

Insect segmentation: Genes, stripes and segments in 'Hoppers'

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Recent work has revealed that orthologues of several segmentation genes are expressed in the grasshopper embryo, in patterns resembling those shown in *Drosophila*. This suggests that, despite great differences between the embryos, a hierarchy of gap/pair-rule/segment polarity gene function may be a shared and ancestral feature of insect segmentation.

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A defining feature of insects — indeed, of all arthropods — is the subdivision of the body into repeating segmental units. Some 20 years of molecular genetics have produced a good understanding of how segments develop in *Drosophila* — but is this valid for all insects, or has the mechanism of segmentation changed radically during their evolution? Now we have intriguing clues from recent studies of grasshopper embryos [1–3], which suggest that the underlying mechanisms of insect segmentation may be more conserved than has been thought to be the case.

The insect egg is typically large, with a central yolk mass within which the nuclei undergo early divisions. They move to the peripheral cytoplasm which is then transformed from a syncytium into the blastoderm cell layer. Insects vary significantly in how the segmented embryo is formed [4]. In long-germ development, as in *Drosophila*, most of the blastoderm contributes to the embryo and the segments form early and more-or-less simultaneously. In short-germ insects, such as the grasshopper, by contrast, the embryo arises from a small region of the cellular blastoderm and forms segments sequentially as its anterior–posterior axis is extended by growth.

Segmentation in *Drosophila*

In *Drosophila*, pattern formation starts during oogenesis, with maternal gene expression and the localisation of transcripts at anterior (*bicoid*) and posterior (*nanos*) ends of the oocyte [5]. Translation and diffusion result in gradients of Bicoid and Nanos protein in the early egg, and these then repress the translation of other maternal transcripts — *caudal* and *hunchback*, respectively — generating reciprocal gradients of those proteins (Figure 1). The gradients act as positional cues which directly regulate a hierarchy of gap, pair-rule and segment polarity genes, the localisation of which progressively subdivides the embryo into segments [5,6].

The gap genes are the first of the segmentation hierarchy to be transcribed. The *hunchback* gene is activated anteriorly, by Bicoid protein, and other gap genes are expressed at the ends of the embryo. The gap proteins, all transcription factors, diffuse in the syncytial cytoplasm of the early embryo, forming a set of overlapping gradients that position other components of gap gene expression, such as a central *caudal* band and a posterior *hunchback* band. Gap proteins control the next phase: the expression of pair-rule genes (Figure 1).

The pair-rule genes are transcribed in seven broad stripes. Some of these genes — for example, *even-skipped* (*eve*) and perhaps *paired* — are controlled directly, by gap protein distribution. Each stripe reflects gene activation, through an independent enhancer, by a different combination of gap protein concentrations. Thus, the periodic pair-rule expression pattern is modular — a regularly spaced montage of specific responses to the set of aperiodic cues. The pair-rule genes mostly encode transcription factors and they modulate each other's expression, so that the pattern of both *eve* and *paired* changes to fourteen narrow segmental stripes (Figure 1).

The stripe patterns of the different pair-rule genes are out of register and, as the blastoderm becomes cellular at around three hours, the overlaps activate segment polarity genes in a narrow stripe within each of the gnathal (mouthpart), thoracic and abdominal segments (Figure 1). The segment polarity genes mostly encode transcription factors — such as the *engrailed* and *gooseberry* gene products — or components of the Wingless and Hedgehog cell signalling pathways. The products of the segment polarity genes, acting within the now-cellular embryo, mediate the cell interactions that establish segment borders and control the anterior–posterior pattern within each segment.

Drosophila and other insects

Orthologues of *Drosophila* segmentation genes have been investigated in several other insects. In general, similar expression patterns have been found in all long-germ embryos that have been studied — such as flies (Diptera) and beetles (Coleoptera) — and even in the well-studied short-germ beetle *Tribolium* [7–9]. The short-germ grasshopper, *Schistocerca* (Orthoptera) was found to express *engrailed* in segmental stripes [10], but orthologues of two pair-rule genes, *eve* [11] and *ftz* [12], were expressed in the posterior tip of the embryo and not in pair-rule stripes. Thus, although the mechanism may be conserved in the more recently derived, holometabolous orders, it seemed that segmentation occurs differently in more basal insects,

Figure 1

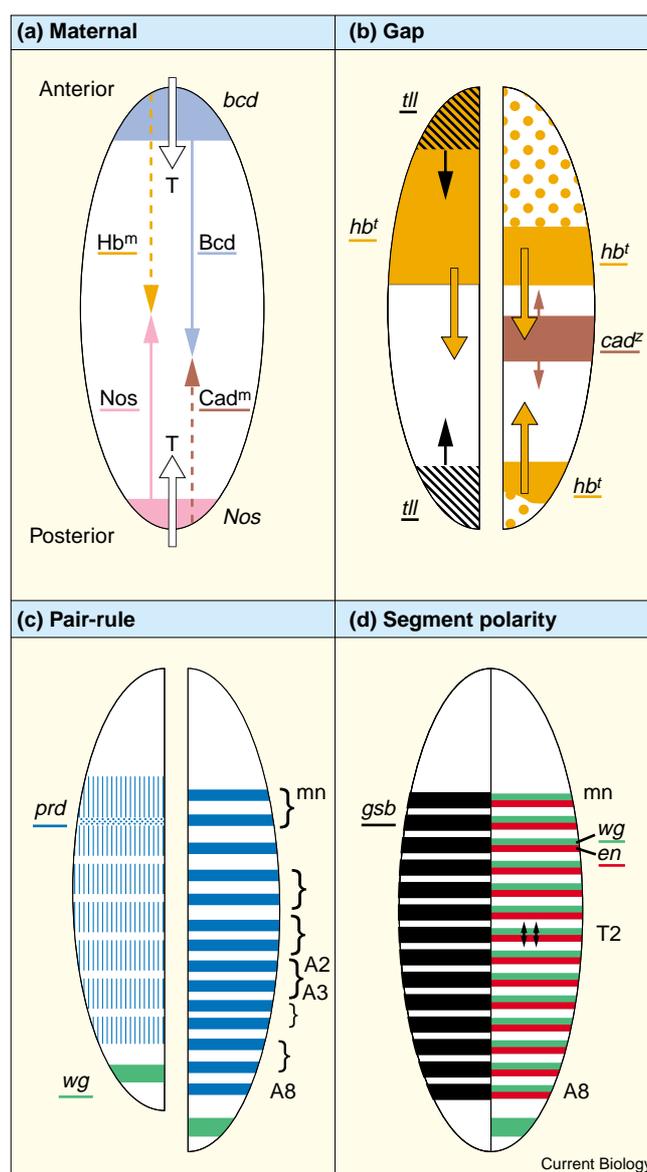
Segmentation gene expression in *Drosophila*. Eggs are shown in a ventral view, with anterior at the top. (a) Maternal gene products at stage 3 (early syncytial blastoderm). Maternal *bicoid* (*bcd*) and *nanos* (*nos*) transcripts were localised to anterior and posterior ends during oogenesis. After translation, diffusion gives anterior and posterior gradients of the Bcd and Nos proteins (arrows). Through specific translational repression, this results in anterior hunchback (Hb^m) and posterior caudal (Cad^m) protein gradients (dashed arrows). Also shown (by thick arrows) are terminal transcription factor gradients (T) resulting from other maternal products localised at both ends of the egg. (b) Gap gene expression at stage 4. Left: in the mid-syncytial blastoderm, *tailless* (*tll*) is activated by the terminal cues, and *hunchback* (*hb^t*) is expressed anteriorly, in response to high levels of bicoid. The resulting proteins diffuse, forming gradients (arrows). Right: later, *caudal* (*cad^z*) is activated medially and *hunchback* posteriorly, by maternal and gap proteins. As before, proteins diffuse in the syncytium. Later, expression of *hunchback* fades in terminal regions (shown by dots). (c) Expression of the pair-rule gene *paired* (*prd*). Left: at stage 5 (cellularisation), *prd* is expressed in seven broad stripes. Also, *wingless* (*wg*) expression appears at the posterior and anterior (not shown) ends of the embryo. Right, later, at stage 6 (early germ band extension), *prd* is expressed in 14 segmental stripes, from the mandibular (mn) to the 8th abdominal (A8) segment. With the exception of the second, each of the pair-rule stripes has resolved into two segmental stripes (bracketed). (d) Segment polarity gene expression at stages 6/7 (extending germ band). Segmental stripes of *gooseberry* (*gsb*) and of *wingless* (*wg*) and *engrailed* (*en*) are shown on the left and right, respectively. Further stripes appear later, in the anterior of the head, without being preceded by pair-rule gene expression. The *wingless* gene encodes a secreted signal that mediates cell interactions, indicated by small arrows on only the mesothoracic (T2) stripe.

such as orthopterans. But this picture is changing as we learn about other segmentation genes.

Segmentation gene expression in the grasshopper

In *Schistocerca*, as in *Drosophila*, both *hunchback* and *caudal* are first transcribed during oogenesis. At least for *hunchback*, translation then occurs and the protein becomes localised posteriorly in the oocyte [1]. In the early egg, cells are formed first at the posterior end, where they aggregate to form the small embryonic primordium; elsewhere, nuclei reach the surface later and the resulting cells form extra-embryonic serosa [13]. Both *hunchback* and *caudal* transcripts are found initially throughout the embryo and then a reciprocal pattern develops: *caudal* expression becomes restricted to the posterior tip, where it remains until segmentation is completed [2], while *hunchback* expression is lost from the posterior end [1].

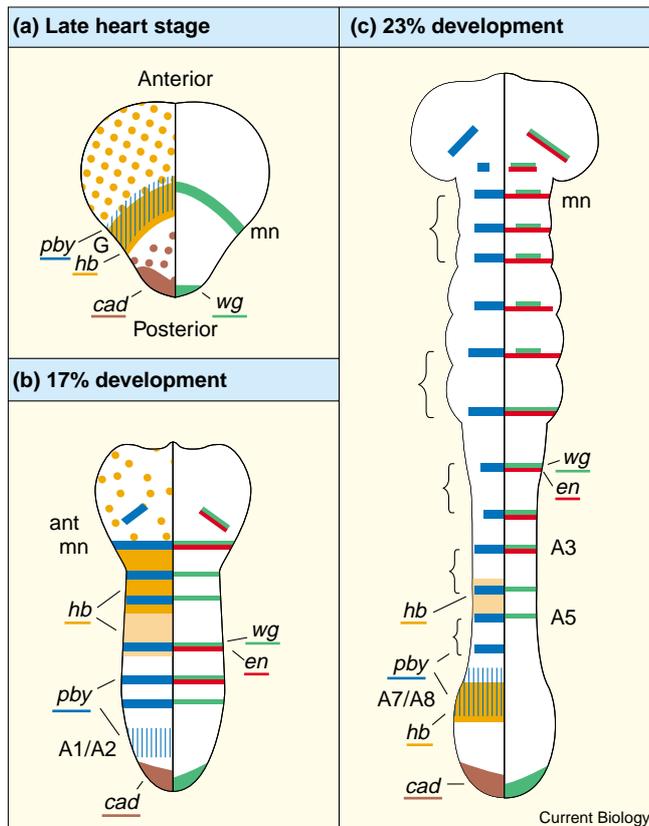
By the ‘heart-stage’ (see Figure 2), embryonic *hunchback* expression is elevated in a broad central arc; subsequently, the levels of *hunchback* transcript (and protein) resolve into two distinct bands, across the future gnathal and prothoracic segments. Later, this anterior expression fades and two further transient bands appear, in the future mid and then posterior abdominal segments [1]. Clearly, *Schistocerca* shows some similarities to *Drosophila* in the maternal and



the embryonic (gap) expression patterns of both *caudal* and *hunchback* (Figures 1,2). Furthermore, unlike *eve* and *ftz*, a grasshopper orthologue of the fly pair-rule gene *paired* does show a pair-rule expression pattern [3].

The *Schistocerca* genes *pairberry1* and *pairberry2* are orthologues of the small *Drosophila* gene family that includes *paired* (and the segment polarity gene *gooseberry*). *Pairberry1* is first expressed at the posterior end of the early embryo and then, at the heart-stage, in a central arc that largely coincides with *hunchback* expression (Figure 2). Subsequently, the arc resolves into a thin stripe in each of the three gnathal segments, and *pairberry1* expression appears in a new prothoracic stripe and in a broad posterior stripe. As the embryo grows, the broad stripe resolves into two thin stripes, in future mesothoracic and metathoracic segments, and another broad posterior stripe appears. This

Figure 2



Segmentation gene expression in the grasshopper *Schistocerca*. Only the embryos are shown, in ventral view, with anterior at the top. (a) At late heart stage, the embryo is already cellular and *hunchback* (*hb*) and *pairberry1* (*pby*) are strongly expressed in arcs in the future gnathal region (G); *hb* is also expressed anteriorly and there is a posterior gradient of *caudal* (*cad*) expression. Right: *wingless* (*wg*) is expressed in a thin arc in the future mandibular segment (mn), at the posterior tip and in an anterior region (not shown). (b) By 17% of development time, *hb* expression has resolved into high and lower level bands (indicated by colour intensity) and *pby* is expressed in thin stripes in antennal (ant), gnathal and thoracic segments, and in a broad posterior stripe (A1/A2) that will resolve later into thin stripes in the 1st and 2nd abdominal segments. Right: further stripes of *wg* have appeared, accompanied in some segments by adjacent expression of *engrailed* (*en*); *wg* and *cad* are still expressed in the posterior tip of the growing embryo. (c) By 23% of development time, the anterior *hb* bands have faded, but there are two further bands of expression in the future abdomen. *pby* is now expressed in thin stripes in segments down to the 6th abdominal, and in a broad posterior stripe (A7/A8). Pairs of segmental stripes originating from one earlier pair-rule stripe are bracketed. In the older stripes, *pby* is restricted to the ventral part (which also expresses the related *pairberry2* gene). Right: segmental stripes of *wg* and *en* now extend to the 5th (A5) and 3rd (A3) abdominal segments, respectively. *wg* and *cad* are still expressed in the posterior tip. (Illustration derived from data from *S. americana* [1,3] and *S. gregaria* [2].)

process is repeated down the developing abdomen, sequentially forming pairs of segmental stripes of *pairberry1* expression [3]. The expression of *pairberry1* in *Schistocerca* thus

closely resembles that in *Drosophila* of *paired* and then *gooseberry* (Figures 1,2), although, curiously, the pair-rule phasing is different — for example, one broad stripe resolves into abdominal stripes 1 and 2 in *Schistocerca*, but stripes 2 and 3 in *Drosophila*.

The *engrailed* orthologue is expressed in segmental stripes in the grasshopper embryo [10], and the same has now been shown for another segment polarity gene, *wingless* [2]. The first (mandibular) stripe appears at heart-stage, at the anterior edge of the *hunchback* and *pairberry1* arcs, and there is also early and persistent *wingless* expression at the posterior tip of the embryo (Figure 2). Further segmental stripes then appear in the thorax, in the other gnathal segments and sequentially down the abdomen — always after *pairberry1*. The *wingless* stripe is immediately anterior to the *engrailed* stripe, as in *Drosophila*, but, in *Schistocerca*, the expression of *wingless* starts well before that of its neighbour [2].

Mechanisms of insect segmentation

Earlier studies [11,12] suggested that *Schistocerca* forms segments without a pair-rule phase of subdividing tissue — perhaps by cycles of short-range cell interactions behind the growing posterior tip [10]. Indeed, as long-germ development occurs only in the more recently derived orders, such as the Dipteran and Coleoptera, pair-rule patterning might have originated during their evolution. The new results [1–3] are very important, however, in demonstrating that orthologues of some *Drosophila* segmentation genes do contribute to a temporal sequence of bands, pair-rule and segmental stripes. This sequence appears, as in the short-germ beetle *Tribolium*, in successively more posterior regions as the embryo grows.

The *Schistocerca* gene expression patterns are certainly suggestive and can mostly be interpreted in terms of the *Drosophila* mechanism. Hence the posterior loss of embryonic *hunchback* expression could follow from translational repression by Nanos (the *hunchback* transcript has an appropriate sequence motif for such control [1] and *Schistocerca* has maternal *nanos* expression [14]). This could restrict *caudal* expression to the posterior end of the embryo. Gap bands of *hunchback* expression could provide spatial cues for pair-rule expression of *pairberry*, which could then be involved in the control of *wingless* and *engrailed*. This interpretation would suggest that the hierarchy of gap/pair-rule/segment polarity gene function is a shared and ancestral feature of insect segmentation. There are, however, a number of caveats to this view.

Firstly, an expression pattern may be consistent with a particular gene function, but proof requires experiments, as in the recent manipulations of *Tribolium eve* (by protein inactivation [15]) and *wingless* (by forced ectopic expression

[16]). Secondly, in the *Drosophila* embryo, elaboration of spatial pattern depends on the diffusion of proteins, such as Hunchback, in the syncytial cytoplasm: a graded distribution forms and new expression borders are set by enhancer response thresholds. For this mechanism to operate, the cellular embryo of *Schistocerca* (or, indeed, *Tribolium*) would require either novel cell junctions to pass large proteins, for which there is no evidence, or an intermediate step whereby a transcription factor affects nearby cells through triggering a signalling pathway [7–9]. It is notable that the *wingless* signal is produced at the *hunchback* gnathal band (and at the posterior tip), and perhaps other ligands signal from other bands of gap gene expression.

Thirdly, even if a basic hierarchy of segmentation genes is common to long-germ and short-germ insects, there are evident differences in the genes involved and their regulatory relationships. Thus *eve*, which has pair-rule expression and function in *Drosophila* and *Tribolium*, is unstriped in *Schistocerca* [11], but expressed in segmental stripes in the related cricket *Acheta* [9]. Similarly, *eve* expression varies greatly between closely related wasp species, again suggesting considerable flexibility in the mechanism of segmentation [17]. Finally, *Schistocerca* is but one short-germ orthopteran whose similarities to the more derived insects could be convergent. Evolutionary conclusions about an ancestral state require the study of segmentation in more members of the Orthoptera and other basal insect orders.

By broad comparative analysis, we can hope to trace the evolution of segmentation. Beyond the insects, it will be fascinating to find shared features in the other arthropods, which segment very differently — with no initial syncytial stage and often with a defined cell lineage [10]. These are very early days, but segmental *engrailed* stripes are known in crustaceans [10] and in a chelicerate, which has also been found to exhibit early, transient and striped expression of orthologs of several *Drosophila* pair-rule genes [18], including *eve*!

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