Evolution of Development: Beyond Bicoid

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 its target genes in a concentration-dependent manner, a morphogenetic gradient. Bcd functions by activating the antero-posterior axis in the syncytial embryo to form a primary anterior determinant. During studies in most animals, extensive evidence about this process in most animals.

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

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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
specifying the most anterior part of the embryo is well conserved in bilaterally symmetrical animals — the bila-
teria — from flies to mammals. Like Bcd, Otd is a home-
domain 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 pro-
teins having DNA binding specificities that are identical and clearly distinct from those of most other homeo-
proteins, 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 ante-ior of the embryo might have been patterned by coop-
erating Hb and Otd, through a mechanism that remains to be fully elucidated. In the fly lineage, the ven gene was duplicated: one paralog gave rise to the bcd pre-
cursor 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 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 anterior-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 common phenotype was the loss of all prepupal head segments, along with the mandibular segment. In the most severe cases, all head and the first thoracic segments, along with the mandibular segment, 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 the two genes 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 phenocopied by injection of double-stranded RNA, indicating a possible interaction of the two genes. Removing maternal and zygotic Tc-hb activity, either alone or in combination with Tc-otd1, indicates 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 arthropod 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