Different ways to make a head

Urs Schmidt-Ott

Summary
In *Drosophila*, the establishment of the larval head and thorax depends on the transcription factors BICOID and HUNCHBACK, and on signalling mediated by the receptor tyrosine kinase TORSO. Genetic experiments described in two recent papers[1,2] demonstrate that these factors can, to a large extent, replace each other, revealing a surprising degree of plasticity in establishing larval anterior structures. The commutability of developmental factors might in part reflect the evolutionary history of the system. *BioEssays* 23:8–11, 2001.
© 2001 John Wiley & Sons, Inc.

Introduction

Insect embryos develop in response to factors spreading from the poles of the patterning system. Although the experimental findings that suggested this general principle in the premolecular era came from insects as diverse as leafhopper, beetles, midges and flies,[3–5] the proposed anterior determinant has since been substantiated only in some flies, where it is encoded by the *bicoid* gene.[6–9] Anteriorly localized maternal *bicoid* mRNA encodes a homeodomain protein (BICOID) that specifies anterior development in a concentration-dependent manner through spatially restricted activation of genes required for segmentation.[10–12] BICOID enhances the expression of the transcription factor HUNCHBACK in the anterior half of the *Drosophila* embryo.[13–15] Both proteins share in activating target genes involved in the specification of distinct portions of the head and thorax.[16] BICOID-dependent gene activation in the most anterior portion of the head is not enhanced by HUNCHBACK but by a signalling pathway (reviewed in Ref. 17), which is dependent on the tyrosine-kinase-receptor TORSO[18–20] (Fig. 1). Two recent papers describe genetic experiments that reveal that BICOID can substitute for TORSO-mediated signalling activity in the head and that HUNCHBACK can partially substitute for BICOID.[1,2]

Thorax formation in the absence of BICOID activity

Wimmer et al.[1] asked to what extent HUNCHBACK activity requires BICOID function. Three *hunchback* enhancers are known: one maternal, one early zygotic, and one late zygotic.[14] Only the early zygotic enhancer/promotor is BICOID-dependent.[11,12,21,22] How do flies deprived of the BICOID-dependent enhancer develop? To address this question *hunchback* rescue constructs without the BICOID-dependent early zygotic enhancer element were engineered (hbP1only) and introduced into flies. Embryos deficient for early zygotic *hunchback* activity lack all three thoracic and the labial segments, if derived from heterozygous *hunchback* mutant mothers (Fig. 2A). However, if the full maternal contribution of HUNCHBACK to the embryos is restored by introducing a BICOID-independent copy of the *hunchback* gene into the mother, the first thoracic and the labial segments are rescued (Fig. 2B). A complete rescue is observed, if maternal *hunchback* is further increased to four copies and *knirps*, coding for a zygotic transcription factor with a repressive effect on *hunchback*, is lowered from two copies to one (Fig. 2C). These experiments demonstrate that, under certain conditions, natural BICOID-independent *hunchback* enhancers can restore essential *hunchback* expression sufficient to cause thoracic and labial segments.

Is this effect of HUNCHBACK due to replacing BICOID function? This question was addressed in a second series of experiments. BICOID-deficient embryos lack the entire head and thorax, which are replaced by posterior abdominal structures in reversed polarity. When such embryos, which show normal maternal *hunchback* expression, were provided with zygotic HUNCHBACK activity in form of a BICOID-like anterior HUNCHBACK gradient, the duplicated posterior structures at the anterior pole of the embryos were almost completely suppressed. In addition, two of the three thoracic segments were rescued. Thus, an increased amount of HUNCHBACK can partially replace BICOID function in the thorax.

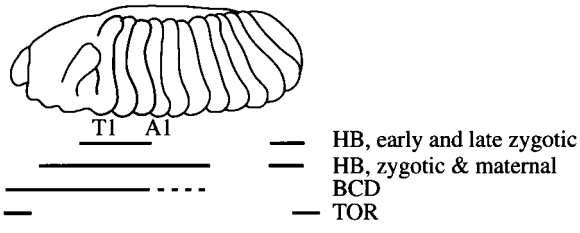

Figure 1. Schematic view of a segmented embryo. Segmental deletions dependent on BICOID (BCD), HUNCHBACK (HB) and TORSO (TOR)-mediated signalling are indicated with bars. T1 and A1 mark the first thoracic and the first abdominal segments, respectively.

Max-Planck-Institut für biophysikalische Chemie, Abteilung für molekulare Entwicklungsbiologie, Am Fassberg 11, D-37077 Göttingen, Germany. E-mail: uschmid@gwdg.de

BioEssays 23:8–11, © 2001 John Wiley & Sons, Inc.

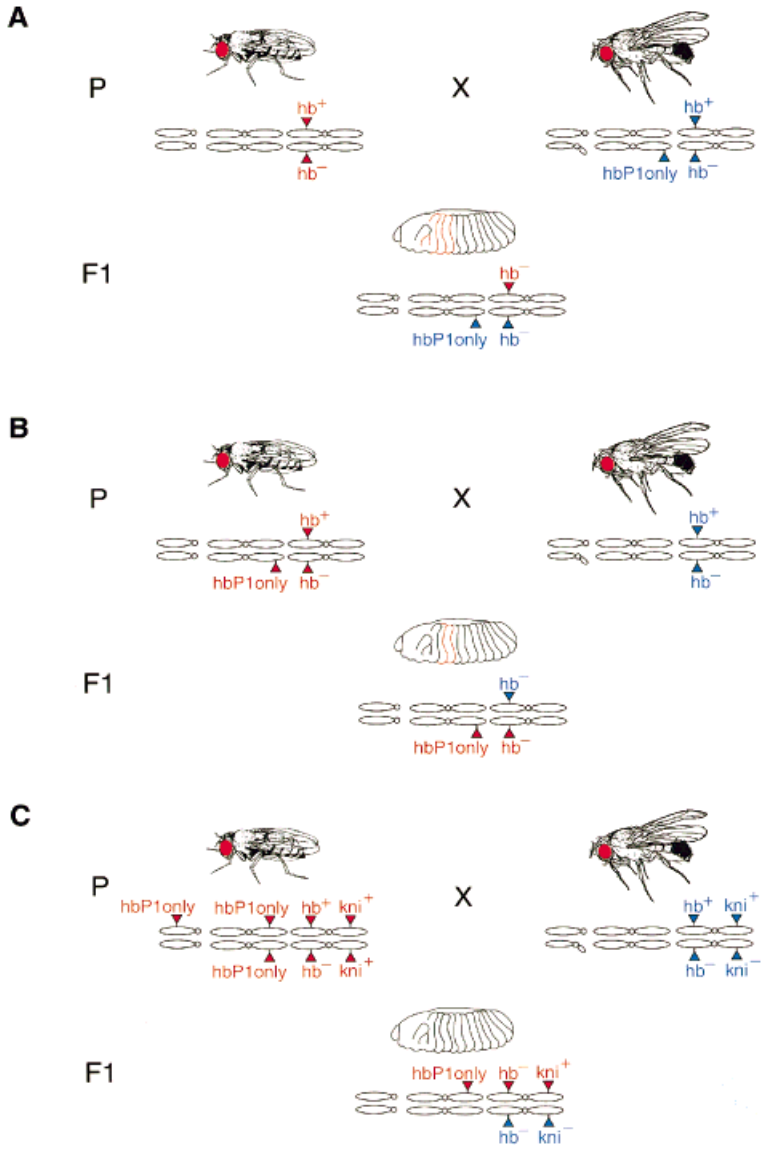

Figure 2. Genetic crosses demonstrating that the Bicoid-independent *hunchback* enhancers can substitute for the Bicoid-dependent *hunchback* enhancer. Females are on the left side. Relevant genotypes are indicated in red (female derived) and in blue (male derived) below each fly/embryo. Deleted segments in the F_1 embryos are marked in red. For further explanations, see text.

Head formation in the absence of TORSO-mediated signalling

Drosophila embryos deficient in TORSO-mediated signalling fail to develop head structures such as the clypeolabrum and parts of the cephalopharyngeal skeleton.[18,23–25] These embryos also show reduced expression of several BICOID target genes.[26–29] This observation indicates a link between the BICOID- and TORSO-dependent patterning systems. There is good evidence that both systems act independently on common target genes. For example, TORSO-mediated signalling does not alter reporter gene expression driven by a defined BICOID-dependent enhancer, supporting the notion that TORSO-mediated signalling does not act on BICOID target genes by modifying the BICOID protein but acts in parallel and independently on common target genes.[2] When Schaeffer et al.[2] studied various *bicoid* rescue constructs in embryos deficient in *bicoid*- and TORSO-mediated signalling, they made the surprising observation that, in one of the transgenic lines, not only the *bicoid* phenotype but also the phenotype caused by suppression of TORSO-mediated signalling was rescued. The rescue was due to increased levels of BICOID protein. By increasing the normal *bicoid* copy number from two up to six copies, a significant portion of the TORSO-mediated signalling mutants were rescued. The

results clearly show that BICOID can compensate for deficiencies in the TORSO-mediated signalling pathway. In summary, anterior patterning in the *Drosophila* embryo relies on several factors, which not only complement each other but, to some extent, can functionally replace each other.

Evolutionary considerations

Failure to identify *bicoid* in insects other than the monophyletic cyclorrhaphan flies[30] led to the hypothesis that a different gene or genes functionally replace *bicoid* in these species. How was the newcomer *bicoid* inserted into the patterning system already in place? Two general scenarios can be envisioned. First, BICOID inherited functions from an ancestral gene.[9] Second, BICOID took over functions from another factor (or factors) that carried a major role in setting up the anterior patterning process[1,16,31–33] The second scenario involves (at least transiently) partial genetic redundancies where BICOID and its functional precursor(s) share functions as described above. Thus, one might speculate that, in an ancestral state lacking *bicoid*, maternal HUNCHBACK instead of BICOID controls early zygotic *hunchback* activity (1). However, such a scenario can only be part of the story, since a localized determinant, which—like Bicoid—spreads from the anterior pole of the patterning system has been postulated not only for higher flies but for most insects including leafhopper, beetles and, in particular, for chironomid midges (Nematocera).[3,4] Yet, transcripts of different *hunchback* homologs are not maternally localized and the respective proteins do not spread from the anterior pole like BICOID.[34,35] Thus, an additional factor is required to explain the experimental findings. Furthermore, in *Drosophila*, TORSO-mediated signalling and BICOID rather than HUNCHBACK are required for patterning most anterior structures of the larval head. At this point, one might ask whether TORSO-mediated signalling in concert with HUNCHBACK constitutes the ancestral anterior determinant of insects. The fact that TORSO-mediated signalling spreads from the anterior pole and acts in parallel to regulate BICOID target genes might support this view. The germbands of most insects, however, develop in only part of the blastoderm, the anterior portion giving rise to extraembryonic tissue. In fact, anterior TORSO-mediated signalling in the red flour beetle *Tribolium* is restricted to a region from which only the extraembryonic serosa emerges.[36] In such cases, a role of TORSO-mediated signalling in segmentation is unlikely to be direct, leaving the option that a player different from TORSO, HUNCHBACK and BICOID, with head patterning features is still to be hunted.

Conclusions

Tinkering with factors involved in anterior patterning of the *Drosophila* embryo reveals a high degree of developmental flexibility. It is, however, difficult to interpret these data in evolutionary terms, and to draw conclusions about the "who is who" in insects other than higher flies. A better understanding of the evolution of anterior patterning in insects will require a comparative approach, which integrates phylogenetic relationships, comparative functional embryology and molecular data in order to define the conditions under which BICOID emerged. A central line of research towards this goal will be to map precisely the emergence of *bicoid* on the phylogenetic tree of insects.

Acknowledgments

I thank Herbert Jäckle, Michael Stauber, Ralf Pflanz, Alexander Prell, Wendy Gerber and Gordon Dowe for comments on the manuscript.

References

1. Wimmer EA, Carleton A, Harjes P, Turner T, Desplan C. *bicoid*-independent formation of thoracic segments in *Drosophila*. Science 2000;287: 2476–2479.
2. Schaeffer V, Killian D, Desplan C, Wimmer EA. High Bicoid levels render the terminal system dispensable for *Drosophila* head development. Development 2000;127:3993–3999.
3. Sander K. Specification of the basic body pattern in insect embryogenesis. Adv Insect Physiol 197;12:125–238.
4. Kalthoff K. Analysis of a morphogenetic determinant in an insect embryo (*Smittia Spec.*, *Chironomidae, Diptera*). In Subtelny S, Konigsberg IR. ed; *Determinants of spatial organization*. New York: Academic Press. 1979. p. 79–126.
5. Sander K. Variants of embryonic patterning mechanisms in insects: Hymenoptera and Diptera. Seminars in Dev Biol 1996;7:573–582.
6. Berleth T, Burri M, Thoma G, Bopp D, Richstein S, Frigerio G, Noll M, Nüsslein-Volhard C. The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. 1988;7:1749–1756.
7. Sommer R, Tautz D. Segmentation gene expression in the housefly *Musca domestica*. Development 1991;113:419–430.
8. Schröder R, Sander K. A comparison of transplantable *bicoid* activity and partial *bicoid* homeobox sequences in several *Drosophila* and blowfly species (Calliphoridae). Roux's Arch Dev Biol 1993;203:34–43.
9. Stauber M, Jäckle H, Schmidt-Ott U. The anterior determinant *bicoid* of *Drosophila* is a derived Hox class 3 gene. Proc Natl Acad Sci USA 1999; 96:3786–3789.
10. Driever W, Nüsslein-Volhard C. A gradient of bicoid protein in *Drosophila* embryos. Cell 1988;54:83–93.
11. Driever W, Nüsslein-Volhard C. The bicoid protein is a positive regulator of *hunchback* transcription in the early *Drosophila* embryo. Nature 1989; 337:138–143.
12. Struhl G, Struhl K, Macdonald PM. The gradient morphogen bicoid is a concentration-dependent transcriptional activator. Cell 1989;57:1259–1273.
13. Tautz D, Lehmann R, Schnürch H, Schuh R, Seifert E, Kienlin A, Jones K, Jäckle H. Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes. Nature 1987;327:383–389.
14. Tautz D. Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centers. Nature 1988;332:281–284.
15. Struhl G, Johnston P, Lawrence PA. Control of *Drosophila* body pattern by the *hunchback* morphogen gradient. Cell 1992;69:237–249.
16. Simpson-Brose M, Treisman J, Desplan C. Synergy between the *hunchback* and *bicoid* morphogens is required for anterior patterning in *Drosophila*. Cell 1994;78:855–865.
17. Perrimon P, Desplan C. Signal transduction in the early *Drosophila* embryo: when genetics meets biochemistry. Trends Biol Sci 1994;19: 509–512.
18. Klingler M, Erdelyi M, Szabad J, Nüsslein-Volhard C. Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. Nature 1988;335:275–277.

19. Sprenger F, Nüsslein-Volhard C. Torso receptor activity is regulated by a diffusible ligand produced at the extracellular terminal regions of the *Drosophila* egg. Cell 1992;71:987–1001.
20. Casanova J, Struhl G. The *torso* receptor localizes as well as transduces the spatial signal specifying terminal body pattern ion *Drosophila*. Nature 1993;362:152–155.
21. Schröder C, Tautz D, Seifert E, Jäckle H, Differential regulation of the two transcripts from the *Drosophila* gap segmentation gene *hunchback*. EMBO J 1988;7:2881–2887.
22. Margolis JS, Borowsky ML, Steingrímsson E, Wha Shim C, A. LJ, Posakony JW. Posterior stripe expression of *hunchback* is driven from two promoters by a common enhancer element. Development 1995;121: 3067–3077.
23. Schüpbach T, Wieschaus E. Germline autonomy of maternal-effect mutations altering the embryonic body pattern in *Drosophila*. Dev Biol 1986;113:443–448.
24. Stevens LM, Frohnhöfer HG, Klingler M, Nüsslein-Volhard C. Localized requirement for torso-like expression in follicle cells for development of terminal anlagen of the *Drosophila* embryo. Nature 1990;346.
25. Schmidt-Ott U, González-Gaitán M, Technau GM. Analysis of neural elements in head-mutant *Drosophila* embryos suggests segmental origin of the optic lobes. Rouxs Arch Dev Biol 1995;205:31–44.
26. Grossniklaus U, Cadigan KM, Gehring W. Three maternal coordinate systems cooperate in the pattering of the *Drosophila* head. Development 1994;120:3155–3171.
27. Wimmer EA, Simpson-Brose M, Cohen SM, Desplan C, Jäckle H. Trans- and cis-acting requirements for blastodermal expression of the head gap gene *buttonhead*. Mech Dev 1995;53:235–245.
28. Gao Q, Wang Y, Finkelstein R. *Orthodenticle* regulation during embryonic head development in *Drosophila*. Mech Dev 1996;56: 3–15.
29. Brönner G, Jäckle H. Regulation and function of the terminal gap gene *huckebein* in the *Drosophila* blastoderm. Int J Dev Biol 1996;40: 157–165.
30. McAlpine JF. Phylogeny and classification of the Muscomorpha. In McAlpine JF. ed; Manual of Nearctic Diptera. Hull: Canadian Government Publishing Centre. 1989. p.1397–1518.
31. Irish V, Lehmann R, Akam M. The *Drosophila* posterior-group gene *nanos* functions by repressing *hunchback* activity. Nature 1989;338: 646–648.
32. StJohnston D, Nüsslein-Volhard C. The origin of pattern and polarity in the *Drosophila* embryo. Cell 1992;68:201–220.
33. Sander K, The evolution of insect patterning mechanisms: a survey of progress and problems in comparative molecular embryology. Development 1994;(Suppl):187–191.
34. Wolff C, Schröder R, Schulz C, Tautz D, Klingler M. Regulation of the *Tribolium* homologues of *caudal* and *hunchback* in *Drosophila*: evidence for maternal gradient systems in a short germ embryo. Development 1998;125:3645–3654.
35. Rohr KB, Tautz D, Sander K. Segmentation gene expression in the mothmidge Clogmia albipunctata (Diptera, Pschodidae) and other primitive dipterans. Dev Genes Evol 1999;209:145–154.
36. Schröder R, Eckert C, Wolff C, Tautz D. Conserved and divergent aspects of terminal patterning in the beetle *Tribolium castaneum*. Proc Natl Acad Sci USA 2000;97:6591–6596.