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'De-evolution' of *Drosophila* toward a more generic mode of axis patterning

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Establishing the primary axes (anterior-posterior and dorsal-ventral) is one of the first steps in patterning bilateral animals. In *Drosophila,* this process is well understood at the molecular level. One of the molecules that have been shown to be absolutely critical in patterning wild type embryos is the homeoprotein, Bicoid (Bcd) (St Johnston *et al*., 1989). Loss of *bicoid (bcd)* function results in embryos that lack all anterior structures, including the head, thorax, and some anterior abdominal segments (Frohnhofer and Nusslein-Volhard, 1986; St Johnston, 1995). However, the overall polarity of the remaining abdominal segments is retained. The *bcd* message is deposited into the egg by the mother, and factors binding to its 3'UTR localize it to the anterior pole of the egg (Berleth *et al*., 1988), via a migration along a polarized array of microtubules (Gonzalez-Reyes *et al*., 1995; Schnorrer *et al*., 2000). Translation of the *bcd* mRNA generates an anterior-to-posterior (A-P) concentration gradient of the Bcd homeodomain protein (Driever and Nusslein-Volhard, 1988). The resulting Bcd morphogenetic gradient differentially activates specific effector genes. The highest concentrations activate the head gap genes (*e.g. orthodenticle)* (Gao *et al*., 1996), while lower levels activate the thoracic gene *hunchback (hb)* (Tautz, 1988; Driever and Nusslein-Volhard, 1989; Struhl *et al*., 1989), and even lower levels activate the abdominal genes *Krüppel (Kr)* (Hulskamp *et al*., 1990;Hoch *et al*., 1991; Struhl *et al*., 1992) and activate *knirps (kni)* (Rivera-Pomar *et al*., 1995). The ability of Bcd to differentially target genes provided the first molecular explanation as to how a transcription factor functions as a morphogen. Further, Bcd is not only a transcriptional regulator, but also represses the translation of the ubiquitously distributed maternal *caudal* mRNA (Dubnau and Struhl, 1996; Rivera-Pomar *et al*., 1996; Chan and Struhl, 1997), thus generating an opposite posterior to anterior gradient of the Caudal protein. Bcd performs this function by directly binding to the 3'UTR of the *caudal mRNA* with its homeodomain (Niessing *et al*., 1999; Niessing *et al*., 2000). Therefore, *bcd* has multiple roles in activating the head (*otd*), thoracic (*hb*) and abdominal (*Kr* and *kni*) gap genes, as well as in blocking the function of the posterior determinant *cad*. This places *bcd* in a central position for patterning the A-P axis of the fly embryo (Fig. 1).

Bicoid as a unique patterning system of higher Diptera

Despite the absolutely critical role for *bicoid* in patterning the *Drosophila* embryo, evidence has mounted indicating that the Bicoid gradient is a relatively recent addition to the developmental toolkit of insects, and that it may be unique to higher flies.

Considering first a comparison to more basal insects, the morphology of the *Drosophila* embryo, occupying the entire egg, allows for the formation of an anterior morphogenetic center where spatial determinants can be localized (*e.g. bcd* mRNA and *tor* activity) and can diffuse posteriorly. However, an anterior

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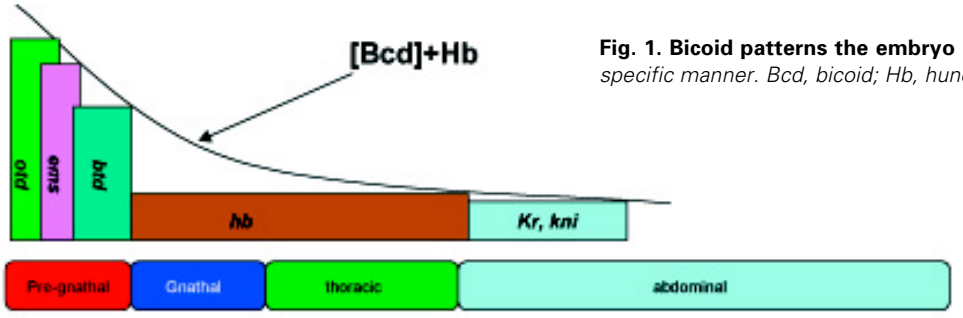

Fig. 1. Bicoid patterns the embryo *by activating target genes in a concentration specific manner. Bcd, bicoid; Hb, hunchback.*

patterning system would be unfeasible in insect species that develop with the head anlagen located at the posterior end of the egg, as in the more ancestral *Schistocerca* (Fig. 2). Since the *Schistocerca* embryo is at a considerable distance from the anterior pole of the egg, it is unlikely that an anterior morphogenetic center could pattern the head of the embryo through such a distance(Sander, 1975). However, a posterior morphogenetic center acting via degradation of a uniform factor could function in these conditions, like for example the regulation of maternal *hb* (*hb*^{mat}) mRNA translation by *nanos* (Curtis *et al.*, 1995).

For the beetle *Tribolium canstaneum (Tc)* (Fig. 2), recent contradictory studies have provided arguments both for and against the existence of a *bcd*-like function: when the *Tc-cad* mRNA, whose translation is repressed at the anterior of the *Tc* embryo, is placed in *Drosophila*, translation of its mRNA is blocked at the anterior of the embryo in a *bcd*-dependent manner. On the other hand, sequencing of the genomic region encompassing the *Tc* Hox region indicates that there is no *bcd* gene in the *Hox3/zen* region where it is found in *Drosophila* (Brown *et al.*, 2001). To reconcile these observations with the model that *bcd* is absent from *Tribolium*, it is possible that the regulation of *Tc-cad* mRNA translation is performed by another factor. For example, a homologue to the C. elegans *mex-3* gene (sharing no homology with Bcd) which regulates translation of the *C. elegans cad* homologue, *pal-1* (Hunter and Kenyon, 1996) could play a similar role in *Tribolium*, and Bcd might recognize the same regulatory element when placed in *Drosophila*. *Tc-cad* mRNA translation is also repressed in anterior regions of the *Tribolium* embryo (Wolff *et al.*, 1998).

Finally, no genes homologous to *bcd* have been identified outside higher Diptera (Schroder and Sander, 1993; Bonneton *et al.*, 1997; Stauber *et al.*, 1999), despite its homeobox and genomic position in the Hox cluster (Berleth *et al.*, 1988). Moreover, *bcd* shows an unusually high divergence for a homeobox gene (Sommer and Tautz, 1991; Stauber *et al.*, 1999), and its function is not even conserved within higher Diptera (Schroder and Sander, 1993; Bonneton *et al.*, 1997).The most distant species in which *bcd* has been found is the basal cyclorrhaphan (a monophyletic clade of highly derived dipterans that includes *Drosophila* and houseflies, among others) fly *Megaselia* (Fig. 2). In *Megaselia*, *bcd* and *hb* appear to play similar roles as in *Drosophila*. Surprisingly, the phenotype of RNAi experiments with *Megaselia bcd* is significantly more severe than that of *Drosophila bcd*, suggesting that, in this species, *bcd* has taken even more roles than in the *Drosophila* embryo. Alternatively, *hb*^{mat}, whose function patterns some of the axis of the embryo in *bcd* mutants, might have a lower contribution in *Megaselia* (Stauber *et al.*, 2000).

Despite an intense search, *bcd* homologs have not been found in any insects outside the cyclorrhapha, even in the lower Dipteran *Clogmia*, or in the completely sequenced genome of the mosquito *Anopheles* (Fig. 2) (Sommer and Tautz, 1991; Schroder and Sander, 1993; Stauber *et al.*, 1999; Stauber *et al.*, 2000). Therefore, although Bcd is normally absolutely required for the patterning of most of the anterior-posterior axis in *Drosophila*, other factors must play its roles in other species.

The origin of Bicoid

The combined molecular and embryological data strongly indicate that the Bcd morphogen gradient is not universally employed among insects. How, then, could an anterior patterning system based on an anteriorly centered Bcd morphogen gradient evolve? *bcd* is present in the Hox complex (Antp-C), next to the genes *zerknuellt* (*zen*) and z2, a recent duplication of the *zen* gene. Genes at this Hox3 paralogous position have a tendency to duplicate and diverge (Falciani *et al.*, 1996), and *bcd* is likely to have arisen through an earlier duplication of *zen*. *bcd* has diverged extensively

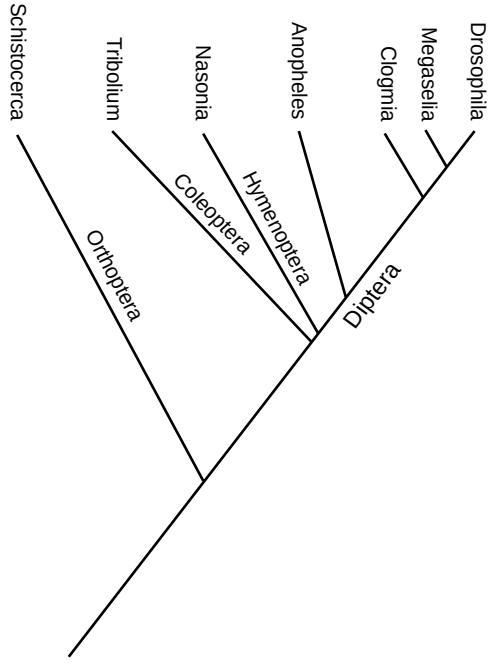

Fig. 2. Phylogenetic relationships of insects discussed in text *(Wheeler* et al., *2001; Friedrich and Tautz, 1997).*

along the lineage leading to *Drosophila* such that a close molecular relationship with zen has been obscured. However, the *bcd* gene found in *Megaselia* is clearly recognizable as being highly similar to *zen* (Stauber *et al.*, 1999). In ancestral short germband insects, *zen* has two components of expression. It has a maternal component which is ubiquitous throughout the egg, and a zygotic component which is restricted to the anterior and dorsal regions of the egg and coincides with the extraembryonic membrane anlage (Falciani *et al.*, 1996; Dearden, 2000). Along the dipteran lineage, the embryo came to occupy the entire length of the egg, with the extraembryonic membranes restricted mostly to the dorsal side. This is reflected in the expression of *zen* in lower diptera such as *Clogmia* (Stauber *et al.*, 2002). In the ancestor of cyclorrhaphan flies, the *zen* locus was duplicated, with one of the paralogs maintaining the maternal aspect of expression, that evolved to become *bcd*, and the other keeping the zygotic, dorsal component that is the actual function of *Drosophila zen*. The maternal paralog began diverging rapidly, gaining a unique binding specificity, the ability to translationally repress *caudal*, and most importantly, the localization of its mRNA at the anterior pole, allowing for the formation of a protein morphogenetic gradient. These changes allowed the nascent *bcd* ancestor to transcriptionally control and usurp some of the patterning functions of the components of the ancestral patterning system. Eventually, Bcd became indispensable for conferring long range polarity on the embryo (Fig. 3).

The question then becomes: How can axis formation be accomplished in the absence of the Bicoid gradient? One hypothesis is that genes downstream of Bicoid in *Drosophila* ancestrally had more significant roles in patterning the A-P axis, which became redundant upon the advent of Bicoid acquiring control of long range polarity of the embryo. One good candidate for an ancestral patterning is the gap gene *hb.*

Hunchback as an A-P patterning gradient

hb encodes a zinc finger transcription factor that specifies anterior development while preventing posterior development (Hulskamp *et al.*, 1990). As a zygotic gap gene, hb^{zyg} is expressed in response to *bcd* in the anterior half of the early embryo. The binding of Bcd to high affinity sites in the *hb* P2 promoter (Driever and Nusslein-Volhard, 1989; Driever *et al.*, 1989; Struhl *et al.*, 1989) directs expression of the anterior hb^{zyg} domain, and has been documented in much detail. This system serves as a paradigm for the functioning of a transcriptional morphogen (Driever and Nusslein-Volhard, 1989; Struhl *et al.*, 1989). *hb* is also provided maternally (hb^{mat}) as a ubiquitously distributed mRNA whose translation is blocked by the posterior gene *nanos* (Sander and Lehmann, 1988; Lehmann and Nusslein-Volhard, 1991), thereby generating another A-P Hb protein gradient (Hulskamp *et al.*, 1990; Struhl *et al.*, 1992). Thus, two different and independent mechanisms lead to similar hb^{mat} and hb^{zyg} expression patterns (*i.e.* high at the anterior and low at the posterior), although hb^{zyg} expression is stronger and persists longer than hb^{mat}. Interestingly, the simultaneous deletion of *nanos* and hb^{mat} does not result in embryonic pattern defects (Hulskamp *et al.*, 1989; Irish *et al.*, 1989; Struhl, 1989), indicating that hb^{mat} is non-essential, and that the only embryonic function of *nanos* is to remove hb^{mat} function from the posterior. This way to generate an Hb gradient, although redundant with the *bcd*-dependent gradient, might be the ancestral pathway.

hb is also expressed as a stripe that initiates at the late blastoderm stage and overlaps the progenitor region of parasegment 4 (hb^{PS4}). This hb^{PS4} stripe does not depend directly on *bcd* but is autoregulated by early, *bcd*-dependent hb^{zyg}. hb^{PS4} is responsible for limiting the expression of posterior Hox genes to the abdominal regions (Zhang and Bienz, 1992). It has been shown that hb^{PS4} is essential for thoracic development (Wimmer *et al.*, 2000) and represents the most critical domain of hb^{zyg} function. Finally, *hb* has a posterior stripe of expression that patterns segments A_7 and A_8 (Hulskamp *et al.*, 1990). Although it is difficult to reconcile this function with the necessity to clear hb^{mat} from this region earlier in development, this might be due to the later developmental stage. The P1 promoter controls maternal and later zygotic expression.

Embryos homozygous for hb^{zyg} lack the labial and all three thoracic segments (Lb & T_1-T_3) (Lehmann and Nusslein-Volhard, 1987). They also show fusion of abdominal segments A_7-A_8 due to the absence of the later posterior stripe of hb^{zyg} expression. Although embryos lacking hb^{mat} have no apparent phenotype, hb^{mat} clearly can play a very important role: Embryos that lack *bcd* function (and thus have no hb^{zyg} expression but have kept normal hb^{mat}) still have normal A-P polarity of the remaining segments. However, the simultaneous removal of both *bcd* and hb^{mat} generates near-'bicaudal' embryos that have reversed polarity (Gavis and Lehmann, 1992). Therefore, hb^{mat} has morphogenetic properties, and can create A-P polarity in the absence of *bcd* (Hulskamp *et al.*, 1990; Struhl *et al.*, 1992). Interestingly, many embryos totally lacking $hb^{mat+zyg}$ exhibit a

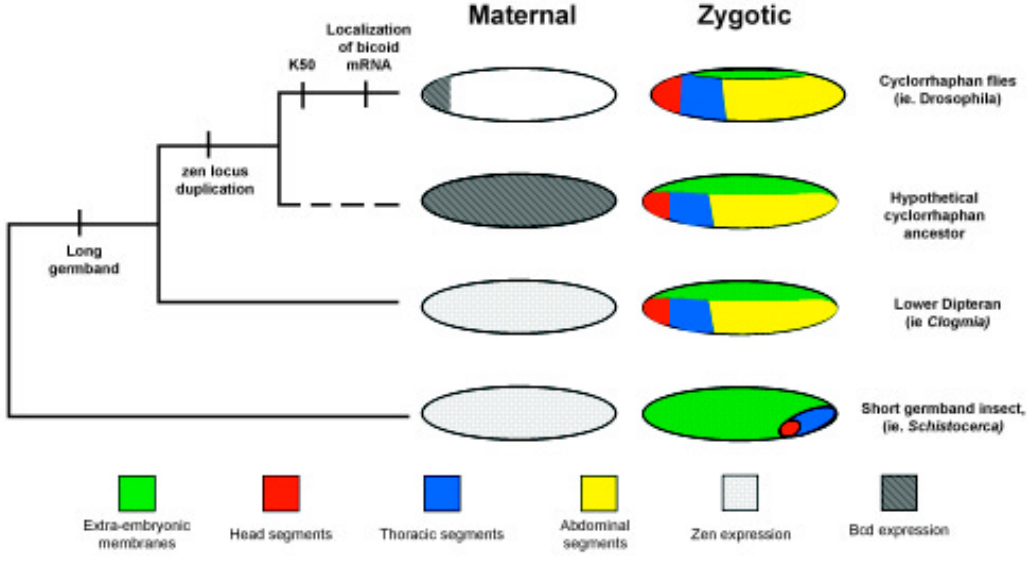

Fig. 3. Model for the evolution of Bicoid dependent patterning. *(See text for details).*

near bicaudal phenotype as well, in spite of the presence of *bcd* (Simpson-Brose *et al.*, 1994). This phenotype is reminiscent to that of embryos lacking both *bcd* and *hb*, which are completely bicaudal with a duplicated telson replacing the labrum. This phenotype indicates that *bcd* is not able to create correct long-range polarity in the absence of *hb*, and emphasizes the crucial early patterning role of *hb* (Simpson-Brose *et al.*, 1994).

Ancestral patterning role of *hb* for patterning of the thorax

In the abdomen, flies still contain an ancestral patterning system that is redundant with *bcd* since *hb* and *cad* can replace *bcd* function (Hulskamp *et al.*, 1990; Struhl *et al.*, 1992; Schulz and Tautz, 1994; Rivera-Pomar *et al.*, 1995). However, similar experiments have failed to characterize the relative contributions of *hb* and *bcd* in patterning the head and thorax. This results from the fact that activation of hb^{zyg} is *bcd*-dependent. Thus, whenever *bcd* activity is altered, hb^{zyg} activity is also changed (Driever and Nusslein-Volhard, 1989; Struhl *et al.*, 1989). To circumvent this problem, a system was developed that allows the study of the two morphogens (Bcd and Hb) independently of each other. *bcd*-dependent expression of hb^{zyg} is mediated by the *hb* P2 promoter; whereas maternal and late blastoderm expression of *hb* is initiated by the *hb* P1 promoter (Margolis *et al.*, 1995). Therefore, a functional *hb* transgene (*hbP1*) was constructed that does not mediate early *bcd*-dependent zygotic expression. However, *hbP1* is able to direct maternal as well as late zygotic expression, in particular the intense stripe of hb^{PS4}. Therefore, this transgene uncouples the direct link between *bcd* and *hb*.

Experiments employing the *hb*P1 promoter construct show that the standard "zygotic" phenotype of embryos born from heterozygous *hb* parents (*i.e.* loss of lb, T_1, T_2 & T_3) is in part caused by a decrease in the dose of hb^{mat}. In fact, by restoring full maternal hb^{mat} expression(*i.e.* two copies, by placing one *hb-P1* transgene in the mother), the mutant phenotype of hb^{zyg} is less severe, leading to the deletion of only the T2 and T3 segments while lb and T_1 segments are restored (Fig. 4). Therefore, in the presence of a normal hb^{mat} dosage, hb^{zyg} is necessary only for the development of T_2 and T_3, two segments that exactly overlap the domain of hb^{PS4} expression.

Based on these results it can be hypothesized that: 1) the lb and T1 segments depend on a high maternal contribution of *hb* and not on hb^{zyg}, and 2) The real hb^{zyg} defects are due to the late hb^{zyg} stripes, such that the T_2-T_3 deletion seems to result from the failure to activate the hb^{PS4} stripe and the fusion of A7/A8 depends on the posterior stripe. This strongly suggests that the early *bcd*-dependent hb^{zyg} domain does not play a fundamental role. As the hb^{PS4} stripe is autoregulated (in hb^{zyg} mutants, this stripe is absent), the only role of *bcd*-dependent hb^{zyg} might be to drive the high levels of *hb* expression necessary to 'kick in' this autoregulation. Thus, if strong hb^{mat} contribution could be driven, the *bcd*-dependent hb^{zyg} could be made obsolete

To test this model, a situation where Bcd is no longer able to activate hb^{zyg} (*hb*P1only in an hb^{zyg} mutant background) was generated. In order to restore normal hb^{PS4} expression, the dose of its activator, hb^{mat} was increased via 4 *hb-P1* transgenes; and the dose of its repressor, *kni* (Pankratz *et al.*, 1989), was reduced to one copy. In this context, most embryos develop thoracic structures posterior to T_1; and a significant proportion exhibit complete rescue of the lb, T_1, T_2 and T_3 segments (Wimmer *et al.*, 2000) (Fig. 4). Therefore, the role of *bcd* in activating hb^{zyg} can be replaced by adding higher levels of hb^{mat} to activate hb^{PS4} and form T_2 and T_3. Concomitant with the rescue of T_2 and T_3, the hb^{PS4} stripe reappears (as assayed by *hbP1-lacZ* expression).

These results demonstrate that the Bcd and Hb morphogenetic systems do not need to be directly linked in *Drosophila*, which is consistent with the fact that *bcd* is unlikely to exist in other insects. Further, they support the argument that the control of *hb* by *bcd* has been recently acquired phylogenetically and can be bypassed for proper thoracic segmentation.

Although thoracic development can be recovered when *bcd* control over *hb* is eliminated, *bcd* may still be required for functional synergy with *hb* to form the thoracic segments. This model can be tested in a situation where embryos lack *bcd* but have high levels of hb^{zyg} provided by an alternative mechanism. A maternally expressed gene was generated with a maternal promoter fused to the coding sequences of the Gal4 DNA binding domain and three copies of the yeast GCN4 activation domain (Janody *et al.*, 2000). The mRNA is localized to the anterior pole of the embryo by the *bcd* 3'UTR. Translation of this mRNA creates an A-P concentration gradient of the artificial Gal4-GCN4 transcription factor. By crossing *bcd* mutant females containing this construct with males bearing a UAS-*hb*

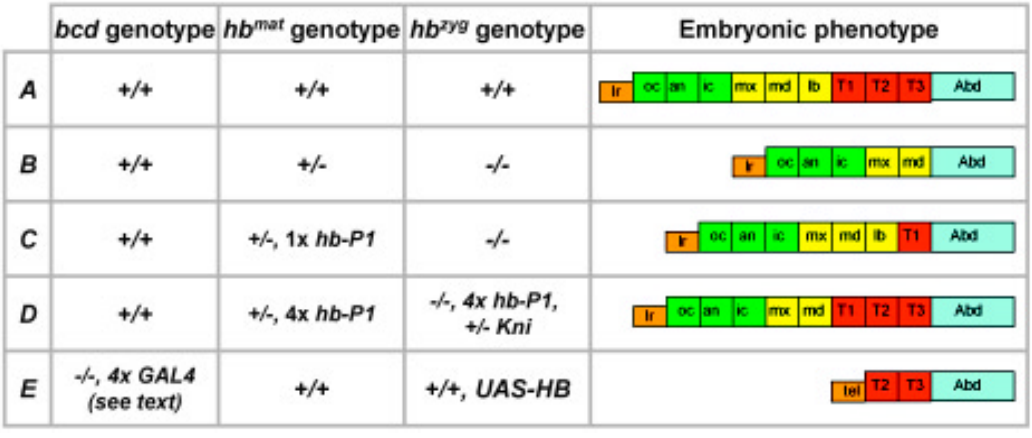

Fig. 4. Phenotypes of embryos in various *hb* and *bcd* backgrounds. **(A)** *Wildtype,* **(B)** *Zygotic* hb *mutant phenotype (with 1/2 maternal contribution).* **(C)** *Zygotic* hb *mutant with normal maternal contribution restored by addition of one* hb *copy driven by P1 promoter.* **(D)** *Nearly complete rescue of* hb *phenotype via 4 copies of hb-P1, and reduction of* Kni. **(E)** *Rescue of thoracic segments lost in* bcd *mutant by driving* hb *expression under control of artificial transcription factor. Abbreviations: Abd, abdomen; an, antennal; ic, intercalary; lb, labial; lr, labrum; md, mandibular; mx, maxillary; oc, ocular; T1-3, thoracic segments 1-3 respectively; tel, telson (posterior terminal structure).*

transgene, a strong gradient of hb^{zyg} expression is created in the absence of *bcd* function. This genetic combination results in embryos that develop normal T_2 and T_3 segments (Wimmer *et al.*, 2000). This T_2-T_3 rescue is likely due to the observed rescue of hb^{PS4} expression that is absent in *bcd* mutants. Therefore, T_2 and T_3 can form in the total absence of *bcd*, as long as hb^{PS4} is activated. However, the lb and T_1 segments, which were shown to depend on high levels of hb^{mat}, do not form in this situation (Fig. 4). Although there are high levels of *hb* in this region of the embryo that gives rise to lb and T1, there is a delay in reaching high levels of Hb protein expression from UAS-*hb* and this expression is transient. It is likely that the formation of lb and T_1, like the more anterior head segments, requires a synergy between Bcd and Hb as discussed below.

Can *hb* control head development?

The above described experiments show that *hb* is able to pattern thoracic and abdominal segments in the absence of *bcd*, and may thus control patterning of these regions in embryos with a more ancestral patterning system. However, since the pre-gnathal head segments are never rescued by manipulating *hb* levels, at least one other factor must be invoked. The head gap gene *orthodenticle* (*otd*) has been proposed to fulfill this role.

otd is a head gap gene that defines metamerization of head segments and specifies their identity. In the absence of *otd* function, the ocular and antennal segments are missing (Cohen and Jurgens, 1990). The role of *otd* as a determinant of head structures is highly conserved in evolution. There are four *otx* genes in vertebrates that specify forebrain structures and the eye (Bally-Cuif and Boncinelli, 1997) and are expressed in anterior regions. Even in hydra, where there is no A-P axis, *otd* is expressed around the mouth (along the oral-aboral axis) (Smith *et al.*, 1999). In vertebrates, one of the functions of Otx is to antagonize the function of Cdx, the homologue of Cad (Isaacs *et al.*, 1999), mostly at the transcriptional level. In *Drosophila*, *otd* is activated by high levels of Bcd (Fig. 1) through low affinity Bcd binding sites in its regulatory sequences (Gao and Finkelstein, 1998). *otd* encodes a HD protein characterized by the presence of a lysine (K) residue at the critical position 50 in its HD that defines its DNA binding specificity (Finkelstein *et al.*, 1990). This K_{50} residue is also found in the Bcd HD and in very few other HD proteins. As it imparts the same DNA binding specificity to Otd and Bcd (Treisman *et al.*, 1989), this makes it possible that Bcd and Otd share targets and functions, and that Bcd might use sites ancestrally used for the regulation of *otd* targets, including *otd* autoregulation. Therefore, the Bcd gradient is likely not a general feature of insect A-P patterning. It is hypothesized that in ancestral insects, some of the orthologs of current Bcd targets were the main determinants of A-P polarity. *hb* is one of these determinants. However, since *hb* does not have the capability to pattern the most anterior regions, another factor must be involved. Based on expression pattern, ancestral role in head patterning, and sharing a unique binding specificity with Bcd, this additional factor might be Otd.

Synergy between *hb* and *otd* for anterior patterning

The conclusions reached by manipulations of the *Drosophila* embryo have been substantiated by experiments in other insects, where techniques for manipulating embryos have been recently developed. In *Tribolium* the functions of *hb* and *otd* have been tested by knocking down their respective messages with RNAi (Schroder, 2003) (Fig. 5). In this organism, double stranded RNA can be delivered to embryos through the mother, termed parental RNAi (pRNAi), resulting in a knock down of both maternal and zygotic components of expression (Bucher *et al.*, 2002). This is important since, unlike in *Drosophila*, *Tc-otd1* is expressed maternally as well as zygotically. When *Tc-otd1* is knocked down in this manner, the embryos exhibit a range of phenotypes, the strongest being the loss of all head structures. This is much more severe than what is seen in

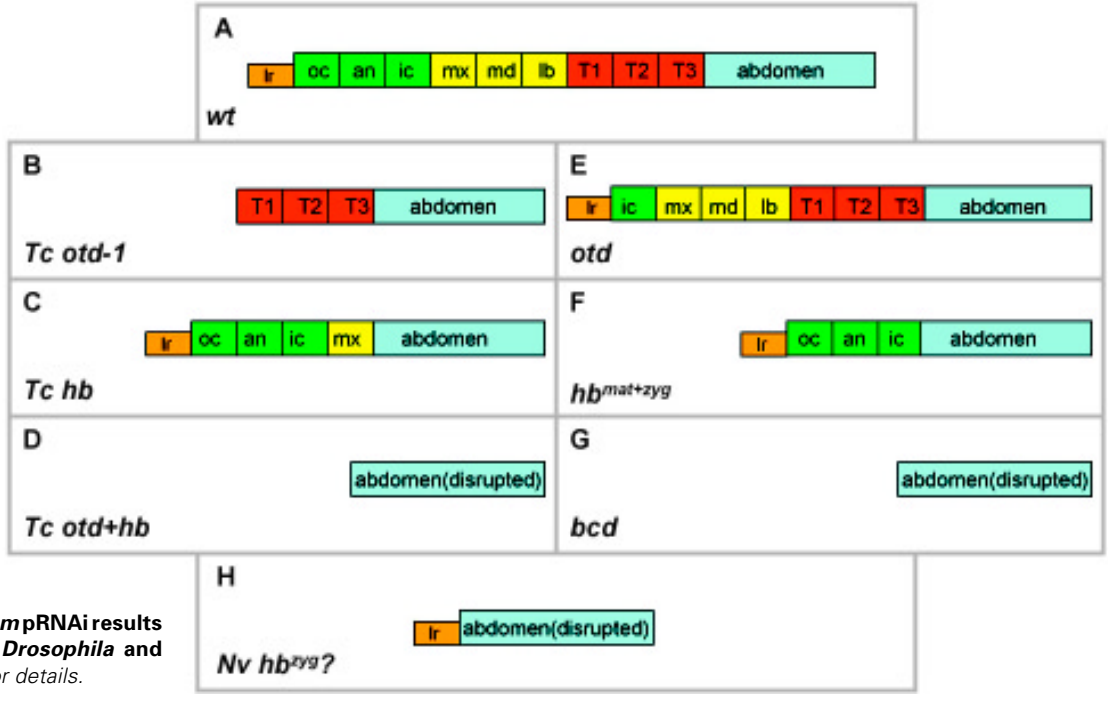

Fig. 5. Comparison of *Tribolium* pRNAi results and mutant phenotypes of *Drosophila* and *Nasonia* mutants. *See text for details.*

Drosophila otd null mutants, which only lose the ocular and antennal segments. In fact, this phenotype is more reminiscent of a weak *bcd* mutant. There is a second *Tc-otd* paralog, *otd2*, which does not appear to play an important role in the early embryo, but rather act like the late *Dm otd* gene.

The *Tc-otd1* pRNAi phenotype is not as strong as what is seen in *bcd* mutant, indicating that another factor combines with *otd* to replace *bcd* function in the beetle embryo. The pRNAi phenotype of *Tc-hb* is consistent with this role: the more extreme cases are missing all thoracic segments as well as some of the gnathal head segments. The overlap of the two phenotypes indicates that *hb* and *otd* cooperate in setting up the axis of the *Tribolium* embryo.

Interestingly, there appears to be some lability in this proposed ancestral patterning mechanism. A zygotic loss of function mutation in the *hb* ortholog of the wasp *Nasonia vitripennis* causes a loss not only of the thoracic and gnathal head segments, but also most of the pre-gnathal segments (Pultz *et al*., 1999). This phenotype is more severe than both the loss of maternal and zygotic function in *Drosophila* and the *Tribolium* pRNAi experiments (Fig. 5). This result indicates that the *Nasonia* embryo relies more heavily on input from *hb* in patterning the anterior than either the fly or the beetle. The role of *otd* in this organism is currently being examined.

In conclusion, by manipulating the expression of genes in the context of the highly derived embryogenesis of the fly *Drosophila*, it is possible to gain insight into the mechanisms employed by insects of a more ancestral type. The application of emerging techniques such as RNAi (Hughes and Kaufman, 2000; Stauber *et al*., 2000; Schoppmeier and Damen, 2001; Schroder, 2003;) and germline transformation (Horn and Wimmer, 2000; Peloquin *et al*., 2000; Grossman *et al*., 2001; Hediger *et al*., 2001; Kokoza *et al*., 2001; Heinrich *et al*., 2002) for manipulating the embryos of insects and arthropods other than *Drosophila* will allow hypotheses generated by these *Drosophila* experiments to be tested in a wide variety of organisms. Thus, the depth of knowledge obtained from the *Drosophila* research program can be supplemented with a dimension of breadth, allowing for a much clearer understanding of the evolution of developmental mechanisms in this incredibly diverse animal Phylum.

Summary

The genetics of the establishment of the primary axes of the early embryo have been worked out in great detail *Drosophila*. However, evidence has accumulated that *Drosophila* employs a mode of patterning that is not shared with most insects. In particular, the use of the morphogenic gradient of the Bicoid homeoprotein appears to be a novel addition to the fly developmental toolkit. To better understand the ancestral mode of patterning that is probably more widely used by insects, several groups have used Evo-Devo approaches as well as sophisticated genetic manipulations of *Drosophila* to achieve some form of 'de-evolution' of this derived insect. Genetic manipulations of the beetle *Tribolium* and the wasp *Nasonia* have validated most of these results.

KEY WORDS: *AP patterning, Evolution of Development*

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