

# Evolution of Development: Beyond Bicoid Dispatch

Jeremy Lynch and Claude Desplan

The Bicoid-based anterior patterning system of *Drosophila* embryogenesis appears to be unique to higher dipterans. A new study suggests how this may have evolved out of an alternative mechanism based on cooperating Orthodenticle and Hunchback proteins, the two mechanisms intersecting at the level of downstream target genes.

One of the first steps in the development of an organism with a bilaterally symmetrical body plan is the establishment of the antero-posterior axis. Although little is known about this process in most animals, extensive studies in *Drosophila* have identified Bicoid (Bcd) as the primary anterior determinant. During *Drosophila* oogenesis, *bcd* mRNA is provided by the mother and localized at the anterior pole of the egg. Upon fertilization, *bcd* mRNA is translated and the Bcd protein diffuses along the antero-posterior axis in the syncytial embryo to form a morphogenetic gradient. Bcd functions by activating its target genes in a concentration-dependent manner, and by preventing translation of *caudal* (*cad*) mRNA at the anterior end of the embryo [16]. In the absence of Bcd, anterior segments — the head, thorax and some abdominal segments — fail to develop, and anterior terminal structures are transformed to a posterior fate as a result of ectopic Cad activity.

Despite the absolute requirement for Bcd in setting up the antero-posterior axis in *Drosophila*, there is growing evidence that Bcd is a unique character possessed only by a highly derived clade of dipteran flies [1–6]. The ability to test hypotheses about the evolution of development afforded by the wide applicability of RNA interference (RNAi) has now been exploited by Schröder [7] to investigate how the equivalent functions to those mediated by Bcd in *Drosophila* are carried out in other insects. Schröder [7] has found that orthologs of two identified targets of the Bcd gradient in *Drosophila* cooperate to perform most of Bcd's functions in the red flour beetle *Tribolium castaneum*.

Despite the elegance of the Bcd system for setting up the antero-posterior axis in *Drosophila* and related flies — which have what is known as a 'long germband' style of development — such an anterior morphogenetic gradient would not be a practical mechanism in many insect groups. For example, in insects that undergo 'short germband' development, the cells fated to become the embryo are located at the posterior pole. Furthermore, in these species, most patterning takes place in a cellular environment, rather than a syncytium

as in *Drosophila*, so that a gradient emanating from the anterior pole, such as that formed by Bcd, would be highly inefficient.

It is now believed that Bcd only occurs in a highly derived clade of higher dipterans. The most convincing evidence for this comes from genomic data. The *bcd* locus resides near *zen* in the *Drosophila* Hox complex, where *Hox3* paralogous genes are normally found [8]. The *bcd* gene appears to have arisen by tandem duplication of an ancestral *zen*-like gene somewhere along the lineage leading to higher flies (Figure 1) [6]. No *bcd* ortholog can be found at the equivalent position in the *Tribolium* Hox complex [9] or even in lower flies [3]. Furthermore, *bcd* appears to be absent from the genome of the lower dipteran, *Anopheles gambiae*. These data strongly indicate that *bcd* is a recent addition to the lineage leading to *Drosophila*. The implication is that another mechanism for setting up the antero-posterior axis must exist in the rest of the insects [2,4].

Insights into the nature of this mechanism came from experiments in which *Drosophila bcd* mutants were rescued by manipulating the levels of other conserved maternal genes that normally play minor roles in this species. For example, maternal *hunchback* (*hb*) activity is able to rescue loss of *bcd* function in the abdomen and the thorax [10,11], and Hb protein also strongly contributes to the activation of the anterior genes by synergistic interaction with Bcd [1]. But maternal *hb* is clearly not sufficient to replace *bcd* function in the head. This suggests that *hb* is part of the ancestral patterning system, but that other factors must contribute to head formation. In *Drosophila*, *bcd* has likely taken over the functions of the ancestral morphogens, perhaps by directly controlling their zygotic expression as in the case of *hb*.

A prime candidate for this additional factor is Orthodenticle (Otd). In *Drosophila*, *otd* is the most anterior head gap gene, and is expressed only zygotically under the control of the Bcd gradient. The role of *otd* in

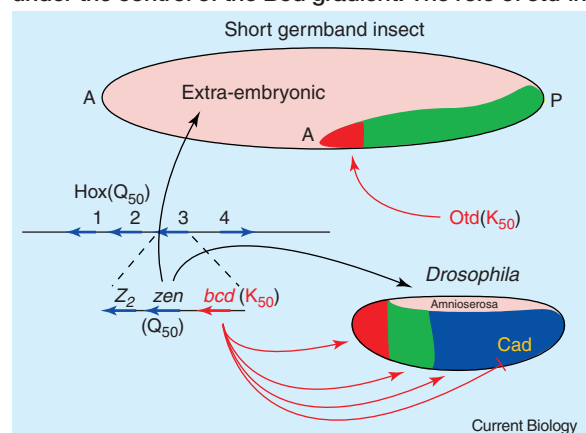


Figure 1. A model for the evolutionary replacement of an Otd-dependent anterior patterning mechanism by Bcd in higher flies (see text for details).

Department of Biology, New York University, New York, New York 10003, USA.  
E-mail: claude.desplan@nyu.edu

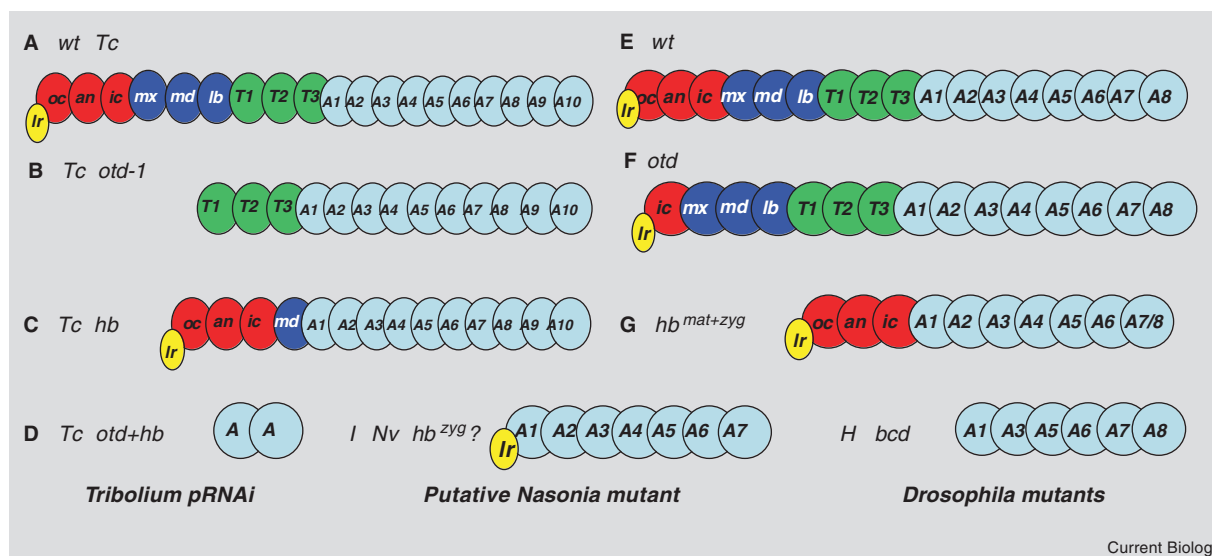


Figure 2. A comparison of the effects of inactivating *otd* and *hb* by mutation or RNAi in *Tribolium*, *Drosophila* and *Nasonia*. (A,E) Wild-type body plans of *Tribolium* and *Drosophila*, respectively. (B–D) Severe classes of *Tribolium* RNAi experiments. (F–H) Phenotypes of *Drosophila* mutants. (I) Phenotype of a putative zygotic loss-of-function mutant in the wasp *Nasonia*. Abbreviations: Ir, labral; oc, ocular; ic, intercalary; mx, maxillary; md, mandibular; lb, labial.

specifying the most anterior part of the embryo is well conserved in bilaterally symmetrical animals — the bilateria — from flies to mammals. Like Bcd, Otd is a homeodomain transcription factor, although from a different family as it is not derived from the Hox complex. But Bcd and Otd both have a lysine residue at position fifty in their homeodomain, and this results in the two proteins having DNA binding specificities that are identical and clearly distinct from those of most other homeoproteins, including Zen, which bear a glutamine at this position.

A model can be proposed for the evolution from an anterior patterning system based on *otd* and *hb* to one dependent on *bcd* (Figure 1). In lower flies and short germband insects, extraembryonic membranes arise from nuclei in the dorsal and anterior portions of the egg, whereas they are relegated to the extreme dorsal region in *Drosophila*. In the ancestral patterning system, these membranes might have been specified by maternally and zygotically provided Zen, while the anterior of the embryo might have been patterned by cooperating Hb and Otd, through a mechanism that remains to be fully elucidated. In the fly lineage, the *zen* gene was duplicated: one paralog gave rise to the *bcd* precursor gene which maintained the maternal aspect of the expression pattern, while the other — the extant *zen* gene — took on the zygotic ‘extraembryonic’ function, represented in *Drosophila* by its role in forming the amnioserosa [6,8].

In flies, the embryo eventually came to occupy the entire length of the egg, giving the *bcd* ancestor the opportunity to pattern embryonic tissue. A point mutation resulting in the substitution of glutamine by lysine at position 50 of the homeodomain would have caused a dramatic switch in DNA-binding specificity, allowing Bcd to usurp the function of Otd — as the the new form

of Bcd has the same DNA-binding specificity as Otd it can now activate Otd’s same target genes. Bcd became indispensable for anterior patterning by controlling the zygotic expression of both *hb* and *otd*, which then lost much of their maternal morphogenetic potential.

The beetle *Tribolium* has many features that make it a good organism in which to test the hypotheses implicit in the above model. *Tribolium* has well developed genetics, and many of its orthologs of *Drosophila* patterning molecules have been cloned and analyzed in great detail. One of the two *Tribolium otd* orthologs, *Tc-otd1*, is expressed in a similar manner to its *Drosophila* counterpart at blastoderm stage, forming an anterior stripe. But Schröder [7] found that, unlike *Drosophila otd*, *Tc-otd1* is expressed maternally throughout the embryo. Tc-Otd1 protein is lowered at posterior end of the *Tribolium* embryo, so as to form an antero-posterior gradient, by a mechanism that has not yet been elucidated, but which is reminiscent of the translational regulation of *hb* mRNA by Nanos protein observed at the same stage in flies. This posterior reduction of Tc-Otd1 may well be mediated by *Tribolium* Nanos, as a putative Nanos response element has been identified in the 3’ untranslated region (UTR) of *Tc-otd1* mRNA.

*Tc-hb* has an expression pattern somewhat reminiscent of *hb* in *Drosophila*. Maternal *Tc-hb* expression is uniform throughout the embryo, but at the blastoderm stage, zygotic *Tc-hb* expression resolves into two broad domains: an anterior cap and a posterior gap domain. The gap domain later splits into two stripes that lie within the gnathal and thoracic regions of the embryo [12]. The anterior cap domain coincides with tissue fated to become extraembryonic membranes, indicating that Tc-hb plays a role in patterning these structures. This finding is consistent with what is seen with

the *Schistocerca* and *Drosophila hb* orthologs [13]. Although such observations cannot definitively establish function, they fit nicely with the idea that Otd and Hb play a role in patterning the *Tribolium* embryo in the absence of a *bcd* gene.

RNAi has become a powerful technique for inactivating specific genes, and it appears to work well in *Tribolium*. Several *Tribolium* mutations have been phenocopied by injection of double-stranded RNA, allowing functional testing of the significance of expression patterns not associated with mutations. Furthermore, the ability to introduce double-stranded RNA into embryos by injecting it into the abdominal cavity of the pupae of their mothers — ‘parental RNAi’ [14] — allowed Schröder [7] to inactivate the very early function of the *Tc-hb* and *Tc-otd1* genes, including their maternal component. This type of experiment with *Tc-otd1* led to a high rate of defective embryos: The most commonly observed phenotype was the loss of all pre-natal head segments, along with the mandibular segment. In the most severe cases, all head and the first thoracic segments were missing. These phenotypes are much more severe than those seen in *Drosophila otd* mutants, and are more similar to those of weak *bcd* mutants (Figure 2).

As Hb strongly synergizes with Bcd in *Drosophila*, Schröder [7] also tested the phenotype caused by removing maternal and zygotic *Tc-hb* activity, either alone or in combination with *Tc-otd1*. When *Tc-hb* alone was disrupted, the most common phenotype was the loss of two gnathal head segments, as well as the three thoracic segments, with no effect on more anterior head segments, a phenotype reminiscent of that caused by loss of *hb* function in *Drosophila*. This phenotype overlapped with the most severe effects of *Tc-otd1* RNAi, indicating a possible interaction of the two factors in patterning head segments. When *Tc-otd1* and *Tc-hb* were inactivated simultaneously, most embryos were completely headless, lacking thorax and with only two to six abdominal segments left. The effect of the double knockdown is more severe than the sum of effects of inactivating the two genes separately, indicating that Tc-Otd1 and Tc-Hb have a synergistic relationship in patterning the axis of *Tribolium*, much as Bcd and Hb synergize in *Drosophila* axis formation.

It had previously been asserted that *Tribolium* must have a *bcd* ortholog, primarily based on the observation that a *Tc-cad* transgene is regulated in a Bcd-dependent manner in *Drosophila* [17]. Schröder’s [7] work shows that the morphogen function of Bcd is replaced by the combination Otd and Hb in *Tribolium*. Another factor must be invoked to perform the function of excluding Cad from the anterior of the *Tribolium* embryo, however, because posterior terminal structures are not seen at the anterior end in any of the RNAi experiments

This work demonstrates the critical importance of experiments with non-model systems, and in particular the power of RNAi, which means that those interested in the evolution of development can now test their hypotheses, rather than having to rely on mere inferences from comparisons of expression patterns. RNAi has been shown to be applicable to a number of arthro-

pod species, and so breadth, along with depth, can be added to our understanding of the patterning mechanisms in this spectacularly diverse phylum. It will be interesting to see how much of what is seen in *Tribolium* holds in other insects. Some data already point to the existence of diverse mechanisms in early patterning. For example, the loss of zygotic *Hb* function in the wasp *Nasonia vitripennis* results in the loss of most head and all thoracic segments ([15] and M. A. Pultz, personal communication), pointing to the critical importance of the zygotic component of Hb, which may act in synergy with Otd. This indicates a change in the relative patterning requirement for *hb* and *otd* between wasp and beetle. Only when we have information from a much wider sample of the arthropods will it be possible to make well-informed hypotheses about the ancestral state of the system that generates long-range polarity of the embryo.

#### References

1. Simpson-Brose, M., Treisman, J. and Desplan, C. (1994). Synergy between the hunchback and bicoid morphogens is required for anterior patterning in *Drosophila*. *Cell* 78, 855-865.
2. Dearden, P. and Akam, M. (1999). Developmental evolution: Axial patterning in insects. *Curr. Biol.* 9, R591-R594.
3. Stauber, M., Jackle, H. and Schmidt-Ott, U. (1999). The anterior determinant bicoid of *Drosophila* is a derived Hox class 3 gene. *Proc. Natl. Acad. Sci. USA* 96, 3786-3789.
4. Wimmer, E.A., Carleton, A., Harjes, P., Turner, T. and Desplan, C. (2000). Bicoid-independent formation of thoracic segments in *Drosophila*. *Science* 287, 2476-2479.
5. Brown, S., Fellers, J., Shippy, T., Denell, R., Stauber, M. and Schmidt-Ott, U. (2001). A strategy for mapping bicoid on the phylogenetic tree. *Curr. Biol.* 11, R43-R44.
6. Stauber, M., Prell, A. and Schmidt-Ott, U. (2002). A single *Hox3* gene with composite bicoid and zerknullt expression characteristics in non-Cyclorrhaphan flies. *Proc. Natl. Acad. Sci. USA* 99, 274-279.
7. Schröder, R. (2003). The genes orthodenticle and hunchback substitute for bicoid in the beetle *Tribolium*. *Nature* 422, 621-625.
8. Falciani, F., Hausdorf, B., Schroder, R., Akam, M., Tautz, D., Denell, R. and Brown, S. (1996). Class 3 Hox genes in insects and the origin of *zen*. *Proc. Natl. Acad. Sci. USA* 93, 8479-84.
9. Brown, S.J., Fellers, J.P., Shippy, T.D., Richardson, E.A., Maxwell, M., Stuart, J.J. and Denell, R.E. (2002). Sequence of the *Tribolium castaneum* homeotic complex: the region corresponding to the *Drosophila melanogaster* antennapedia complex. *Genetics* 160, 1067-1074.
10. Hulskamp, M., Pfeifle, C., Tautz, D. (1990). A morphogenetic gradient of hunchback protein organizes the expression of the gap genes *Kruppel* and *knirps* in the early *Drosophila* embryo. *Nature* 346, 577-580.
11. Struhl, G., Johnston, P. and Lawrence, P.A. (1992). Control of *Drosophila* body pattern by the hunchback morphogen gradient. *Cell* 69, 237-249.
12. Wolff, C., Sommer, R., Schroder, R., Glaser, G. and Tautz, D. (1995). Conserved and divergent expression aspects of the *Drosophila* segmentation gene hunchback in the short germ band embryo of the flour beetle *Tribolium*. *Development* 121, 4227-4236.
13. Patel, N.H., Hayward, D.C., Lall, S., Pirkil, N.R., DiPietro, D. and Ball, E.E. (2001). Grasshopper hunchback expression reveals conserved and novel aspects of axis formation and segmentation. *Development* 128, 3459-3472.
14. Bucher, G., Scholten, J. and Klingler, M. (2002). Parental RNAi in *Tribolium* (Coleoptera). *Curr. Biol.* 12, R85-R86.
15. Pultz, M.A., Pitt, J.N. and Alto, N.M. (1999). Extensive zygotic control of the anteroposterior axis in the wasp *Nasonia vitripennis*. *Development* 126, 701-710.
16. Chan, S.K. and Struhl, G. (1997). Sequence-specific RNA binding by bicoid. *Nature* 388, 634.
17. Wolff, C., Schroder, R., Schulz, C., Tautz, D., Klingler, M. (1998) Regulation of the *Tribolium* homologues of caudal and hunchback in *Drosophila*: evidence for maternal gradient systems in a short germ embryo. *Development* 125, 3645-3654.