The spatial and temporal deployment of Dfd and Scr transcripts throughout development of Drosophila

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Summary

In Drosophila, the Deformed (Dfd) and Sex combs reduced (Scr) genes determine the developmental pathways followed by the most anterior metameric units. Using in situ hybridization, we have monitored the spatial distributions of transcripts from these two genes. Dfd mRNA accumulates in parasegments 0 and 1; Scr RNA accumulation shows a dynamic pattern spanning parasegments 2 and 3. The expression of Dfd and Scr seems to change from parasegmental to segmental during formation of the gnathal appendages. Both genes are transcribed during imaginal development: Dfd in a portion of the eye–antennal disc; Scr in the labial and prothoracic discs. In addition, we find Scr RNA in the adepithelial cells of all mesothoracic discs.

Key words: homeotic genes, in situ hybridization, parasegments, Drosophila, pattern formation.

Introduction

The body plan of all arthropods consists of repeat units called segments (Snodgrass, 1938); changes in the number and morphology of these units underlie the generation and diversity of species (Bateson, 1894). In Drosophila, differences between segments depend on the activity of homeotic selector genes (Lewis, 1951, 1978; Garcia-Bellido, 1975, 1977). During embryogenesis, those phenotypic differences are foreshadowed by the ordered and spatially restricted expression of selector genes (Akam, 1983; Levine, Hafen, Garber & Gehring, 1983; McGinnis et al. 1984; Harding, Weeden, McGinnis & Levine, 1985; Akam & Martinez-Arias, 1985; Kuroiwa, Kloter, Baumgartner & Gehring, 1985; Martinez-Arias, 1986).

The selector gene hypothesis (Garcia-Bellido, 1975) proposes that homeotic selector genes are activated around cellular blastoderm in precisely bounded domains along the anteroposterior axes of the animal and that these domains are propagated through cell divisions to the adult (Garcia-Bellido, 1975; Lawrence & Morata, 1976; Garcia-Bellido, Lawrence & Morata, 1979). Detailed studies on the expression of two of these genes, Ultrabithorax (Ubx) (Akam, 1983; Akam & Martinez-Arias, 1985) and Antennapedia (Antp) (Levine et al. 1983; Martinez-Arias, 1986; Carroll et al. 1986; Martinez-Arias, Scott & Akam, in preparation), while confirming the broad principle of selective expression of homeotic genes, have also revealed unexpected complexities. The main features of these complexities are (a) cells of a given lineage may change their state of selector gene expression as development proceeds; (b) a given gene may show different spatial and temporal patterns of expression in different germ layers and (c) non-metameric boundaries of gene expression are observed within some metameres, particularly at later stages of development.

Here, we study the expression, during embryogenesis and imaginal development, of two further selector genes: Deformed (Dfd) and Sex combs reduced (Scr) both of which are located in the ANT-C (Kaufman, Lewis & Wakimoto, 1980; Lewis, Kaufman, Denell & Tallerico, 1980a; Lewis, Wakimoto, Denell & Tallerico, 1980b; Wakimoto & Kaufman,
1981; Hazelrigg & Kaufman, 1983). The Dfd locus is characterized by dominant mutant phenotypes affecting the eyes and maxillary palps (Sinclair, 1977; Lewis et al. 1980a,b) and recessive lethal phenotypes in which the mandibular and maxillary segments (Turner & Kaufman, in Hazelrigg & Kaufman, 1983; W. McGinnis, personal communication) as well as the antennal sense organ (Kaufman, 1983) are defective. The Scr locus is located between Dfd and Antp (Lewis et al. 1980a,b; Hazelrigg & Kaufman, 1983; Scott et al. 1983) and is required, both in the larva and the adult, for the development of the labial and first thoracic segments (Lewis et al. 1980a,b; Wakimoto & Kaufman, 1981; Struhl, 1981a,b, 1982, 1983; Kaufman & Abbot, 1984; Sato, Haynes & Denell, 1985). In situ hybridization studies with cloned sequences from each locus, suggest patterns of expression in agreement with the genetic data (Harding et al. 1983; Kuroiwa et al. 1985).

We have used specific probes from each locus to monitor the expression of these genes throughout development. Our results indicate that the spatial, temporal and germ layer controls acting on these genes are analogous to those regulating Ubx and Antp. We also report large differences in the deployment of the transcripts from one of these genes, Scr, between the blastoderm and the imago.

Materials and methods

In situ hybridization

Drosophila embryos and larvae were collected, fixed, sectioned and hybridized with single-stranded DNA or RNA probes as described previously (Akam, 1983; Akam & Martinez-Arias, 1985; Ingham, Howard & Ish-Horowicz, 1985a; Martinez-Arias, 1986). For Dfd, the probe used was a 5 kb EcoRI genomic fragment from -1 to +4 (all coordinates are according to Scott et al. 1983); this probe contains the homeobox (McGinnis et al. 1984). For Scr, two probes were used: the first one is a cDNA clone (X-111) carrying 2 kb of sequences from the 3' exon centred at -30 kb (F. Storfer & M. Scott, unpublished data), the second, an EcoRI genomic fragment from a 5' exon (+42 to +47). The Dfd and 5' Scr probes were used as RNA probes; the 3' Scr probe was used as a single-stranded DNA probe. All Scr probes detect similar patterns except at blastoderm (see text). The pair rule pattern of Scr described in Fig. 1 was observed with only certain batches of 5' probe; other batches prepared from the same template labelled only the primordium of PS2. This suggests that the more posterior stripes are detected only with sequences close to the 5' end of the gene; such sequences, distant from the priming site, may not be efficiently copied in all probe syntheses.

Results

The Dfd and Scr loci: probes used

The Dfd and Scr loci lie in the proximal portion of the ANT-C (Lewis et al. 1980a,b; Hazelrigg & Kaufman, 1983). The Dfd gene appears to encode a single 2-8 kb RNA throughout development (McGinnis et al. 1984; Regulski et al. 1985). The Scr gene encodes a single 4 kb RNA from exons that lie 20 kb apart (Kuroiwa et al. 1985; Storfer & Scott, unpublished data). To detect the spatial distribution of these transcripts we have used three probes. For Dfd we have used a 5 kb probe, from coordinates -1 to +4 (Scott et al. 1983), which spans most of the Dfd locus (W. McGinnis, personal communication). For Scr, we have used two probes, one from the 5' exon (+42—+47) and another from the 3' exon (+29—+31). (For details, see Materials and methods.)

Spatial expression during blastoderm formation

In the cellular blastoderm, transcripts from the Dfd gene accumulate in a band six to seven cells wide, which spans the whole circumference of the egg and is located between 65—75 % EL (McGinnis et al. 1984; Fig. 1A). Scr transcripts accumulate preferentially in a band adjacent and posterior to the cells which express Dfd (Fig. 1B,C). This band, three to four cells wide, spans dorsal and lateral regions of the blastoderm, but does not extend into the midventral region (presumptive mesoderm). More posteriorly, 5' probes for Scr reveal lower levels of Scr RNA accumulating in six additional bands spaced at double segment intervals (Fig. 1B,C); the 3' Scr probe detects transcripts only in the band adjacent to the cells expressing Dfd and not in these more posterior stripes.

To define more precisely the primordia expressing these genes, we have used the domains of expression of the engrailed, en gene (Fjose, McGinnis & Gehring, 1985; Kornberg, Siden, O'Farrell & Simon, 1985) to locate P compartments in the late cellular blastoderm (Weir & Kornberg, 1985). At this stage, the principal expression of Scr overlies and extends posterior to the second engrailed stripe (Fig. 1D,E); hence it lies in parasegment 2 (Martinez-Arias & Lawrence, 1985). The posterior Scr stripes are likely to correspond to the remaining even-numbered parasegments. The posterior margin of Dfd expression lies anterior to this second engraved stripe and therefore includes PS1 (data not shown). We notice, however, that the Dfd stripe is broader than the anlage for other parasegments.
The formation of germ layers during gastrulation and the ensuing cell proliferation (Poulson, 1950; Campos-Ortega & Hartenstein, 1985) are accompanied by changing patterns of Scr and Dfd expression. We describe these patterns for two stages, before and after formation of the gnathal buds (early stage 10 and late stage 11, respectively, in the staging of Campos-Ortega & Hartenstein, 1985) (Figs 2, 3). In order to determine the identity of the labelled zones, we have used as reference points, both morphological landmarks (Martinez-Arias & Lawrence, 1985; Akam & Martinez-Arias, 1985) and the location of cells that express the engrailed gene (Fjose et al. 1985; Kornberg et al. 1985; Ingham, Martinez-Arias, Lawrence & Howard, 1985b). Before the gnathal buds are formed (stages 9, 10), Dfd is expressed in two regions. In PS1, which at this stage spans the cephalic furrow, both ectodermal and mesodermal cells accumulate Dfd RNA. An adjacent region, anterior to PS1, accumulates lower levels of Dfd RNA; this unit may be termed 'parasegment O' since it extends forwards to include the first postoral cells that express engrailed (data not shown) and thus lies in the premandibular (Ingham et al. 1985) or hypopharyngeal (Jürgens, Lehmann, Schardin & Nüsslein-Volhard, 1986) region (Figs 2A,C, 3A).

Scr transcripts accumulate in ectodermal cells of PS2, but not in the overlying mesoderm. However, Scr transcripts do accumulate in the mesoderm overlying PS3 (Figs 2B,C, 3B). This out-of-register pattern is observed from the time that Scr expression is first visible in the mesoderm shortly after gastrulation and is detected with both 5' and 3' probes. Since the patterns of expression of ftz (Hafen, Kuroiwa & Gehring, 1984; Carroll & Scott, 1985), en (Weir & Kornberg, 1985 and unpublished observations) and Ubx (Akam & Martinez-Arias, 1985), reveal no sign of relative movement between ectoderm and mesoderm at this stage (Martinez-Arias & Lawrence, 1985), we believe that the lack of registration reflects a real difference in the metameric regulation of Scr in the ectoderm and the mesoderm.

By late stage 11, the ectoderm has given rise to the epidermis and the neural precursors, and cellular proliferation has taken place in all germ layers.

**Fig. 1.** Expression of the Deformed (Dfd) and Sex combs reduced (Scr) genes in the cellular blastoderm. (A) Dfd transcripts accumulate in a single band 6-7 cell diameters in width. (B,C) At the same stage, Scr transcripts accumulate at high levels in a band 3-4 cell diameters wide just posterior to the site of Dfd expression. In addition, there is lower level accumulation extending posteriorly in a periodic pattern resembling that of the pair-rule segmentation gene ftz (Hafen et al. 1984). This is emphasized by the width of the most posterior band which, like that of ftz, is wider than the rest. (C,D) Details of late cellular blastoderms hybridized with both engrailed (en) and Scr 5 probes. The Scr RNA accumulates preferentially over the nuclei and the en RNA is distributed equally between nuclei and cytoplasm. The second en stripe (large arrowhead) accumulates over the most anterior nucleus expressing Scr and this suggests that the Scr domain is PS2 (small arrowheads).
Fig. 2. Parasegmental and segmental expression of Dfd and Scr in stages 10 and 11. (A) Dark-field image of a sagittal section through a stage-10 embryo hybridized with both en and Dfd. The numbers refer to the en stripes, which lie at the anterior margin of every parasegment (Ingham et al. 1985b). The Dfd RNA accumulates at low levels in PS0 and at high levels in PS1 (see also C) within the domains bounded by the arrowheads. (B) As A but hybridized with the Scr 5' probe. Notice Scr RNA accumulation in PS2. (C) Detail of a section through late-stage-10 embryo hybridized with the Dfd probe showing Dfd RNA accumulation in PS0 and PS1. Notice the higher levels of RNA in PS1 and the sharp limit of expression at the parasegmental boundary (arrowheads). Dfd RNA is detected in the epidermis (ep) and neuroectoderm (nec) of both parasegments, but only in the mesoderm (mes) of PS1. (D) Detail of a similar section as in C hybridized with the Scr 3' probe. Notice the sharp boundaries delimiting PS2 and the mesodermal label in PS3. Abbreviations as in C. (E) Detail of a lateral section through a stage-11 embryo hybridized with the Dfd gene. Gnathal buds are visible and the maxilla (mx) shows high levels of Dfd RNA. The mandibula also accumulates Dfd RNA but is not visible in this section (see Fig. 3E). (F) As E hybridized with the Scr 5' probe. The labial appendage (lb) and part of the first thoracic segment (T1) accumulate Scr RNA; by contrast none of the maxillary (mx) compartments do.

(Poulson, 1950; Campos-Ortega & Hartenstein, 1985). Parasegmental grooves are still clearly visible in the midventral epidermis, but in parasegments 0–3, the mandibular, maxillary and labial buds have formed on the ventrolateral regions. These gnathal buds are the first clearly segmental (rather than parasegmental) structures and engrailed is expressed as expected, along the posterior margin of both the maxillary and labial buds (DiNardo, Kuner, Theis & O'Farrell, 1985).

Midventrally, a sharp boundary coinciding with the parasegmental groove separates the domains of Dfd
Spatial expression of the \textit{Dfd} and \textit{Scr} genes in \textit{Drosophila}

Fig. 3. Expression of the \textit{Dfd} and \textit{Scr} genes in sagittal sections of the extended and shortened germ bands. (A) Sagittal section of early stage-10 hybridized with the \textit{Dfd} probe. Notice the existence of two distinct regions of RNA accumulation: the more posterior one corresponds to PS1, and contains RNA in both mesoderm and ectoderm. The more anterior one corresponds to the premandibular or hypopharyngeal region. These assignments are corroborated by double-label experiments with \textit{en} (see Fig. 2). (B) Sagittal section of late-stage-9 embryo hybridized with the \textit{Scr} 5' probe. PS2 accumulates \textit{Scr} RNA in the ectoderm. The label in the mesoderm lies in PS3. Notice that behind a region without detectable levels of transcripts (PS4 and PS5), \textit{Scr} RNA accumulates from PS6 to PS12, although at much lower levels than in PS2. (C) Midsagittal section through a late-stage-11 embryo hybridized with the \textit{Dfd} probe. Notice, in the nervous system (nec), the existence of two metameres which accumulate \textit{Dfd} RNA, PS0 and PS1; in the epidermis (ep), at this stage, only PS1 does. (D) Sagittal section of an embryo similar to that of C hybridized with the 5' \textit{Scr} probe. In the epidermis (ep), PS2 and PS3 accumulate \textit{Scr} RNA. In the developing nervous system (nec), only PS2 transcribes \textit{Scr}. The label in the mesoderm (mes) corresponds to cells derived from PS3 and represent both, somatic and visceral mesoderm. (E) Lateral section of a stage-11 embryo showing \textit{Dfd} RNA accumulation in the mandibular (mb) and maxillary (mx) lobes. (F) Lateral section similar to E hybridized with the 5' \textit{Scr} probe. The labial (lb) lobe and part of the prothoracic segment (T1) accumulate \textit{Scr} RNA. (G) Horizontal section of a stage-13 embryo hybridized with 5' \textit{Scr} probe. Transcripts are detectable in the myoblasts of segment T1 (arrow) and also in the visceral mesoderm around the anterior midgut (arrowheads). (H) Sagittal section of embryo similar to that in G hybridized with \textit{Scr} probe. Note the absence of transcripts in the salivary gland (sg) and presence of transcripts in the visceral mesoderm around the anterior and posterior midgut (arrowheads).
and Scr expression in both the epidermis and the neural cells. Laterally, however, the boundary between Scr and Dfd expression lies between gnathal buds. The entire mandibular and maxillary buds express Dfd (Figs 2E, 3E) and the entire labial bud expresses Scr (Figs 2F, 3F). Thus, by the criterion of engrailed expression, cells in the P compartment of parasegment 2 are expressing Dfd. At this stage, Scr expression in the epidermis also extends more posteriorly than it did at stage 10, into parasegment 3. In any single embryo, there is a gradient of Scr transcripts across parasegment 3, giving the appearance that the Scr gene turns on first (or most strongly) in those cells adjacent to parasegment 2. At the same time, transcripts of Dfd become much less abundant in the epidermal cells anterior to PS1. Thus, Dfd expression in the midventral epidermis clearly defines a single parasegmental unit, PS1.

There are no equivalent changes of gene expression in the nervous system, the pattern at stage 11 being essentially equivalent to that of the ectoderm during stage 10 (Fig. 3C, D). Neural derivatives of PS1 express Dfd whilst those of PS2 (but not PS3) express Scr. Anterior to PS1, there are neural cells that express Dfd; we consider these to be the neural derivatives of parasegment 0.

Dfd is not expressed posterior to PS1 (excepting the maxilla). However, Scr is expressed posterior to PS3: PS4 and PS5 do not accumulate Scr transcripts, but PS6–12 accumulate some Scr RNA (Fig. 3B, D, H). The signal is considerably lower than the signal in PS2 and PS3, is more prominent with the 5' probe than with the 3' probe and shows a gradient spanning each parasegment.

In the mesoderm of stage 11, only one metamere can be identified as expressing Scr; this is PS3 (Fig. 3D). Scr transcripts are detected in both the somatic and visceral components of this unit. This is seen more clearly in the shortened embryo (stages 12, 13) when all myoblasts of T1 (presumably derived from PS3) and a small portion of the mesoderm surrounding the anterior midgut express Scr (Fig. 3G). At this stage, we cannot unambiguously identify mesoderm anterior to PS3.

So far as we can tell, the patterns of Dfd and Scr expression established by stage 11 persist largely unchanged until hatching. However, we note that the salivary glands, although derived from the labial segment, do not accumulate Scr RNA at any time after they have invaginated (Fig. 3H) (we have not been able to identify the salivary placode in stage-11 embryos). Late in organogenesis some cells of the visceral mesoderm in the posterior midgut accumulate Scr transcripts (Fig. 3H).

**Larval and imaginal structures**

We have also observed the patterns of expression of these genes in the tissues of third instar larvae. The

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**Fig. 4. Expression of Dfd in the eye antennal disc.** (A, B) Details of adjacent sagittal sections through a third instar larva, hybridized with the Dfd probe. Part of the eye antennal disc is labelled, the part corresponds to the primordium of the maxillary palp (large arrow, A), and the peripodial membrane (small arrows, A, B). (C) Drawings of the eye–antennal disc in sagittal (upper) and horizontal views; (Gehring, 1966; Bryant, 1978) anterior to the left. The line in the latter indicates the approximate plane of sectioning in A, B. The different primordia are indicated: maxillary palp (mp); peripodial membrane (pm); antenna (ant).
**Fig. 5.** Expression of *Scr* in the imaginal discs of the third instar larva. These results were obtained with the 3' exon probe but essentially the same patterns were observed with the 5' probe. (A–C) Low-power micrograph of the anterior end of a third instar larva. A, bright field; B, section map; C, dark field. Labelled structures include the labial (lb), first and second leg (L1, L2) and humeral (h) discs, and part of the ventral ganglion (vg), probably parasegment 2. The eye-antennal (ea) and wing discs (w), the salivary gland duct (sd) and the anterior spiracle (as) are unlabelled. (D–F) Detail of a nearly horizontal section of a similar larva, presented as above showing the pairs of first (L1) and second (L2) leg discs. All cells from the first leg discs accumulate *Scr* RNA, as do some cells (arrowhead) of the second leg discs. From the position of these cells in the basal folds of the cuboidal epithelium, we infer that they are adephithelial cells. Similarly labelled cells are seen in other sections of the wing disc.

CNS shows RNA accumulation comparable, in position, to that observed in the embryonic CNS (Fig. 5). In addition, some imaginal discs are labelled.

*Dfd* is expressed in a sharply bounded region of the eye-antennal disc and in no other disc (Fig. 4). Most of these cells correspond to the primordium of the maxillary palp according to the fate map of the disc (Gehring, 1966; Bryant, 1978); part of the peripodial membrane also accumulates *Dfd* RNA.

*Scr* RNA accumulates in all or most cells of the labial, first leg and humeral disc (Fig. 5); differences in labelling intensity are observed between different parts of the discs. In addition, some cells in the wing and second leg disc transcribe *Scr*; these cells are not epithelial and their position and structure suggests that they are adephithelial cells, i.e. precursors of adult muscle (Poodry & Schneiderman, 1970; Ursprung, Conscience-Egli, Fox & Walliman, 1972) (Fig. 5).
Discussion

The areas expressing Dfd and Scr in the embryo and the imago of Drosophila melanogaster include the regions affected by mutations at these loci. Whilst patterns of transcription cannot alone reveal the function of these genes, they do enable us to follow the ways in which a comparatively simple blastoderm fate map is translated into the complex organization of a first instar larva or an adult fly. It is in this context that we discuss the expression of Scr and Dfd.

Cellular blastoderm

In the cellular blastoderm, transcripts from both Dfd and Scr accumulate in well-defined regions. Dfd transcripts are detected in and anterior to PS1. Scr transcripts are found predominantly in PS2. We suspect that these two transcription zones abut, but the techniques we have used do not allow us to say that this is precise at the level of single cells. In addition, the Scr 5′ probe detects low levels of transcripts more posteriorly, in stripes which correspond approximately with even-numbered parasegments. This pattern parallels that of the segmentation gene fushi tarazu in its periodicity, location and relative width of the bands. Similar ‘pair rule modulations’ have been observed for another homoeotic gene, Ubx (Akam & Martinez-Arias, 1985; Akam, 1985) and suggest a very close relationship between segmentation and homoeotic genes (Akam, 1985; Ingham, Ish-Horowicz & Howard, 1986; Ingham & Martinez-Arias, 1986; Martinez-Arias, 1987). In the case of Scr, this relationship may be mediated by the upstream sequences that Scr shares with the ftz promoter (see Scott et al. 1983; Kuroiwa et al. 1985 for cis relationship between ftz and Scr). Dfd, by contrast, shows no such modulation and is expressed throughout the maturation of the blastoderm only in a single, localized band.

Unfolding of the blastoderm fate map

The selector gene hypothesis (Garcia-Bellido, 1975) proposes that homoeotic selector genes become active at around cellular blastoderm, endowing groups of cells (polyclones) with an anatomical identity; afterwards, lineal descent will transfer these ‘genetic states’ to the differentiated cells in the mature animal. The early localized expression of selector genes strongly endorses this model. However, differences in the expression of Ubx between the embryo and the imago (Akam, 1983; Akam & Martinez-Arias, 1985; White & Wilcox, 1985a, b) challenge this view and indicate a progressive refinement of pattern based upon continuing changes in the expression of homoeotic genes. The patterns of Dfd and, particularly, Scr reinforce this dynamic view and provide examples of significant changes in deployment of selector genes (summarized in Fig. 6). Thus at blastoderm, Scr is preferentially expressed in PS2, but in the developing adult it is expressed in the labial disc (PS2/3) in all T1 discs (PS3/4) and in the adepi-thelial cells of T2 (PS4). This pattern involves both segmental and parasegmental deployment of Scr, the extension of its expression along the anteroposterior axis as development proceeds and an elaboration of tissue specific differences.

Parasegmental and segmental expression

The boundary between Dfd and Scr expression in the blastoderm and early germ band is probably parasegmental. It is clearly parasegmental in the midventral epidermis and nervous system of the stage-11 embryo, lying between PS1 (Dfd) and PS2 (Scr) and it remains unchanged in the nervous system throughout embryogenesis. In the gnathal appendages, however, the corresponding boundary lies between segments. This is consistent with the effects of Dfd and Scr mutations on the appendages: Dfd mutations affect mandibular and maxillary structures, Scr mutations affect labial structures.

We consider that the segmental expression in the gnathal appendages reflects a respecification of cells.
in the P compartment of parasegment 2, such that they switch from Scr to Deformed expression as the buds emerge. Unambiguous confirmation of this will require effective double labelling techniques for Scr and Dfd gene products. The evidence that cells in the epidermis of parasegment 3 are respecified is more compelling. In the stage-10 embryo, the entire epidermis of parasegment 3 expresses Antp from the P2 promoter (Martinez-Arias et al. in preparation). During stages 10 and 11, Scr expression extends into this region and, thereafter, Antennapedia expression is much reduced (Martinez-Arias et al. in preparation). Indeed, genetic data suggest that, during adult development, Scr expression in the epidermis is necessary not only throughout parasegment 3, but also in T1p, that is, in part of parasegment 4.

Analogous redeployments occur in the expression of another selector gene, Ubx. In the early embryo, Ubx is expressed (Akam & Martinez-Arias, 1985) and required (Morata & Kerridge, 1981) in cells of parasegment 5 that will give rise to the epidermis of T2p. However, after 10 h, the Ubx gene is no longer required in the progeny of these cells that will contribute to the adult (Morata & Kerridge, 1981). By the time these cells form imaginal discs in the mature larva, Ubx is either not expressed or is expressed at very low levels in T2p. By contrast, Ubx is expressed at high levels in both compartments of T3 discs (Akam, 1983; White & Wilcox, 1985a,b) and is absolutely required for their normal development. These transitions in Scr and Ubx expression suggest that the development of certain segments is controlled by a sequence of different homeotic genes and not only by a static 'combinatorial code' (Garcia-Bellido, 1975; Struhl, 1982).

Changing patterns of homeotic gene expression challenge the significance of segments or parasegments as absolute units of metameric identity. The significance of the segmental unit is defined by the functional anatomy of the arthropod (Snodgrass, 1938). The parasegment, however, is significant primarily as a unit in the internal representation of the fly (Martinez-Arias & Lawrence, 1985), even though its origin may relate to the functional organization or developmental strategy of some ancient metameric ancestor (Martinez-Arias, 1987), vestiges of which may still be retained in the development of the mesoderm.

The embryonic mesoderm and ectoderm

The patterns of expression of Ubx (Akam & Martinez-Arias, 1985) and Antp (Martinez-Arias, 1986) imply that selector genes are under different controls in different germ layers. The expression of Dfd and Scr reinforces this observation and, in addition, indicates that different controls might also operate in the epidermis and the nervous system.

Shortly after gastrulation, Scr is expressed in the ectoderm of PS2 and the mesoderm of PS3. In the young embryo, the latter are the only mesodermal cells expressing Scr and after germ band shortening they will give rise to the muscles of T1 and to a region of the visceral mesoderm. Dfd is expressed in both the ectoderm and the mesoderm of PS1, thus leaving the mesoderm of PS2, without expression of a known selector gene. Both Scr and Dfd are also differentially expressed within the two major derivatives of the ectoderm, the epidermis and the nervous system. This becomes apparent after the major phase of neuroblast segregation (Hartenstein & Campos-Ortega, 1984) when the epidermis of PS3 expresses Scr, but not the developing nervous system. A related switch occurs in PS0, where the epidermis stops expressing Dfd whereas the neural tissue continues to transcribe this gene.

Imaginal discs

In general, the pattern of Scr expression in imaginal discs conforms with the structures affected by mutations (Lewis et al. 1980a,b; Kaufman & Abbot, 1984) or clones (Struhl, 1981a,b) of Scr mutant alleles. One exception is the expression of Scr in the aepithelial cells of the T2 discs. Little is known about the effect of homeotic mutations on the mesoderm and, therefore, the function of these transcripts remains enigmatic.

The expression of Dfd in a small region of the eye antennal disc is interesting, for it points to a boundary of expression of a selector gene where no lineage boundary exists. In the eye antennal disc, the only lineage restriction refers to anterior and posterior compartments (Morata & Lawrence, 1979). The variety of morphological structures derived from this disc suggests that it results from a fusion of several metameric primordia (Morata & Lawrence, 1979; Jürgens et al. 1986). Struhl (1981) argued against this possibility, on the grounds that all structures of the eye–antennal disc cluster closely on a gynandromorph fate map of the blastoderm. His data suggest that the maxillary palp and the adult eye–antenna derive from a single blastoderm primordium and that this primordium lies very anterior, adjacent to the primordium of the clypeolabrum. If so, the expression of Dfd in the embryo and the developing adult must occur in linearly unrelated cells, nonetheless in each case it specifies structures that morphologists identify as maxillary. It is now clear, however, that the larval 'antennal' and 'maxillary' sense organs are very closely juxtaposed on the blastoderm fate map, at about 70% egg length (Jürgens et al. 1986) in the region where Dfd is expressed. If these are any
guide to the derivation of the homonimous adult structures, the cells of the eye–antennal disc that express Dfd may derive from part of the embryonic primordia of parasegments 0/1.

We thank P. Lawrence for comments on the manuscript, and M. Bate and I. Dawson for discussions throughout the course of the work. This work was supported by a long-term EMBO fellowship to AMA and by the Medical Research Council of Great Britain.

References


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Note added in proof

R. Chadwick and W. McGinnis (1987, EMBO J. 6, 779–789) report patterns of Dfd expression which are compatible with our observations.

(Accepted 7 April 1987)