. (1989b). Expression of *engrailed* during segmentation in grasshopper and crayfish. *Development* **107**, 201-212.

Patel, N. H., Ball, E. E. and Goodman, C. S. (1992). Changing role of *even-skipped* during the evolution of insect pattern formation. *Nature* **357**, 339-342.

Patel, N. H. (1993). Evolution of insect pattern formation: a molecular analysis of short germband segmentation. In *Evolutionary Conservation of Developmental Mechanisms* (ed. A. C. Spradling), pp. 85-110. New York: Wiley-Liss, Inc.

Patel, N. H., Condron, B. G. and Zinn, K. (1994). Pair-rule expression patterns of *even-skipped* are found in both short and long germ beetles. *Nature* **367**, 429-434.

Sander, K. (1976). Specification of the basic body pattern in insect embryogenesis. *Adv. Insect. Physiol.* **12**, 125-238.

Sander, K., Gutzeit, H. O. and Jäckle, H. (1985). Insect embryogenesis: morphology, physiology, genetical, and molecular aspects. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology* (eds. G. A. Kerkut and L. I. Gilbert), pp. 319-385. New York: Pergamon Press.

Sander, K. (1988). Studies in insect segmentation: from teratology to phenogenetics. *Development* **104 Supplement**, 112-121.

Scholtz, G. (1992). Cell lineage studies in the crayfish *Cherax destructor* (Crustacea, Decapoda): germ band formation, segmentation, and early neurogenesis. *Roux's Arch. Dev. Biol.* **202**, 36-48.

Scholtz, G. Dohle, W., Sandeman, R. and Richter, S. (1993). Expression of engrailed can be lost and regained in cells of one clone in crustacean embryos. *Int. J. Dev. Biol.* **37**, 299-304.

Sommer, R. J. and Tautz, D. (1993). Involvement of an orthologue of the *Drosophila* pair-rule gene *hairy* in segment formation of the short germ band embryo of *Tribolium* (Coleoptera). *Nature* **361**, 448-450.

St Johnston, D. and Nüsslein-Volhard, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201-220.

Stuart, J. J., Brown, S. J., Beeman, R. W. and Denell, R. E. (1993). The *Tribolium* homeotic gene *Abdominal* is homologous to *abdominal-A* of the *Drosophila* bithorax complex. *Development* **117**, 233-243.

Tear, G., Bate, C. M. and Martinez Arias, A. (1988). A phylogenetic interpretation of the patterns of gene expression in *Drosophila* embryos. *Development* **104 Supplement**, 135-146.

Tear, G., Akam, M. and Martinez Arias, A. (1990). Isolation of an *abdominal-A* gene from the locust *Schistocerca gregaria* and its expression during embryogenesis. *Development* **110**, 915-925.

Vincent, J. P. and O'Farrell, P. H. (1992). The state of *engrailed* expression is not clonally transmitted during early *Drosophila* development. *Cell* **69**, 923-931.