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CONSERVATION AND DIVERGENCE IN MOLECULAR MECHANISMS OF AXIS FORMATION

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■ **Abstract** Genetic screens in *Drosophila melanogaster* have helped elucidate the process of axis formation during early embryogenesis. Axis formation in the *D. melanogaster* embryo involves the use of two fundamentally different mechanisms for generating morphogenetic activity: patterning the anteroposterior axis by diffusion of a transcription factor within the syncytial embryo and specification of the dorsoventral axis through a signal transduction cascade. Identification of *Drosophila* genes involved in axis formation provides a launch-pad for comparative studies that examine the evolution of axis specification in different insects. Additionally, there is similarity between axial patterning mechanisms elucidated genetically in *Drosophila* and those demonstrated for chordates such as *Xenopus*. In this review we examine the postfertilization mechanisms underlying axis specification in *Drosophila*. Comparative data are then used to ask whether aspects of axis formation might be derived or ancestral.

CONTENTS

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INTRODUCTION

Many organisms manifest polarity at some level; indeed, asymmetry seems essential for promoting meaningful interaction with the environment. Even the simplest unicellular organisms display temporary polarity in response to their environment; for example, in reception and response to chemotactic signals (for example, see 11). In higher animals the body plan is arranged along two axes: the anteroposterior (AP) axis, and the broadly perpendicular dorsoventral (DV) axis.

Breaking symmetry to specify these axes is one of the most basic and earliest processes in the development of higher animals. The early nature of axis formation is clear in *Drosophila melanogaster* where the process has been genetically dissected, and the cues for AP and DV axiation are established during oogenesis. Animals can utilize diverse environmental cues as the basis of axiation, suggesting that axis formation is a variable, plastic process. For instance, the physical process of axis formation can vary between closely related nematodes (46). Furthermore, *Xenopus laevis* can be induced to use gravity rather than sperm entry point as a cue for axis formation; indeed, the dorsalizing organizer forms 180° to sperm entry point only 70% of the time (45). How can an event that provides basic information for patterning the entire body plan be so inconstant?

In this review, we use knowledge about embryonic axis formation in the dipteran *Drosophila melanogaster* (*D. melanogaster*) to address the contention that axis formation is variable, and then to ask how this can be true of such a fundamental process. Variation in axis formation probably has an intimate relationship with alterations in embryonic morphology and changes in life-history. Hence we discuss data suggesting that variation in axis formation within the dipterans correlates phylogenetically with variation in embryonic morphology.

D. melanogaster has four maternal coordinate systems that specify the major body axes (reviewed in 165). Three specify positional information along the AP axis (the anterior, posterior, and terminal systems), whereas the fourth is involved in DV axis formation. We describe the molecular nature of the anterior and DV patterning systems, addressing which of the following might be true:

1. Does a given molecular process play a conserved role in axis formation across phyla, and could it therefore be ancient?
2. Might a particular molecular process have been co-opted into axis formation from another context prior to the origin of insects?
3. Might a given molecular process be a very recent evolutionary innovation?

Examples of all three scenarios can be drawn out of the axis formation mechanisms elucidated in *D. melanogaster*, suggesting that the process of axis formation may well have been elaborated upon multiple times. We discuss the fact that *D. melanogaster* has been shown genetically to display redundancy between two maternal coordinate systems, and suggest that this may facilitate radical changes in axis formation. Finally, we examine recent data suggesting that rapid molecular evolution of axiation can be correlated with gross morphological rearrangements within the dipterans.

Axis Formation in *Drosophila melanogaster*

Genetic screens and subsequent molecular analyses have led to a detailed understanding of the earliest embryonic events in axis formation in *D. melanogaster* (165). All four of the maternal coordinate systems are set up during oogenesis, and recognition of and elaboration upon axial maternal cues are among the earliest events after egg activation and fertilization. The anterior system involves cytoplasmic diffusion of morphogens within the syncytial *D. melanogaster* embryo. In contrast the terminal and dorsal patterning systems require signaling from the extracellular perivitelline space to the embryo. In this review, we focus on the dorsoventral patterning system, as an example of morphogenetic activity set up by extracellular signaling, and the anterior system, as an illustration of cytoplasmic diffusion to form a morphogen gradient. We begin by asking whether such mechanisms are likely to be conserved among other insect orders.

Are Models of Axis Formation Formulated in *Drosophila* Tenable in Other Insects?

D. melanogaster displays a mode of development designated long germ embryogenesis, in which the presumptive head, thoracic, and abdominal cells are present at blastoderm stage in the same proportion as in the hatching larva (reviewed in 140). *D. melanogaster* also has a relatively prolonged syncytial stage. The cues for AP axis formation are initially elaborated upon within a syncytial environment during the first 2.5 h of embryonic development. Short germ insects, on the other hand, have only specified the most anterior segments at the end of blastoderm stage, the posterior segments being generated by subsequent growth [for reviews addressing short and long germ development, see (140, 144, 177)]. Although most insects display superficial cleavage and have a syncytial phase, cellularization occurs well before gastrulation in many short and long germ embryos. For example, grasshopper nuclei cellularize almost as soon as they reach the embryonic cortex, i.e., at the onset of blastoderm stage (55).

Both short germ embryogenesis and early cellularization have profound consequences for axis formation models involving morphogen diffusion. Short germ embryogenesis suggests that patterning of posterior segments may be substantially delayed, whereas early cellularization makes it difficult to envision specification by the diffusion of a morphogen such as the *bicoid* transcription factor in *Drosophila*

(see below). In contrast, systems that elaborate axes via a signaling pathway might more obviously be conserved. The insect orders discussed in this review and their approximate relationships are illustrated in Figure 1*b* (based on 82, 195).

Axis formation mechanisms in *D. melanogaster* are even harder to apply to animals that undergo holoblastic (complete) rather than superficial cleavage after fertilization. In embryos undergoing complete cleavage, diffusion or segregation of a cytoplasmic morphogen must occur during the first embryonic cleavages. Thus, if aspects of axial patterning in *Drosophila* are conserved across species, they will at the very least have been modified in the lineage leading to flies.

THE DORSOVENTRAL SYSTEM

DV Axiation Processes in *D. melanogaster*

Our understanding of DV patterning during early embryogenesis in *D. melanogaster* is based on the genetic dissection of the *Toll/dorsal* pathway (Figure 2) (reviewed in 100). Upon mutation, 12 genes show maternal-effect dorsoventral patterning defects in the embryo, but have normal eggshell patterning (5, 21, 152). These genes are (in putative order of action within the pathway) *windbeutel*, *pipe*, *nudel*, *gastrulation defective*, *snake*, *easter*, *spätzle*, *Toll*, *pelle*, *tube*, *dorsal*, and *cactus* (4, 22, 53, 99, 133). Normal DV development is ultimately evident by the stereotypical arrangement of denticles on the larval cuticle, and more immediately by the correct expression of molecular markers indicating specification of territories along the dorsoventral axis during embryogenesis. The strongest recessive mutations in 11 of the dorsal group genes lead to a larval cuticle that is covered in dorsal-type denticles and lacks ventral tissue such as the mesoderm. The 12th gene, *cactus*, gives the opposite phenotype: a ventralized cuticle (133, 152). Readouts of the *Toll/dorsal* pathway display dose sensitivity genetically, and depend upon differential levels of Dorsal nuclear localization along the DV axis (110, 125, 134, 136, 138, 171, 172). Such evidence suggests that fate along the DV axis depends upon the activity of the *dorsal* transcription factor.

During oogenesis, a molecular cue localized around the oocyte nucleus determines follicle cells lying on one side of the oocyte as dorsal. This leads to the development of follicle cells on the other side of the oocyte as ventral, via the expression of the *pipe* gene. *pipe* encodes a heparan sulfate 2-O-sulfotransferase and has been suggested to make a ventral extracellular modification, or perhaps modify *nudel* ventrally, as the latter behaves nonautonomously and may thus potentially mediate an extracellular signal (109, 155). These cues, set up during oogenesis, initiate a proteolytic cascade, mediated by the proteases *nudel*, *gastrulation defective*, *snake*, and *easter*, in the perivitelline space outside the fertilized embryo (Figure 2*a*) (see 20, 58, 79, 86, 169). Interestingly, *pipe* is not essential for initiating the proteolytic activity of Gastrulation-defective, implying that the situation is more complicated than a simple spatial cue locally activating the protease at the top of a proteolytic hierarchy (86). The proteolytic cascade results in the ventral processing

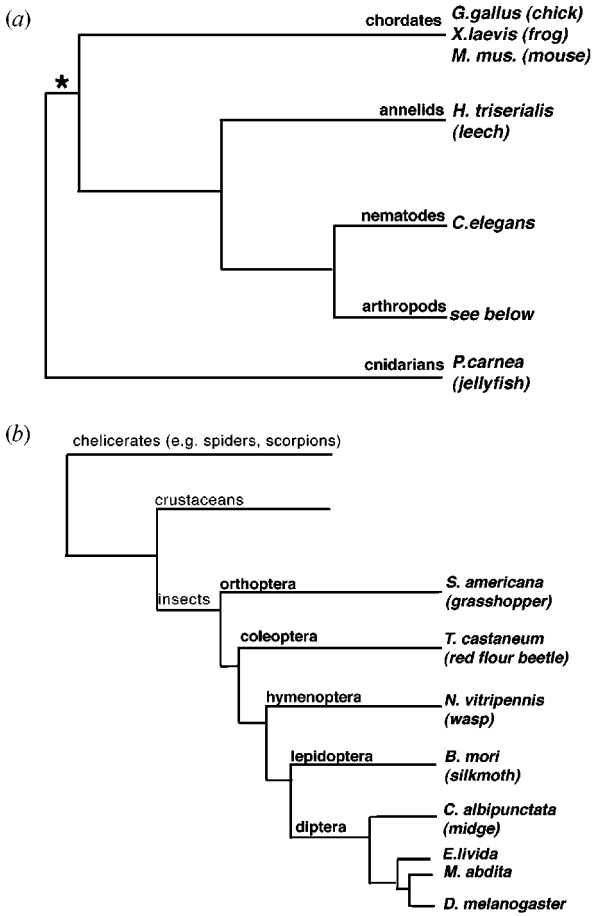

Figure 1 Phylogenetic trees illustrating the positions of the species and phyla discussed in this review. The trees provide a rough framework of (*a*) the positions of the phyla discussed (based on 1), and (*b*) the positions of the insect orders and particular dipterans (flies) used as examples in the text. The asterisk indicates the origin of bilaterians, essentially animals with a body plan arranged along two perpendicular axes. Arthropod species are: *Schistocerca americana*, *Tribolium castaneum*, *Nasonia vitripennis*, *Bombyx mori*, *Clogmia albipunctata*, *Empis livida*, *Megaselia abdita*, and *Drosophila melanogaster*.

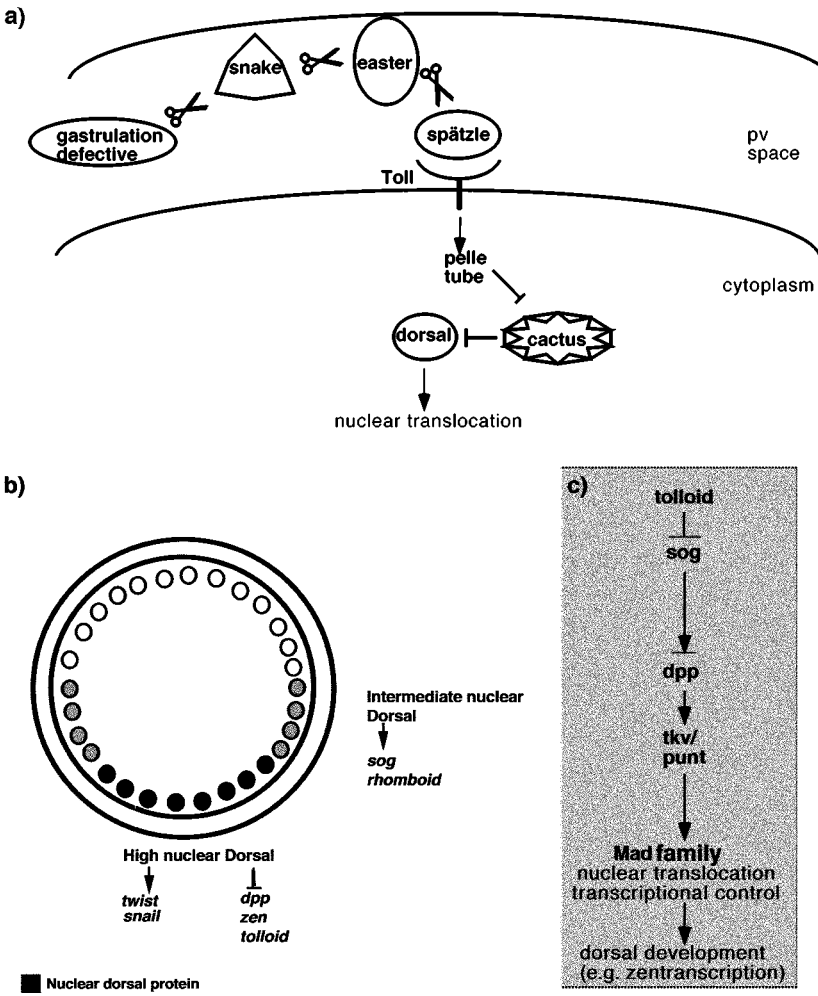

Figure 2 DV patterning in *Drosophila melanogaster*. The pathways illustrate the interactions of genes that act to pattern the DV axis of the embryo without influencing eggshell morphology (*a*). (*a*) shows maternal effect genes that act, within the perivitelline space (pv space) to control the nuclear localization of Dorsal protein along the DV axis. (*b*) Dorsal is found in a nuclear gradient. At high nuclear concentrations Dorsal represses zygotic genes that pattern the dorsal regions of the embryo while activating ventral development through *twist* and *snail* activation. At intermediate levels the Dorsal transcription factor promotes lateral fates (*b*). (*c*) Factors regulating Dpp signaling are illustrated, showing basic interactions. *sog* both antagonizes and potentiates Dpp signaling.

of Spätzle protein to a 23-kD form by Easter (99, 147). When injected into the perivitelline space, cleaved Spätzle activates ventral development in both a site- and concentration-specific fashion (99). Genetic and biochemical evidence suggests that the cleaved and active form of Spätzle then acts as a ligand for the Toll receptor, which is immediately upstream of *Toll* but downstream of *easter* (Figure 2*a*) (see 22). Localized activation of the Toll receptor leads to the stimulation of an intracellular pathway involving *tube* and *pelle*, the end result of which is the phosphorylation and degradation of the IkB orthologue *cactus* (10, 53, 126).

Cactus physically interacts with and thereby inhibits a key gene in dorsoventral axis formation, the morphogenetic transcription factor Dorsal (a *NFκB/rel* homologue; 170). Degradation of Cactus allows Dorsal to enter the nucleus (12, 186). Since Toll is activated ventrally, Dorsal protein enters the nucleus at highest concentration ventrally. Immunostaining against Dorsal protein allows direct and elegant visualization of its nucleocytoplasmic gradient, running ventral to dorsal across the embryo (134, 138, 171).

Activation of the maternal *Toll/dorsal* pathway leads to the expression of zygotic genes at different DV levels of the embryo (Figure 2*b*). At highest nuclear concentration (ventral), Dorsal activates *twist* and *snail*, which are required for specification of ventral fate (mesoderm) and for the inhibition of lateral fates such as neurectoderm (48, 66, 80, 87, 104, 124, 125, 180–182). Laterally, intermediate nuclear concentrations of Dorsal activate a second group of targets including *rhomboid* and *short gastrulation* (*sog*). These genes specify lateral neurectodermal territories and influence the activity of Decapentaplegic (Dpp), respectively (see below; 42, 65). Dorsal also acts as a transcriptional repressor of genes such as *dpp* and *zen*, which specify dorsal fates (32, 64, 68, 125, 136). Thus, higher Dorsal nuclear activity in ventral and lateral regions restricts *dpp* expression to the dorsal side of the embryo. *dpp* acts to pattern ectoderm, specifying different tissue territories such as amnioserosa and dorsal epidermis (39, 187).

Which aspects of these processes are conserved across species? We focus on components with known homologues in other species that can therefore be discussed in an evolutionary context. In particular, we discuss *dpp* and *twist* and examine their potential regulation by the *Toll* signaling pathway outside *D. melanogaster*.

Dpp Signaling May Play a Conserved Role in Axis Formation Across Phyla

dpp is expressed at blastoderm stage, in a longitudinal stripe restricted by Dorsal protein to the dorsal 40% of the embryo (166). Dpp protein (enhanced by the ligand Screw; 106) is responsible for the transcriptional activation of a number of targets in specific dorsal territories including *zerknüllt* (*zen*) (8, 39, 68, 125, 137, 187). Dpp is a ligand for the Tkv/Punt receptor complex (17, 88, 105, 116, 135). Generation of a Dpp activity gradient is also dependent on the *dpp* antagonist, *sog*. Genetic mosaic analysis suggests that *sog* is required ventrolaterally and acts

non-cell autonomously. Moreover, genetic experiments addressing the interaction of *dpp* and *sog* suggest that *sog* antagonizes Dpp function dorsolaterally but intensifies Dpp activity in the extreme dorsal region of the embryo (Figure 2*c*) (see 7, 13, 40, 42, 92, 199). Therefore an antagonistic gradient of Sog emanating from lateral regions of the embryo may help to grade the activity of Dpp in dorsal territories.

Regulation of Dpp signaling is also mediated by the *tolloid* gene. *tolloid* embryos have a ventralized cuticle phenotype, and are missing the most dorsal structures (amnioserosa) as well as some dorsal epidermis (74, 156). *tolloid* is expressed dorsally, encodes a metalloprotease, and has been shown genetically to be upstream of *dpp* (Figure 2*c*). Epistasis analysis in *Drosophila* and second axis induction assays in *Xenopus* suggest that *tolloid* functions upstream of *sog* as an antagonist (92). Tolloid cleaves Sog protein, as evidenced by the fact that Sog cleavage products can be detected in the embryo upon *sog* overexpression, or when Sog and Tolloid are co-incubated in vitro (92, 196). Sog destruction by Tolloid may allow Dpp to bind to its receptor, perhaps by reducing the affinity of the Sog/Dpp interaction and thereby freeing Dpp to function independently of Sog. The isolation of interacting antimorphic mutations was used to suggest a physical interaction between the *tolloid* and *dpp* gene products. These phenotypes have not been reconciled with the current view that *tolloid* acts on *sog* (24, 40, 41, 123). Further regulation of Dpp activity is suggested by recent data concerning the *twisted gastrulation* gene, which though originally proposed to antagonize Sog might actually be an antagonist of Dpp via Sog (for more details, see 132, 196).

Components downstream of the Tkv/Punt receptor complex have also been identified. *Mad* and *Medea* mutants are enhancers of a weak *dpp* phenotype, and mediate the transcriptional response to Dpp signaling (107, 108, 123, 154). Between them, the *Mad/Medea* and *schnurri* DNA binding transcription factors mediate activation and repression of *dpp*-responsive transcriptional targets (27, 61, 77, 93, 107, 108, 123, 154, 184). *Mad* family genes seem to collaborate with a variety of transcriptional cofactors and are more generally required for Dpp signaling than *schnurri*, which functions in part by antagonizing the general repression of *dpp* transcriptional targets by *brinker* (71, 93). Some of the above components have been isolated not only from other insect species, but also from across phyla, suggesting that *dpp* may have played an ancient role in dorsoventral axis formation. We first discuss comparative insect studies which are distinct from chordate studies in focusing on Dpp expression, not activity. We then discuss data from chordates, where the regulation of Dpp signaling activity has been successfully dissected biochemically and genetically.

dpp and *zen* homologues have been isolated from more basal insects, including the beetle *Tribolium castaneum* (*Tc-dpp* and *Tc-zen*) and the grasshopper *Schistocerca americana*. Analysis of the genes has been used to determine whether they are expressed in dorsal tissue (as in *Drosophila*), and whether *dpp* expression is regulated by Dorsal protein. Expression of *Tc-dpp* and *Tc-zen* is observed in serosal cells (23, 37, 139). Serosa is an extraembryonic membrane with a putative

protective function during insect embryogenesis (3). In the higher flies extraembryonic membranes are reduced and referred to as the amnioserosa, a dorsally placed tissue that, in *Drosophila*, also expresses *dpp* and *zen* (2). In more basal insects such as *Tribolium* and *Schistocerca*, the serosa is anteriorly placed in the egg. At first this may suggest that *dpp* and *zen* do not play a role in DV axis formation in basal insects but expression of *dpp* in the serosa of basal insects may still play a role in patterning dorsal tissue, given the position of serosa relative to the dorsal ectoderm. *Tc-dpp* is expressed in serosal cells surrounding the germ anlage [i.e., closest to dorsal ectoderm (72, 139)]. Positionally, this is essentially the same, relative to the dorsal ectoderm, as *dpp* and zen expression in *D. melanogaster* and may therefore constitute "dorsal" expression. Thus the serosal *dpp* domain might still be involved in patterning "dorsal" tissue in the *Tribolium* embryo. Later in development, both *Tribolium* and *Schistocerca dpp* are expressed in the dorsal ectoderm in the abdominal field of the embryo (72, 139). Thus there is potentially a more compelling argument for DV patterning by *dpp* during later development.

Evidence for repression of *Tc-dpp* by Dorsal protein can be inferred from the fact that *Tc-dpp* is not co-expressed in most cells with nuclear Tc-Dorsal (23). Although one could argue against repression of *Tc-dpp* by Tc-Dorsal as they are co-expressed in terminal cells, both *dpp* and nuclear Dorsal are also found in terminal cells of the *D. melanogaster* embryo suggesting differences in *dpp* regulation at the termini in both species. Here we should point out the limits of expression data, in that *Tribolium dpp* expression also potentially overlaps with the anterior factor *hunchback* and with the terminal system gene product *tailless* (23, 148, 193). Thus expression data could just as well be used to argue that *Tc-dpp* is regulated by the anterior or terminal systems. The growing inventory of tools available for gene expression manipulation in *Tribolium* may confirm whether the transcriptional regulation of *dpp* by Dorsal is conserved from flies to beetles.

dpp, one of the *D. melanogaster* homologues of the TGFβ superfamily, is most similar to the BMP2/4 group. Indeed, the *dpp* phenotype can be rescued using a Dpp-BMP4 fusion product (112, 113). Manipulations in *Xenopus* as well as zebrafish genetics suggest that the Dpp signaling pathway plays a conserved role in axial patterning (reviewed in 29, 56). As in *Drosophila*, regulation of Dpp protein activity may be the key to generating positional information along the DV axis in chordates. This has been shown in *Xenopus* by the injection of BMP4, which affects DV patterning in a concentration-dependent fashion: Injection of BMP4 can ventralize mesoderm (promoting fates such as blood, and having antineurogenic effects on ectodermal tissue). Similarly, injection of a dominant negative BMP receptor shows that BMP signaling is required for the development of ventral fates (47). The role of BMPs in DV patterning of zebrafish has been demonstrated genetically, where the *swirl* (BMP2) mutant has a dorsalized phenotype [broadened notochord and expanded somites (51, 78)].

Regulation of BMP signaling is also conserved in chordates in that antagonists of BMPs operate during DV axial patterning. Classical embryological

experiments demonstrate that dorsal fates are promoted by organizer tissue, which upon transplantation leads to dorsalization of ventral host tissue, i.e., an ectopic DV axis (163). The *Xenopus* organizer expresses BMP antagonists including *chordin*, the functional homologue of *short gastrulation* (57, 141). *Xenopus chordin* injections induce a second axis, rescue UV ventralized embryos, and can dorsalize mesoderm by antagonizing BMPs (118, 141). Conversely, the effect of *chordin* injections alone can be mitigated by the co-injection of high concentrations of BMP4, suggesting a competitive binding interaction (189). Physical Chordin/BMP4 interaction has been confirmed by in vitro binding studies that demonstrate interaction with a binding constant sufficient to interfere with receptor binding (118). Genetic data from zebrafish confirm that the interaction of BMP/Chordin mirrors the *Drosophila* Dpp/Sog interaction in patterning the DV axis. Thus the *chordino* (*chordin*) mutant leads to ventralization, a phenotype that is suppressed by the *swirl* (BMP2) mutant (51, 78, 149).

Further levels of conservation are revealed upon examining Chordin regulation. A *Xenopus tolloid* homologue is capable of cleaving Chordin, thereby overriding Chordin inhibition of BMP4 (117). More recently, conservation of the *twisted gastrulation* gene has also been demonstrated. In contrast to initial data, *twisted gastrulation* may be a conserved inhibitor of BMP function. Some evidence indicates that it is responsible for producing a differential cleavage product of Sog/Chordin that may have increased anti-Dpp/BMP activity (19, 111, 132, 153, 196).

Downstream effectors of Dpp signaling also appear to be conserved in chordates, such that signal transduction is mediated by *Xenopus* homologues of the *Mad* transcription factor (for example, see 89). A number of zebrafish mutations leading to DV phenotypes lie in genes encoding members of the BMP signaling pathway, once again suggesting a conserved role for this signal transduction pathway in DV axis formation (for examples, see 9, 14, 54).

Thus the *dpp* ligand, its antagonist (*sog*), potentiator (*tolloid*), and downstream components (*Mad* homologues) all play a role in dorsoventral axis formation in *Drosophila*, *Xenopus*, and zebrafish, suggesting a potentially ancient role for this pathway in DV axis formation. Moreover, injection of *D. melanogaster dpp* and *sog* into *Xenopus* shows that they behave functionally as BMP4 and *chordin* (57, 144). However, not all aspects of DV axis formation are conserved from flies to chordates. Although the protein Noggin binds and antagonizes BMP4 in *Xenopus*, an orthologue has yet to be found in the genomic sequence of the fly (103, 198).

Since Dpp signaling is involved in providing axial information in both *Xenopus* and *D. melanogaster*, one might argue that it is an ancient component in dorsoventral axis formation and perhaps ancestral. However, there is evidence that the axis classically regarded as the dorsoventral axis in *Xenopus* may not actually be so. Reassessment of the position of primitive blood on the *Xenopus* fate map has been used to argue that the classical embryonic DV axis can actually be characterized as an anteroposterior axis (83). This does not necessarily argue for nonconservation, only that the BMP signaling pathway may also control some aspects of

anteriorization along the "DV" axis in *Xenopus*. One could also argue that as *dpp* genetically behaves morphogenetically, it could have been recruited into axis specification in multiple lineages, given that morphogenetic activity is clearly an efficient means of generating positional information.

However, the BMP pathway is tightly coupled to the dorsoventral axis, as demonstrated by the facts that the deuterostome dorsoventral axis seems morphologically inverted when compared to protostomes, but that this inversion occurs with appropriate alterations in the expression patterns of *dpp*/BMP4 and *sog*/*chordin* (56). More specifically, tissue closest to the blastopore and central nervous system expresses *chordin*/*sog*, whereas tissue further away (be it amnioserosa or blood) expresses BMP4/*dpp*. This suggests a tight coupling of the *dpp* homologue to distinct tissue fates, wherever they lie along the DV body axis (dorsal in flies and ventral in frogs), and may reflect an ancestral role of the pathway in restricting neural fates. The intimate relationship between *dpp*/BMP expression and dorsoventral territories across phyla argues that the Dpp signaling pathway played an ancestral role in DV patterning. However, systems involved in the activation of *dpp*/BMP4 transcription have not been shown to be conserved across species in the context of axis formation, and thus may be derived (see below). This may also be true of the system for setting up ventral fates in *Drosophila*.

twist Is Involved in Mesoderm Patterning in Many Animals

twist is essential for specifying the ventral-most territory of the *Drosophila* embryo [fated as mesoderm; (Figure 2*b*)]. Genetic analysis and dissection of the *twist* promoter suggest that it is directly activated by nuclear Dorsal (182). Loss-of-function alleles of *twist* lead to loss of mesoderm, suggesting that *twist* is essential for specification of ventral tissue in flies (180). This role for *twist* seems to be conserved between flies and beetles as the *Tribolium twist* orthologue is expressed at blastoderm stage in a narrow ventral stripe overlapping the ventral embryonic domain of nuclear Dorsal (23, 160). Slightly later in embryogenesis, the *twist* expression domain retracts anteriorly and widens posteriorly in an apparently *Tc-Dorsal*-independent fashion (23). Thus in *Tribolium*, the role of twist as a specifier of ventral fate seems conserved, but its early expression pattern may not be as dependent on *dorsal* as it is in *Drosophila*.

However, unlike *dpp*, *twist* does not appear to play a role in specifying axial fate in the chordates, as evidenced by its lack of involvement in pan-mesodermal specification. Although multiple *twist* orthologues have been isolated from chordates including mouse and *Xenopus*, they are expressed in and seem to activate specific mesodermal derivatives, and may also inhibit some myogenesis (for example, see 59, 191). *twist* may therefore control submesodermal fates rather than acting as a mesoderm or ventral specification factor in the chordates. Interestingly, the jellyfish *twist* orthologue is expressed in nonmesodermal tissue (164). Jellyfish are considered to be diploblastic (i.e., have only two germ layers, lacking true

mesoderm). Thus the expression of *twist* in muscle-like cells in the jellyfish might suggest that *twist* is involved in specification of a mesodermal-like layer. Alternatively, *twist* may play a role in specification of muscle-like cell fate that predates the origin of mesoderm. *twist* may have been independently recruited as an axial patterning output in the arthropod lineage, a likely hypothesis as the data from jellyfish and chordates suggest that the ancestral role of *twist* is not general mesoderm specification. As with *dpp*, regulation of *twist* by the Dorsal transcription factor may be a recent innovation, as the *Toll* signaling pathway may have been recently recruited into DV axis formation in the insects.

The *Toll*/*dorsal* Signaling Pathway May Have Been Co-Opted into Axis Formation from the Immune System

The role of the *Toll* signaling pathway in dorsoventral axis formation may not be ancestral. This is particularly interesting as the *D. melanogaster Toll* pathway is directly responsible for restricting transcriptional activation of *dpp* to the dorsal side of the embryo, and the latter appears to be ancestral for axis formation (see above). As with the *dpp* pathway, the components of the *Toll* signaling pathway have orthologues within the insects and across phyla. Thus *Toll* homologues have been isolated from *Tribolium castaneum*, as well as from mammals (52, 94, 130). The key downstream effector of *Toll* receptor homologues seems functionally conserved across species (*dorsal* in the insects, *NF*κ*B* in mammals).

Expression data indicate that both *Toll* and *dorsal* play a conserved role in DV patterning of *Tribolium* (23, 94). Both *Toll* and *dorsal* are found in gradients in the ventral region of the embryo during early *Tribolium* embryogenesis. Indeed, immunostaining indicates that the *Tribolium* Dorsal protein forms a ventral nuclear localization gradient during blastoderm stage. Interestingly, the *Tribolium Toll* receptor seems at first sight to differ in some respects from its *D. melanogaster* counterpart (94). Rather than being maternally provided and ubiquitously expressed, *Tribolium Toll* is found in a ventral gradient in cells with nuclearly localized *Tc*-Dorsal. This finding was used as the basis of the proposition that the *Toll* gradient may form zygotically in response to nuclear localization of Dorsal (23, 94). Local (as opposed to ubiquitous) injections of *D. melanogaster Toll* can rescue the *Drosophila Toll* phenotype (52). Thus, although it seems important to have enough *Drosophila* Toll receptor to sequester ligand, *Toll* transcript need only be applied (and presumably expressed) relatively locally to form a normal axis. The localized expression of *Toll* in *Tribolium* does not necessarily imply a functional difference to ubiquitous *Toll* expression in *Drosophila*. Furthermore, it has been argued that there has been a shift toward maternal control of axis formation in higher insects such as *Drosophila*, which is consistent with a shift from zygotic to maternal expression of factors such as *Toll* in flies (115, 121). Essentially, the ventral increase in *Toll* levels in putative response to nuclear Dorsal may indicate extensive zygotic refinement of pattern in response to axial signaling. Thus, although the transcriptional regulation of *Tribolium Toll* appears to be different, the

Toll/*dorsal* pathway may play a fundamentally conserved role in axis formation from beetles to flies.

It is unknown whether these factors play a role in axis formation in other phyla. The mammalian *Toll* and *dorsal* homologues were identified for their role in the immune system. Interestingly, *Toll* and *dorsal* in *D. melanogaster* and the *IL-1R*/*NFκB* pathway in mammals both play a role in stimulation of the innate immune response during pathogenic aggression (84, 85, 95). Although *D. melanogaster Toll*, *spätzle*, and *dorsal* can stimulate dorsalization in UV-ventralized *Xenopus* embryos, there is no evidence that the chordate homologues of these genes function, or are expressed, at the correct developmental time to play a role in DV axis formation in these animals (6). Thus one can only hypothesize that the ancestral role of the *Toll*/*dorsal* signaling pathway was in the immune system. The pathway appears to have become involved in axis formation in the lineage leading to insects (Figure 3).

Conservation of the DV Axis Formation Cassette in Its Entirety?

Although the Dpp signaling pathway appears to play a highly conserved role in DV axis formation, this is not necessarily true of either *twist* or *dorsal*. *twist* may have played an ancestral role in some form of mesoderm development (either general

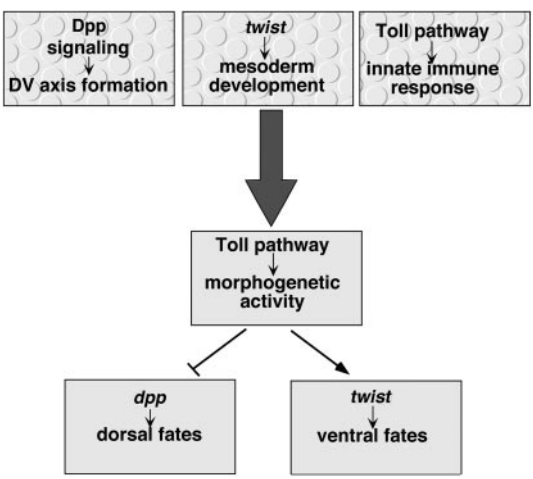

Figure 3 A simplified model for the evolution of DV patterning in *D. melanogaster*. The model considers an ancestral state (*bubbled boxes*, *top row*) where Dpp signaling was involved in DV patterning, *twist* in mesoderm fate specification, and *Toll* signaling in the innate immune response. In the lineage leading to flies (*big arrow*), *dpp* and *twist* fell under the regulation of *Toll* signaling during early development, and thus a single maternal axis formation system.

mesoderm specification or, more likely, specification of muscle percursors) in triploblastic lineages, and perhaps even in more primitive animals. At some point in the lineage leading to higher insects, very early *dpp* and *twist* expression may have fallen under the regulation of the *Toll* signaling pathway. The *Toll/dorsal* pathway could have been recruited from an ancestral role in the immune system for DV axis specification (as suggested by conserved usage of this signaling system in innate immunity from *Drosophila* to mammals). Assumption by *dorsal* of transcriptional regulation of factors such as *dpp* and *twist* would have led to the modern cascade of pathways specifying regions along the DV axis in *D. melanogaster* (Figure 3).

However, the above genes may already form a regulatory cassette in the chick, suggesting that regulation of *twist* and *dpp* by *dorsal* is more ancient than implied in the above scenario (18, 75). During outgrowth of the chick limb bud, *NFκB* (*dorsal*) is expressed in mesenchyme underlying the apical ectodermal ridge (AER). The AER is a morphological ridge along the developing limb bud, which is known from classical embryological experiments to be required for limb outgrowth (142, 175). Decreasing the activity of NFκB using viral overexpression of an *IκB* (*cactus*) mutant that cannot be targeted for degradation leads to defects in limb outgrowth (18, 75). Interestingly, *twist* mutants in mouse and humans lead to similar limb phenotypes, suggesting conservation of this role in limb development across vertebrate species (15, 60). *twist*, which is expressed in chick limb mesenchyme, is downregulated under these experimental conditions, implying that it is under positive regulation by *NFκB* (18, 75). Furthermore, in this experiment the expression of BMP4 is upregulated, suggesting that a *dpp* homologue is negatively controlled by *NFκB* in the context of chick limb development.

Thus the entire regulatory cassette (*dpp* and *twist* under control of a *dorsal* homologue) may actually be an ancient network that in extant animals has become critical for limb development in chordates and DV axiation in *D. melanogaster*. Whether this cassette had a function in axis formation in the chordates is not known since vestiges of *dorsal*-mediated *dpp* regulation during early embryogenesis have not been found outside the arthropods. There is as yet no indication that a *NFκB* homologue is expressed in mouse during early embryogenesis [although not all *dorsal* homologues have been tested, and the transgenic reporter approach used to analyze expression may not recapitulate the complete expression pattern of the gene (see 146)]. The fact that *D. melanogaster spätzle* and *Toll* can induce dorsalization in ventralized *Xenopus* embryos is compelling in this context (6). However, expression analysis and loss-of-function studies are required to show that the *Xenopus Toll* and putative *spätzle* orthologues are expressed at the correct time and can also generate DV phenotypes when overexpressed.

Thus far, we have discussed conservation of signal transduction systems that can easily be envisioned as being conserved in animals with very different embryogenesis from that of *D. melanogaster*. We now examine the anterior system, which is based on the diffusion of molecules in the syncytial environment of the early *Drosophila* embryo, and ask whether such an extreme mechanism for generating a morphogen gradient can be conserved.

THE ANTERIOR SYSTEM

AP Patterning in *Drosophila melanogaster*

Our understanding of the anterior system centers around the archetypal morphogen, *bicoid* (*bcd*) (Figure 4). During oogenesis *bcd* mRNA becomes localized to the anterior of the oocyte in a process depending upon various factors (96). Diffusion of Bcd protein from its anterior location sets up a gradient that determines positional information. The homeoprotein Bcd regulates gene activity through transcriptional and translational control. An example of the former is zygotic *hunchback* (*hb*), which *bcd* transcriptionally activates in an anterior domain (33). Like Bcd, *hb* provides information for anterior patterning through gap, pair-rule, and *hox* genes (reviewed in 127, 157, 173). Bcd is also responsible for the anterior translational repression of ubiquitous maternal *caudal* mRNA leading to a posterior gradient of the homeoprotein transcription factor Caudal (34, 129). *caudal* is involved in posterior patterning through the gap genes and behaves as a homeotic gene in the posterior-most segment of the fly (90, 97, 98, 128, 151). The anterior system involves two factors, *caudal* and *hb,* that are conserved to varying extents in other species.

caudal May Play a Conserved Role in Posterior Patterning Across Phyla

caudal appears to play a role in posterior patterning throughout arthropods and across to the chordates. Within the arthropods, *caudal* has been cloned from, amongst other insects, *Tribolium castaneum* and the silkmoth *Bombyx mori* (150, 194). Both *Tribolium* and *Bombyx caudal* (RNA and protein) form posterior gradients, reminiscent of those observed in the flies, and suggestive of conserved regulation. Furthermore, heterologous expression of the *Tribolium caudal* gene in *D. melanogaster* shows that a *bcd*-dependent gradient can be generated, suggesting conserved translational regulation of *caudal* in the beetle, though a *Tribolium bicoid* homologue has not yet been identified (192). *caudal* is also expressed posteriorly in the grasshopper embryo, suggesting a conserved role in posterior patterning in a more basal insect (31). *caudal* may also play a conserved role in the wasp *Nasonia vitripennis*, where the *head only* mutation greatly resembles the phenotype of the *D. melanogaster caudal* mutant (121). Strikingly, *caudal* homologues may play conserved roles in vertebrate development, as well as in an ascidian and nematode (for example, see 36, 43, 44, 69, 73, 76, 91, 119). For example, *cdxA-C* in chick are expressed in a spatially and temporally graded fashion in posterior neural plate and midline during primitive streak stages, suggesting a conserved role in posterior patterning. Loss of *C. elegans caudal* (*pal-1*) function affects posterior blastomere and adult male tail development (36, 63). Similarly, the *caudal* homologue in an ascidian has been shown to play a functional role in tail development (76). Thus *caudal* likely played a role in posterior patterning in the ancestral insect and perhaps even in the ancestral bilaterian.

hb May Play a Conserved Role in AP Patterning Within the Insects

The status of *hunchback* (*hb*) as an ancestral AP patterning factor is less clear-cut. Orthologues have been cloned across phyla, but there is no evidence for a role in AP patterning outside the insects. As described above, maternal and zygotic *hb* are involved in AP patterning during early *D. melanogaster* development (Figure 4). Flies closely related to *D. melanogaster* (*Drosophila virilis*), as well as basal insects such as the grasshopper *Schistocerca americana*, have *hb* homologues (115, 131, 158, 159, 168, 183, 193). The fact that insect *hb* homologues are expressed maternally and zygotically in an anterior domain suggests a conserved role in AP patterning within the insects. Interestingly, the grasshopper, *S. americana*, expresses *hb* in a cellular environment in blocks of different concentration along the AP axis (115). This may reflect how positional information is conveyed in a cellular environment, as opposed to the gradients observed in the syncytial environment of the *Drosophila* blastoderm. However, outside the insects there is no clear evidence for a role in AP patterning from expression data, although *hb* orthologues are found in conserved domains in the nervous system (and are therefore probably true orthologues). For example, neither *H. triserialis* (annelid) nor *C. elegans* (nematode) *hb* is expressed in an early anterior domain that would indicate a role in AP patterning (38, 70, 143). Perhaps *hb* was co-opted relatively recently from the nervous system into its role as an anterior morphogen within the arthropods. Thus *hunchback* may have played a role in AP axis formation in the lineage leading to insects, but probably not in other phyla. *hb* has orthologues outside the insects. Expression data from the chelicerates and crustaceans should indicate whether *hb* plays a role in AP patterning in all arthropods.

bcd Function May Be a Recent Novelty in AP Patterning

The status of *bcd* in ancestral AP patterning is markedly different. Despite its importance in AP patterning in *Drosophila*, *bcd* homologues have been isolated only from higher dipterans (the cyclorrhaphan flies; 158, 168). There is evidence that *Megaselia abdita bicoid* (*Ma-bcd*) does play a functional role in axis formation: *Ma-bcd* is expressed anteriorly, and dsRNA interference experiments lead to a phenotype that resembles that of the *D. melanogaster bcd* mutant, except that the range of its action extends more posteriorly (168). These data suggest that in contrast to *Drosophila*, *bcd*'s sphere of influence may extend further posteriorly along the AP axis of *Megaselia*. Indirect evidence for the conservation of *bcd* in a more basal insect comes from *Tribolium*. As noted above, *Tc-caudal* transcript can be regulated in a *bcd*-dependent fashion when introduced into flies (192). This suggests the existence of an as yet unidentified *bicoid*-like activity in *Tribolium*. Such data would imply that *bcd* is involved in AP patterning from flies to beetles.

Molecular data, however, suggest that the *bcd* gene arose relatively recently. Phylogenetic analysis of the *Megaselia abdita bicoid* and *zen* orthologues suggests that they are products of a Hox3 duplication in an ancestor of the higher dipterans (167). *bcd* and *zen* reside together in the part of *D. melanogaster* Hox cluster

in the location where one should find a Hox3 orthologue. This adds support to the idea that the two genes are products of a Hox3 duplication. Furthermore, the region of the *Tribolium* hox cluster in which one would expect to find *bcd* has now been sequenced and no *bcd* orthologue was found (16). Although the *Tribolium* orthologue could have moved via a recombination event, the inability to isolate *bcd* from lower insects, as well as the sequence analysis of *Megaselia bcd* and *zen*, are evidence for a relatively recent origin of *bcd* in the lineage leading to higher flies. *bcd* is thus an interesting case in which a gene has arisen recently and yet become a major molecular player in axis formation (whether the ancestor of *bcd* played a role in axis formation is interesting but unresolved). The mechanism by which *bcd* might have become involved in AP axis formation is discussed below and by Schmidt-Ott (145). The Hox3 orthologue has been cloned from two chelicerates, a mite and a spider (28, 179). Hox3 in chelicerates is expressed in a discrete domain of the AP axis, but its anterior border coincides with the Hox gene *proboscopedia* (*pb*). Thus, although Hox3 in chelicerates is found in a hox-like expression domain, it is expressed more anteriorly than expected, suggesting a breakdown in colinear expression of this gene within the arthropods. Overlap with *pb* and the breakdown of colinearity are hypothesized to have allowed Hox3 to lose hox function and take on novel roles in the lineage leading to insects (28, 179).

What does this tell us about the way in which the anterior patterning system in *D. melanogaster* evolved? The expression of *hb* outside the insects suggests that its role in nervous system development is highly conserved. The ancestral patterning system in insects may have involved a posterior *caudal* gradient and an anterior *hb* gradient. The duplication of an ancestral Hox3 gene may have given rise to *bcd*, which in the modern fly plays a dual role as translational regulator of maternal *caudal*, and transcriptional activator of zygotic *hb*. In this scheme a novel gene, *bcd*, has become a major organizer of the AP axis (Figure 5) (reviewed in 30). Thus anterior patterning in *D. melanogaster* displays the same properties as the DV system: Potentially ancient (*caudal*) and more recently recruited (*hb*) regulatory factors have fallen under the global coordination of a novel gene (the anterior *bcd* gradient) (Figure 5).

EVOLUTION OF AXIS FORMATION MECHANISMS

The maternal coordinate systems in *Drosophila* appear to be a melange of molecular processes elaborated upon multiple times in evolutionary history. Modern *D. melanogaster* utilizes evolutionarily ancient factors such as *dpp* and *caudal*, which play a conserved role in axial patterning in chordates. *Drosophila* also use molecules such as *zen* and *bicoid*, which may have arisen relatively recently, in the lineage leading to higher dipterans. This suggests that axis formation in *D. melanogaster* is a plastic and rapidly evolving process at the molecular level and is constantly taking advantage of new cues and innovations. However, innovation does not necessarily mean the displacement of pathways, although the cues

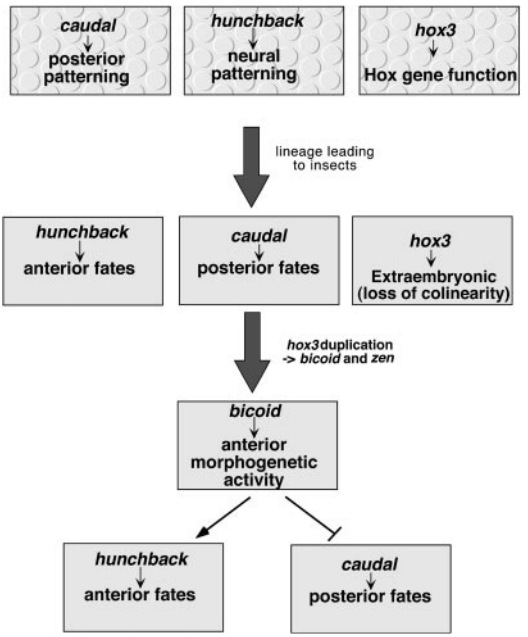

Figure 5 A potential, simplified scheme for the origin of the *D. melanogaster* anterior system. The scheme envisages an ancestral state (*bubble boxes*, *top row*) where *hunchback* was involved in neural patterning, *caudal* in posterior patterning, and a Hox3 gene in segmental identity along the AP axis. In the lineage leading to insects, *hunchback* and *caudal* may both have been involved in axial patterning, whereas Hox3 became expressed in a spatial pattern that is no longer colinear with other Hox genes (*middle row*). In the lineage leading to the higher dipterans, the duplication of Hox3 may have led to *bicoid*, which acts in the modern fly as a major player in AP axis formation through the regulation of *hunchback* and *caudal*.

regulating them may have changed, as exemplified by the deep-set link between Dpp signaling and DV axiation. Also, it is not simply axial cues that change, but downstream specification factors can also fall under the control of axial patterning systems. This is exemplified by *twist*, which plays a role in the development of mesoderm and its derivatives in all systems studied, but may only constitute a global mesoderm specification factor within the insects.

REDUNDANCY IN AXIS FORMATION

How can an event as important as axis formation evolve rapidly? One mechanism would be to retain old pathways in a redundant fashion. *bcd* may still be rapidly changing at the sequence level, and its role in AP axiation might have arisen relatively recently. Interestingly, the anterior system has long been known to show

redundancy with the posterior maternal coordinate system. Such large-scale redundancy may be crucial to plasticity in axis formation.

Both the anterior and posterior systems lead to the same outcome, although this outcome is slightly temporally displaced: Both systems result in a Hb protein gradient with highest levels at the anterior (Figure 4) (see 33, 62, 67, 173, 176). Hb protein is detectable as a plateau until about 50% egg length, where it begins to taper off (176). As mentioned above, the Bcd gradient is crucial to generating the zygotic *hb* pattern. The earliest differential *hb* expression is, however, due to the translational repression of a uniform maternal pool of *hb* transcript (Figure 4) (see 176, 178). This translational repression is mediated by *nanos*, and its cofactor *pumilio* (62, 67, 102). Pumilio recognizes a sequence (NRE) in the 3′UTR of *hb* transcript, and forms a multiprotein complex in vitro, which includes the NRE, Nanos protein, and Pumilio itself (161, 162, 188). Since *nanos* transcript is maternally localized to the posterior of the developing oocyte and translationally repressed anteriorly, a Nanos protein gradient forms emanating from the posterior (Figure 4) (26, 185). Hence, maternal *hb* transcript is translationally repressed at the posterior, leading to an anterior gradient of Hb protein.

Thus two systems, an anterior and a posterior system, independently generate an anterior Hb gradient. Furthermore, elegant genetic data indicate that the two gradients of *hb* are redundant. Genetically, the *nanos* phenotype, in which abdominal segments are entirely lost, can be rescued by eliminating maternal *hb* transcript (62, 67). The *nanos* axis formation phenotype is therefore due to the ectopic activity of maternal *hb* in the posterior of the embryo. Furthermore, zygotic *hb* compensates entirely for loss of the maternal transcript. Therefore, the posterior maternal coordinate system can be eliminated and AP axis formation occurs normally, with only the anterior system operating.

More recent evidence suggests the converse may also be true. Elimination of *bcd* activity cannot be compensated for by the posterior system as it stands (190). Phenotypes are still observed in T2/T3 (parasegment 4), so that levels of maternal *hb* seem to be unable to compensate for the lack of zygotically activated *hb* in this domain. However, if extra copies of *hb* are supplied, and the levels of *hb* further increased by reducing levels of a transcriptional repressor of zygotic *hb* (*knirps*), the need for *bcd* in thoracic development is abrogated (190). Thus *hb* can almost entirely compensate for the anterior system during early AP axis formation (almost entirely, since the extreme anterior of the *bcd* cuticle phenotype cannot be rescued in this way).

Why does *D. melanogaster* have two virtually redundant systems carrying out such similar functions? The answer may be that one system was derived more recently than the other. Nanos response elements are found in *hb* orthologues in many insects (for example, see 158, 183, 193). Furthermore, *nanos* and *pumilio* have been cloned from multiple phyla including chordates, nematodes, and annelids (e.g., 25, 81, 101, 120, 174, 197). *nanos* may even play a role in axis specification in comparatively basal insects (S.L. & N.H.P., unpublished observation). Thus while the *bcd* system seems a recent innovation in axis formation, setting up a gradient of Hb using translational repression by Nanos seems to be a more

ancestral mechanism. What we may be observing in *D. melanogaster* is an intermediate in the displacement of an ancient AP patterning system (*nanos* regulated) by a more recently innovated one (*bcd* regulated). Redundancy of the two systems could constitute a safety net that protects the integrity of AP patterning as axis formation rapidly evolves.

LINKING MORPHOLOGICAL CHANGES IN EMBRYOGENESIS TO EVOLUTION OF AXIS FORMATION

Recent data from dipterans suggest a correlation between the origin of *bcd* as an anterior patterning factor and a profound and potentially linked change in developmental morphology. Less derived insects have two extraembryonic membranes, the amnion and the serosa, that have distinct morphologies and derivations during embryogenesis (3). However, *D. melanogaster* has a fused and reduced single extraembryonic tissue called the amnioserosa (2). The link between the amnioserosa in *D. melanogaster* and the amnion and the serosa in less derived insects is clear from the expression of extraembryonic markers such as *zen*, as well as from the fate of these tissues in later embryogenesis. However, whereas *D. melanogaster* amnioserosa lies in the DV axis of the egg and is clearly specified by the DV axis formation pathway (under the control of *dpp*), serosa in basal insects originates from tissue anterior to the embryo proper. A major change may thus have occurred in specification of extraembryonic material since it has moved into a different egg axis.

Examination of the status of extraembryonic membranes within dipterans suggests that the fused and reduced amnioserosa is a character of the higher dipterans (cyclorrhaphan flies). Thus the basal cyclorrhaphan fly *Megaselia abdita* has a single extraembryonic membrane, whereas a representative species from a sister taxon, the *Empidoidea* (*E. livida*), has a separate amnion and serosa (145, 167). These data place the origin of amnioserosa and its DV axis location in the same phylogenetic position as the hypothesized duplication of a Hox3 group gene to give *bcd* and *zen* (167). This gene duplication may also have been essential for relocation of extraembryonic material from the anterior region of the egg to the DV axis under control of the *Toll* signaling system. The appearance of a novel anterior patterning factor, *bcd*, may have been key to allowing full spatial repositioning of amnioserosa, along with *zen* expression, into the DV axis of the egg (145).

Interestingly, there are also major changes in head morphology at the base of the cyclorrhaphan flies. In particular, the cyclorrhaphan flies have reduced feeding structures (the cephalopharyngeal skeleton) and larvae hatch with involuted heads (195). Although the morphology of head characters is variable amongst the cyclorrhaphan flies, displacement of an ancestral anterior patterning system by *bcd* may have been essential for (or even responsible for) these changes. Early patterning by *bcd* may have a profound effect on later head morphology, and the fact that *Megaselia* has a markedly different range of *bcd* activity may indicate that even within the cyclorrhaphan flies *bcd* has varying roles or effects (168).

Thus the molecular evolution of axis formation systems in the higher flies appears to correlate with major changes in morphology. This would suggest that rapid evolution of axis formation is potentially tied to the diversity of morphology before and perhaps even after the phylotypic stage, i.e., the stage where embryos of different species within a phylum converge on a very similar morphology.

CONCLUDING REMARKS

Recent data, such as those examining *bicoid* and *zen* in more basal insects, suggest that some factors involved in axis formation in *D. melanogaster* are derived (167). Along with data examining the morphology of axis specification in nematodes, these findings suggest that axis formation is a variable and plastic process (46). This is consistent with models that view developmental processes as an hourglass with variability at the base leading to a highly conserved phylotypic stage, followed by increasing diversity in adult body plan (see 35, 122). This analogy may understate the remarkable underlying conservation of axis formation (for example in the use of Dpp signaling in DV axis formation and *caudal* in AP patterning).

The recent data that correlate changes in morphology with molecular evolution of axis formation are particularly exciting, as they indicate the types of morphological change that rapid evolution of axis formation might allow. Early developmental processes have been investigated in the light of extreme modifications in life history, for example, in the context of the endoparasitic wasp *Copidosoma* (reviewed in 49). *Copidosoma* undergoes polyembryonic development, where early cleavage events give rise to 2000 randomly oriented embryos. Here axis formation may differ from the mechanisms elucidated in *D. melanogaster* (50). Future data from *Copidosoma* may shed light on the molecular evolution of axis formation during a derived and fundamentally different form of embryogenesis.

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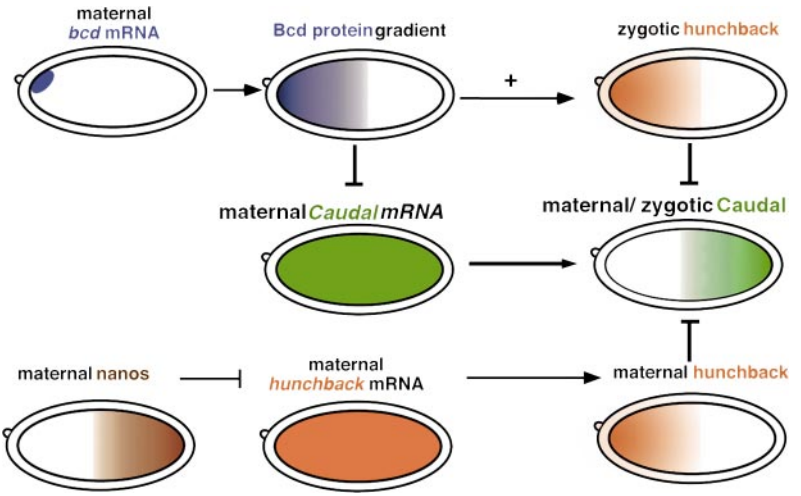

Figure 4 AP patterning in *D. melanogaster*. The diagram illustrates the interconnections between the anterior and posterior maternal co-ordinate systems. The anterior system (key gene *bicoid*) leads to the localized activation of zygotic *hb* transcription and maternal *caudal* translational control. The posterior system involves the translational repression of maternal *hunchback* transcript. The resulting Hunchback protein gradient has an effect on zygotic *caudal* transcription. Anterior is to the left, posterior to the right, and shading indicates gradient vs ubiquitous localization.