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The molecular genetics of head development in *Drosophila melanogaster*

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Key words: head development, *Drosophila*, segmentation.

(I) Introduction

> 'It would be too bad if the question of head segmentation ever should be finally settled; it has been for so long such fertile ground for theorizing that arthropodists would miss it as a field for mental exercise'.
>
> Snodgrass, 1960 (cited by Rempel, 1975)

The development of the *Drosophila* embryo has provided an excellent model system for the analysis of embryonic pattern formation. In little more than a decade, many of the elements of the molecular genetic cascade underlying early development have been identified. However, these studies have focused primarily on the development of the central, overtly segmented region of the fruitfly embryo. As the influential insect morphologist R. E. Snodgrass pointed out, understanding of the formation of the complex embryonic head region has advanced quite slowly. Recently, however, substantial progress has been made in the identification and analysis of the genes determining head development. These studies are generating increasing hope that both the structure of the head and how it is formed will soon be significantly clearer. What is perhaps most interesting is that there are already a number of hints that the rules governing head formation may differ from the paradigm established for the central region of the embryo.

In this review, we will focus on the process of embryonic head development in *Drosophila melanogaster*. First, we will describe the evolution of the head, its formation, and some of the difficulties involved in analyzing its structure. We will then discuss what has been learned about the genes responsible for embryonic head development. Finally, the implications of this molecular genetic data for models of head development will be explored.

(A) *Head evolution and morphogenesis*

The dipteran *Drosophila* embryo is one of the most highly evolved of the arthropod embryos. Arthropods, annelids, and other members of the articulate group (see Fig. 1) all show marked similarities in body plan. Most obviously, both arthropods and annelids are metameric in structure, consisting of a series of segmental units. An early definition of a metameric unit was proposed by Snodgrass (1935) as simply a body division of the embryo. This definition, however, was rather crude and was later supplanted by a series of more precise criteria. Rempel (1975), for example, summarized the attributes of a metamere as: (1) a pair of mesodermal somites (which give rise to the muscles) (2) a pair of appendages (3) a pair of apodemes (inner projections of the ectoderm which form muscle attachment sites) and (4) a neuromere (which produces a ganglion and its associated lateral nerves). Although these characteristics are easily recognizable in the metameres of most annelids, in many arthropod segments they are often less evident. In particular

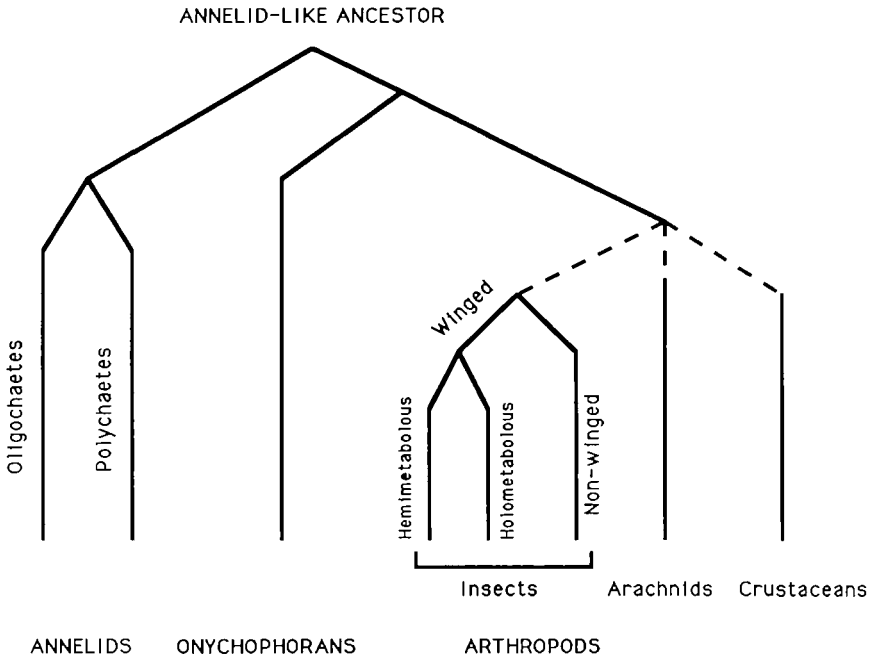

Fig. 1. The evolutionary origin of present day annelids, onychophorans and arthropods, which constitute the articulate group. Controversy regarding the derivation of the arthropod line is indicated by dashed lines. The characteristics of the annelid-like ancestor of the articulates have been deduced largely from homologies among modern representatives of this group (adapted from Strecker and Lengyel, 1988).

regions of arachnids and crustaceans, for example, metamerism is quite difficult to detect. This loss of segmental attributes (which as we shall see is an important issue in *Drosophila* head development) has occurred in several ways. In a number of cases, particular segments have been lost or fused together during evolution. In various species, segmental appendages are greatly reduced and apodemes and neuromeres difficult to discern.

In addition to a metameric body plan, both arthropods and annelids have other similarities in general body structure. Both groups have dorsal hearts and related nervous systems, consisting of an anterior, dorsally located brain and a ventral nerve cord formed of a series of ganglia. Because of these structural similarities, present day articulates are thought to have evolved from a primitive annelid-like organism (see Fig. 1 and, for example, Anderson, 1973). This hypothesized ancestor consisted of an array of metameric units, which showed little evidence of functional or structural specialization. These metameres, as in present-day annelids, were likely to have been generated sequentially from a central growth zone. The ends of this animal were primitive non-segmented structures. The anterior end was probably most similar to the anterior 'prostomial' region of modern annelids, housing a simple brain (archicerebrum). This 'head' area was involved in sensory and feeding functions. The posterior 'tail' region most likely consisted of a non-segmented area surrounding the anal opening.

The evolutionary process of head formation involved the progressive incorporation of structures into the head region (see Fig. 2). In animals even more primitive than the annelids (e.g. the Platyhelminthes), there is already an increasing concentration of sensory and feeding structures (eyes, ganglia and tentacles) in the anterior region. A critical step in this process of 'cephalization' occurred when several of the anterior-most trunk segments became incorporated into the beginnings of a clearly recognizable head region. This process is already evident in certain annelid embryos (e.g. the polychaete *Nereis*) in which the head includes regions called the prostomium (anterior to the mouth opening) and the peristomium (surrounding the mouth). Although the precise origin of the peristomium is unclear, it is generally thought to include one or more former trunk segments. One piece of evidence for this is that the peristomium contains pairs of cirri, which are likely the remnants of the parapodia, the appendages present on annelid trunk metameres. Cephalization continued as evolution proceeded and is evident in present-day myriapods (centipedes and millipedes). The insect head, consisting of an asegmental terminal region and cephalized trunk segments, first appeared in primitive form during the Devonian period (Smart and Hughes, 1972). However, because of the scarcity of fossil material from this period, its structural form has been deduced largely through the comparative analysis of more recent species.

As cephalization occurred, several of the anterior-most trunk segments shifted forward, so that they came to lie in front of the mouth opening. These segments became increasingly specialized and capable of performing more 'head specific' roles. For example, the specialization and anterior position of the antennal segment allowed it to acquire an important sensory function. The preoral domain of the head is often referred to as the procephalon. It is in this region that segmental identities are most difficult to establish. Behind the mouth (postoral), is the section of the head known as the gnathocephalon. In this region, cephalized trunk segments have become adapted for feeding functions. As evolution proceeded, the remaining trunk segments became subdivided into thoracic and abdomi-

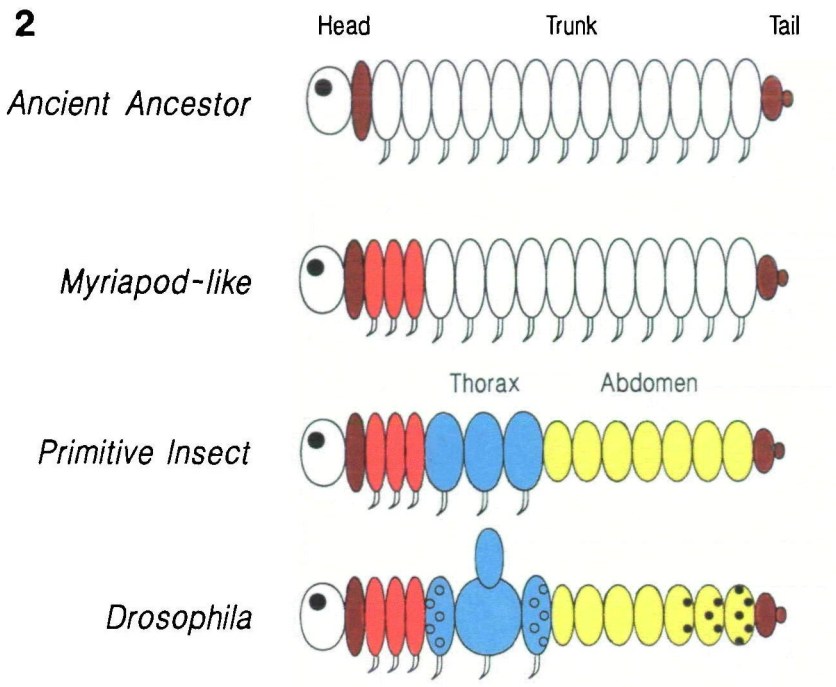

Fig. 2. A schematic representation of the evolutionary progression leading to the *Drosophila* body plan (see text). In the early articulate ancestor, the segmental units of the trunk were structurally equivalent. The recruitment of anterior trunk segments into the head (cephalization) generated an evolutionary intermediate probably resembling modern myriapods. Further regional specialization resulted in the highly distinctive units comprising the head, thorax, abdomen, and tail of present day *Drosophila* (adapted from Akam *et al.* 1988).

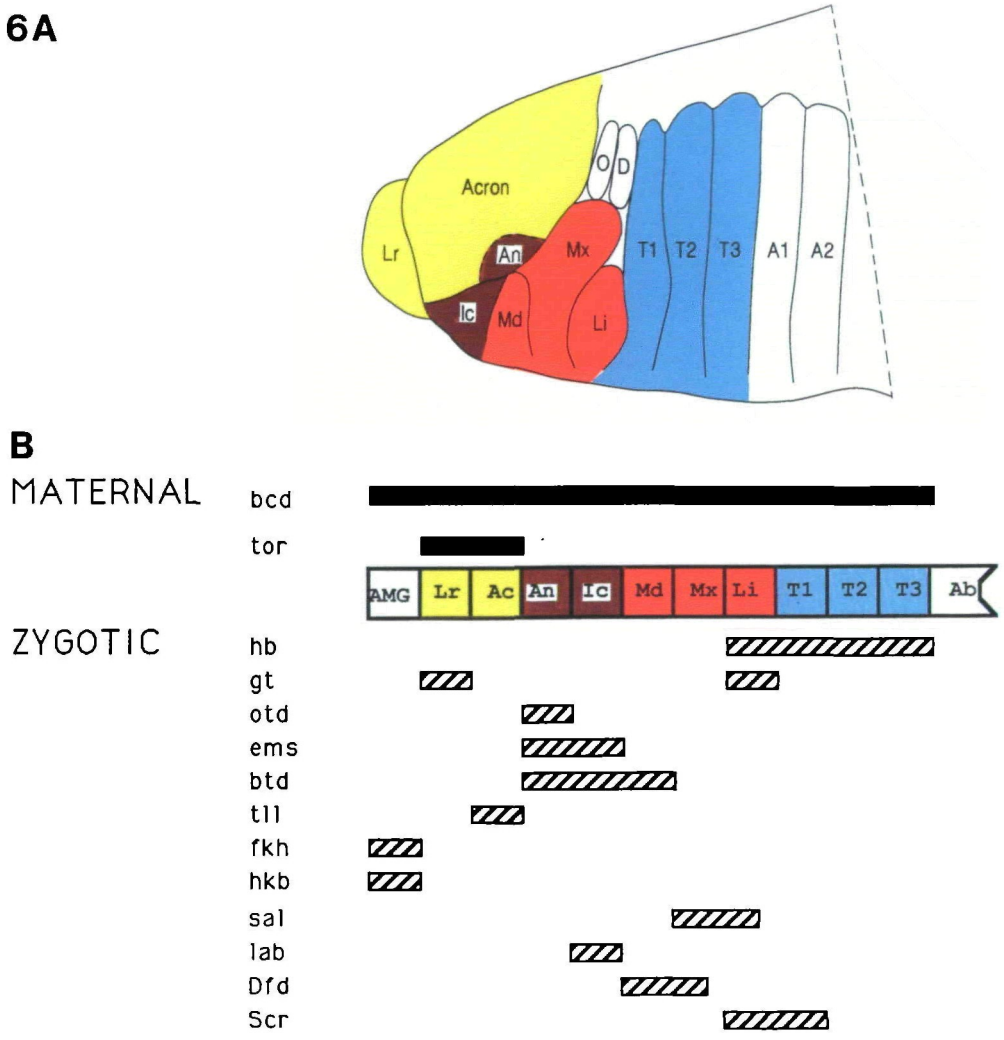

Fig. 6. A is a drawing of the anterior end of a segmented *Drosophila* embryo after the germ band has shortened. The labels shown refer to the segmental regions from which various structures have been shown to derive. B is a summary of the regions deleted or transformed in embryos lacking various maternal or zygotic gene products. For each mutation, what appears to be the primary domain affected is shown, rather than defects which are thought to be secondary (e.g. resulting from the failure of head involution). The limits of the regions deleted or transformed are in some cases approximate (see specific descriptions in text). In addition, proposed preantennal segments (see Fig. 5) are not shown here, and only the anterior midgut is indicated. Abbreviations: AMG, anterior midgut; Ab, abdominal region. All other abbreviations as described above.

nal units with clearly distinct functional roles. Further regional specialization throughout the embryo led to the complex *Drosophila* body plan.

In the *Drosophila* embryo, part of this process of head specialization has been the evolution of a series of complex morphological movements which occur during hours 9–12 of development. During this time window, a parallel series of complex events (which will not be discussed here) also occurs in the posterior anal region (Turner and Mahowald, 1977). Much of our understanding of the process of head formation described below stems from the detailed scanning electron microscopy (SEM) studies of Turner and Mahowald (1979). Initially, the 'germ band' of the embryo is fully extended and segmental furrows are clearly visible (stage 12; Campos-Ortega and Hartenstein, 1985). In the head region, a series of six lobes can be identified. These include the procephalic, clypeolabral and hypopharyngeal lobes as well as three gnathal lobes (mandibular, maxillary and labial). At this time, the germ band begins to retract and a series of rotations and fusions of the head lobes begins that culminates with the process of 'head involution'. During head involution, the gnathal lobes move closer to the stomodeal opening at the anterior end of the embryo and eventually become largely internalized (see Fig. 3). As a result, the labial lobe becomes the floor of the mouth, the maxillary and mandibular lobes fuse and form the lateral sides of the mouth, and the internalized clypeolabrum forms the roof of the mouth. The completed head structures then retract beneath the first thoracic segment. This highly specialized process of head involution is a relatively recent evolutionary development. It results in the acephalic appearance of the *Drosophila* larva, a consequence of the fact that most of the head structures are brought to positions inside the anterior end of the embryo.

Following head involution, a layer of epidermal cells contained within the involuted head region secretes a variety of cuticular and sensory structures (see Fig. 4). These structures include various easily recognizable 'skeletal' elements (which occur mostly in pairs and constitute the cephalopharyngeal skeleton), as well as the mouth hooks, labrum, cirri and several segment-specific sensory organs. In the head of the first instar larva, these structures can be relatively easily identified in cuticular preparations. This has two important implications. First, it permits fate mapping studies to be performed in which regions of the early embryo are disrupted, and the subsequent effects on head development monitored. Second, the effects of mutations that perturb head development on the formation of head structures can be determined. Both these approaches have been critical to understanding the structure of the head domain.

(B) *The structure of the head region*

What Rempel (1975) referred to as the 'endless dispute' about insect head development involves the determination of the number and nature of the segments included in the head. This problem has been approached using several different methodologies. Traditional analyses have involved the comparative morphological study of the heads of a wide variety of insects. Such investigations have produced numerous hypotheses to describe head segmentation (summarized in Rempel, 1975). There have been two critical points of disagreement in these models. The first issue is whether

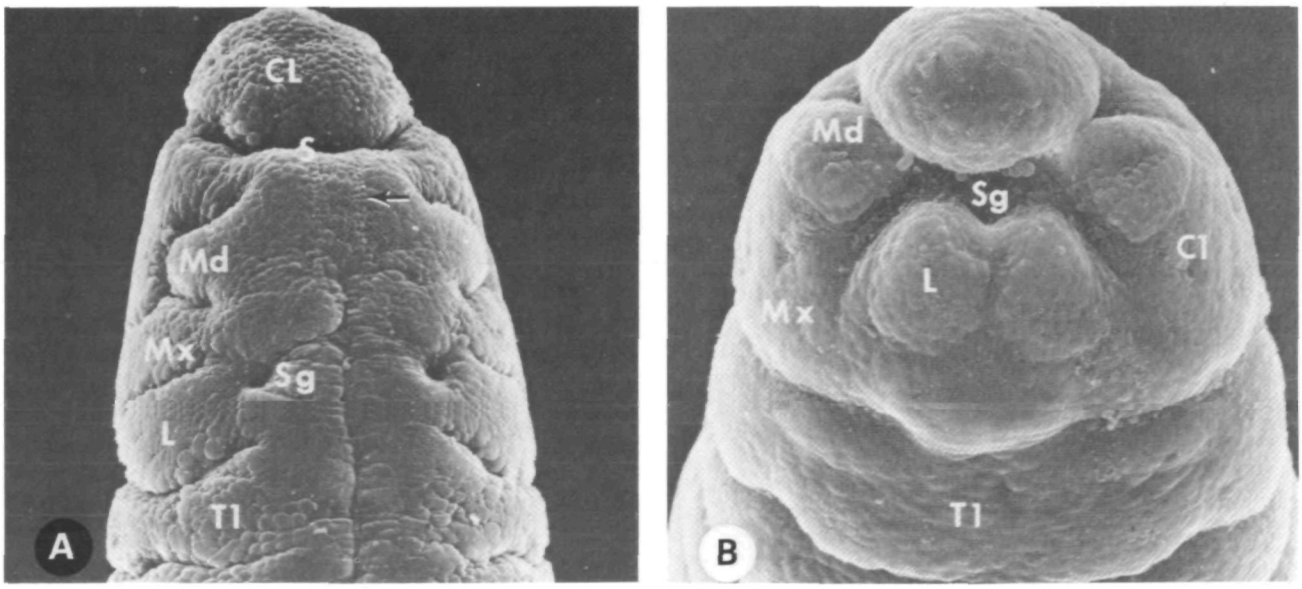

Fig. 3. Electron micrographs showing the head region of the *Drosophila* embryo before (A) and during (B) the process of head involution (see text). Panel A is a ventral view of the anterior end of the embryo. The gnathal lobes (Md, mandibular; Mx, maxillary; L, labial) are clearly visible anterior to the first thoracic segment (T1). In B, head involution is almost complete. Note how the labial lobes from each side of the embryo have begun to fuse. In addition, the other structures shown in A have moved inside of or adjacent to the stomodeal opening (S). Other abbreviations: Sg, salivary gland invagination. (Photographs from Turner and Mahowald, 1979).

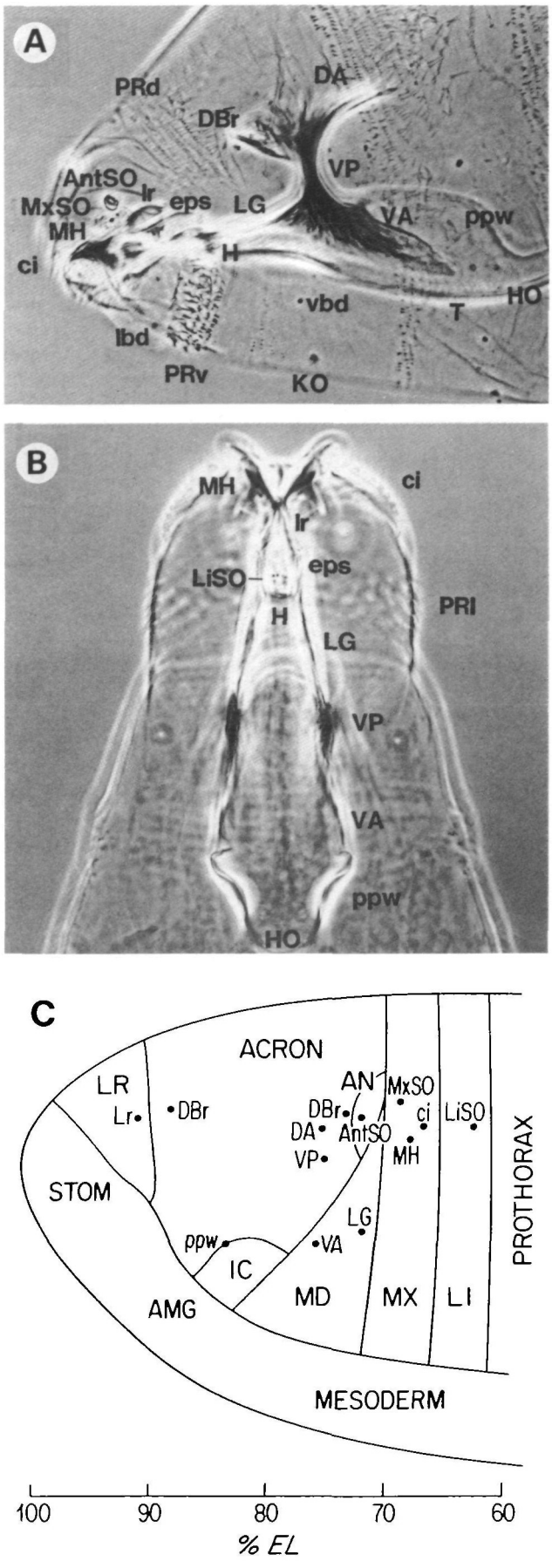

Fig. 4. A and B are lateral and frontal views of cuticle preparations of the head of the first instar larva. A variety of sensory and cuticular structures, which are secreted by epidermal cells within the involuted head, can be seen. The maxillary and antennal sense organs (MxSO and AntSO) are visible near the anterior end of the head. In addition, the darkly pigmented cephalopharyngeal skeleton [composed of the dorsal arms (DA), dorsal bridge (DBr), vertical plates (VP), ventral arms (VA), and lateralgraten (LG)] is clearly evident. These skeletal elements form the structural base for the muscles which open and close the lumen of the pharynx. For other abbreviations, see Jurgens *et al.* 1986. C shows the blastoderm fate map of larval head structures and the organization of segments at this stage. Notice that the three most anterior head segments (LR, labral; AN, antennal; IC, intercalary) occupy relatively small regions of the embryo. Selected abbreviations: STOM, stomodeum; AMG, anterior midgut; MD, mandibular segment; MX, maxillary segment; LI, labial segment. (Adapted from Jurgens *et al.* 1986).

there is a domain of the embryonic head that is in fact unsegmented in structure. Although most current hypotheses propose that there is an asegmental region (referred to as the acron), there have been observers who maintain that the insect head is entirely metameric in composition. The second controversy, which is clearly related to the first, has involved the number of segments present in the head. This number has been estimated to be as low as three and as high as seven (e.g. Roonwal, 1938). There has been no disagreement about the gnathocephalon, which is composed of three easily visible segments. These gnathal subdivisions, the mandibular, maxillary and labial segments, clearly meet the requirements for metameric identity discussed in the previous section. In the procephalon, however, segmental identities are more difficult to establish. More recent morphological studies (Scholl, 1969; Rempel and Church, 1971) have proposed that the anterior region is composed of the asegmental acron and three distinct segments. From anterior to posterior, these include the labral, antennal and intercalary (premandibular) segments. However, establishing the metameric nature of the labral and intercalary segments in particular has been difficult. In addition, some observers have hypothesized that the posterior region of the acron is in fact an additional pre-antennal segment (e.g. Roonwal, 1938).

In *Drosophila*, the highly evolved form of the procephalon has made segmental identification particularly difficult by morphological methods. As a result, various other approaches have been utilized. By histological analysis of staged embryos, Poulson (1950) was able to assign various larval organs to specific positions on the blastoderm fate map. More recently, Campos-Ortega and colleagues (Hartenstein and Campos-Ortega, 1985; Technau and Campos-Ortega, 1985; Hartenstein *et al.* 1985) have refined this fate map by following individual cells injected with horseradish peroxidase (HRP). This technique has been particularly useful in determining the pattern of mitoses in various embryonic primordia, including the cephalic region.

Specifically, HRP injection has allowed better definition of the boundaries of the gnathal segments and of the size of the blastoderm anlagen that give rise to them. In particular, it was shown that as one proceeds in the anterior direction, each gnathal segment is derived from an increasingly small region of the blastoderm fate map. In addition, a population of about 80 cells was defined as constituting the procephalic neurogenic region. This region shows a pattern of mitotic behavior independent of that of the more well-characterized ventral neurogenic domain. Although the procephalon was demonstrated to consist of several distinct groups of cells in this study, specific segmental identities were not proposed.

Another approach to the structural analysis of the head has been genetic. This method has relied on the analysis of marked clones of cells to deduce the regions of the blastoderm that give rise to adult, rather than embryonic head structures. The adult head of *Drosophila* is formed from three pairs of imaginal discs which develop during larval stages. Most head structures, including the head capsule, eyes, antennae and maxillary palp are derived from the eye–antennal discs (Bryant, 1978). The proboscis and the clypeolabrum are formed from the labial and cibarial discs respectively. By analyzing clones of cells in wild-type and mutant flies, Morata and Lawrence (1978, 1979) showed that the eye–antennal disc, like other imaginal discs, is divided into anterior and posterior compartments. This division was shown to require the activity of the *engrailed* gene. However, the relative positions of the anterior and posterior compartments of this disc were shown to be reversed, with the posterior compartment giving rise to the extreme anterior region of the adult head. Struhl (1981) extended this analysis using gynandromorph fate mapping. He was able to show that the eye–antennal disc, which is presumably derived from a single blastoderm (antennal) segment, becomes spatially reversed as a result of a rotation that occurs after the blastoderm stage. This gynandromorph analysis also yielded two important results relevant to head segmentation. The first is that the clypeolabrum of the adult head is derived from a region anterior to the antennal segment, supporting the existence of a labral segment. The second finding is that, assuming segments initially arise from equally sized regions of the blastoderm, there is sufficient 'room' on the fate map for three segments between the antennal and labial primordia. Therefore, this genetic analysis supports the existence of the intercalary segment, and is consistent with a six segment model of the insect head.

The final technique that has been used to study head structure is analyzing the effects of destroying cells at the blastoderm stage of development. This has been done mechanically (Bownes and Sang, 1974*b*; Underwood *et al.* 1980), by microcautery (Bownes and Sang, 1974*a*), and by UV-laser irradiation (Lohs-Schardin *et al.* 1979; Jurgens *et al.* 1986). We will discuss the work of Jurgens and colleagues, since it has produced the highest level of resolution. In these experiments, *Drosophila* embryos were irradiated at either the cellular blastoderm stage or at the end of the extended germ band stage (approximately 9 h of development). In each case, irradiation was performed in small designated regions of the presumptive head region (the anterior 40% of the embryo). By scoring the cuticle defects that occurred in the heads of the larvae that developed from irradiated embryos, the regions of the blastoderm and the extended germ band embryo that give rise to particular head structures could be determined. It should be noted however, that in this study, only epidermal derivatives of the head and not internal tissues were considered. The detailed blastoderm fate map derived from this work is shown in Fig. 4. The information in this fate map was combined with the results of irradiating regions within the morphologically recognizable head lobes in the extended germ band embryo. It was concluded that the *Drosophila* larval head is derived from the unsegmented acron and six segmental anlagen present at the cellular blastoderm stage. Not all of these proposed segments are composed of symmetric, circumferential strips of embryonic cells. The most anterior segments in particular consist of small localized regions of the blastoderm (see Fig. 4).

In general, the current evidence can be summarized as follows. The *Drosophila* head, like the 'generalized' insect head, is bipartite in structure. The first region is the asegmental acron, which gives rise to the brain (including the optic lobes) and several of the elements of the cephalopharygeal skeleton. The remaining paired cuticular and sensory structures shown in Fig. 4 are derived from the second, segmented region of the head. This region comprises six segments which are, from anterior to posterior, the labral, antennal, intercalary, mandibular, maxillary and labial segments. There are two areas about which some controversy still exists. The first involves the acron. As some investigators have postulated, there may be regions of the acron that are in fact the remnants of ancestral segments. The second area about which there is still some dispute is the existence of the six head segments. There remains some doubt about whether the labral region is truly a metamere and whether the intercalary segment still exists in the highly evolved *Drosophila* head. As we will see, molecular genetic studies have provided new information relevant to these issues of head structure.

(II) Molecular genetics of the head region

In the *Drosophila* embryo, pattern formation is specified during the first few hours of development (for review, see Akam, 1987; Ingham, 1988). The 'coordinates' of the embryo are established by four classes of maternal gene products. These gene products are encoded by the terminal, anterior, posterior and dorsal/ventral groups of genes. In the central trunk region, maternal information from the anterior and posterior groups is translated into the metameric pattern of the embryo through the activation of a

cascade of zygotic genes. The gap, pair-rule, and segment polarity genes subdivide the embryo into parasegments, the initial, transient units of segmentation. The identities of these parasegments are specified by the homeotic genes of the Antennapedia complex (ANT-C) and Bithorax complex (BX-C). Parasegmental divisions are ultimately replaced by segments, which persist throughout embryogenesis.

As noted above, the head is composed of two parts, the asegmental acron and a segmental domain. These two regions are primarily specified by the genes of the terminal and anterior classes, respectively. As will be described below, however, the labral segment is in fact included in the region of the head specified by the terminal group of genes. In this section, we will review what is currently known about the genes in these two classes that are required for head development. For convenience, we will group these genes using the categories that have been established for the trunk region of the embryo. However, as will be discussed, the functions of these genes are not necessarily analogous in the head region.

(A) *The anterior terminal domain*

(1) *Maternal genes*

The termini of the embryo are established by a signal transduction pathway which translates a localized signal to generate the formation of various structural derivatives. The maternal genes involved in this pathway include *torso*, *torsolike*, *trunk*, *fs(1)Nasrat*, *fs(1)polehole* and *l(1)polehole* (reviewed in Manseau and Schupbach, 1989). Mutations in any of these genes result in the loss of terminal structures, which in the anterior head region include the acron and the derivatives of the labral segment. In each case, posterior terminal elements are also deleted. The *torso* (*tor*) gene encodes a putative tyrosine kinase receptor which is uniformly distributed throughout the embryo (Sprenger *et al.* 1989; Casanova and Struhl, 1989). Upon being activated by a presumably localized ligand, the tor protein transmits its signal *via* a downstream serine-threonine kinase encoded by the *l(1)polehole* gene, which is the *Drosophila* homologue of the *raf* oncogene (Nishida *et al.* 1988; Ambrosio *et al.* 1989*a*,*b*). This mode of activation appears to be similar to the mammalian signal transduction pathway involving the PDGF receptor and the c-*raf* gene.

(2) *Zygotic genes*

Recently, several genes have been identified that are potential zygotic elements of the *torso* signaling pathway. Each of these genes is involved in the specification of both ends of the embryo, but we will focus primarily on their anterior functions.

(a) tailless *and* huckebein *(terminal domain gap genes).* Loss of function mutations at the *tailless* (*tll*) locus result in the deletion of a subset of the structures that are derived from the anterior terminal domain. Specifically, *tll* mutant embryos are missing most of the brain (including the supraesophageal ganglion and optic lobe, but probably not the most anterior region of the brain) and parts of the cephalopharyngeal skeleton (the dorsal bridge and dorsal arms). These derivatives originate from the posterior part of the anterior terminal region. *huckebein* (*hkb*) appears to be required for the establishment of the extreme poles of the embryo. *hkb* mutations in fact affect the anterior midgut, which is a structure outside the anterior limits of the *tor*-requiring region. Although *tll* and *hkb* are together probably sufficient for the development of the posterior terminal domain, they are probably not the only zygotic genes required for establishing the anterior terminus. This can be deduced from the fact that the labrum, which is absent in maternal mutants of the *tor* class, is present in both *tll* and *hkb* embryos.

The *tll* gene has been isolated by Pignoni and colleagues (1990) and shown to encode a protein that is quite similar to the members of the family of steroid hormone receptors. This similarity extends over both the putative DNA-binding zinc finger region and the ligand binding domain. Although it is initially transcribed in mirror-image terminal 'caps', anterior *tll* RNA expression quickly becomes localized to a smaller region which probably roughly coincides with its domain of function. This domain includes cells that appear to be procephalic neuroblasts which give rise to the developing brain.

The homology to steroid hormone receptors suggests a role for the *tll* gene product as a transcriptional regulatory molecule. This is consistent with the fact that *tll* expression is required for transcription of the *caudal*, *hunchback* and *fork head* genes in particular terminal regions (Mlodzik and Gehring, 1987; Schroder *et al.* 1988; Weigel *et al.* 1990). In addition, in its anterior domain, *tll* represses the expression of *hb*, *fushi tarazu* (*ftz*) and *Deformed*, and may thereby function as an 'anti-segmentation' gene (Reinitz and Levine, 1990). The *tll* gene thus appears to be a critical element (downstream of *torso* and *l(1)polehole*) in the pathway that induces the formation of the unsegmented ends of the embryo. The *hkb* gene has also recently been isolated and shown to encode a zinc finger-containing protein (G. Bronner and H. Jackle, personal communication), again suggesting a function in transcriptional regulation.

(b) fork head *and* spalt *('region specific' homeotic genes).* Recently, two loci have been identified which appear to represent a novel class of homeotic genes. These genes, *fork head* (*fkh*; Jurgens and Weigel, 1988) and *spalt* (*sal*; Jurgens, 1988), function outside the trunk region, where parasegmental identities are established by the homeotic selector genes of the ANT-C and BX-C. In addition, the activities of both *fkh* and *sal* appear to be genetically independent of those of the homeotic selector genes. Although the *sal* gene actually affects the segmented domain of the head, we will include it here because of its functional similarities to *fkh*.

fkh and *sal* mutations affect both the head and the tail regions of the embryo. In *fkh* mutant embryos, unsegmented terminal regions (both anterior and

posterior) become transformed into segmental derivatives. At the anterior end, the structures affected include preoral head derivatives (the dorsal bridge and the labrum) and the foregut (including the esophagus and proventriculus, which are derived from the stomodeum). *fkh* mutations result in the transformation of these asegmental structures into segmented, postoral head elements. Analogous transformations also occur at the posterior end of the embryo. In both cases, loss of *fkh* activity appears to produce transformations directed towards the center of the embryo. Although the posterior *fkh* domain lies within the region affected by the *tor* group, the anterior domain, which extends to the tip of the embryo, includes anterior parts of the gut beyond the *tor* region. Therefore, the anterior domain must require additional maternal inputs, perhaps from the *bcd* system.

Mutations in *sal* also produce centrally directed homeotic transformations near each end of the embryo. Compared to *fkh*, the regions affected are more posterior (in the head) and more anterior (in the tail). Specifically, posterior head structures (derived mostly from the labial segment) are changed into anterior thoracic structures while anterior tail segments become posterior abdominal segments.

The functions of *fkh* and *sal* are distinct from those of the homeotic selector genes. This independence is supported by the genetic relationship between these mutations and mutations in the *Polycomb* (*Pc*) group. Genes in the *Pc* class have been shown to regulate negatively the genes of the ANT-C and BX-C (Struhl and Akam, 1985; Wedeen *et al.* 1986). In *Pc* group mutant embryos, these homeotic genes are ectopically expressed, causing a range of phenotypic defects. However, the domain of *sal* action is not similarly expanded in such embryos, demonstrating that the region affected by *sal* mutations is distinct from the trunk (Jurgens, 1988). An equivalent genetic analysis showed that the *fkh* domain is not expanded in *Pc* embryos (Jurgens and Weigel, 1988). The functional difference between *sal* and *fkh* and the genes of the ANT-C and BX-C is also supported by molecular analysis. The *fkh* gene encodes a nuclear protein which has no homeodomain but may have a novel DNA-binding motif (Weigel *et al.* 1989; Weigel and Jackle, 1990). *sal* is predicted to encode a small protein with interesting repetitive stretches but also lacking a homeodomain (Frei *et al.* 1988). The expression patterns of the *fkh* protein and *sal* RNA during embryogenesis include the regions that give rise to the structures affected by each mutation. Further experiments will be required to determine the modes of action of each predicted gene product.

(B) *The segmented head domain*

(1) *Maternal genes*

The central trunk domain of the embryo (including the thoracic and abdominal segments) derives from the region of the blastoderm extending from 20–60 % EL (parasegments 3–13). As mentioned earlier, a hierarchy of genes has been shown to be responsible for the progressively more refined subdivision of the embryo. In the anterior region of the trunk, the critical maternal morphogen has been shown to be *bicoid* (Frohnhofer and Nusslein-Volhard, 1986). The *bicoid* mRNA is initially localized by the maternal products of the *swallow* and *exuperantia* genes (Berleth *et al.* 1988; Stephenson *et al.* 1988). bicoid protein subsequently forms a concentration gradient (Driever and Nusslein-Volhard, 1988) that declines in the posterior direction. However, in embryos lacking maternal *bcd* product, not only the thoracic segments, but the entire head region is deleted and replaced by a duplication of asegmental tail structures. This indicates that *bcd* is also the key maternal requirement for the establishment of all the head segments. The zygotic components of this process will be discussed in the following sections.

(2) *Gap genes*

(a) hunchback *and* giant. The only zygotic gene that has been demonstrated to be a direct transcriptional target of *bcd* is *hunchback* (*hb*; Driever and Nusslein-Volhard, 1989*a*). In embryos homozygous for strong *hb* mutations, two embryonic domains are deleted (Lehmann and Nusslein-Volhard, 1987). These are the labial and thoracic segments (anteriorly) and the 8th and part of the 7th abdominal segments (posteriorly). The deletion of the labial anlagen, the most posterior of the head segments, is indicated by the absence of the labial sense organ and the H-piece, a component of the cephalopharyngeal skeleton (see Fig. 4). All the more anterior head segments appear to be present in mutant embryos. The *hb* gene has been shown to encode a putative zinc finger-containing transcription factor which is initially expressed in the anterior half of the blastoderm (Tautz *et al.* 1987).

A second mutation with a gap-like phenotype that affects head segments is *giant* (*gt*). As in the case of *hb*, loss-of-function mutations in this gene affect more than one region of the embryo. In the head, structures derived from the labral and labial segments are deleted (Mohler *et al.* 1989; Petschek *et al.* 1990). Posteriorly, abdominal segments 5–7 are affected although not all the cuticular tissue derived from this region is lost. Perhaps as a consequence of the anterior deletions, head involution fails to occur properly, resulting in parts of the head skeleton being extruded from the embryo. The *gt* gene has recently been isolated and shown to encode a protein containing a 'leucine zipper' motif (V. Pirrotta, personal communication), suggesting that it functions (perhaps in conjunction with other gene products) as a transcriptional regulator of downstream segmentation genes. The expression pattern of *gt* RNA during early embryogenesis is interesting but somewhat difficult to explain fully. At the cellular blastoderm stage, *gt* is expressed in four stripes, each about 5–6 cells in width. Stripes 1, 3 and 4 are expressed in regions that give rise to labral, labial and abdominal structures, respectively. However, in the case of stripe 2 (at approximately 80 % EL), no corresponding defects can be identified. In addition, stripe 3 covers a significantly wider region than the labial segment

primordium. The function of this seemingly superfluous transcription may be clarified when antibodies to the *gt* protein become available. Some of the interactions between *gt* and other segmentation genes have also recently been identified. These include cross-regulatory effects among *gt* and other gap genes as well as the determination of particular homeotic gene boundaries of expression by *gt* (Reinitz and Levine, 1990).

(b) orthodenticle, empty spiracles *and* buttonhead *(anterior gap genes?)*

As mentioned above, *bcd* mutant embryos lack all head and thoracic structures. Since *hb* is only required for development of the labial head segment, there must be other *bcd*-regulated genes responsible for more anterior head segments. Accordingly, Driever *et al.* (1989) have proposed the existence of an additional gap gene ('gene X') or genes which would be activated at higher bcd concentrations than is *hb*. Loss-of-function mutations at such a locus should result in the deletion of a block of adjacent head segments.

One of the problems involved in attempting to identify candidate head gap genes is the difficulty in scoring head structures in mutant embryos. Mutations that disrupt head involution can result in gross abnormalities in the head cuticle which do not necessarily represent true structural deletions (see for example, the subsequent discussion of the *labial* gene). There are, however, head structures whose presence or absence is relatively easy to assess (e.g. segment-specific sensory organs). In addition, as will be discussed shortly, there are genes whose expression patterns may function as segmental markers in anterior head regions.

Three mutations have recently been characterized which affect anterior head segments. All three loci were originally identified in a large scale screen for zygotically acting mutations affecting segmental patterning (Wieschaus *et al.* 1984; Jurgens *et al.* 1984). The first, *empty spiracles* (*ems*), has been phenotypically and molecularly characterized by Dalton *et al.* (1989). Named because it is required for the development of the tracheal system in abdominal segment 8, *ems* mutations also result in the deletion of specific anterior head structures. The interpretation of the *ems* head phenotype, however, is not entirely straightforward. Dalton and colleagues argue that *ems* is a homeotic selector gene controlling the identities of the antennal and mandibular segments. However, as they point out, no obvious homeotic transformations can be seen to occur. Cohen and Jurgens (1990) have proposed a different segmental interpretation of the *ems* head phenotype, which will be discussed in section III. The *ems* gene has been isolated and shown to contain a canonical homeobox. At the blastoderm stage, the protein is expressed in an anterior circumferential stripe. This stripe is under the regulation of the maternal *bcd* product. Embryos with varying dosage of *bcd* form the *ems* stripe at different anterior–posterior positions. Later, the *ems* protein becomes localized to specific head regions of the extended germ band embryo.

The second candidate for an anterior gap gene is *orthodenticle* (*otd*). *otd* mutations result in the deletion of an overlapping set of head structures more anterior than those affected by *ems* (Finkelstein and Perrimon, 1990). For example, both mutations cause the deletion of the antennal sense organ, while only *otd* deletes the dorsomedial papilla (DMP) and only *ems* deletes the dorsolateral papilla (DLP; the DLP and DMP are peripheral parts of the maxillary sense organ that are not derived from the maxillary segment). As in the case of *ems*, no homeotic transformations can be found in *otd* mutant embryos. The *otd* gene has also been isolated and shown to be expressed in a circumferential anterior stripe at the cellular blastoderm stage. This *otd* expression, like that of *ems*, is under *bcd* control. However, the anterior limit of *otd* expression (at approximately 90 % EL) is determined by the maternal *tor* product which represses *otd* expression in the anterior 10 % of the blastoderm. *otd* also encodes a predicted homeodomain protein (Finkelstein *et al.* 1990). At residue 9 of the 'recognition helix', *otd* is the only homeodomain protein with the same amino acid as *bcd*, which suggests a similar binding specificity for the two proteins (Hanes and Brent, 1989; Treisman *et al.* 1989). *otd* has indeed been shown to bind to consensus bcd-binding sites in the *hb* promoter (M. Simpson and C. Desplan, unpublished observations). The regulatory significance of this binding is not yet clear.

A third mutation that causes deletions in this anterior segmented region is *buttonhead* (*btd*). As in the cases of *ems* and *otd*, head involution fails in *btd* mutant embryos. The structures deleted by *btd* form a third overlapping set that extends more posteriorly than those of *otd* or *ems* (Cohen and Jurgens, 1990). Again, no evidence for homeotic transformations can be found. The *btd* gene has not yet been characterized, so the molecular mechanism of its effects remains unclear.

(3) *Pair-rule genes*

We will discuss this class only briefly, because little is understood about the role of pair-rule genes in terminal and segmental head development. A number of pair-rule genes are expressed in stripes in the gnathal region and are required for the correct establishment of the gnathal segment boundaries. Amorphic *ftz* mutations, for example, result in the absence of the boundary between the maxillary and labial segments (Wakimoto *et al.* 1984). *hairy* (*h*), *paired* (*prd*) and *runt* (*run*) have been shown to be expressed in more anterior regions at the blastoderm stage (Ingham *et al.* 1985; Kilchherr *et al.* 1986; Gergen and Butler, 1988). In each case, anteriormost expression is confined to a dorsal region of the embryo. *hairy*, for example, is expressed in a dorsal region from 85–95 % EL as well as in a circumferential stripe at about 70–75 % EL. Null mutations at the *h* locus affect labral derivatives and delete the mandibular–maxillary boundary. Strong mutations in both the *prd* and *run* genes also cause segmental deletions and fusions (Nusslein-Volhard and Wieschaus, 1980) but the

role of these genes in the pregnathal regions is not yet known.

(4) *Segment polarity genes*

In the trunk region, segment polarity genes act to define and maintain cell fates within segmental units (Martinez-Arias *et al.* 1988). Mutations in any of these genes cause deletions (and often accompanying duplications) of specific intrasegmental pattern elements. Several of the genes in this class are first expressed in narrow stripes one cell in width, which define positions within each parasegment (and ultimately segment). For example, the expression of the *engrailed* (*en*) and *wingless* (*wg*) genes respectively demarcate the anterior and posterior compartments of each parasegment (DiNardo *et al.* 1985; Baker, 1988; van den Heuvel *et al.* 1989).

In addition to their expression in the trunk, *en* and *wg* are expressed in stripes in the three gnathal segments and in discrete patterns in more anterior head regions. This anterior expression occurs in the postulated positions of the intercalary and antennal segmental primordia as well as in preantennal regions. The two segment polarity genes in the *gooseberry* locus (*BSH4* and *BSH9*) also appear to be expressed in all six head segments (Baumgartner *et al.* 1987). As will be discussed, these expression patterns are extremely useful in analyzing these anterior regions. Because the effects of specific mutations on anterior head segments are difficult to assess, segment polarity gene expression is providing an important molecular marker for these segments.

(5) *Homeotic selector genes (of the Antennapedia complex)*

Parasegmental identities in the trunk region are specified by the homeotic genes of the ANT-C (thoracic region) and BX-C (abdominal region). In addition to several other genes, the ANT-C contains five homeotic genes: *labial* (*lab*), *proboscipedia* (*pb*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*) and *Antennapedia* (*Antp*). Three of these genes (*lab*, *Dfd* and *Scr*) are required for the embryonic head formation and will be discussed below. It should first be noted, however, that these three genes were originally labelled 'homeotic' because of the segmental transformations of *adult* head structures caused by particular alleles. Only in the case of *Scr*, however, is it clear that similar transformations can be seen in the embryonic head region.

(a) labial. The *lab* mutation was originally named because two of the affected structures (the H piece and salivary glands) are thought to be derivatives of the labial segment. In mutant embryos, head involution fails, and several other larval head structures (derived from the gnathal segments) are also disrupted (Merrill *et al.* 1989). As with other mutations discussed previously, no obvious homeotic transformations can be seen. *lab* embryos develop normally until the onset of head involution, when the required fusions and movements of the head lobes do not occur correctly.

The molecular analysis of *lab* has been quite important in the interpretation of the mutant phenotype. The *labial* protein contains a homeodomain and is the most anteriorly expressed of the ANTP-C genes (Diederich *et al.* 1989). It is not expressed in any of the gnathal lobes (including the labial lobe) from which the phenotype was thought to derive. Instead, it is expressed in more anterior regions, including the lateral margins of the procephalic lobe. Diederich and colleagues propose that *labial* expression occurs in regions necessary for correct head involution. A critical region in this process may be the intercalary segment, for which *labial* could be a marker. This argument is partly based on a comparison of the position of *lab* protein expression in the *Drosophila* embryo with the position of the intercalary segment in more primitive insect heads. The expression pattern of the *lab* protein suggests that some of the head defects are a secondary consequence of the failure of head involution.

(b) Deformed. *Dfd* was originally identified as a dominant mutation that affects the formation of the adult head (Lindsley and Grell, 1968). Subsequently, recessive loss of function alleles were recovered that were shown to affect embryonic head development. However, the interpretations of the head phenotype of embryos lacking *Dfd* function differ. Merrill *et al.* (1987) found that mutant embryos have disrupted head involution and that larval head structures of maxillary, and to a lesser extent, mandibular and antennal origin are deleted or perturbed. A second group (Regulski *et al.* 1987) saw similar deletions, but also reported the duplication of an anterior portion of the larval head skeleton. This homeotic transformation was only seen in a single allelic combination though, and may not reflect the true loss of function phenotype. However, ectopic expression of the *Dfd* gene driven by a heat shock promoter does induce homeotic transformations of many head and thoracic segments towards a maxillary identity (Kuziora and McGinnis, 1988).

The predicted *Dfd* gene product also contains a homeodomain (Regulski *et al.* 1987). The protein is expressed in a circumferential stripe at the blastoderm stage and later becomes concentrated in the region of the mandibular and maxillary segments. Elegant analyses by Jack and colleagues (1988, 1990) have shown that the initial stripe of *Dfd* expression requires input from maternal, gap and pair-rule genes for its correct establishment.

(c) Sex combs reduced. The *Scr* gene is necessary for the formation of the labial segment, as well as for the prothoracic segments of the embryo (Wakimoto and Kaufman, 1981). In mutant embryos, a partial transformation of the labial segment to a maxillary identity is suggested by the duplication of part of the maxillary sense organ in the labial segmental region (Sato *et al.* 1985). The *Scr* gene has been shown to contain a homeobox (LeMotte *et al.* 1989) and to be expressed in the regions of the embryo affected by mutations. In the head region, the protein is first expressed at gastru-

lation and becomes increasingly concentrated in the labial lobes (Riley *et al.* 1987; LeMotte *et al.* 1989). As in the case of *Dfd*, *Scr* expression is under combinatorial control and has been shown to be altered by mutations in different classes of segmentation genes (Riley *et al.* 1987).

(III) Conclusions: Implications of the molecular genetic data

How does pattern formation in the *Drosophila* head compare with the development of the central region of the embryo? In principle, two basic types of models of head development are possible. The first model is that head formation follows the same hierarchy of genetic interactions that govern the trunk. In this model, the roles played by the various classes of regulatory genes (coordinate, gap, pair-rule, segment polarity, homeotic) would be equivalent in the head and trunk. Only the structural difficulties in identifying head segments make these roles difficult to assess. The second model of head development is that, in order to promote a higher degree of specialization, a different, or modified, system of genetic control has evolved in the head region. Such a model might be expected to be more combinatorial in nature in order to permit a higher degree of flexibility. In addition, it might include new classes of gene products as well as not utilizing all the classes functioning in the trunk.

The current molecular and genetic evidence supports the second type of model of head formation. To begin with, both morphological and genetic studies demonstrate that the head is composed of two distinct domains. The anterior terminal domain (which includes the acron and labral segment) is specified primarily by a genetic system (the *torso* class) which is quite different from that used in the trunk. This system is initiated by what appears to be a phosphorylation cascade that ultimately activates zygotic transcription factors. Within the terminal class, there are novel genetic activities (i.e. the *fork head* gene) which promote terminal development over a segmental ground state. Such functions, as discussed by Jurgens and Weigel (1988), are quite ancient, since they would be required even in the annelid-like ancestor of the articulates (see

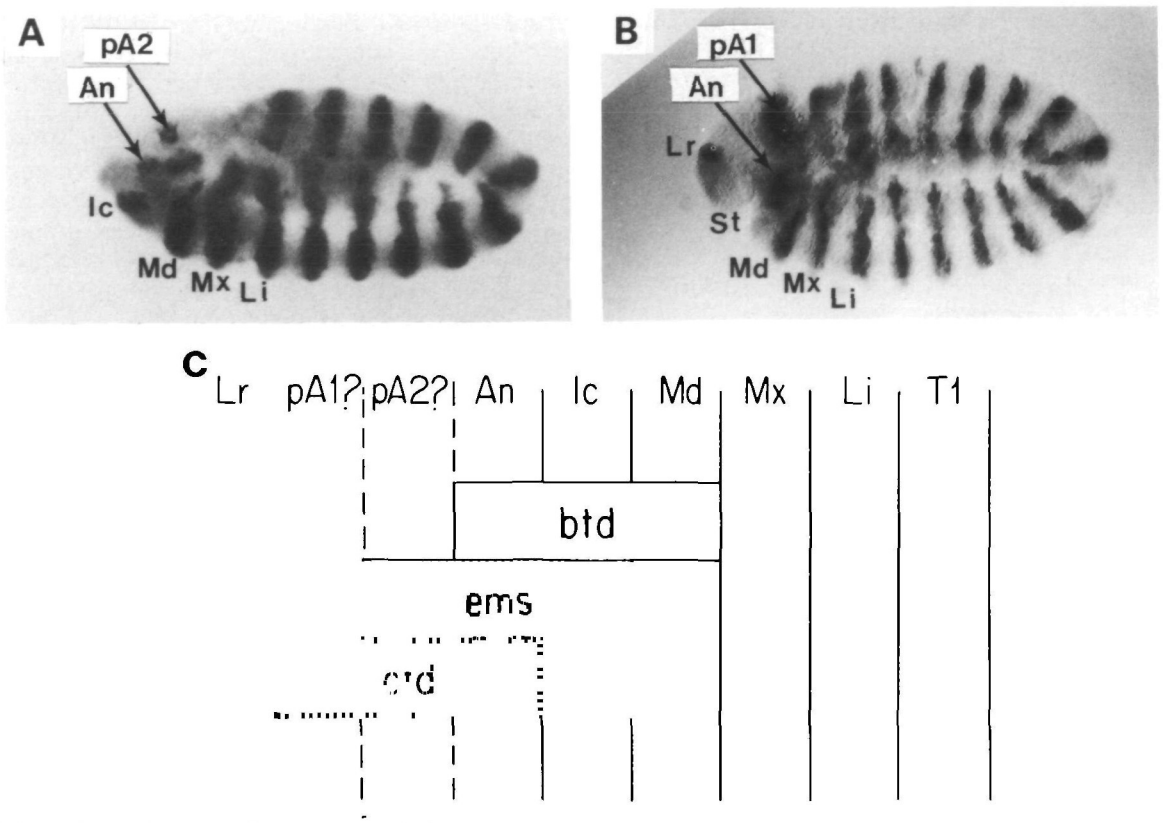

Fig. 5. The embryonic expression patterns of the segment polarity genes *engrailed* (*en*) (A) and *wingless* (*wg*) (B), at the extended germ band stage of development. In addition to the stripes marking the gnathal (Md, Mx, Li), thoracic and abdominal segments, each of these genes is also expressed in discrete anterior head regions. These include the labral (Lr) and intercalary segments (Ic; not visible in the focal plane of panel (B)). In addition, expression can be seen in the antennal segment (arrows An) and preantennal regions (arrows pA1 and pA2). For both *en* and *wg*, expression was monitored using *lacZ* insertion strains that accurately reproduce the embryonic patterns of protein expression (see Finkelstein and Perrimon, 1990). Panel C depicts the model of Cohen and Jurgens (1990) of overlapping gap genes for head development. *orthodenticle*, *empty spiracles* and *buttonhead* are each proposed to be required for the development of blocs of three head segments (shaded bars). These include two hypothesized preantennal segments (pA1 and pA2) which are demarcated by the expression patterns of *en* and *wg* (pA1 in B, pA2 in A).

section I). In addition, the terminal head domain requires input from the *bicoid* system to specify its anterior character. In the absence of maternal *bicoid* activity, the anterior region is replaced by posterior terminal structures.

The second domain of the head, the segmented region, is specified by the anterior (*bicoid*) class of genes. However, currently available evidence suggests that head segmentation is governed by different, or modified, rules compared with segmentation in the trunk region. Cohen and Jurgens (1990), for example, have proposed a novel model for the establishment of head segmentation. Through a comparative study of *otd*, *ems* and *btd* mutant embryos, they have postulated that these three genes form an overlapping set of head gap genes. This analysis was undertaken in an attempt to understand the regulation of the *Distal-less* gene, which is also expressed in a segmental pattern throughout the embryo (Cohen, 1990). In addition to analyzing cuticular structures, they demonstrated that each of these mutations deletes three adjacent segments, as defined by the *en/wg* expression pattern (Fig. 5). The deletions, which include two postulated preantennal 'segments', are each out of register by one segment, such that *btd*, *ems* and *otd* act in increasingly anterior domains. In addition, the deletions appear (based on the *en/wg* pattern) to be in segmental, rather than parasegmental register. These investigators proposed that, in the head, these genes may be required not only to establish contiguous blocs of segments, but also to specify segmental identity. If this is true, *otd*, *ems* and *btd* would be functioning simultaneously as gap and homeotic selector genes. In such a combinatorial model, the identity of each head segment could be specified by the combination of (three) 'gaplike' genes expressed within its boundaries. It will be important to determine if there are additional overlapping gap genes that are required in the posterior head segments. It is also possible, however, that specification of the gnathal segments is more similar to that of the trunk region.

It also appears that other classes of genes that function in the trunk are used differently in the head region. The analysis by Mahaffey and colleagues (1989) is interesting in this regard. By examining the protein distribution patterns of three of the genes from the ANTP-C, they found that these genes are expressed in non-overlapping domains in the head. This differs sharply from the overlapping expression of homeotic selector genes in the trunk. In addition, the various pair-rule genes are either not expressed in anterior head regions, or else appear to show less specificity of expression. It may be, if the overlapping gap gene model discussed earlier is correct, that pair-rule gene expression is not required for specifying segments in this region.

Finally, the 'region specific' activity of the *spalt* gene appears to have evolved during the process of head specialization. *sal*, which promotes head segmental development above a trunk ground state, would be required for the cephalization of anterior trunk segments (the first evolutionary step in Fig. 2). This function represents the real beginning of the development of a more complex head. Future studies will determine if there are other members of this class of genes required to specify the head (and tail) of the embryo as distinct from the central trunk domain.

The molecular genetic data is also relevant to the controversial area of metameric identity in the head. The expression patterns of the segment polarity genes are proving useful in defining head segments. It is reasonable to assume that these genes, which mark intrasegmental compartments in the trunk, are also expressed in segmental patterns in the head. The expression patterns of, for example, the *engrailed* and *wingless* genes, support the existence of all six head segments described in section I. In addition, these patterns suggest that there is cryptic segmentation in preantennal regions. These preantennal 'segments' are no longer true metameres, but their possible existence is significant in interpreting the phenotypes of mutations that affect head formation.

In order to establish just how different development is in the head and trunk, it will be critical to determine the precise hierarchy of gene regulation. For example, are the putative head gap genes *btd*, *ems* and *otd* directly regulated by *bcd*? Is pair-rule gene activity required in the head to establish segment polarity gene expression? How does the molecular cascade in the terminal domains differ in the head and tail? As in the trunk region, the availability of this kind of information should clarify our models of head formation. It should also be emphasized that there are other mutations that affect head development that we have not discussed (e.g. *Distal-less*), as well as additional loci perhaps not yet identified.

An area not discussed in this review is the development of the adult head in *Drosophila*. This process (which occurs during the larval stages from imaginal disc primordia) is essentially independent of embryonic head formation, but should nevertheless cast increasing light on the events discussed here. For example, many of the genes mentioned here play important roles in the development of the adult head. In several cases, the absence of expression of these genes during adult head development results in clear homeotic transformations not seen during embryonic stages. It will be important to determine whether such differences represent true variations in function, or simply reflect current inadequacies in our understanding.

It is likely that understanding *Drosophila* head formation will have important implications for higher organisms. Recently, for example, an increasing amount of evidence indicates that the vertebrate hindbrain develops as a series of segments or rhombomeres (for review, see Lumsden, 1990). Furthermore, *in situ* hybridization experiments have demonstrated that various homeobox and zinc-finger genes appear to be expressed in patterns that respect rhombomere borders. For example, there are vertebrate homologues of the genes of the Bithorax and Antennapedia complexes which are arranged in clusters on the chromosome resembling those in *Drosophila*. The

anterior limits of expression of these 'Hox' genes, as in the fruitfly, appear to correlate with their positions within these clusters (Graham *et al.* 1989; Wilkinson *et al.* 1989). It is not yet clear whether the vertebrate forebrain and midbrain are also segmentally organized. In this regard, it will be important to determine whether there are vertebrate homologues of the fly genes discussed here which are expressed in more anterior head regions. Our increasing understanding of the rules governing *Drosophila* head development is certain to contribute to models of our own morphogenesis.

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References

Akam, M. (1987). The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101**, 1–22.

Akam, M., Dawson, I. and Tear, G. (1988). Homeotic genes and the control of segment diversity. *Development* **104** (Supplement), 123–133.

Ambrosio, L., Mahowald, A. P. and Perrimon, N. (1989*a*). *l(1)pole hole* is required maternally for pattern formation in the terminal regions of the embryo. *Development* **106**, 145–158.

Ambrosio, L., Mahowald, A. P. and Perrimon, N. (1989*b*). Requirement of the *Drosophila raf* homologue for *torso* function. *Nature* **342**, 288–291.

Anderson, D. T. (1973). *Embryology and Phylogeny in Annelids and Arthropods*. Oxford: Pergamon.

Baker, N. E. (1988). Localization of transcripts from the *wingless* gene in whole *Drosophila* embryos. *Development* **103**, 289–298.

Baumgartner, S., Bopp, D., Burri, M. and Noll, M. (1987). Structure of two genes at the *gooseberry* locus related to the paired gene and their spatial expression during *Drosophila* embryogenesis. *Genes Dev.* **1**, 1247–1267.

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M. and Nusslein-Volhard, C. (1988). The role of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J.* **7**, 1749–1756.

Bownes, M. and Sang, J. H. (1974*a*). Experimental manipulation of early *Drosophila* embryos. I. Adult and embryonic defects resulting from microcautery at nuclear multiplication and blastoderm stages. *J. Embryol. exp. Morph.* **32**, 253–272.

Bownes, M. and Sang, J. H. (1974*b*). Experimental manipulation of early *Drosophila* embryos. II. Adult and embryonic defects resulting from the removal of blastoderm cells by pricking. *J. Embryol. exp. Morph.* **32**, 273–285.

Bryant, P. J. (1978). Pattern formation in imaginal discs. In Ashburner, M., Wright, T.R.F. *The Genetics and Biology of Drosophila*, vol. 2c. Academic Press, London, New York, San Francisco.

Campos-Ortega, J. A. and Hartenstein, (1985). *The embryonic development of Drosophila melanogaster*. Springer-Verlag, Berlin.

Casanova, J. and Struhl, G. (1989). Localized surface activity of *torso*, a receptor tyrosine kinase, specifies terminal body pattern in *Drosophila*. *Genes Dev.* **3**, 2025–2038.

Cohen, S. M. (1990). Specification of limb development in the *Drosophila* embryo by positional cues from segmentation genes. *Nature* **343**, 173–177.

Cohen, S. M. and Jurgens, G. (1990). Gap-like segmentation genes that mediate *Drosophila* head development. *Nature* **346**, 482–485.

Dalton, D., Chadwick, R. and McGinnis, W. (1989). Expression and embryonic function of *empty spiracles*: a *Drosophila* homeobox gene with two patterning functions on the anterior–posterior axis of the embryo. *Genes Dev.* **3**, 1940–1956.

Diederich, R. J., Merrill, V. K. L., Pultz, M. A. and Kaufman, T. C. (1989). Isolation, structure, and expression of *labial*, a homeotic gene of the Antennapedia Complex involved in *Drosophila* head development. *Genes Dev.* **3**, 399–414.

DiNardo, S., Kuner, J. M., Theis, J. and O'Farrell, P. (1985). Development of embryonic pattern in *D. melanogaster* as revealed by accumulation of the nuclear *engrailed* protein. *Cell* **43**, 59–69.

Driever, W. and Nusslein-Volhard, C. (1988). A gradient of *bicoid* protein in *Drosophila* embryos. *Cell* **54**, 83–93.

Driever, W. and Nusslein-Volhard, C. (1989*a*). The bicoid protein is a positive regulator of *hunchback* transcription in the *Drosophila* embryo. *Nature* **337**, 138–143.

Driever, W., Thoma, G. and Nusslein-Volhard, C. (1989). Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature* **340**, 363–367.

Finkelstein, R. and Perrimon, N. (1990). The *orthodenticle* gene is regulated by *bicoid* and *torso* and specifies *Drosophila* head development. *Nature* **346**, 485–488.

Finkelstein, R., Smouse, D., Capaci, T. M., Spradling, A. C. and Perrimon, N. (1990). The *orthodenticle* gene encodes a novel homeodomain protein involved in the development of the *Drosophila* nervous system and ocellar visual structures. *Genes Dev.* **4**, 1516–1527.

Frei, E., Schuh, R., Baumgartner, S., Burri, M., Noll, M., Jurgens, G., Seifert, E., Nauber, U. and Jackle, H. (1988). Molecular characterization of *spalt*, a homeotic gene required for head and tail development in the *Drosophila* embryo. *EMBO J.* **7**, 197–204.

Frohnhofer, H. G. and Nusslein-Volhard, C. (1986). Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. *Nature* **324**, 120–125.

Gergen, J. P. and Butler, B. A. (1988). Isolation of the *Drosophila* segmentation gene *runt* and analysis of its expression during embryogenesis. *Genes Dev.* **2**, 1179–1193.

Graham, A., Papalopulu, N. and Krumlauf, R. (1989). The murine and *Drosophila* homeobox gene complexes have common features of organization and expression. *Cell* **57**, 367–378.

Hanes, S. D. and Brent, R. (1989). DNA specificity of the Bicoid activator protein is determined by homeodomain recognition helix residue 9. *Cell* **57**, 1275–1283.

Hartenstein, V. and Campos-Ortega, J. A. (1985). Fate-mapping in wild-type *Drosophila melanogaster* I. The spatio-temporal pattern of embryonic cell divisions. *Roux Arch. dev. Biol.* **194**, 181–195.

Hartenstein, V., Technau, G. M. and Campos-Ortega, J. A. (1985). Fate-mapping in wild-type *Drosophila melanogaster* III. A fate map of the blastoderm. *Roux Arch. dev. Biol.* **194**, 213–216.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**, 25–34.

Ingham, P. W., Howard, K. R. and Ish-Horowicz, D. (1985). Transcription pattern of the *Drosophila* segmentation gene *hairy*. *Nature* **318**, 439–445.

Jack, T. and McGinnis, W. (1990). Establishment of the *Deformed* expression stripe requires the combinatorial action of coordinate, gap and pair-rule proteins. *EMBO J.* **9**, 1187–1198.

Jack, T., Regulski, M. and McGinnis, W. (1988). Pair-rule segmentation genes regulate the expression of the homeotic selector gene *Deformed*. *Genes Dev.* **2**, 635–651.

Jurgens, G. (1988). Head and tail development of the *Drosophila* embryo involves *spalt*, a novel homeotic gene. *EMBO J.* **7**, 189–196.

JURGENS, G., LEHMANN, R., SCHARDIN, M. AND NUSSLEIN-VOLHARD, C. (1986). Segmental organization of the head in the embryo of *Drosophila melanogaster*. *Roux Arch. devl Biol.* **195**, 359–377.

JURGENS, G. AND WEIGEL, D. (1988). Terminal *versus* segmental development in the *Drosophila* embryo: the role of the homeotic gene *fork head*. *Roux Arch. dev. Biol.* **197**, 345–354.

JURGENS, G., WIESCHAUS, E., NUSSLEIN-VOLHARD, C. AND KLUDING, M. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. II. Zygotic loci on the third chromosome. *Roux Arch. dev. Biol.* **193**, 283–295.

KILCHHERR, F., BAUMGARTNER, S., BOPP, D., FREI, E. AND NOLL, M. (1986). Isolation of the *paired* gene of *Drosophila* and its spatial expression during early embryogenesis. *Nature*. **321**, 493–499.

KUZIORA, M. A. AND MCGINNIS, W. (1988). Autoregulation of a *Drosophila* homeotic selector gene. *Cell* **55**, 477–485.

LEHMANN, R. AND NUSSLEIN-VOLHARD, C. (1987). *hunchback*, a gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. *Devl Biol.* **119**, 402–417.

LEMOTTE, P. K., KUROIWA, A., FESSLER, L. I. AND GEHRING, W. J. (1989). The homeotic gene *Sex combs reduced* of *Drosophila*: gene structure and embryonic expression. *EMBO J.* **8**, 219–227.

LINDSLEY, D. L. AND GRELL, E. H. (1968). *Genetic variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **627.**

LOHS-SCHARDIN, M., CREMER, C. AND NUSSLEIN-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 *Devl Biol* **73**, 239–255.

LUMSDEN, A. (1990). The cellular basis of segmentation in the developing hindbrain. *Trends Neurosc.* **13**, 329–335.

MAHAFFEY, J. W., DIEDERICH, R. J. AND KAUFMAN, T. C. (1989). Novel patterns of homeotic protein accumulation in the head of the *Drosophila* embryo. *Development* **105**, 167–174.

MANSEAU, L. J. AND SCHUPBACH, T. (1989). The egg came first of course! Anterior–posterior pattern formation in *Drosophila* embryogenesis and oogenesis. *Trends Genet.* **5**, 400–405.

MARTINEZ-ARIAS, A., BAKER, N. AND INGHAM, P. W. (1988). Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development* **103**, 157–170.

MERRILL, V. K. L., DIEDERICH, R. J., TURNER, F. R. AND KAUFMAN, T. C. (1989). A genetic and developmental analysis of mutations in *labial*, a gene necessary for proper head formation in *Drosophila melanogaster*. *Devl Biol.* **135**, 376–391.

MERRILL, V. K. L., TURNER, F. R AND KAUFMAN, T. C. (1987). A genetic and developmental analysis of mutations in the *Deformed* locus in *Drosophila melanogaster*. *Devl Biol.* **122**, 379–395.

MLODZIK, M. AND GEHRING, W. J. (1987). Hierarchy of the genetic interactions that specify the anteroposterior segmentation pattern of the *Drosophila* embryo as monitored by *caudal* protein expression. *Development* **101**, 421–435.

MOHLER, J., ELDON, E. D. AND PIRROTTA, V. (1989). A novel spatial transcription pattern associated with the segmentation gene, giant, of *Drosophila*. *EMBO J.* **8**, 1539–1548.

MORATA, G. AND LAWRENCE, P. A. (1978). Anterior and posterior compartments in the head of *Drosophila*. *Nature* **274**, 473–474.

MORATA, G. AND LAWRENCE, P. A. (1979). Development of the eye–antenna imaginal disc of *Drosophila*. *Devl Biol.* **70**, 355–371.

NISHIDA, Y., HATA, M., AYAKI, T., RYO, H., YAMAGATA, M., SHIMIZU, K. AND NISHIZUKA, Y. (1988). Proliferation of both somatic and germ cells is affected in the *Drosophila* mutants of *raf* proto-oncogene. *EMBO J.* **7**, 775–781.

NUSSLEIN-VOLHARD, C. AND WEISCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801.

PETSCHEK, J. P. AND MAHOWALD, A. (1990). Different requirements for *l(1) giant* in two embryonic domains of *Drosophila melanogaster*. *Dev. Genet.* **11**, 88–96.

PIGNONI, F., BALDARELLI, R. M., STEINGRIMSSON, E., DIAZ, R. J., PATAPOUTIAN, A., MERRIAM, J. R. AND LENGYEL, J. A. (1990). The *Drosophila* gene *tailless* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell* **62**, 151–163.

POULSON, D. F. (1950). Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster*. In Demerec, M. (ed) *Biology of Drosophila*. John Wiley, New York, 267–274.

REGULSKI, M., MCGINNIS, N., CHADWICK, R. AND MCGINNIS, W. (1987). Develomental and molecular analysis of *Deformed*; a homeotic gene controlling *Drosophila* head development. *EMBO J.* **6**, 767–777.

REINITZ, J. AND LEVINE, M. (1990). Control of the initiation of homeotic gene expression by the gap genes *giant* and *tailless* in *Drosophila*. *Devl Biol.* **140**, 57–72.

REMPEL, J. G. (1975). The evolution of the insect head: the endless dispute. *Quaestiones Entomologicae*. **11**, 7–25.

REMPEL, J. G. AND CHURCH, N. S. (1971). The embryology of *Lytta viridana* Le Conte (Coleoptera, Meloidae). V. The blastoderm, germ layers, and body segments. *Can. J. Zool.* **47(6)**, 1157–1171.

RILEY, P. D., CARROLL, S. B. AND SCOTT, M. P. (1987). The expression and regulation of *sex combs reduced* protein in *Drosophila* embryos. *Genes Dev.* **1**, 716–730.

ROONWAL, M. L. (1938). Some recent advances in insect embryology, with a complete bibliography of the subject. *Journal of the Asiatic Society of Bengal, Science* **4**, 17–105.

SATO, T., HAYES, P. H. AND DENNELL, R. E. (1985). Homoeosis in *Drosophila* : roles and spatial patterns of expression of the *Antennapedia* and *sex combs reduced* loci in embryogenesis. *Devl Biol.* **111**, 171–192.

SCHOLL, G. (1969). Die Embryonalentiwicklund des Kopfes und Prothorax von *Carausius morosu* Br. (Insecta, Phasmida) 2. Zeitschrift fuer Morphologie der Tiere **65**, 1–142.

SCHRODER, C., TAUTZ, D., SEIFER, E. AND JACKLE, H. (1988). Differential regulation of the two transcripts from the *Drosophila* gap segmentation gene *hunchback*. *EMBO J.* **7**, 2881–2887.

SMART, J. AND HUGHES, N. F. (1972). The insect and the plant: progressive palaeoecological integration *Symposium on insect/plant relationships of the Royal Entomological Society of London*. **6**. Blackwell Scientific Publication, Oxford.

SNODGRASS, R. E. (1935). *Principles of Insect Morphology*. McGraw-Hill, N.Y.

SPRENGER, F., STEVENS, L. M. AND NUSSLEIN-VOLHARD, C. (1989). The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. *Nature* **338**, 478–483.

STEPHENSON, E. C., CHAO, Y. AND FACKENTHAL, F. D. (1988). Molecular analysis of the *swallow* gene of *Drosophila melanogaster*. *Genes Dev.* **2**, 1655–1665.

STRECKER, T. R. AND LENGYEL, J. A. (1988). Anterior–posterior pattern formation: an evolutionary perspective on genes specifying terminal domains. *Bioessays* **9**, 3–7.

STRUHL, G. (1981). A blastoderm fate map of compartments and segments of the *Drosophila* head. *Devl Biol.* **84**, 386–396.

STRUHL, G. AND AKAM, M. (1985). Altered distributions of *Ultrabithorax* transcripts in *extra sex combs* mutant embryos of *Drosophila*. *EMBO J.* **4**, 3259–3264.

TAUTZ, D., LEHMANN, R., SCHNURCH, H., SCHUH, R., SEIFERT, E., KIENLIN, A., JONES, K. AND JACKLE, H. (1987). Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes. *Nature* **324**, 383–389.

TECHNAU, H. M. AND CAMPOS-ORTEGA, J. A. (1985). Fate-mapping in wild-type *Drosophila melanogaster*. II. Injections of horseradish peroxidase in cells of the early gastrula stage. *Roux Arch. devl Biol.* **194**, 196–212.

TREISMAN, J., GONCZY, P., VASHISHTHA, M. AND DESPLAN, C. (1989). A single amino acid can determine the DNA-binding specificity of homeodomain proteins. *Cell* **59**, 553–562.

TURNER, F. R. AND MAHOWALD, A. P. (1977). Scanning electron microscopy of *Drosophila melanogaster* embryogenesis; II. Gastrulation and segmentation. *Devl Biol* **57**, 403–416.

TURNER, F. R. AND MAHOWALD, A. P. (1979). Scanning electron microscopy of *Drosophila melanogaster* embryogenesis;

III. Formation of the Head and Caudal Segments. *Devl Biol* **68**, 96–109.

UNDERWOOD, E. M., TURNER, F. R. AND MAHOWALD, A. P. (1980). Analysis of cell movements and fate mapping during early embryogenesis in *Drosophila melanogaster*. *Devl Biol* **74**, 286–301.

VAN DEN HEUVEL, M., NUSSE, R., JOHNSTON, P. AND LAWRENCE, P. (1989). Distribution of the *wingless* gene product in *Drosophila* embryos: a protein involved in cell–cell communication. *Cell* **59**, 739–749.

WAKIMOTO, B. T. AND KAUFMAN, T. C. (1981). Analysis of larval segmentation in lethal genotypes associated with the *Antennapedia* gene complex in *Drosophila melanogaster*. *Devl Biol.* **81**, 51–64.

WAKIMOTO, B. T., TURNER, F. R. AND KAUFMAN, T. C. (1984). Defects in embryogenesis in mutants associated with the Antennapedia gene complex of *Drosophila melanogaster*. *Devl Biol.* **102**, 147–172.

WEDEEN, C., HARDING, K. AND LEVINE, M. (1986). Spatial regulation of Antennapedia and bithorax gene expression by the *Polycomb* locus in *Drosophila*. *Cell* **44**, 739–748.

WEIGEL, D. AND JACKLE, H. (1990). The *fork head* domain: a novel DNA binding motif of eukaryotic transcription factors? *Cell* **63**, 455–456.

WEIGEL, D., JURGENS, G., KLINGER, M. AND JACKLE, H. (1990). Two gap genes mediate maternal terminal pattern information in *Drosophila*. *Science* **248**, 495–498.

WEIGEL, D., JURGENS, G., KUTTNER, F., SEIFERT, E. AND JACKLE, H. (1989). The homeotic gene *fork head* encodes a nuclear protein and is expressed in the terminal regions of the *Drosophila* embryo. *Cell* **57**, 645–658.

WIESCHAUS, E., NUSSLEIN-VOLHARD, C. AND JURGENS, G. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. III. Zygotic loci on the X chromosome and the fourth chromosome. *Roux Arch. dev. Biol.* **193**, 296–307.

WILKINSON, D. G., BHATT, S., COOK, M., BONCINELLI, E. AND KRUMLAUF, R. (1989). Segmental expression of *Hox-2* homeobox-containing genes in the developing mouse hindbrain. *Nature* **341**, 405–409.

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