Playing by pair-rules?

Gregory K. Davis[1] and Nipam H. Patel[2]*

Summary

Although in *Drosophila* pair-rule genes play crucial roles in the genetic hierarchy that subdivides the embryo into segments, the extent to which pair-rule patterning is utilized by different arthropods and other segmented phyla is unknown. Recent data of Dearden et al.[(1)] and Henry et al.,[(2)] however, hint that a pair-rule mechanism might play a role in the segmentation process of basal arthropods and vertebrates. *BioEssays* 25:425–429, 2003. © 2003 Wiley Periodicals, Inc.

Introduction

In *Drosophila*, gradients of maternal information act at the top of a hierarchy involving the sequential activation of the zygotic gap, pair-rule, and segment polarity genes. While segment polarity genes are defined by their loss-of-function phenotypes in which pattern defects are segmentally repeated in the embryonic cuticle, pair-rule genes are instead defined by deletions of the cuticle occurring with a two-segment periodicity.[(3)] In order to gain insight into how segmentation is controlled in other organisms, as well as to have a clearer understanding of the evolution of the *Drosophila* segmentation hierarchy, a number of studies have examined the expression of orthologues of *Drosophila* segment polarity and pair-rule genes in various arthropods.

A dozen years of such comparisons have certainly yielded one major conclusion: at least some aspects of the segment polarity level of the hierarchy appear to be well conserved among all extant arthropods. The segment polarity genes that have been most widely studied outside of *Drosophila* are *wingless* (*wg*) and *engrailed* (*en*). Consistent with their phenotypes, most segment polarity genes are expressed in *Drosophila* just before and throughout the morphologically segmented germ-band stage in a segmentally reiterated pattern. *wg* and *en* are each expressed as single ectodermal stripes within each individual segment such that every *wg* stripe lies adjacent and anterior to an *en* stripe. Each *wg* and *en* stripe demarcates the posterior and anterior limits, respectively, of adjacent units known as parasegments. Although each parasegment is one-segment wide, parasegmental boundaries are slightly out of phase with segmental boundaries so that each parasegment contains approximately the posterior one third to one quarter of one segment and the anterior two thirds to three quarters of the adjacent segment.

Thus far, similar patterns of *wg* and *en* have been found in all four of the major arthropod groups: hexapods (including insects),(reviewed in Ref. 4) crustaceans,[(5–8)] myriapods (millipedes and centipedes)[(9)] and chelicerates (spiders, mites, scorpions and horseshoe crabs)[(10–12)] (Fig. 1). In all cases, *wg* stripes lie adjacent and anterior to stripes of *en* and these observations, together with functional studies in the flour beetle,[(13)] suggest that in all these groups the *wg–en* interaction, and hence the parasegment, is conserved. Some interesting differences are observed, such as the apparent spatial division of the roles played by *wg* in *Drosophila* among multiple *Wnt* genes in some crustaceans and spiders,[(6,11,14)] but the fact that expression patterns of *wg* and *en* appear to be shared by all four groups strongly suggests that at least certain aspects of the segment polarity level of the *Drosophila* segmentation hierarchy were part of the ancestral arthropod segmentation mechanism.

Such conservation has not yet been observed at the pair-rule level of the hierarchy. In contrast, variation in the expression of particular pair-rule genes, both subtle and gross, is found among even insects (see below). Before discussing this variation, it is instructive to ask what is meant when it is claimed that a particular gene is ''pair-rule''. The answer, it turns out, depends on whether the claim refers to the gene's expression pattern, its function, or both.

As mentioned above, ''pair-rule'' was originally a genetic classification based on a gene's loss-of-function.[(3)] The subsequent cloning and characterization of the pair-rule genes revealed that, consistent with their phenotypes, most of these genes are expressed in stripes of a two-segment periodicity in both the syncytial and cellular blastoderm. Such ''pair-rule patterns'' of expression represent the first periodic gene expression in the developing *Drosophila* embryo and are set up by the spatial pattern of maternal coordinate and gap gene expression.

The pair-rule genes that have thus far been examined outside of *Drosophila* are *even-skipped* (*eve*), *hairy, runt, fushi-tarazu* (*ftz*) and *paired* (*prd*). In *Drosophila, hairy* and *runt* are expressed in the early blastoderm in complementary

[1]Department of Ecology and Evolutionary Biology, Princeton University.
[2]Howard Hughes Medical Institute, Dept. of Organismal Biology & Anatomy, University of Chicago.
*Correspondence to: Nipam H. Patel, HHMI MC 1028, University of Chicago, 5841 S. Maryland Ave., Chicago, IL 60637.
E-mail: npatel@midway.uchicago.edu
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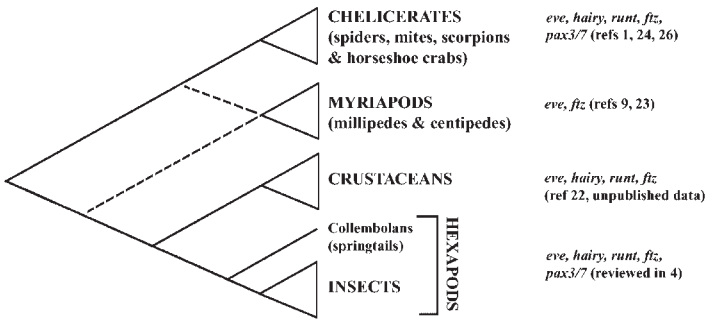

Figure 1. A phylogeny of the four major arthropod groups based on molecular studies,[38–40] indicating the pair-rule orthologues that have been investigated in each group. Molecular phylogenies differ over whether myriapods are more closely related to crustaceans + hexapods or to chelicerates.

patterns, each consisting of seven stripes of a two-segment periodicity. Similarly, *eve* and *ftz* are also found in complementary seven-stripe patterns. Unlike *hairy* and *runt*, the *eve* and *ftz* stripes loosely obey the boundaries of future parasegments and are centered on the odd- and even-numbered parasegments, respectively. Finally, *prd* is also found in seven pair-rule stripes in the early blastoderm and, like *ftz*, these stripes are centered on even-numbered parasegments, but in this case extend across the position of future parasegmental boundaries. Although the "pair-rule pattern" of expression is intuitively consistent with a pair-rule phenotype, it is important to note that, following gastrulation, *eve*, *runt* and *prd* are additionally expressed in stripes of a one-segment periodicity, coinciding temporally with the early expression of segment polarity genes.

Pair-rule genes in insects other than *Drosophila*

Among holometabolous insects (including flies, moths, bees and beetles), pair-rule genes are expressed in mostly conserved patterns. (reviewed in Ref. 4) Based on such pair-rule patterns of expression, the majority of studies have tended to infer, either explicitly or implicitly, that a particular gene also performs a pair-rule function. Any such inference to function, however, must be made with caution. In the flour beetle *Tribolium*, for example, a deletional mutant of the Hox complex that includes *ftz* does not exhibit any obvious segmentation defects,[15] indicating that at least this gene is functioning differently to its *Drosophila* orthologue. Despite this result, we know that pair-rule patterning is likely to be a critical aspect of segmentation in *Tribolium*. Genetic screens using cuticle preparations have yielded at least one and perhaps two pair-rule mutants,[16,17] and, in the case of *Tribolium eve*, chromophore-assisted laser inactivation of Eve protein results in a pair-rule phenotype, indicating that the pair-rule function of this gene is conserved.[18]

Among hemimetabolous insects (regarded as phylogenetically primitive relative to holometabolous insects), evidence of pair-rule patterning has thus far been limited to the short-germ grasshopper *Schistocerca*, in which a *prd* homologue, *pairberry1* (*pby1*), is transiently expressed in stripes of a two-segment periodicity before resolving into a segmental pattern[19] (Fig. 2C). In contrast, orthologues of *eve* and *ftz* are not expressed in periodic stripes in the embryo, but in broad posterior domains,[20,21] suggesting that these genes play altogether different roles in grasshoppers.

Pair-rule genes in non-insect arthropods?

Among non-insect arthropods, divergent expression of pair-rule orthologues appears to be more prevalent. *ftz* expression has thus far been examined in the barnacle crustacean *Sacculina carcini*,[22] the centipede *Lithobius atkinsoni*,[23] and the mite *Archegozetes longisetosus*.[24] In contrast to insects, *ftz* in mites is expressed not in stripes, but in a Hox-like domain consistent with the position of this gene in the arthropod Hox cluster. In centipedes, *ftz* is expressed in a similar Hox-like pattern, as well as a posterior domain that gives rise to transient segmental stripes. Thus, with regard to *ftz*, there seems to have been an evolutionary transition from a Hox-like pattern to a striped pattern of expression.[23,25] As yet, however, there is no convincing evidence of a pair-rule expression pattern for this gene outside of insects.

The additional data that we have concern orthologues of the pair-rule genes *eve*, *runt* and *hairy*. In crustaceans, these genes are expressed in segmental stripes, but without any obvious pair-rule pattern (N. H. Patel, M. Duman-Scheel, W. E. Brown, M. Gerberding, unpublished data). In the spider *Cupiennius salei*, *eve*, *runt* and *hairy* all show some form of striped expression, suggesting an ancient role in segmentation for these genes. In particular, *eve* and *runt* are both transiently expressed in stripes that arise in newly formed segments at the posterior, while *hairy* is expressed in a broad posterior domain that is periodically cleared, resulting in stripes.[26] Importantly, it is not yet clear whether these stripes exhibit any sort of two-segment periodicity, though this has been suggested.[26] The pattern of *hairy* expression in the spider is similar in several respects to *eve* expression in the centipede *L. atkinsoni*, where a broad posterior domain in

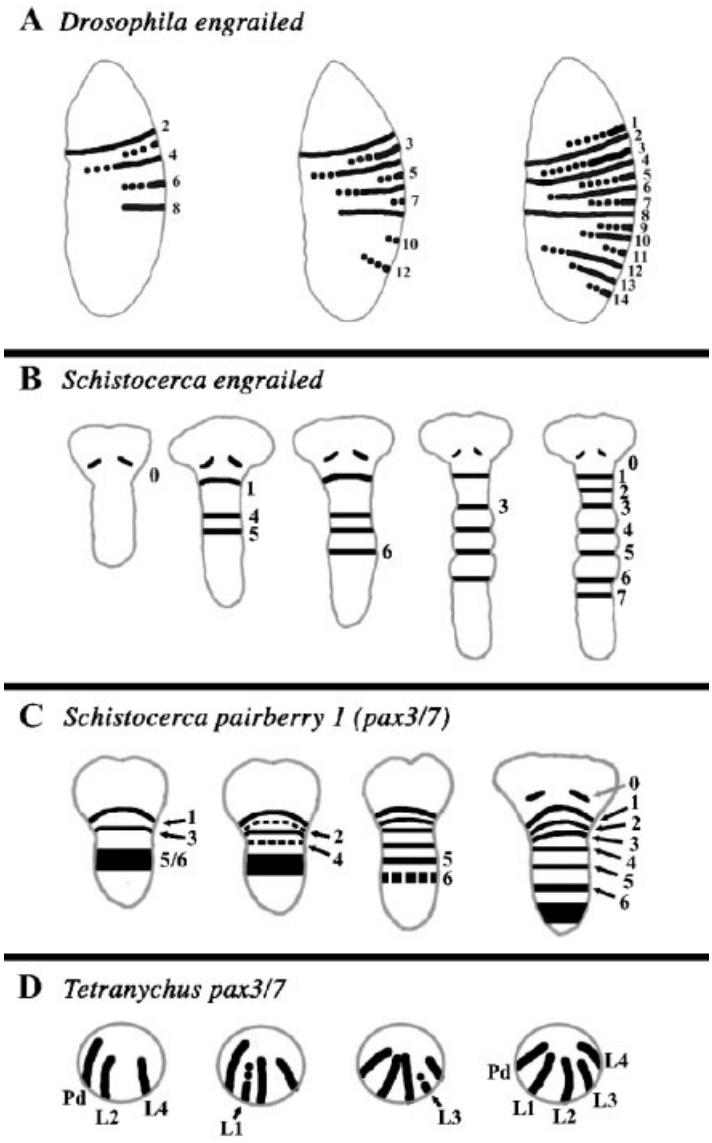

Figure 2. The order of appearance of early stripes of the segment polarity gene *en* and the *pax 3/7* genes. **A:** *en* stripes in blastoderm embryos of *Drosophila* appear in an anterior-to-posterior progression with a temporal pair-rule modulation presumably reflecting regulation by pair-rule genes. **B:** The order of appearance of *en* stripes 1–6 in early embryos of the grasshopper *Schistocerca*[19] (17–20% of development) deviates from the strict anterior-to-posterior sequence found in the abdomen (stripes 7–16, not shown), though in this case the deviation is not obviously pair-rule. **C:** Stripes of the *pax 3/7* gene *pby1* in *Schistocerca*[19] (16–17% of development). From T2 (stripe 5) on posterior, there is a clear pair-rule pattern to *pby1* expression, with broad pair-rule stripes resolving into segmental stripes (only shown for the pair-rule stripe giving rise to segmental stripes 5 and 6). In the anterior part of the embryo, however, there is a temporal modulation with stripes 2 and 4 appearing later than stripes 1 and 3. **D:** *Tu-pax3/7* in blastoderm embryos of the mite *Tetranychus urticae*.[1] Posterior to the L4 segment (stripe 5), Tu-pax 3/7 stripes are segmental and appear in an anterior-to-posterior sequence (not shown). In the anterior portion of the early embryo, however, the stripes of L1 and L3 are delayed in a manner that may reveal underlying pair-rule patterning. *Drosophila* embryos are oriented with anterior up, dorsal to the left, *Schistocerca* embryos are ventral views with anterior up, and mite embryos are oriented with dorsal up, anterior to the left. For *Drosophila* and *Schistocerca*, 1, mandibular; 2, maxillary; 3, labial; 4–6, thoracic segments T1–T3; 7–16, abdominal segments A1–A10. For the mite, Pp, pedipalp segment; L1–L4, walking leg segments.

the growth zone resolves into stripes that persist transiently in newly formed segments.[9] Although the latter is reported not to exhibit a pair-rule pattern,[9] both this and the expression of spider *hairy* warrant closer analysis.

Despite the "negative evidence" described above, it would be premature to assert that pair-rule patterning is altogether absent from basal arthropods. Indeed, a recent examination of *runt* and a *prd*-like gene in the two-spotted spider mite *Tetranychus urticae* may suggest the opposite.[1] In this mite, both genes are expressed in segmental ectodermal stripes. Prior to its striped pattern, *Tu-runt* is also expressed in bilateral rings that surround the presumptive limb buds. Most intriguingly, the *prd*-like gene *Tu-pax3/7* is expressed in prosomal stripes that exhibit a temporal pair-rule modulation. That is, the appearance of stripes in segments of the 1st and 3rd walking legs (likely homologues of the gnathal segments in the insect head) are delayed relative to stripes in adjacent segments[1] (Fig. 2D).

While not implying a pair-rule patterning function for *Tu-pax3/7* per se, such a pair-rule modulation may reflect regulation by yet unidentified genes acting in true pair-rule fashion.[1] Indeed, the pattern of initiation of many segment polarity genes in *Drosophila* shows a pair-rule periodicity. For example, even-numbered stripes of *en* appear slightly before adjacent odd-numbered stripes along the length of the *Drosophila* embryo (Fig. 2A). This pattern in *Drosophila* is thought to reflect the underlying regulation of segment polarity genes by pair-rule genes. In this regard, it is worth mentioning

that *Tu-pax3/7* appears to be an ancestral member of the Pax3/7 family of transcription factors, which in *Drosophila* includes not only the pair-rule gene *prd*, but also the segment polarity gene *gooseberry*. It should be noted, however, that the delay of two alternate stripes in the spider mite head does not reflect a general trend across the entire body axis and thus may be insufficient grounds upon which to infer an underlying pair-rule mechanism. In insects and crustaceans, stripes of the head, as opposed to the thorax and abdomen, typically do not appear in strict anterior-to-posterior sequence. Examples include the expression of *en*, *wg* and *pby1* in grasshopper[19,27] (Fig. 2B,C), the exact order of appearance of *en* stripes in particular having been shown to be evolutionarily labile. However, the fact that *Tu-pax3/7* is not expressed in stripes of a two-segment periodicity along the entire body axis does not necessarily preclude a pair-rule function for this gene. For example, in *Drosophila*, *prd* is responsible for activating and defining the posterior border of odd-numbered *en* stripes,[28,29] but it remains unclear whether this pair-rule function derives from the early pair-rule pattern of *prd*, or is instead restricted to the odd-numbered stripes of *prd*'s later segmental pattern.[30]

Taken together, the observations in various arthropods suggest that orthologues of some, but not all, *Drosophila* pair-rule genes are likely to have played an ancestral role in arthropod segmentation. What is not yet clear is whether this ancestral role included the pair-rule patterning function observed in some insects.

Pair-rule genes in vertebrates?

In 1996 a good deal of excitement was generated when it was reported that, in zebrafish embryos, transcripts of *her1*, a homologue of the *Drosophila* pair-rule gene *hairy*, localize to presumptive alternating somites in the presomitic mesoderm.[31] This report of a pair-rule expression pattern in zebrafish subsequently led to the suggestion that the common ancestor of protostomes and deuterostomes might have been segmented.[32,33] A more recent study of *her1* expression[34] revealed that the gene is expressed in a cyclical pattern similar to the expression of another *hairy*-related gene from chick, *c-hairy1*.[35] Using *MyoD* as a marker, it was determined that the stripes resulting from this cyclical pattern in fact correspond to every somite, rather than every other somite.[34]

Controversy, however, is often resilient. *her1*, it turns out, is expressed in largely overlapping patterns with a closely related gene, *her7*.[2,36] Disruption of either *her1* or *her7* function using antisense morpholinos results in segmentation defects, while both morpholino-mediated double "mutants" and a deficiency removing both genes show even more dramatic phenotypes, suggesting that *her1* and *her7* are partially redundant.[2,36,37] Detailed descriptions of aberrant somites in *her1*+*her7*-deficient embryos by Henry et al. reveal enlarged somites with a periodicity of one and a half to two somites relative to wild-type embryos.[2] These enlarged somites are often interrupted by weak somite boundaries, suggesting that these large somites might be equivalent to pairs of normal somites. Indeed, similar large somites are found in *her7*-deficient embryos and, in these embryos, it was observed that, although the normal number of somite boundaries begin to form, abnormally large somites are produced because only alternate boundaries are strengthened[2] (Fig. 3).

Although weak boundaries and strong boundaries tend to alternate, the pattern is by no means strictly pair-rule: normal somites are sometimes found between two large somites and large somites are not always the same size. Furthermore, because zebrafish vertebrae are relatively uniform, it is not yet possible to confirm that, like *Drosophila* pair-rule mutants, it is alternating segment identities that are missing.[2] Nevertheless, this intriguing result suggests that, in addition to other mechanisms such as the oscillator, there may be pair-rule mechanisms at work in the zebrafish.

Concluding remarks

Thus far, there is no conclusive evidence in favor of pair-rule patterning in basal arthropods. At present, we still favor the hypothesis that pair-rule patterning is a derived mode of segmentation utilized by insects. The relative scarcity of gene expression data and especially of functional data from basal arthropod groups, however, requires that the hypothesis be provisional, knowing that the pace of work in this area will soon lead to more definitive conclusions.

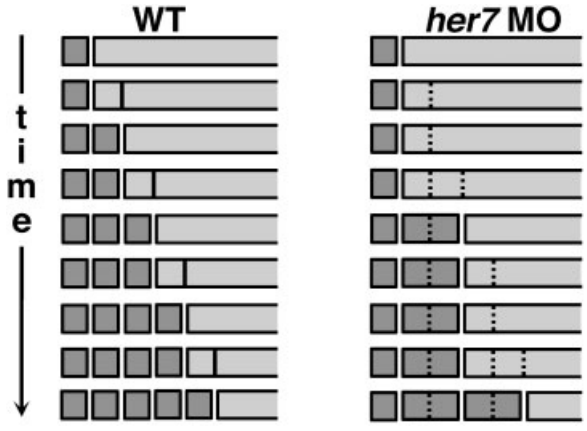

Figure 3. Formation of normal somites in wild-type embryos versus aberrant somites in zebrafish embryos injected with *her7* morpholino. In *her7*-deficient embryos, all somite boundaries initiate, but only alternate boundaries are strengthened and completed.[2] Somites are dark grey, presomitic mesoderm is light grey. Solid and dashed lines indicate forming strong and weak somite boundaries, respectively. In *her7*-deficient embryos, the somites that do form are roughly the size of two wild-type somites.

While the exact nature of *her1* expression seems to be resolved in favor of a segmental, rather than pair-rule, pattern, this does not necessarily preclude all pair-rule function for this gene. The "alternate segments strengthened" result of Henry et al. does suggest that zebrafish *her* genes may play a role reminiscent of the pair-rule patterning observed in *Drosophila*, though it is still unclear whether this is an independently evolved function, or constitutes evidence for the shared ancestry of vertebrate and arthropod segmentation. This debate is certain to continue for some time.

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