

Insect embryogenesis – what is ancestral and what is derived?

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SUMMARY

The systematic genetic analysis of *Drosophila* development has provided us with a deep insight into the molecular pathways of early embryogenesis. The question arises now whether these insights can serve as a more general paradigm of early development, or whether they apply only to advanced insect orders. Though it is too early to give a definitive answer to this question, we suggest that there is currently no firm reason to believe that the molecular

mechanisms that were elucidated in *Drosophila* may not also apply to other forms of insect embryogenesis. Thus, many of the *Drosophila* genes involved in early pattern formation may have comparable functions in other insects and possibly throughout the arthropods.

Key words: evolution, insect embryogenesis, oogenesis, segmentation

INTRODUCTION

There is a long tradition of research in comparative insect embryology. Representative taxa of almost all insect orders were studied in detail and inferences were made on ancestral and derived traits of embryogenesis. Among these, *Drosophila* clearly represents a derived mode of insect embryogenesis. However, the choice of *Drosophila* as an embryological system was entirely governed by the unique suitability of this organism for genetic analysis. This genetic approach to embryogenesis (Nüsslein-Volhard and Wieschaus, 1980) turned out to be very successful. There is now an almost complete understanding of the principles of early *Drosophila* development at the molecular level (reviewed in Bate and Martinez Arias, 1993). It is therefore time to ask which of these processes may also be utilized in other insect orders and which may be special to the *Drosophila* mode of development.

The key to such studies is the possibility of using the *Drosophila* genes as molecular tools to isolate homologs of segmentation genes from other insects and to study their expression pattern in these species. The patterns can then be related to the patterns known from *Drosophila*. Thus, comparative insect embryology can now be done at the molecular level. A number of genes have already been studied in this way and the results have recently been summarized (Tautz and Sommer, 1994). Here we want to review the literature on previous work of comparative insect embryogenesis and to reassess it in the light of the new molecular results. We feel that this may serve as a basis for developing new ideas and new experimental directions in the future.

PHYLOGENY

To discriminate ancestral from derived traits, one needs a reliable phylogeny in order to carry out the necessary outgroup

comparisons (Kitching, 1992). The phylogeny of major insect groups, as it is supported by morphological analysis (Kristensen, 1991) is depicted in Fig. 1. We found that the same tree is also supported by comparisons of ribosomal RNA sequences from representative taxa (Friedrich and Tautz, unpublished data). Thus, there is little doubt about the correct grouping of these insect orders. The picture is however less clear for the more primitive entognathan hexapods. These relationships are therefore left unresolved in Fig. 1. The following discussions will deal mainly with the ectognathan insects, since the relationships among these are most clearly resolved.

LONG, INTERMEDIATE AND SHORT GERM EMBRYOS

The most obvious difference among the embryos of different insect taxa is the way in which the early germ band is formed. Krause (1939) introduced a classification according to the length of the early germ band, whereby he has used two different descriptive terms, namely *Kleinkeim* versus *Grosskeim* (small germ versus large germ) and *Kurzkeim* versus *Langkeim* (short germ versus long germ). The former terms describe merely how large the germ anlage is with respect to the size of the egg. In contrast, the terms short or long germ were meant to imply functional differences, namely a germ anlage which, among other criteria, does, or does not show a secondary growth process after blastoderm stage (Fig. 2). Krause also used the term *halblang* (semi-long) which is now more frequently called intermediate germ. Short and intermediate germ embryos are found in the more primitive, hemimetabolous insect orders, such as Orthoptera and Ephemeroptera, while long germ embryos are restricted to the more advanced holometabolous orders, such as Hymenoptera and Diptera. In long germ embryos, all segments are already defined at blastoderm stage, while in intermediate and short

germ embryos, the more posterior segments are produced during a secondary segmental growth process. In extreme cases, for example, in *Schistocerca* (Orthoptera), the early germ anlage at blastoderm stage shows only the head lobes and a growth zone from which the remainder of the segments will be generated. These extremely short germ embryos thus share a superficial similarity with Trochophora larvae that are characteristic for taxa with spiral embryogenesis, such as annelids and molluscs. Therefore, it seemed reasonable to conclude that these extremely short-germ type embryos represent the ancestral mode of insect embryogenesis (Krause, 1939; Sander, 1983). This interpretation is, however, not unequivocal for two reasons. First, not all Orthopteran species show the extreme short germ mode seen in *Schistocerca* (Kanelis, 1952, and see below), and second, more primitive insect groups such as the Odonata are of the intermediate germ type (see below). Similarly, in the bristle tail *Petrobius* (Archaeognatha), the head lobes and at least the mandibular segments are found in the early germ anlage (Larink, 1969). Thus, it seems possible that the extremely short germ embryos are not ancestral, but represent a secondary specialization (Anderson, 1973).

The long germ mode exemplified by *Drosophila* becomes particularly clear when one looks at the expression pattern of early segmentation genes. Crucial for the following discussion is the class of pair rule genes that are responsible for a transient double segmental organization of the developing embryo (Nüsslein-Volhard and Wieschaus, 1980). In *Drosophila*, the pair rule genes are usually expressed in seven stripes at blastoderm stage (Ingham, 1988), corresponding to the three mandibular, three thoracic and eight abdominal segments that will eventually be formed. The expression pattern of the pair rule genes can thus be taken as a direct molecular marker for the blastoderm fate map.

Such a molecular fate map has also been created for the moth *Manduca sexta* (Lepidoptera). *Manduca* is phylogenetically close to the dipterans (Fig. 1), but morphologically, *Manduca* looks more like a short or intermediate germ type. Only the headlobes and a region that looks superficially like a growth zone become visible at the end of blastoderm stage. However, rather than undergoing a secondary growth process, the originally rather broad germ anlage elongates via tissue reorganization (Broadie et al., 1991). Homologues of *Drosophila* gap, pair rule and segment polarity genes were cloned and their early expression pattern was studied (Kraft and Jäckle, 1994). It was found that the expression of these genes was very similar to that in *Drosophila*. In particular, the pair rule gene *runt* was seen to be expressed in eight stripes and the segment polarity gene *wingless* in sixteen stripes in the early embryo. This suggests that all sixteen segments (three mandibular, three thoracic and ten abdominal) may already be specified at blastoderm stage, even though they become morphologically visible only later and form in the progressive manner typical of a short germ embryo. Thus, from the molecular point of view, *Manduca* shows clearly a long germ embryogenesis (Kraft and Jäckle, 1994). Interestingly, this conclusion is

also supported by UV irradiation experiments in the related lepidopteran *Tineola*. By destroying certain cell groups at blastoderm stage with UV-light, Lüscher (1944) found that specific larval structures were affected at later stages. Most importantly, he found that practically all larval pattern elements could be destroyed in this way, depending on which region of the egg was treated with the UV-light. This allowed him to conclude that all segments were already specified at blastoderm stage. These types of destructive fate mapping experiments were also applied to *Drosophila* where basically the same results were obtained (Lohs-Schardin et al., 1979). However, there are some conceptual caveats about the use of destructive methods for fate mapping. On the one hand, it is possible that cells that had already become specified may be replaced by other cells after they were destroyed. In this way, an early specification would be missed. On the other hand, cells that are not yet committed may not be destroyed completely, but only lose their capability to respond to the signals that would normally specify them at later stages. In this way, an early determination would be incorrectly assumed. However, even though these possibilities may exist, at least in *Drosophila*, but apparently also in lepidopteran embryos, the destructive fate maps seem to conform very closely with the molecular results that were obtained later. Thus, fate maps constructed in this way appear to be reliable indicators for the underlying molecular principles, at least in insects. Therefore, we are going to use arguments that are based on such fate maps in the following discussion.

While the dipterans and the lepidopterans have apparently a very similar molecular fate map, the situation is entirely

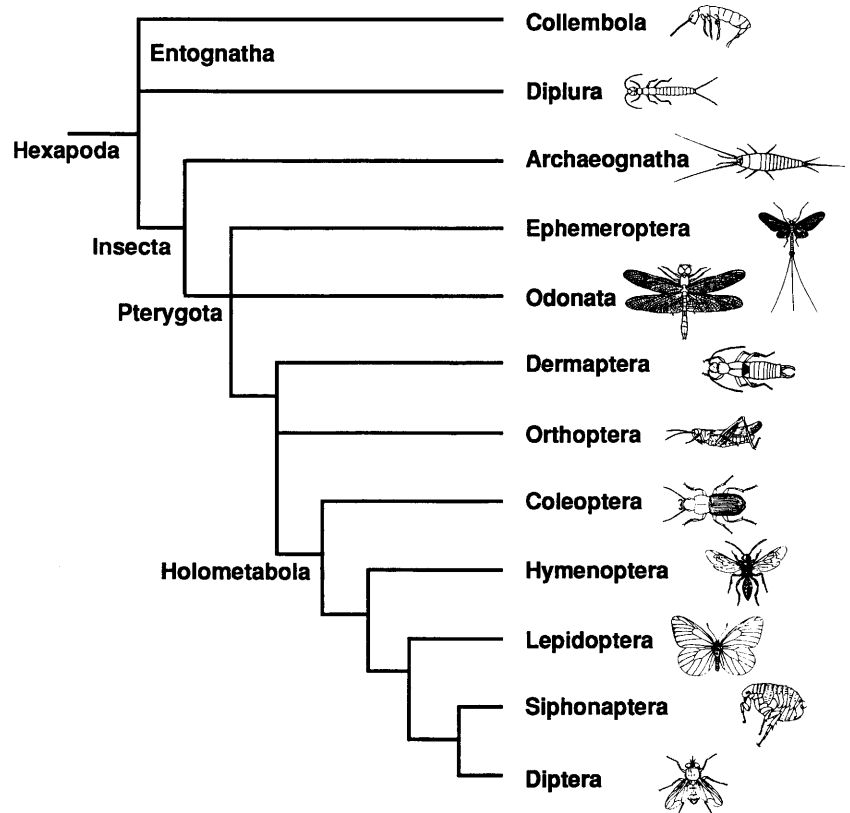


Fig. 1. Phylogeny of the insect orders discussed in the text (after Kristensen, 1991).

different in beetles (Coleoptera). Again, it is the expression pattern of the pair rule genes that shows this most clearly. In the flour beetle *Tribolium*, only three stripes of the pair rule genes *hairy* and *even skipped* are formed at blastoderm stage (Sommer and Tautz, 1993; Patel et al., 1994). According to the definitions of Krause (1939), *Tribolium* belongs to the intermediate germ type of embryo, where the three gnathal and the three thoracic segments become specified at blastoderm stage. Thus, six segments need to be defined, which is in line with the three pair rule stripes observed. Most interestingly, a striped pair rule gene expression is also evident after blastoderm stage, during the secondary segmental growth process (Sommer and Tautz, 1993; Patel et al., 1994). This suggests that these genes are involved in defining the segments even at these later stages.

Only one pair rule gene, *even skipped*, has so far been analysed in the Orthopteran *Schistocerca*, which shows the extremely short germ mode of embryogenesis. Pair rule stripes were not found in these embryos, either at blastoderm stage, or during the secondary segmental growth process, though *even skipped* is transiently expressed in the growth zone (Patel et al., 1992; Patel et al., 1994). It was therefore suggested that pair rule gene activity may not be present in the more primitive hemimetabolous insect orders (Patel et al., 1992). However, it is still possible that another one of the several pair rule genes known from *Drosophila* has substituted for the function of *even skipped* in *Schistocerca*. An alternative interpretation could be that *Schistocerca* represents a secondary reduction of the intermediate germ type (see above). In this case one would not necessarily have to postulate that pair rule genes play a role in these types of embryos, since they could have become secondarily lost. This assumption is supported from a comparison of the mode of embryogenesis in *Gryllus domesticus* (Orthoptera). This species is relatively closely related to *Schistocerca*, yet shows an intermediate germ as can be inferred from a blastoderm fate map (Kanelis, 1952). Moreover, staining with an antibody against *even skipped* (Patel et al., 1994) shows that stripes are formed in the growth zone of these embryos (unpublished results). However, it is not yet clear whether these are organized in double segmental units and might thus represent a pair rule activity, or whether they are segmentally reiterated and might thus have a different function. Still, one can conclude that the situation in *Schistocerca* may be derived and that the answer to how segmentation is achieved in this species may be less relevant for assessing which of the molecular mechanisms are more ancestral.

To find an answer to the question of the ancestral or derived status of the pair rule genes, it will be necessary to look in the oldest insect orders that show the intermediate type of embryogenesis. Particularly well studied in this respect is the damselfly *Platycnemis* (Odonata) (Seidel, 1935). The blastoderm fate map of this organism, as it was derived from experimental embryology, is depicted in Fig. 3A. On the basis of the arguments given above, namely that experimentally produced fate maps concur with

molecular fate maps in those cases where this has been analysed, we should like to use the *Platycnemis* fate map to make the equivalent inferences. It appears that at least six segments are laid down at very early stages (Fig. 3A), very similar to *Tribolium*. It seems therefore reasonable to predict that this pattern is generated by similar molecular mechanisms, i.e. that three pair rule stripes should appear at blastoderm stage.

The *Platycnemis* fate map can in fact be taken as representative of a more general fate map of intermediate germ insects (Anderson, 1973). Thus, an archetypic fate map for insects might look like the one depicted in Fig. 3B. This intermediate germ type is characterized by two separate phases of segmentation, namely one at blastoderm stage and one during the germ band extension phase. Since the *Tribolium* results show that the same genes are utilized during both of these phases, it is easy to see how the two derived forms of embryogenesis, the long and the extremely short germ mode, may have evolved. The extremely short germ forms would have discarded the blastoderm stage phase of segmentation and accordingly, some gene functions required at this stage, such as the pair rule genes, may have become lost, or their function may have become modified. The long germ embryos on the other hand, would have replaced the secondary growth phase by an extension of the blastoderm stage phase of segmentation without the need to recruit additional gene functions, at least at the level of the pair rule genes. Thus, in this interpretation, the *Drosophila* mode of segmentation is likely to have retained ancestral gene functions.

OOGENESIS AND MATERNAL GENES

Not only the mode of segmentation, but also the mode of oogenesis subdivides the insects. Egg production can be either meroistic or panoistic, i.e. with or without nurse cells. In a first view, this subdivision appears to be correlated with the phylogenetic relationships of the insect orders. The more ancestral, hemimetabolous orders show panoistic oogenesis, suggesting

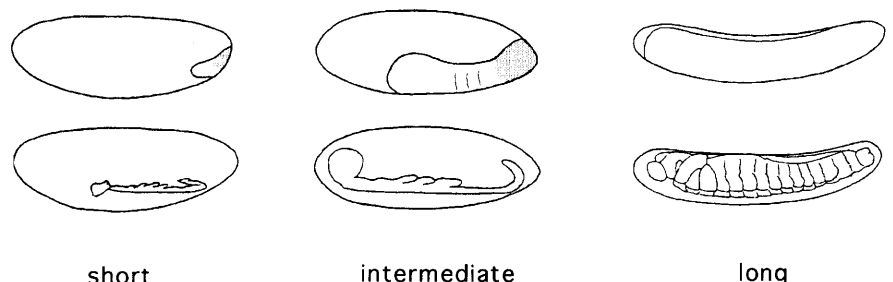


Fig. 2. Germ types in insects. The top row represents blastoderm stages, the bottom row stages during which the first segments become visible. Anterior is to the left and ventral is up. The areas that represent the early germ band are indicated in the top row. The stippled areas represent the regions of the growth zone in the short germ and intermediate germ embryos. The stippled lines in the intermediate germ embryo indicate the fact that some segments are already specified at this stage. This is in contrast to the short germ embryo, where only the headlobes and the growth zone are formed. Note that the total length of the germ band in relation to the egg is not the decisive criterium for classifying short and long germ embryos, but is rather the basis for a second classification system, namely small and large germ embryos (see text; modified after Krause and Sander, 1962).

that this is the ancestral mode of oogenesis (King and Büning, 1985). However, the picture is not so clear. Meroistic modes can be found in phylogenetically basal orders, such as the Ephemeroptera (Gottanka and Büning, 1993) and Dermaptera (King and Büning, 1985) and panoistic modes are seen in rather advanced orders such as the Siphonaptera (King and Büning, 1985). While the latter ones have been classified as secondarily panoistic (Büning and Sohst, 1988), this is less clear for the situation in the older orders. Meroistic oogenesis can also be found among the entognathan hexapod orders, the Collembola and the Diplura (Stys and Bilinski, 1990). These observations suggest that the mode of oogenesis is not a stable evolutionary character and may have changed independently several times.

Why does the mode of oogenesis matter for embryonic development? In *Drosophila* it was found that both, the antero-posterior and the dorsoventral axis become originally specified by maternally localized factors. The means by which this localization is achieved is however rather different for the different factors. Four sets of genes are involved, the anterior group (key gene *bicoid*), the posterior group (key gene *nanos*), the terminal group (key gene *torso*) and the dorsoventral group (key gene *dorsal*) (St. Johnston and Nüsslein-Volhard, 1992).

Among these, only the localization of the anterior factor *bicoid* seems to depend on the mode of oogenesis. *bicoid* RNA is synthesized in the nurse cells and delivered to the growing oocyte where it becomes trapped at the anterior pole by gene products that are homogeneously distributed in the oocyte (St. Johnston et al., 1989). Thus, the fact that the *bicoid* RNA becomes anteriorly localized would be due to the anterior-posterior polarity information caused by the asymmetric localization of the nurse cells. In fact when the nurse cell clusters are aberrantly located on both sides of the oocytes, as occurs in the *Drosophila* mutant *dicephalic*, one can observe a duplication of head structures (Lohs-Schardin, 1982; Bohrmann and Sander, 1987)

The localization of the posterior determinant *nanos* relies on a different mechanism. *nanos* RNA is also produced in the nurse cells and delivered into the oocyte. However, it then becomes trapped by a specific receptor at the posterior pole (Wang and Lehmann, 1991). The localization signal for this receptor might in turn be determined by a signal from the follicle cells that surround the oocyte and would therefore be nurse cell independent. A similar mechanism applies to the realization of the terminal information, as well as to the dorso-ventral axis formation where again the follicle cells are involved in providing the respective primary signal (St. Johnston and Nüsslein-Volhard, 1992). Intriguingly, in the latter case it was shown that the follicle cells themselves receive the signal from a gene product that is produced by the oocyte nucleus (Neumann-Silberberg and Schüpbach, 1993).

Thus, it appears that only the localization of *bicoid* would be a conceptual problem in the panoistic mode of oogenesis. However, a

reassessment of the way in which *bicoid* becomes localized in the *Drosophila* embryo suggests that even this process could be nurse cell independent. The protein involved in anchoring the *bicoid* mRNA appears to be capable of actively transporting the *bicoid* RNA along a microtubule scaffold (Pokrywka and Stephenson, 1991; St. Johnston and Nüsslein-Volhard, 1992). Such a localization mechanism could of course also work in a panoistic oocyte. Asymmetric localization of an RNA within a single cell is in fact also seen for other genes (Ding and Lipshitz, 1993) and is obviously no particular problem for a cell.

Thus, none of the *Drosophila* results imply directly that embryonic axis formation has to occur differently in species with meroistic or panoistic oogenesis. However, unequivocal homologs of the genes involved in primary axis determination in *Drosophila* have not yet been recovered from more distantly related insects. On the other hand, there is at least indirect evidence for a similar mode of dorso-ventral axis formation (Sommer and Tautz, 1994), a terminal activity (Nagy and Carroll, 1994; and see below) as well as for a *nanos*-like activity in *Tribolium* (Wolff et al., unpublished data).

CONSERVED PATTERNING DECISIONS IN INSECTS

The differences seen in the mode of oogenesis and segmentation among the insect taxa conceal, somewhat, the fact that other modes of patterning decisions are much more conserved.

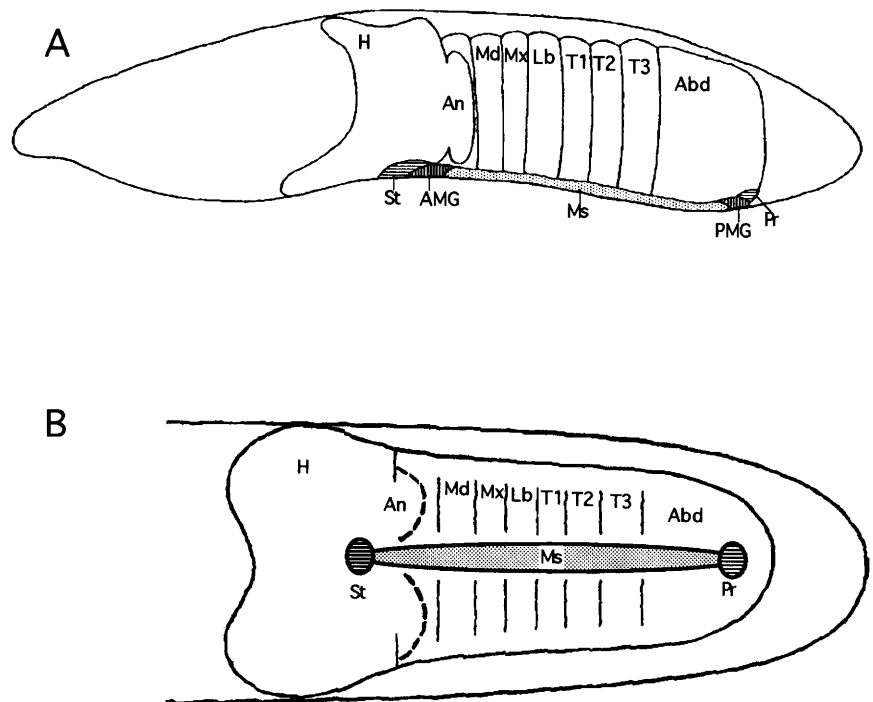


Fig. 3. Blastoderm fate maps of (A) *Plactynemis* (after Seidel, 1935) and (B) a generalized fate map for the intermediate germ type (see text). Anterior is left, the embryo in A is viewed from the lateral side, the one in B from the ventral side. The anlagen of the mesoderm and the gut are indicated by the stippled and hatched areas respectively. H, head; An, antennae; Md, mandible; Mx, maxilla; Lb, labium; T1-T3, thorax 1-3; Abd, abdominal growthzone; St, stomodaeum; Pr, proctodaeum; Ms, mesoderm; AMG, anterior midgut; PMG, posterior midgut.

These concern head segment formation, mesoderm formation, formation of the gut and the formation of the nervous system. Two of these processes, namely mesoderm formation and gut formation, are particularly pertinent to the question of determination by maternal positional cues and shall therefore be discussed in more detail here.

Mesoderm formation

The mesoderm in *Drosophila* is formed in response to maternal dorso-ventral cues and involves the specific expression of the genes *twist* and *snail* at the ventral side at blastoderm stage (St. Johnston and Nüsslein-Volhard 1992; Leptin, 1991). The expression domains of these two genes are directly regulated by the maternal *dorsal* gene product, which forms a gradient of nuclear localization in the early embryo. *twist* and *snail* determine the mesodermal fate of cells in which they are expressed and these cells invaginate during gastrulation. The expression pattern of *twist* and *snail* was also studied in *Tribolium* and found to be basically the same as in *Drosophila*. Both genes are expressed at the ventral side and the cells expressing them are the ones that will invaginate (Sommer and Tautz, 1994). Thus, this establishes a link between morphology and gene expression pattern and, it suggests also that the underlying maternal mode of dorso-ventral specification may be conserved.

What happens in the other insects? The mesoderm invagination (called the "gastral groove") occurs always in a very similar manner along the whole germ anlage, independent of the length of the germ (Anderson, 1972a,b). In the short germ embryos, the invagination continues until all segments are formed and in *Tribolium* it was in fact found that *snail* and *twist* expression persist until the process is completed (Sommer and Tautz, 1994). Thus, the morphological and genetic mode of mesoderm invagination appear to be very similar among insects. Most importantly however, the mesodermal anlagen can be defined on the blastoderm fate map (Fig. 3B) even in the most primitive insects, including the Archaeognathans (Jura, 1972). It seems possible therefore that the maternal mode of the definition of the mesoderm via the regulation of the zygotic genes *twist* and *snail* is the ancestral one, at least in insects. It should be noted, however, that there are some observations that would argue against this assumption. In several insect species it is possible to produce "parallel twins", i.e. twinned embryos along the longitudinal axis, by different types of experimental manipulation (reviewed by Sander, 1976). Clearly, the dorso-ventral axis for these additional embryos must have become specified in a way that is difficult to reconcile with a maternal specification. However, these experiments do not exclude the possibility that at least the zygotic pathway, as it is reflected in the expression of *twist* and *snail*, is conserved. Interestingly, homologues of *twist* and *snail* are also expressed in vertebrates in the developing mesoderm (Hopwood et al., 1989; Smith et al., 1992), though the mode of mesoderm formation is apparently rather different and nothing is known about maternal influences.

Formation of the gut

The gut is formed from several primordia in insects. The stomodaeum at the anterior and the proctodaeum at the posterior are derived from ectodermal tissues and the midgut is of endodermal origin. The genes defining the posterior anlagen of the

gut in *Drosophila* are regulated by the maternal terminal system (Weigel et al., 1990). Though homologues of these genes have not yet been analysed in more primitive insects, it is nonetheless evident, that similar blastoderm anlagen of the gut can be defined (Fig. 3B), even in the most basic orders (Anderson, 1973). Thus, this suggests, indirectly, that a maternal system equivalent to the terminal class of genes is also ancestral, though this speculation has to be verified by data. It is, however, interesting to note that even the further development of the gut occurs in a fairly stereotypic manner in most insects. In *Drosophila*, the stomodaeum and the proctodaeum begin development by forming epithelial tubes during gastrulation, after the completion of the segmentation process. The midgut does not form a tube, but consists at first of two lateral strands of cells that migrate from the ends of the ectodermal parts of the gut towards the middle of the embryo. Once they have met, they spread out ventrally and dorsally and eventually engulf the remaining yolk. The same course of events, sometimes with modifications, is basically found in all insects. The details of this process are beginning to be studied at the genetic level in *Drosophila* (Reuter et al., 1993) and it will be interesting to see which of the genes involved in the process can also be found in other insects.

ARTHROPOD EMBRYOGENESIS

Comparison with representatives from the other arthropod classes (myriapoda, chilopoda, crustacea and chelicerata) may also be helpful in identifying ancestral features of hexapod embryogenesis. An extensive effort in this direction was made by Anderson (1973). Unfortunately, his studies were strongly influenced by Manton's (1973) theory of a polyphyletic origin of the arthropods and some of his inferences have to be treated with caution. Today there is a consensus, based on morphological (Lauterbach, 1973) and molecular data (Turbeville et al., 1991; Ballard et al., 1992), that arthropods do share a common ancestor, although, due to the long time of evolutionary separation, most extant arthropod taxa are presumably very derived with respect to embryological features. Nonetheless, at least some inferences can be made, since there are general similarities among the different forms of arthropod embryogenesis. One concerns the mode of blastoderm formation. Though some taxa begin their development with a total cleavage of the egg, almost all (exception: lower crustacean orders) also form a syncytial blastoderm stage later on (Anderson, 1973), as is characteristic for insects. Given the importance of the syncytial blastoderm for the early patterning decisions in insects, this suggests strongly that similar decisions may be necessary in the embryos of all arthropods. Another intriguing parallel is the fact that a large part of the anterior segment pattern may become specified at, or shortly after blastoderm stage, while the remainder of the segments are generated in a secondary growth process (Anderson, 1973). We have seen above that a similar mode of development may be the ancestral form of embryogenesis in insects. However, direct comparisons of embryogenesis between the classes are difficult, since the morphological details can look rather different. Nonetheless, this does not preclude that similar molecular mechanisms could be at work, since it has also become clear from the work in insects that morphologically

different embryonic forms may be generated by the very similar molecular mechanisms (see above). Moreover, we think that it has become clear that the blastoderm fate map is a reliable indicator of the underlying molecular processes and it is in fact the blastoderm fate map that suggests the parallels among arthropods (Anderson, 1973). At least some homologs of early segmentation genes have already been recovered from representatives of the other arthropod classes (Sommer et al., 1992) and it will, therefore, be highly interesting to study their expression pattern in the future.

THE CONCEPT OF THE PHYLOTYPIC STAGE

The concept of the phylotypic stage was proposed because of a seeming paradox of embryogenesis. The very earliest stages of development, namely egg production, blastoderm formation, gastrulation and secondary growth processes seem to be fairly dissimilar between different taxa and, as discussed above, may not even be related to the phylogeny of the respective taxa. Yet these early events all seem to channel into a highly stereotypic stage at which the full segmental pattern becomes morphologically visible. Intriguingly, this stage looks very similar between different taxa, not only among insects, but even among the arthropods as a whole and thus represents the general bauplan of the phylum. This stage was therefore called the "Körpergrundgestalt" or the phylotypic stage (Seidel, 1960; Sander, 1983). This phenomenon is not merely restricted to the arthropods, but is also seen in the other animal phyla, for example among the vertebrates. Furthermore, comparative analysis of the expression pattern of homologs of the homeotic genes known from *Drosophila* at this phylotypic stage in different taxa has shown that they are expressed in a comparable spatial and temporal order as in *Drosophila* (McGinnis and Krumlauf, 1992). This provides a strong argument for the universality of the animal bauplan (Slack et al., 1993).

However, homeotic genes are not themselves involved in generating the segment pattern. Rather, they depend on the information from the preceding levels of the gene hierarchy for delimiting their own segmentally organized expression domains. Thus, it seems reasonable to propose that the gene network regulating the expression pattern of the homeotic genes should also be more or less conserved between organisms (for an alternative view see Sander, 1983). This way of reasoning, together with the molecular results mentioned above, suggest that there may be no paradox after all with respect to the phylotypic stage. Though it remains true that the morphological pathways towards the phylotypic stage may look rather diverse, there is currently no reason to believe that the regulatory genetic pathways may not be conserved to some degree.

The way to analyse this experimentally would be to take the regulatory regions from homeotic genes and to test them in different animals to see whether they are regulated in a similar manner. So far, one such experiment has been successfully performed. It has been shown that a particular regulatory element of a homeobox gene expressed in the head (*Deformed*) can be functionally interchanged between vertebrates and *Drosophila* (Awgulewitsch and Jacobs, 1992; Malicki et al., 1992). However, the element analysed in these studies is essen-

tially only necessary for autoregulation of the gene. Thus, the conservation of the underlying regulatory network has not been finally proved with this experiment. However, these are clearly the kinds of experiments that have to be done to obtain an insight into the general degree of conservation of the regulatory hierarchy and thus eventually into the evolution of the general animal bauplan.

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REFERENCES

- Anderson, D. T. (1972a). The development of hemimetabolous insects. In *Developmental Systems: Insects* (ed. S. J. Counce and C. H. Waddington), pp. 96-163. London: Academic Press.
- Anderson, D. T. (1972b). The development of holometabolous insects. In *Developmental Systems: Insects* (ed. S. J. Counce and C. H. Waddington), pp. 166-242. London: Academic Press.
- Anderson D. T. (1973). *Embryology and Phylogeny in Annelids and Arthropods*. Oxford: Pergamon Press.
- Awgulewitsch, A. and Jacobs, D. (1992). *Deformed* autoregulatory element from *Drosophila* functions in a conserved manner in transgenic mice. *Nature* **358**, 341-344.
- Ballard, J. W. O., Olsen, G. J., Faith, D. P., Odgers, W. A., Rowell, D. M. and Atkinson, P. W. (1992). Evidence from 12S ribosomal RNA sequences that onychophorans are modified arthropods. *Science* **258**, 1345-1348.
- Bate, M. and Martinez Arias, A. (1993). *The Development of Drosophila melanogaster*. New York: Cold Spring Harbour Laboratory Press.
- Bohrmann, J. and Sander, K. (1987). Aberrant oogenesis in the patterning mutant *dicéphalic* of *Drosophila melanogaster*: time-lapse recordings and volumetry in vitro. *Roux's Arch. Dev. Biol.* **196**, 279-285.
- Büning, J. and Soht, S. (1988). The flea ovary: ultrastructure and analysis of cell clusters. *Tissue Cell* **20**, 783-795.
- Broadie, K. S., Bate, M. and Tublitz, N. J. (1991). Quantitative staging of embryonic development of the tobacco hawkmoth, *Manduca sexta*. *Roux's Arch. Dev. Biol.* **199**, 327-334.
- Ding, D. and Lipshitz, H. D. (1993). Localized RNAs and their functions. *BioEssays* **15**, 651-658.
- Gottanka, J. and Büning, J. (1993). Mayflies (Ephemeroptera) the most "primitive" winged insects, have telotrophic meroistic ovaries. *Roux's Arch. Dev. Biol.* **203**, 18-27.
- Hopwood, N. D., Pluck, A. and Gurdon, J. B. (1989). A *Xenopus* mRNA related to *Drosophila twist* is expressed in response to induction in the mesoderm and the neural crest. *Cell* **59**, 893-903.
- Ingham, P. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**, 25-34.
- Jura, C. (1972). Development of apterygote insects. In *Developmental Systems: Insects* (ed. S. J. Counce and C. H. Waddington), pp. 49-94. London: Academic Press.
- Kanelis, A. (1952). Anlagenplan und Regulationserscheinungen in der Keimanlage des Eies von *Gryllus domesticus*. *Wilhelm Roux Arch. EntwMech. Org.* **145**, 417-461.
- King, R. C. and Büning, J. (1985). The origin and function of insect oocytes and nurse cells. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology* vol. 1. (ed. G. A. Kerkut and L. I. Gilbert), pp. 37-82. Oxford: Pergamon Press.
- Kitching, I. J. (1992). The determination of character polarity. In *Cladistics: a practical course in systematics* (ed. P. L. Forey et al.), pp. 22-42. Oxford: Clarendon Press.
- Kraft, R. and Jäckle, H. (1994) *Drosophila* mode of metamerization in the embryogenesis of the intermediate germ-band insect *Manduca sexta* (Lepidoptera). *Proc. Natl. Acad. Sci. USA* **91**, 6634-6638.
- Krause, G. (1939). Die Eitypen der Insekten. *Biol. Zentralblatt* **59**, 495-536.
- Krause, G. and Sander, K. (1962). Ooplasmic reaction systems in insect embryogenesis. *Adv. Morphogen.* **2**, 259-303.

- Kristensen, N. P.** (1991). Phylogeny of extant hexapods. In *The insects of Australia* 2nd ed. (ed. I. D. Naumann et al.) pp. 125-140, CSIRO, Melbourne University Press, Melbourne.
- Larink, O.** (1969). Zur Entwicklungsgeschichte von *Petrobius brevistylus* (Thysanura, Insecta). *Helgoländer wiss. Meeresunters.* **19**, 111-155.
- Lauterbach, K. E.** (1973). Schlüsselereignisse in der Evolution der Stammgruppe der Euarthropoda. *Zool. Beitr. N.F.* **19**, 251-299.
- Leptin, M.** (1991). *twist* and *snail* as positive and negative regulators during *Drosophila* development. *Genes Dev.* **5**, 1568-1576.
- Lohs-Schardin, M., Cremer, C. and Nüsslein-Volhard, C.** (1979). A fate map for the larval epidermis of *Drosophila melanogaster*: localized cuticle defects following irradiation of the blastoderm with an ultraviolet laser microbeam. *Dev. Biol.* **73**, 239-255.
- Lohs-Schardin, M.** (1982). *dicéphalic* - a *Drosophila* mutant affecting polarity in follicle organization and embryonic patterning. *Roux's Arch. Dev. Biol.* **191**, 28-36.
- Lüscher, M.** (1944). Experimentelle Untersuchungen über die larvale und die imaginale Determination im Ei der Kleidermotte (*Tineola biselliella* Hum.). *Revue suisse Zool.* **51**, 531-627.
- Malicki, J., Cianetti, C., Peschle, C. and McGinnis, W.** (1992). A human HOX4B regulatory element provides head-specific expression in *Drosophila* embryos. *Nature* **358**, 345-347.
- Manton, S. M.** (1973). Arthropod phylogeny - a modern synthesis. *J. Zool. Lond.* **171**, 111-130.
- McGinnis, W. and Krumlauf, R.** (1992). Homeobox genes and axial patterning. *Cell* **68**, 283-302.
- Nagy, L. M. and Carroll, S.** (1994). Conservation of *wingless* patterning functions in the short-germ embryos of *Tribolium castaneum*. *Nature* **367**, 460-463.
- Neumann-Silberberg, F. S. and Schüpbach, T.** (1993). The *Drosophila* dorsoventral patterning gene *gurken* produces a dorsally localized RNA and encodes a TGF α -like protein. *Cell* **75**, 165-174.
- Nüsslein-Volhard, C. and Wieschaus, E.** (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795-801.
- Patel, N. H., Ball, E. E. and Goodman, C. S.** (1992). Changing role of *even-skipped* during the evolution of insect pattern formation. *Nature* **357**, 339-342.
- Patel, N. P., Condrón, B. G. and Zinn, K.** (1994) Pair rule expression patterns of *even-skipped* are found in both short- and long-germ beetles. *Nature* **367**, 429-434.
- Pokrywka, N. J. and Stephenson, E. C.** (1991). Microtubules mediate the localization of *bicoid* RNA during *Drosophila* oogenesis. *Development* **113**, 55-66.
- Reuter, R., Grunewald, B. and Leptin, M.** (1993). A role for the mesoderm in endodermal migration and morphogenesis in *Drosophila*. *Development* **119**, 1135-1145.
- Sander, K.** (1976). Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* **12**, 125-238.
- Sander, K.** (1983). The evolution of patterning mechanisms: gleanings from insect embryogenesis and spermatogenesis. In *Development and Evolution: The Sixth Symposium of the British Society for Developmental Biology*. (ed. B. C. Goodwin, N. Holder and C. C. Wylie), pp. 137-159. Cambridge: Cambridge University Press.
- Seidel, F.** (1935) Der Anlagenplan im Libellenkeim, zugleich eine Untersuchung über die allgemeinen Bedingungen für defekte Entwicklung und Regulation bei dotterreichen Eiern. *Wilhelm, Roux Arch. EntwMech. Org.* **132**, 671-751.
- Seidel, F.** (1960). Körpergrundgestalt und Keimstruktur. Eine Erörterung über die Grundlagen der vergleichenden experimentellen Embryologie und deren Gültigkeit bei phylogenetischen Überlegungen. *Zool. Anz.* **164**, 245-305.
- Slack, J. M. W., Holland, P. W. H. and Graham, C. F.** (1993). The zootype and the phylotypic stage. *Nature* **361**, 490-492.
- Smith, D. E., Franco Del Amo, F. and Gridley, T.** (1992). Isolation of SNA, a mouse gene homologous to the *Drosophila* genes *snail* and *escargot*: its expression pattern suggests multiple roles during postimplantation development. *Development* **116**, 1033-1039.
- Sommer, R. J., Retzlaff, M., Görlich, K., Sander, K. and Tautz, D.** (1992). Evolutionary conservation pattern of zinc-finger domains of *Drosophila* segmentation genes. *Proc. Natl. Acad. Sci. USA* **89**, 10782-10786.
- Sommer, R. J. and Tautz, D.** (1993). Involvement of an orthologue of the *Drosophila* pair rule gene *hairy* in segment formation of the short germ band embryo of *Tribolium* (Coleoptera). *Nature* **361**, 448-450.
- Sommer, R. J. and Tautz, D.** (1994). The expression patterns of *twist* and *snail* in *Tribolium* (Coleoptera) suggest a homologous formation of mesoderm in long and short germ band insects. *Dev. Genet.* **15**, 32-37.
- St. Johnston, D. and Nüsslein-Volhard, C.** (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201-219.
- St. Johnston, D., Driever, W., Berleth, T., Richstein, S. and Nüsslein-Volhard, C.** (1989). Multiple steps in the localization of *bicoid* RNA to the anterior pole of the *Drosophila* oocyte. *Development Supplement* **107**, 13-19.
- Stys, P. and Bilinski, S.** (1990). Ovariole types and the phylogeny of hexapods. *Biol. Rev.* **65**, 401-429.
- Tautz, D. and Sommer, R. J.** (1994). Evolution of segmentation genes in insects. *Trends Genet.* (in press).
- Turbeville, J. M., Pfeiffer, D. M., Field, K. G. and Raff, R. A.** (1991). The phylogenetic status of arthropods as inferred from 18S rRNA sequences. *Mol. Biol. Evol.* **8**, 669.
- Wang, C. and Lehmann, R.** (1991). *nanos* is the localized posterior determinant in *Drosophila*. *Cell* **66**, 637-647.
- Weigel, D., Jürgens, G., Klingler, M. and Jäckle, H.** (1990). Two gap genes mediate maternal terminal pattern information in *Drosophila*. *Science* **248**, 495-498.