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Role of the esc^+ gene product in ensuring the selective expression of segment-specific homeotic genes in *Drosophila*

By GARY STRUHL[1]

From the Medical Research Council Laboratory of Molecular Biology

SUMMARY

The product of the *extra sex combs*$^+$ (*esc*$^+$) gene is required during embryogenesis for the correct determination of segments in *Drosophila*. If this product is absent, most segments develop like the normal eighth abdominal segment. Here, I extend previous results (Struhl, 1981*a*) showing that this phenotype results in large part from indiscriminate expression of the *bithorax*-complex genes which are normally active only in particular segments of the thorax and abdomen. In addition, I test whether the *esc*$^+$ gene product is required for the correct expression of other homeotic genes. First, I have examined two genes of the *Antennapedia*-complex (*Sex combs reduced*$^+$ and *Antennapedia*$^+$): I find that both genes are normally required in only some of the body segments, but that in the absence of the *esc*$^+$ gene product, both appear to function adventitiously in other segments. Second, comparing *esc*$^+$ and *esc*$^-$ embryos lacking both these genes as well as the *bithorax*-complex, I find that additional homeotic genes (possibly those normally involved in specifying head segments) appear to be expressed indiscriminately when the *esc*$^+$ gene product is absent. Finally, I present evidence that the products of the *esc*$^+$ gene and the *Polycomb*$^+$ gene (a second gene required for the correct regulation of the *bithorax*-complex) act independently. On the basis of these results, I propose a tentative outline of the roles and realms of action of all of these genes.

INTRODUCTION

Insect segments come into being around the cellular blastoderm stage as a series of adjacent cell groups of similar size (reviewed in Lawrence, 1981). During subsequent development, these primordia diversify dramatically, giving rise to the unique segments of the larva and the adult. In *Drosophila*, this process requires a small number of 'homeotic' genes which act in combination to specify the particular developmental pathway followed by each segment (Garcia-Bellido, 1977; Lewis, 1978; Struhl, 1982).

Homeotic genes can be classified according to where, and when, their products are required. For example, several genes appear to function selectively in particular segments, and are essential during both embryogenesis and subsequent development [called 'selector' genes by Garcia-Bellido (1975): *viz*, genes of the *bithorax*-complex (Lewis, 1963, 1978; Morata & Garcia-Bellido, 1976), the

[1] *Author's present address:* Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Ave., Cambridge, Mass., 02138, U.S.A.

Antennapedia$^+$ (*Antp*$^+$) gene (Denell, Hummels, Wakimoto & Kaufman, 1981; Wakimoto & Kaufman, 1981; Struhl, 1981*b*, 1982), and the *Sex combs reduced*$^+$ (*Scr*$^+$) gene (Lewis, Kaufman, Denell & Tallerico, 1980*a*; Lewis, Wakimoto, Denell & Kaufman, 1980*b*; Wakimoto & Kaufman, 1981; Struhl, 1982)]. A second class of genes is required in all segments during both embryonic and larval development, and appears necessary to maintain the correct expression of selector genes [*viz*, the *Polycomb*$^+$ (*Pc*$^+$) gene (Lewis, 1978; Struhl, 1981*a*; Duncan & Lewis, 1982), the *Polycomblike*$^+$ (*Pcl*$^+$) gene (Duncan, 1982), and possibly the *Regulator of bithorax*$^+$ (*Rg-bx*$^+$) gene (Ingham & Whittle, 1980; Ingham, 1981; Capdevila & Garcia-Bellido, 1981; Duncan & Lewis, 1982)]. Finally, the product of at least one gene, *extra sex combs*$^+$ (*esc*$^+$) has a discrete early role in the process of initiating the correct combinations of active and inactive selector genes in each segment (Struhl, 1981*a*; Struhl & Brower, 1982). These three classes suggest a hierarchy of genetic control in which the correct combinations of active and inactive selector genes are first established in each segmental primordium, and then maintained during subsequent development.

As a prerequisite to studying the roles of homeotic genes at the molecular level, it is helpful to understand as much as possible about the interactions between members of these different classes. Here, I examine the role of the product of the *esc*$^+$ gene in the process of initially activating, or inactivating, other homeotic genes. In particular, I extend previous results (Struhl, 1981*a*) showing that the *esc*$^+$ gene product is required for the correct expression of the genes of the *bithorax*-complex, and provide evidence that the *esc*$^+$ gene product is also required for the correct expression of the *Scr*$^+$ gene, the *Antp*$^+$ gene, and possibly other segmental selector genes. Finally, I outline how the products of all these genes might act in concert to specify segmental determination throughout the body.

METHODS

a) Preparation and analysis of embryos

Eggs from appropriate parents were collected on agar plates over 12 h periods, and allowed to mature for a subsequent 24 h. They were then rinsed, dechorionated with dilute hypochlorite, fixed by incubation in glycerol-acetic acid (1: 4) for 30 min at 60 °C, and then mounted in Hoyer's mixture according to the procedure of Van der Meer (1977). After incubation of mounted preparations overnight at 60 °C, most of the internal tissues are dissolved allowing the cuticular features to be studied under bright field, phase, Nomarski, or dark field optics. When necessary, embryos were dissected out from the vitelline membrane prior to fixing in glycerol–acetic acid.

b) Cuticular morphology and segmentation of the first instar larva

The cuticle of the first instar larva is secreted approximately 12–14 h after

fertilization (about halfway through embryogenesis), and has been described in fine detail by Lohs-Schardin, Cremer & Nusslein-Volhard (1979) and Turner & Mahowald (1979). The features which are of value for assessing segmental determination are specialized sense organs unique to particular segments or sets of segments (e.g., the Keilin's organs, and the ventral and dorsal pits found only on the thoracic segments), and the characteristic patterns of ventral hairs on the thoracic and abdominal segments. The assignment of cuticular structures to the thoracic and first seven abdominal segments (shown in Fig. 1) is widely accepted (e.g., Herth & Sander, 1973; Lohs-Schardin *et al.*, 1979; Turner & Mahowald, 1979). The eighth abdominal segment is thought to bear the rectangular band of ventral hairs posterior to the seventh abdominal segment, but there is no clear consensus on whether the 'terminalia' (the posterior spiracles, anal pads, tuft, and associated sense organs) also belong to this segment or to other more posterior segments (e.g., Lohs-Schardin *et al.* 1979; Turner & Mahowald, 1979). Because homeotic mutations transforming the eighth abdominal segment alter not only the ventral and dorsal hair patterns but also the posterior spiracles and some of the sense organs (Lewis, 1978; Struhl, unpublished), whereas mutations which appear to affect the reverse transformation of more anterior segments to eighth abdominal segments add these structures back (Struhl, 1981*a*, Duncan & Lewis, 1982, and here (Figs 1, 2, 5 and 6)), I will assume that these structures normally derive from the eighth abdominal segment (the anal pads, tuft, and remaining sense organs are unaffected by either kind of mutation suggesting that they arise from other, more posterior segments, or are non-segmental). The segmental status of most of the head structures is uncertain. Fig. 1 shows the head structures, as well as tentative assignments of their segmental status according to Schoeller (1964), Lohs-Schardin *et al.* (1979) and Turner & Mahowald (1979).

c) Genotypes employed

In order to study the normal requirement for a particular gene, it is helpful to use mutations of that gene which remove most if not all of its wild-type activity. Aside from deficiencies which are visible cytologically, there are at present no direct genetic means of establishing that particular mutations are indeed null alleles. However, the mutations used here were isolated under conditions which should allow null alleles to be obtained, and at frequencies consistent with them being simple knock-out mutations in structural genes. Moreover, several mutations of each gene were obtained, and in each case, a subset of these mutations caused similar homeotic phenotypes at both 17 °C and 29 °C, whether in *trans* to a known deficiency of the locus or, when tested, in *trans* to each other. Such behaviour is expected of mutations which greatly reduce or eliminate a gene's wild-type function. Consequently, I have treated these mutations as null mutations.

(i) *esc*$^-$ mutations: The isolation and initial characterization of new mutations of the *esc* locus have been described previously (Struhl, 1981*a*). In this study, the

mutations esc^2, esc^5, esc^6, and esc^{10} have been used interchangeably and are referred to as esc^-.

(ii) Pc^- mutations: The dominant phenotype associated with apparent point mutations (Pc^1, Pc^2, and Pc^3), and deficiencies of the *Pc* locus can be accounted for simply as a consequence of carrying only one, as opposed to the normal two, copies of the wild-type gene (Duncan & Lewis, 1982). During screens to generate new, EMS-induced mutations of the *esc* locus, I also obtained eight new alleles of the *Pc* locus (named $Pc^{C1,C2,\ldots,C8}$) which show a dominant *Pc* phenotype in adults. These mutations arose with a frequency of approximately 1/2000 mutagenized chromosomes. Moreover, comparison of the embryonic phenotypes of each of these mutations (as well as of the Pc^1, Pc^2, and Pc^3 mutations) in *trans* to a deficiency of the locus kindly provided by G. Jürgens did not reveal conspicuous differences whether at 17 °C or 29 °C. For these reasons, it seems reasonable to treat all of these mutations as null alleles. In order to study the phenotypes of esc^- embryos carrying four copies of the Pc^+ gene I used a tandem duplication of the *Pc* locus, *Dp(3;3)C126*, kindly provided by E. B. Lewis.

(iii) *bithorax*-complex deficiencies, duplications, and mutations: All of the *bithorax*-complex deficiencies and duplications used here were kindly provided by E. B. Lewis (see Lindsley & Grell (1968), Lewis (1978, 1980), and Morata & Garcia-Bellido(1976) for descriptions). During screens to obtain new EMS-induced mutations of the *esc* locus, four new alleles of the *Ultrabithorax* (*Ubx*) locus (named $Ubx^{C2,C3,C4,C5}$) were also obtained. Embryos carrying each of these mutations in trans to *Df(3R)P9* appeared indistinguishable in phenotype from each other and from $Df(3R)bxd^{100}/Df(3R)P9$ embryos. I therefore treat these mutations as null alleles of the *Ubx* locus.

(iv) $Antp^-$ and Scr^- mutations: The isolation and characterization of apparent null mutations of the *Antp* locus used here have been described previously (Struhl, 1981*b*). Two mutations, $Antp^{Ns+RC3}$ and $Antp^{Ns+RC4}$, were used interchangeably.

In a systematic screen for third chromosome mutations altering the segmental pattern of the embryo, C. Nüsslein-Volhard, G. Jürgens, and E. Wieschaus obtained four new mutations of the *Scr* locus ($Scr^{7F,8B,9G,13A}$) which they kindly provided for these experiments. All show the characteristic adult and embryonic phenotypes previously described for apparent null mutations of this locus (Lewis *et al.* 1980*a*,*b*; Wakimoto & Kaufman, 1981). Moreover, comparisons of the embryonic phenotypes of these mutations in *trans* to *Df(3R)4Scb* (a small deficiency eliminating both the $Antp^+$ and Scr^+ genes kindly provided by G. Jürgens) did not reveal conspicuous differences either at 17 °C or 29 °C suggesting that they eliminate most or all of the Scr^+ gene function. Two mutations, Scr^{9G} and Scr^{13A}, have been used interchangeably in this study. In order to study the phenotype of embryos lacking both the $Antp^+$ and Scr^+ genes, a chromosome carrying apparent null mutations at both loci was constructed as follows. Males carrying the $Antp^{Ns+RC3}$ mutation were mutagenized with EMS and outcrossed

to wild-type females under conditions in which progeny carrying the $Antp^{Ns+RC3}$-bearing chromosome could be identified. F1 $Antp^{Ns+RC3}/+$ males were screened for the reduction of sex comb teeth associated with haplo-insufficiency for the *Scr* locus (Kaufman, Lewis & Wakimoto, 1980; Lewis *et al.* 1980*a*,*b*). One such male was obtained, and on subsequent outcrosses, passed on a new mutation at the *Scr* locus (Scr^{C1}). This mutation appears similar in its genetic properties to the other *Scr* mutations described above, and has been treated as a null mutation of the Scr^{+} gene.

d) Preparing esc^{-} *embryos which bear mutations or altered copy numbers of other homeotic genes*

(i) Unless otherwise stated, all mutations and chromosomal aberrations used are described in Lindsley and Grell (1968).

(ii) General procedure for generating esc^{-} adults: The phenotype of esc^{-} progeny depends critically on the genotype of the mother. When the mother is hemi- or homozygous for an esc^{-} mutation, esc^{-} zygotes develop into first instar larvae in which most of the segments appear transformed into eighth abdominal segments (such transformed embryos die as pharate first instar larvae). However, if the mother is $esc^{-}/+$, homozygous esc^{-} zygotes develop into normal first instar larvae which, in turn, are able to develop into adults. It became apparent during the course of experiments described here that such 'rescued' esc^{-} adults were not always fully viable or fertile. This problem was overcome by generating homozygous esc^{-} adults from mothers which carried one mutant, and two wild-type copies of the *esc* locus. Either of two duplications of the esc^{+} gene were used for this purpose: *Dp(2;3)17* of Ising which is an insertion of the region 31B-34D into 76C on the 3L (see Struhl, 1981*a*), and *Dp(2;2)GYL* (isolated by G. Yanopoulis and described by M. Ashburner) which is an insertion of the region 33B1,2-35C1,3 into 50A,B on the 2R. Because this study is concerned exclusively with the cuticular phenotypes of esc^{-} progeny obtained from esc^{-} mothers, such embryos are here referred to simply as esc^{-} embryos.

(iii) Generating esc^{-} embryos which are further transformed by other homeotic mutations: esc^{-} parents heterozygous for particular homeotic mutations on the third chromosome were generated by standard genetic methods (e.g., esc^{-}; $Df(3R)Ubx^{109}/+$ parents were generated as follows. Virgin *CyO*, dp^{1v} esc^{2} *pr* cn^{2}/dp *b cn Dp(2;2)GYL*, esc^{+} *bw* females were crossed to esc^{5} $cn/+$; $Df(3R)Ubx^{109}/+$ males, and male and virgin female *CyO*, dp^{1v} esc^{2} *pr* cn^{2}/esc^{5} *cn*; $Df(3R)Ubx^{109}/+$ progeny selected on basis of their Cy, esc, cn, and Ubx phenotypes.). Embryos derived from such parents were prepared as described in *a*) and embryos of the correct genotype (*i.e.*, homozygous for the appropriate third chromosome mutations) were identified on the basis of their homeotic phenotype which differed significantly from that of standard esc^{-} embryos. Generating esc^{-} embryos carrying *Df(3R)P9* which is thought to delete the entire *bithorax*-complex (Lewis, 1978)) involves more complex genetic

crosses to obtain the appropriate *esc*$^-$ parents, because this deficiency must always be carried in *trans* to at least two copies of the *bithorax*-complex (*Df(3R)P9/+* flies of both sexes are sterile).

(iv) Generating *esc*$^-$ embryos of known third chromosome genotype without reference to homeotic phenotype. In several cases, it was necessary to examine the phenotype of *esc*$^-$ embryos carrying homeotic mutations in the third chromosome without reference to the resulting homeotic phenotype (e.g., when comparing the phenotype of *esc*$^-$ embryos bearing different numbers of copies of the *bithorax*-complex, or of the *Pc*$^+$ gene). To this end, *esc*$^-$ parents were generated which were homozygous for the cuticle colour mutation *yellow* (*y*) and which carried the appropriate homeotic mutation or gene duplication in *trans* to a *TM3*, *e*^{s} *Ser y*$^+$ chromosome (kindly provided by E. Wieschaus). For example, *esc*$^-$ embryos homozygous for *Dp(3;3)P5* (a tandem duplication of the entire *bithorax*-complex (Lewis, 1980)) were prepared as follows: *y*; *dp esc*^{6} *stc pr cn/+*; *Dp(3;3)P5/TM2*, *Ubx*^{130} *e*^{s} males were crossed to virgin *y*; *esc*^{10} *b pr/ In(2L)Cy*, *dp*^{lv}; *TM3*, *e*^{s} *Ser y*$^+$*/Dp(2;3)17*, *esc*$^+$ females, and *y*; *dp esc*^{6} *stc pr cn/esc*^{10} *b pr*; *Dp(3;3)P5*/TM3, *e*^{s} *Ser y*$^+$ progeny selected on the basis of their y$^+$, esc, pr, e$^+$, and Cy$^+$ phenotype. *esc*$^-$ embryos obtained from such flies were prepared as described in *a*) and then screened initially under bright-field illumination to identify phenotypically yellow, and hence genotypically *Dp(3;3)P5/ Dp(3;3)P5*, specimens. This method of independently marking *esc*$^-$ embryos bearing a particular third chromosome genotype was used for generating *esc*$^-$ embryos with the following third chromosome genotypes: *Df(3R)P9/+*, *Dp-(3;3)P5/Dp(3;3)P5*, *Pc*^{2}*/+*, *Pc*^{2}*/Pc*^{2}, *Dp(3;3)C126/Dp(3;3)C126*, *Antp*$^{Ns+RC3}$*/ Df(3R)4Scb*, and *Scr*^{13A}*/Df(3R)4Scb*.

RESULTS

The major aim of these experiments is to examine the extent to which the product of the *extra sex combs*$^+$ (*esc*$^+$) gene is required for the selective expression of other homeotic genes. To this end, the phenotype of embryos lacking the *esc*$^+$ gene product is described in detail, and then, this standard phenotype is compared with the phenotypes of *esc*$^+$ and *esc*$^-$ embryos which lack other homeotic genes, or which carry an altered number of copies of these other genes.

The standard esc$^-$ *phenotype*

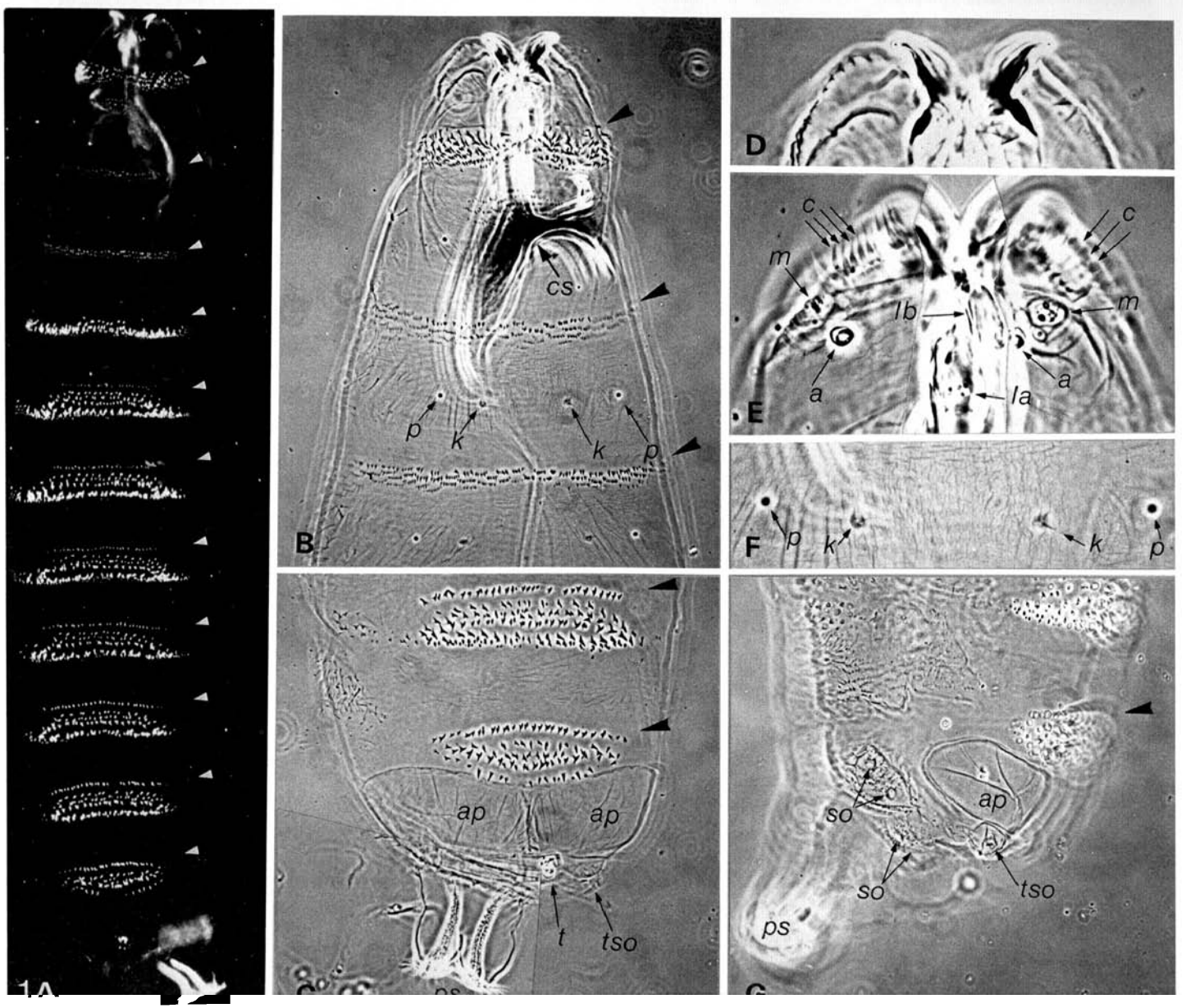
The phenotypes caused by recessive mutations of the *esc* locus depend critically on the genotype of both the mother and the zygote. Homozygous *esc*$^-$ embryos obtained from heterozygous females develop into normal first instar larvae which in turn are able to develop into adults. Such adults show a variable transformation of the distal second and third legs into distal first legs (Slifer, 1942; Tokunaga & Stern, 1965; Struhl, 1981*a*), as well as occasional, weak transformations of antenna to leg, and wing to haltere (Struhl, unpublished). In

contrast, *esc*$^-$ embryos obtained from homozygous *esc*$^-$ females develop into first instar larvae in which most of the segments appear similar to the eighth abdominal segment of wild-type larvae (Struhl, 1981*a*). The cuticle of the first instar larva is secreted about half way through embryogenesis; hence the cuticular pattern is treated as a direct reflection of the embryonic phenotype at the time of cuticle deposition. Because this study is concerned only with the cuticular phenotypes of *esc*$^-$ progeny derived from *esc*$^-$ mothers, such progeny will be referred to simply as *esc*$^-$ embryos.

The phenotype of *esc*$^-$ embryos is characterized by the transformation of at least one cephalic and two gnathal segments, all three thoracic segments, and the first nine abdominal segments into segments resembling to varying degrees the normal eighth abdominal segment. The wild-type eighth abdominal segment bears several unique characteristics which distinguish it from all other segments. These include (i) a square pattern of five to six rows of ventral hairs, (ii) posterior spiracles (both internal *Filzkorper* and external sensory structures), and (iii) unique sensory organs (see Methods and Fig. 1). In *esc*$^-$ embryos (Fig. 2), all three thoracic segments as well as the first seven abdominal segments bear the square pattern of ventral hairs, as well as some of the unique sensory organs characteristic of the eighth abdominal segment. In addition, rudimentary posterior spiracles (both internal and external portions) are frequently found on the more posterior abdominal segments, and sometimes on the more anterior abdominal and thoracic segments. The eighth abdominal segment itself appears indistinguishable from that of wild-type embryos. Thus, the cuticular patterns of the thoracic and first seven abdominal segments approach that of the normal eighth abdominal segment. In addition to these segments, four others also appear to be transformed towards a normal eighth abdominal segment, but here, the transformation is less complete. These are a cephalic segment (tentatively called the antennal segment – see Fig. 5), two gnathal segments immediately anterior to the thorax (tentatively called the labial and maxillary segments – see below), and a ninth abdominal segment. The antennal segment is consistently transformed so that it bears a band of ventral-type hairs similar to that of the eighth abdominal segment. However, these hairs are formed on the dorsal side of the head suggesting the possibility that the transformed segment has rotated such that originally ventral cells now lie dorsally. Occasionally what appears to be a trail of these ventral-type hairs remains and links the dorsally positioned hairs to the ventral side of the embryo (Fig. 2). These remaining hairs lie immediately anterior to the remnants of the ventral portion of the cephalopharyngeal skeleton. The extent to which the labial, maxillary and ninth abdominal segments are transformed is variable; the pattern of ventral hairs formed by these segments ranges from a small tuft of hairs to three to five rows of hairs arranged in a square pattern. None of these three segments exhibit posterior spiracles or sensory organs characteristic of the eighth abdominal segment. Although no sense organs unique to the thoracic segments remain in *esc*$^-$ embryos, remnants

of most of the normal head structures (e.g., separate left and right halves of the labial sense organ, the maxillary cirri and associated sense organs, mouth hooks, maxillary and antennal sense organs, cephalopharyngeal skeleton, labral hatching

Fig. 1. Wild-type first instar larva (see Lohs-Schardin *et al.* 1979, Turner & Mahowald, 1979, and Methods for terminology and assignments of cuticular structures to particular segments). A) Ventral aspect of a wild-type first instar larva. The body is composed of the head, carrying the derivatives of the gnathal and cephalic segments (B, D,E), the thorax, composed of three segments (B, F) the abdomen, composed of at least eight segments (C, G), and the terminalia (C, G). The three thoracic and first eight abdominal segments each bear a characteristic pattern of ventral hairs near the anterior margin. The white arrow heads mark the approximate positions of the anterior margins of the thoracic and first eight abdominal segments (Szabad, Schupbach & Wieschaus, 1979). B) The head and thorax. The anterior margins of the pro-, meso-, and metathorax are marked by black arrow heads. Compared to the other thoracic segments, the prothorax carries a relatively broad band of hairs at the anterior margin, and a second squarish band of fine hairs posterior to the first band (see also Fig. 5A). Note that the anterior-most hairs of the anterior band are thicker than both the remaining hairs on the prothorax, and the hairs on the meso- and metathorax. The mesothorax carries a single thin band of fine hairs, and the metathorax, a single thin band of slightly thicker hairs. All three thoracic segments carry three pairs of bilaterally symmetric sense organs (see also F): the Keilin's organs (or 'trihairs'; *k*) the ventral pits (*p*), and the dorsal pits (not shown here; see Fig. 5A). No other segments bear these sense organs. The head structures apparent in this photograph are the mouth hooks (see also D), and the cephalopharyngeal skeleton (*cs*) which lies inside the body of the larva, underneath the anterior thorax. C) The posterior abdominal segments and terminalia: The anterior margins of the seventh and eighth abdominal segments are marked by black arrow heads. Note that the eighth abdominal segment carries a square pattern of five to six rows of thick hairs. Just posterior to these hairs are the bilaterally symmetric anal pads (*ap*), and between them, the anus, a vertical slit. Just posterior to the anus is a small tuft of hairs (*t*; see also A), and to the right of the tuft, the base of one of two bilaterally symmetric sense organs (*tso*) associated with the anal pads (see also G). As described in Methods and Results, the anal pads, anus, tuft, and terminal sense organ do not appear to belong to the eighth abdominal segment; their segmental status is unknown. Dorsal to all of these structures are the paired posterior spiracles (*ps*) (see G). The tips of the posterior spiracles each bear a crown of long slender hairs; internally the spiracles terminate in a refractile mesh-like structure (the *Filzkorper*). D) Mouth hooks. The base of one of the combs of cirri (see E) can be seen to the left of the left mouth hook. E) Sense organs of the head. Two combs of sensory hairs, the cirri (*c*), are associated with the mouth hook. A small sense organ (not shown here; see Fig. 6A) is also associated with the cirri on each side of the head. The cirri, as well as the mouth hook itself are thought to be derivatives of the maxillary segments (Schoeller, 1964). On the dorsal surface are two pairs of bilaterally symmetric sense organs: the maxillary sense organs (*m*), each a cluster of small circular sensilli having a granular appearance, and the antennal sense organs (*a*), bulb-like, highly refractile sensilli. Just inside the mouth are the labral hatching tooth (*lb*), and the labium (*la*). This second structure carries several sensilli which appear as dots in this photograph. F) The Keilin's organs (*k*) and ventral pits (*p*) of the mesothorax of the larva in B. The Keilin's organs are tight clusters of three sensory hairs; the ventral pits appear as small, highly refractile circles. G) Lateral aspect of the posterior abdominal segments and terminalia (labelled as in C). The left and right sides of the eighth segment each bear two pairs of wart-like sensory organs (*so*; the arrows point to one pair which is in focus and to the position of the second pair which is out of focus). Magnifications: A = ×120; B, C, G, = ×200; D, E, F, = ×400.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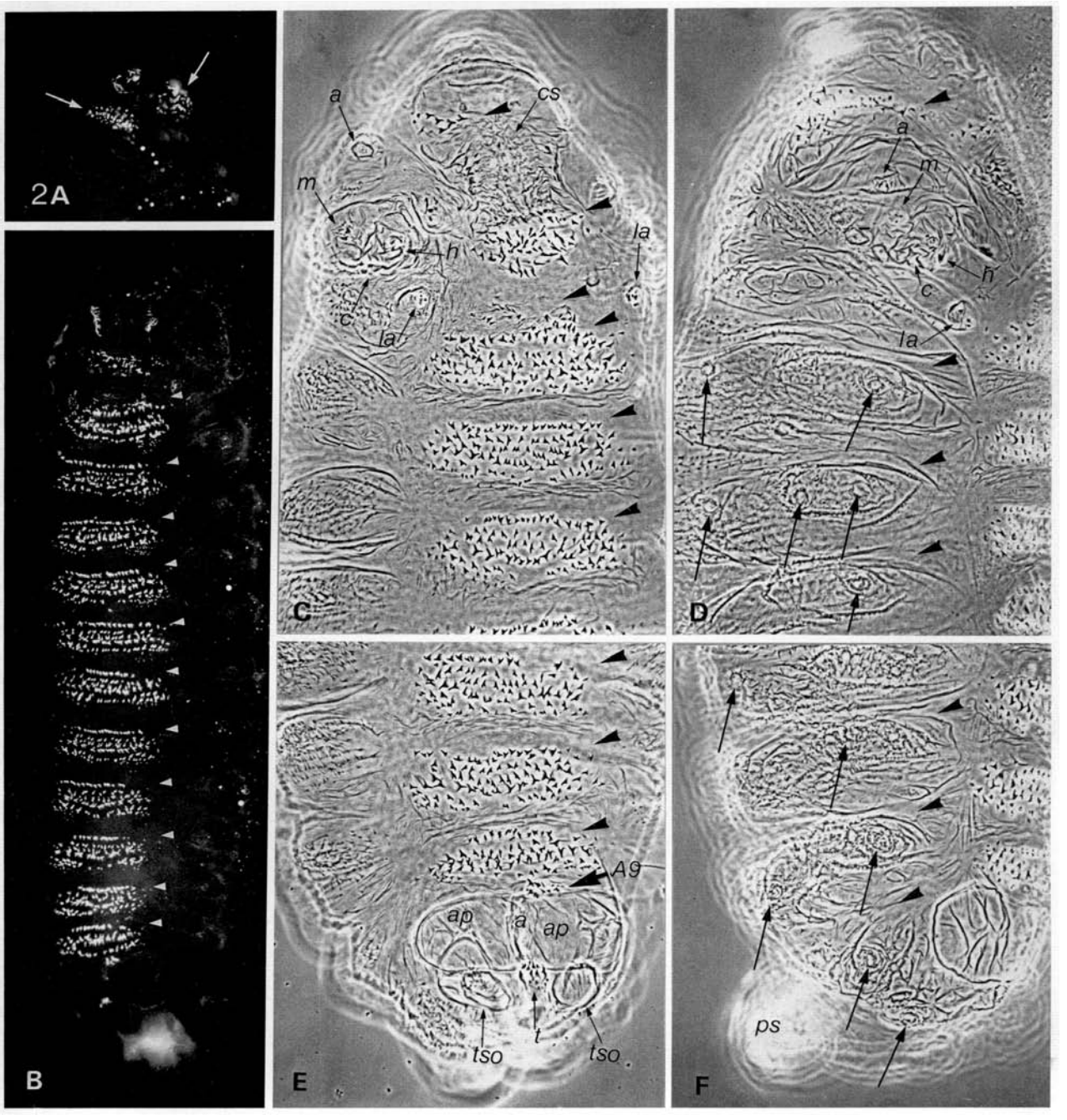
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tooth) can usually be identified. Because *esc*$^-$ embryos fail to undergo head involution, these structures remain on the outside. Several structures appear unaffected in *esc*$^-$ embryos. These are the anal pads, a tuft of ventral hairs posterior to the anal pads, and a set of sense organs immediately posterior to the anal pads.

Dependence of the esc$^-$ *phenotype on the number of copies of the* bithorax-*complex genes*

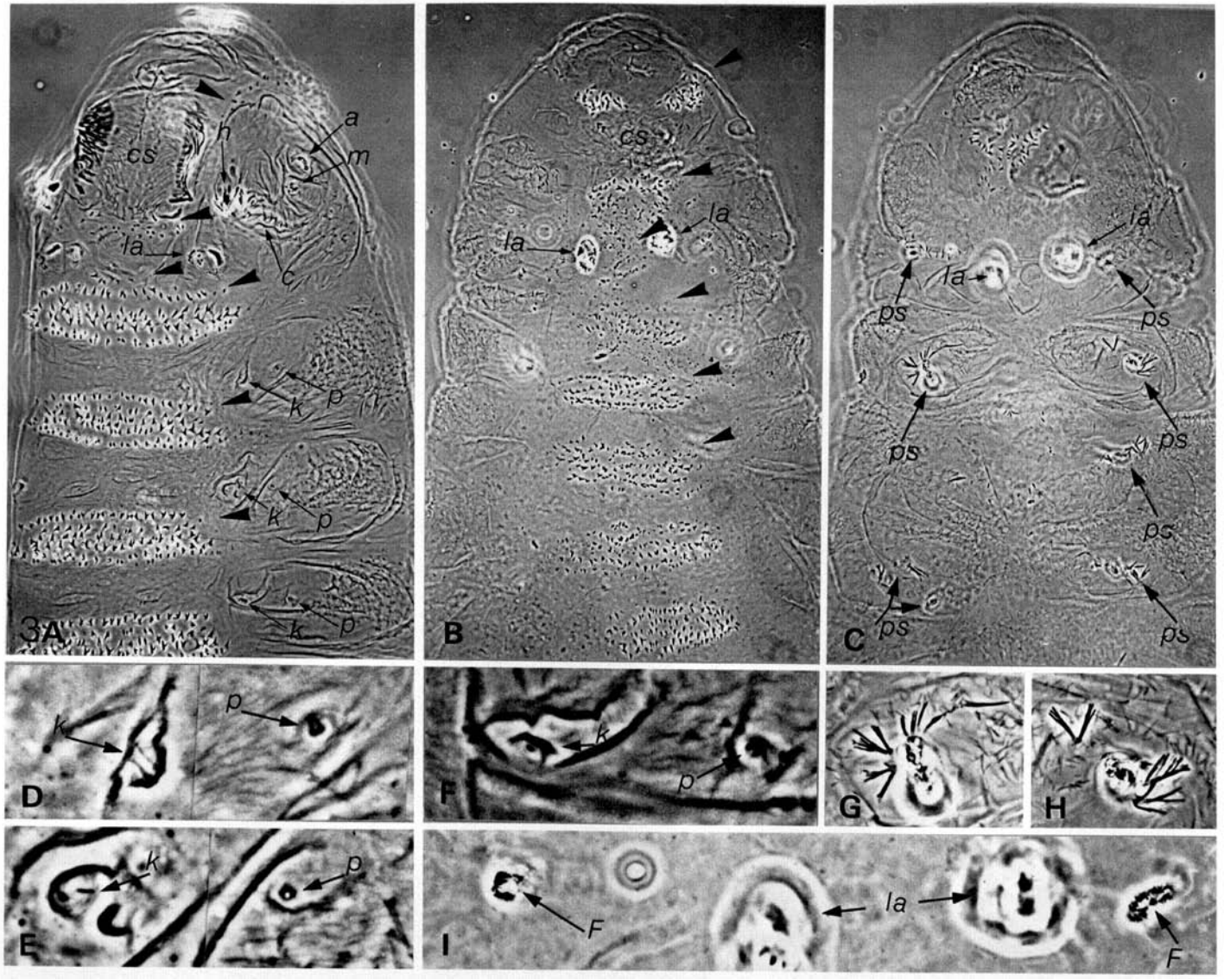
esc$^-$ embryos carrying one, or four copies of the *bithorax*-complex have been generated, and their phenotypes compared with the standard *esc*$^-$ phenotype in which two copies of the *bithorax*-complex are present. *esc*$^-$ embryos carrying only one copy of the complex show a less-extreme transformation than standard *esc*$^-$ embryos (Fig. 3). For example, rudimentary Keilin's organs and ventral pits remain in all three thoracic segments (these sensory organs are normally present in the thoracic segments of wild-type embryos, but absent in standard *esc*$^-$ embryos). *esc*$^-$ embryos carrying four copies of the *bithorax*-complex show a more extreme transformation than that of standard *esc*$^-$ embryos (Fig. 3). For example, rudimentary posterior spiracles appear in most or all of the abdominal and thoracic segments, and in addition, the remnants of the sense organs of the head appear further depauperated. Thus, the expression of the *esc*$^-$ phenotype depends on the number of copies of the *bithorax*-complex. As the number of

Fig. 2. Standard *esc*$^-$ phenotype. A and B) Dorsal anterior and ventral aspects of an *esc*$^-$ embryo. The anterior margins of the thoracic and first eight abdominal segments are marked with white arrow heads as in Fig. 1A. All of the segments bear a square band of thick hairs resembling that of the normal eighth abdominal segment. Note that a segment of the head on the dorsal side (A) bears an incomplete band of thick hairs (arrows). C) Ventral aspect of the head and thorax. Black arrow heads mark (from top to bottom) the anterior margins of a cephalic segment, two gnathal segments, and the three thoracic segments. The remnants of the cephalopharyngeal skeleton (*cs*), labium (*la*; note that the labium is normally formed by the fusion of left and right halves which remain apart in *esc*$^-$ embryos), mouth hooks (*h*), antennal sense organs (*a*), maxillary sense organs (*m*) , and cirri (*c*) are indicated. Note the absence of Keilin's organs and ventral pits in the thoracic segments. D) Lateral aspect of the head and thorax: Black arrow heads mark the dorsal anterior margins of the transformed cephalic and thoracic segments. Remnants of the head structures are labelled as in C. Note the presence of wart-like sensory organs (arrows) as well as the protuberant cuticular morphology (both features characteristic of the normal eighth abdominal segment). Note also that a trail of hairs appears to link the dorsally and ventrally positioned hairs of the transformed cephalic segment marked by the anterior-most arrow head in C and D). E) Ventral aspect of the posterior abdominal segments. Black arrow heads mark the anterior margins of the sixth, seventh, and eighth abdominal segments. The transformed ninth abdominal segment (*A9*) is indicated by an arrow, and the remaining terminal structures labelled as in Fig. 1C. F) Lateral aspect of the posterior abdominal segments. Black arrow heads mark the dorsal anterior margins of the sixth, seventh, and eighth abdominal segments. Note the presence of wart-like sensory organs as well as the protuberant cuticular morphology on all three segments. Magnifications: A, B = ×135; C, D, E, F = ×250.

copies rises from one to two to four, the transformation of all segments into eighth abdominal segments appears progressively more complete.

The dependence of the *esc*$^-$ phenotype on the dosage of particular portions of the *bithorax*-complex has been studied by comparing the phenotypes of *esc*$^-$ embryos carrying one or two copies of specific fragments of the complex. For example, *Df(3R)Ubx*^{109} deletes a centromere–proximal portion of the complex, leaving the distal portion intact. *esc*$^+$ embryos homozygous for this deficiency, or carrying it in *trans* to *Df(3R)P9* (which is thought to be a deletion for the entire *bithorax*-complex (Lewis, 1978)) show a complete transformation of the metathorax and first four or five abdominal segments into mesothoracic segments (Fig. 4). In addition, the remaining posterior segments appear partially transformed into thoracic segments, though the eighth segment still bears rudimentary posterior spiracles and lacks ventral pits and Keilin's organs found on all of the more anterior abdominal segments (Lewis, 1978; Struhl, 1981*a*). The phenotype of *esc*$^-$ embryos carrying *Df(3R)Ubx*^{109} depends on whether or not the deficiency is hemi- or homozygous. In *esc*$^-$; *Df(3R)Ubx*^{109}/*Df(3R)P9* embryos (Fig. 4), the thoracic and first seven abdominal segments are transformed into segments resembling the eighth abdominal segment of *Df(3R)Ubx*^{109}/*Df(3R)P9* embryos. The transformation is not complete; there are rudimentary Keilin's organs and ventral pits in all of these segments. However, in *esc*$^-$; *Df(3R)Ubx*^{109}/*Df(3R)Ubx*^{109} embryos (Fig. 4) the Keilin's organs and ventral pits are now absent in all of the thoracic and abdominal segments. Similar results have been obtained by comparing the phenotypes of *esc*$^-$ embryos that are hemi- or homozygous for two other partial deficiencies of the *bithorax*-complex

Fig. 3. *esc*$^-$ embryos carrying one, or four copies of the entire *bithorax*-complex. A) One copy. Black arrow heads mark the anterior margins of the cephalic and two gnathal segments, and the thoracic segments. Remnants of the head sense organs are labelled as in Fig. 2C and 2D. Note the presence of rudimentary Keilin's organs (*k*) and ventral pits (*p*) in each of the thoracic segments (shown in higher magnification in D, E, and F). Note also that the gnathal segments are transformed to a less extreme extent (i.e., they bear fewer rows of thick hairs) and also that the remaining head structures are more prominent than in standard *esc*$^-$ embryos (Figs 2C and 2D). B) Four copies; ventral aspect. Black arrow heads mark the anterior margins of the cephalic, gnathal and thoracic segments as in A. Note that the remnants of most of the head sense organs are no longer apparent. C) Four copies; dorsal aspect. At least one gnathal segment, as well as each of the thoracic segments carry rudimentary posterior spircles (*ps*: both external clusters of slender hairs, and the refractile internal *Filzkorper* (see Fig. 1C) are present in most cases). The remnants of the left and right halves of the labium are marked as in B. D, E, F) Rudimentary Keilin's organs and ventral pits formed in the pro- (D), meso- (F), and metathoracic (G) segments of the embryo in A. G, H) Left and right rudimentary posterior spiracles formed in the dorsal prothorax of the embryo shown in B and C. Note presence of both external hairs, and internal *Filzkorper*. I) Internal *Filzkorper* (*F*) associated with rudimentary posterior spiracles formed in the gnathal segment of embryo in B and C. Remnants of the labium (*la*) are shown as in C. Magnifications: A,B,C = ×200; D, E, F = ×900; G, H, I = ×600.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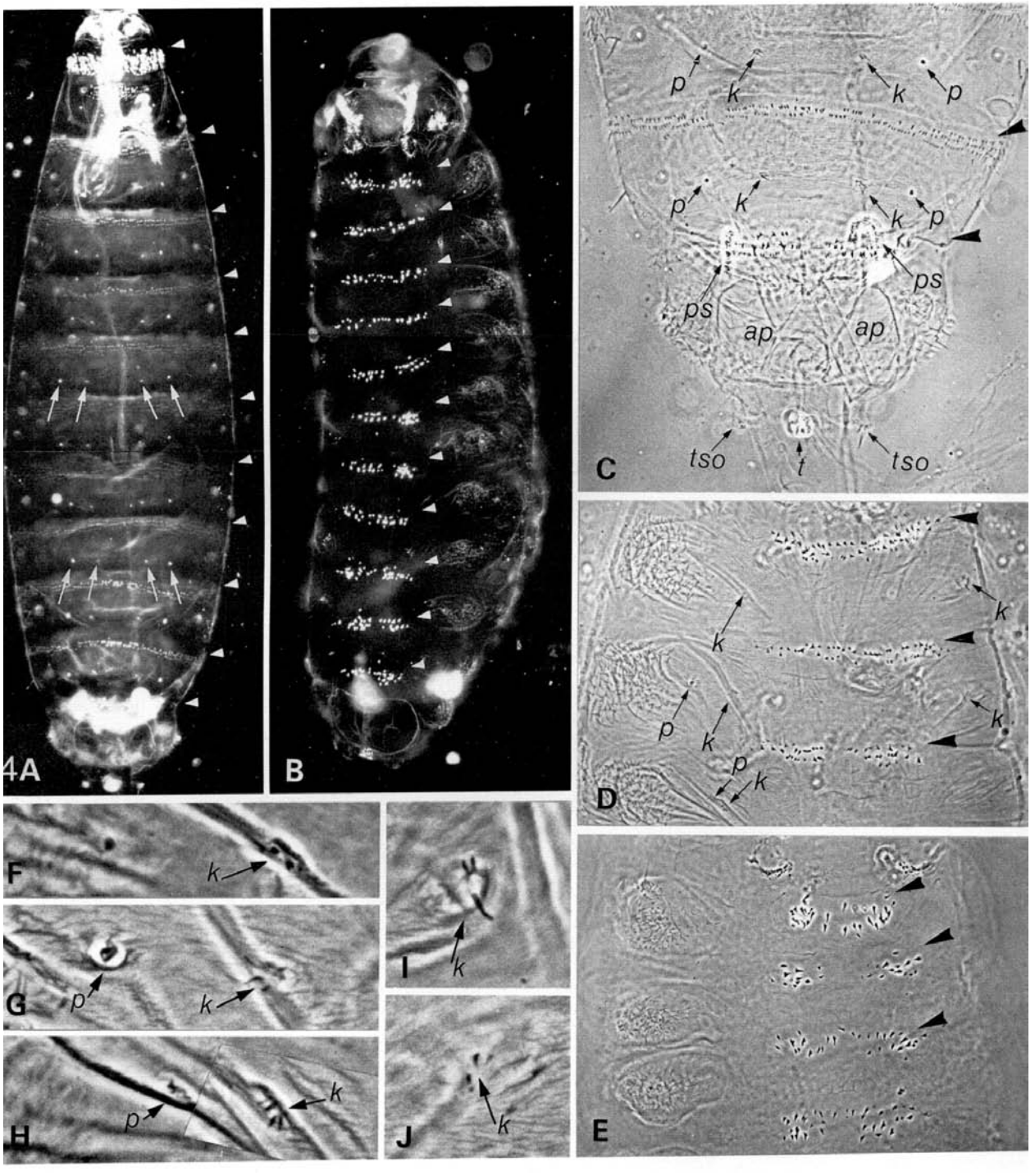
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(*Df(3R)bxd*^{100}, and *Df(3R)P10*). Thus, the *esc*$^-$ phenotypes appear to depend on the numbers of copies of both the entire *bithorax*-complex as well as particular fragments of the complex.

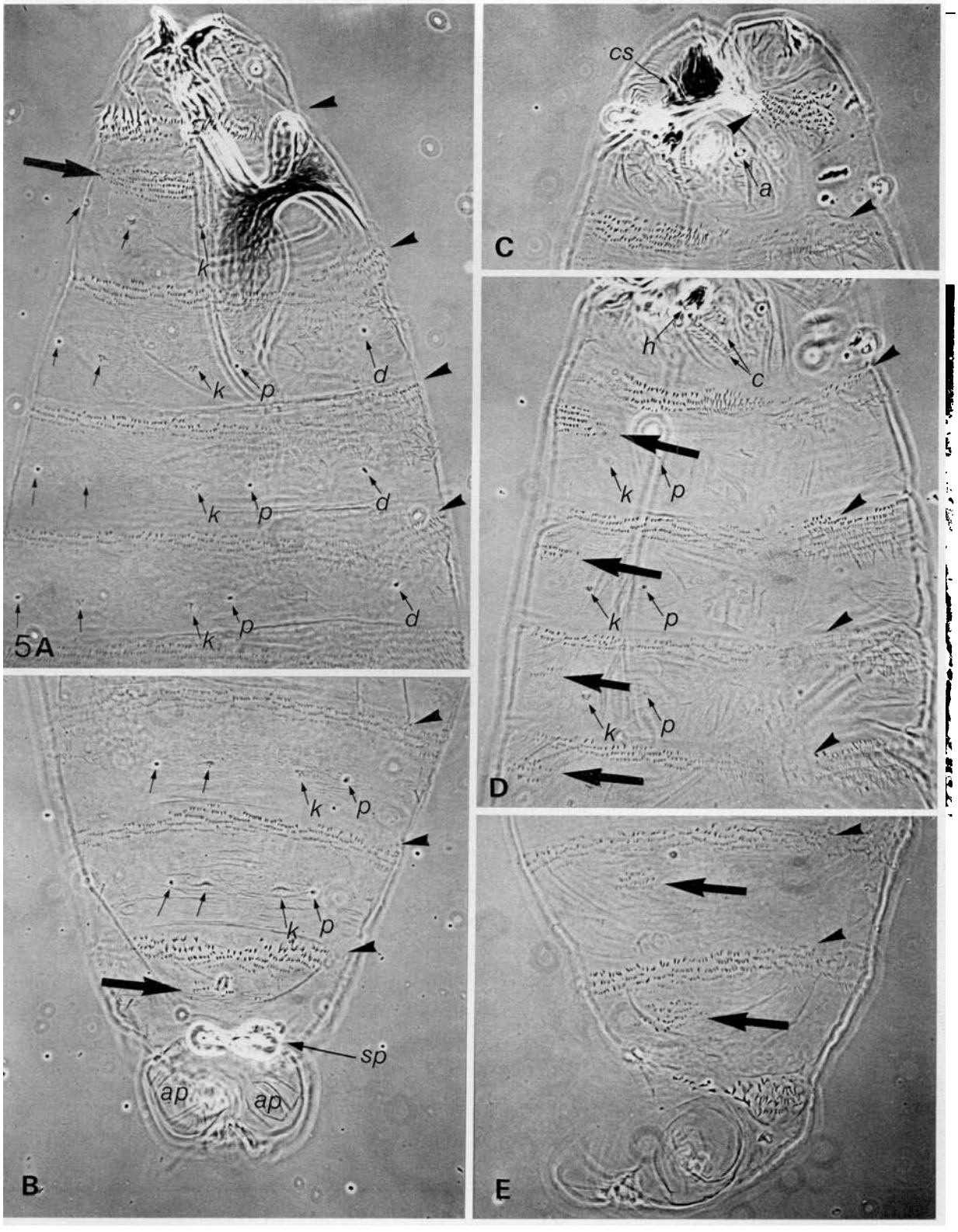
Dependence of the esc$^-$ *phenotype on the number of copies of the* Pc$^+$ *gene*

Embryos homozygous for small deficiencies or point mutations of the *Polycomb* (*Pc*) locus show an embryonic lethal phenotype similar to, though less extreme, than that of the standard *esc*$^-$ phenotype (Lewis, 1978; Duncan & Lewis, 1982). However, unlike the *esc*$^+$ gene product which appears to have a discrete early role (Struhl, 1981*a*; Struhl & Brower, 1982), the product of the *Pc*$^+$ gene appears to play the same role throughout development (Struhl, 1981*a*;

Fig. 4. *esc*$^-$ embryos carrying one or two copies of a centromere-distal fragment of the *bithorax*-complex. A) *esc*$^+$ embryo carrying *Df(3R)Ubx*^{109}/*Df(3R)P9* (*DF(3R)Ubx*^{109} deletes a large proximal portion of the *bithorax*-complex, but leaves a distal portion of the locus intact (Lewis, 1978); *Df(3R)P9* deletes the entire complex). White arrow heads mark the anterior margins of the thoracic and first eight abdominal segments. Note that the head, pro, and mesothorax appear normal (compare with Fig. 1A), but that the metathorax and first several abdominal segments develop like the mesothorax (i.e., they bear the mesothoracic pattern of ventral hairs at the anterior magin, and also Keilin's organs, and ventral pits (e.g., white arrows in the second and sixth abdominal segments)). The eighth abdominal segment bears paired, rudimentary posterior spiracles which obscure the pattern of hairs at the anterior margin of the segment (see C). B) An *esc*$^-$; *Df(3R)Ubx*^{109}/*Df(3R)P9* embryo. White arrow heads mark the anterior margins of the thoracic and first eight abdominal segments. Note that all of these segments develop like the eighth abdominal segment of the *Df(3R)Ubx*^{109}/*Df(3R)P9* embryo in A and C. C) Posterior abdominal segments and terminalia of the embryo in A. Black arrow heads mark the anterior margins of the seventh and eighth segments. Note the presence of Keilin's organs (*k*) and ventral pits (*p*) remaining in the sixth and seventh segments. The hairs at the anterior margins of these two segments appear a little thicker and sparser than in the mesothorax (compare with Fig. 5B) suggesting that they are only partially transformed towards the mesothorax. The eighth abdominal segment bears a band of 1–3 rows of thick hairs which appears like an abbreviated version of the normal eighth abdominal segment. This segment also bears rudimentary posterior spiracles (*ps*) and a pattern of dorsal hairs approaching that of the normal eighth abdominal segment; it does not carry either Keilin's organs or ventral pits. Posteriorly, the anal pads (*ap*), tuft (*t*) and terminal sense organ (*tso*) are normal. D) Thorax of an *esc*$^-$; *Df(3R)Ubx*^{109}/*Df(3R)P9* embryo. Black arrow heads mark the anterior margins of the three thoracic segments. Note that rudimentary Keilin's organs and ventral pits remain in each segment (shown in higher magnification in F, G, H, I, and J). E) Thorax of an *esc*$^-$; *Df(3R)Ubx*^{109}/*Df(3R)Ubx*^{109} embryo. Black arrow heads mark the thoracic segments as in D. Note that Keilin's organs and ventral pits are absent and also that the pattern of ventral hairs appears more square-like than in D. F–J) Rudimentary Keilin's organs (*k*) and ventral pits (*p*) on the left and right sides of the pro- (F, I), meso- (G, J), and metathorax (H) of the embryo in D. It is appropriate here to correct an omission in the description of the phenotype of *Dp(3;2)P10 esc*$^-$/*esc*$^-$; *Df(3R)P9* embryos in Struhl, 1981*a*, fig. 5. Though not indicated in that figure, such embryos show rudimentary Keilin's organs and ventral pits in the three thoracic segments. Magnifications: A, B = ×140; C, D, E. = ×280; F, G, H, I, J = ×1100.

Duncan & Lewis, 1982). The possibility that the product of the esc^+ gene is required for the correct expression of the Pc^+ gene has been examined by comparing the phenotypes of esc^- embryos carrying zero, one, two, or four copies of the *Pc* locus.

Fig. 5. esc^+ and esc^- embryos lacking the entire *bithorax*-complex. A) Ventrolateral aspect of the head and thorax of an esc^+ embryo lacking the *bithorax*-complex (i.e., homozygous for *Df(3R)P9)*. Black arrow heads mark the anterior margins of the three thoracic and first abdominal segments. The head, pro-, and mesothorax appear normal, but the metathorax and first abdominal segment appear like the mesothorax (compare with Fig. 1). Note that the prothorax bears two bands of hairs: an anterior band composed of several rows of hairs, the more anterior rows carrying thicker hairs than in the posteriorly situated rows, or in the meso- or metathorax (see also Fig. 1B). Also, it carries a squarish band of fine hairs (large arrow). The Keilin's organs (*k*), ventral pits (*p*) and dorsal pits (*d*) are indicated by letters and arrows on the right side; on the left side, the bilaterally symmetric Keilin's organs and ventral pits are marked only with arrows. B) Ventral aspect of the posterior abdominal segments of the embryo in A. Black arrow heads indicate the anterior margins of the sixth, seventh, and eighth abdominal segments. Note that the sixth and seventh segments appear identical to the mesothorax in A. However, the eighth segment appears intermediate in its hair pattern between the mesothorax and prothorax. Note especially that it bears two bands of hairs, the anterior-most carrying thickened hairs resembling those found in the anterior rows of the anterior band of the prothorax, and the remaining band (large arrow) appearing like an abbreviated version of the posteriorly situated band found in the prothorax. Finally, note that bilaterally symmetric plates of sclerotized cuticle (*sp*) are found at the posterior margin of the segment. The anal plates (*ap*) remain intact as do the tuft and terminal sense organs (out of the plane of focus; see Fig. 6D). C) Lateral aspect of the head of an esc^- embryo lacking the entire *bithorax*-complex. Black arrow heads mark the anterior margins of a transformed cephalic segment, and of the prothorax. Note that the cephalic segment bears a band of mesothoracic-type ventral hairs on the dorsal portion of the head. It is curious to note that this segment appears to be of reversed polarity (i.e., the hairs point anteriorly, not posteriorly). These properties suggest that the transformed head segment corresponds to a rotated thoracic segment. Since the eye-antennal segment of the adult is known to undergo such a relative rotation during development (Struhl, 1981*c*), it is posible that this head segment is in fact the antennal segment. (Remnants of the antennal sense organ (*a*) and the cephalopharyngeal skeleton (*cs*) are indicated). D) Lateral aspect of the thorax and anterior abdomen of the embryo in C. Black arrow heads mark the anterior margins of the thoracic segments and the first abdominal segment. Note that all four segments bear a second band of hairs (large arrows) similar to, though smaller than, the posteriorly situated band formed in the normal prothorax. Note also that the hairs of the anterior band of the prothorax are thinner than in the prothorax of the esc^+ embryo in A (see also C). Thus, all four segments bear similar patterns of ventral hairs which appear to be intermediate between the normal pro- and mesothorax. Remnants of the mouth hooks (*h*), and cirri (*c*) are indicated as are Keilin's organs (*k*) and ventral pits (*p*). E) Lateral aspect of the posterior abdominal segments of the embryo in C and D. Black arrow heads mark the anterior margin of the seventh and eighth abdominal segments . Note as in D that both segments bear a second band of hairs (large arrows) as in the normal prothorax. Note also that the anterior band of hairs in the eighth segment carries uniformly fine hairs (as in the normal mesothorax) unlike the corresponding band of hairs in the eighth abdominal segment of esc^+ embryos lacking the *bithorax*-complex (B). Also it lacks the bilaterally symmetric plates of sclerotized cuticle. Magnifications: A, B, C,D, E, =×330.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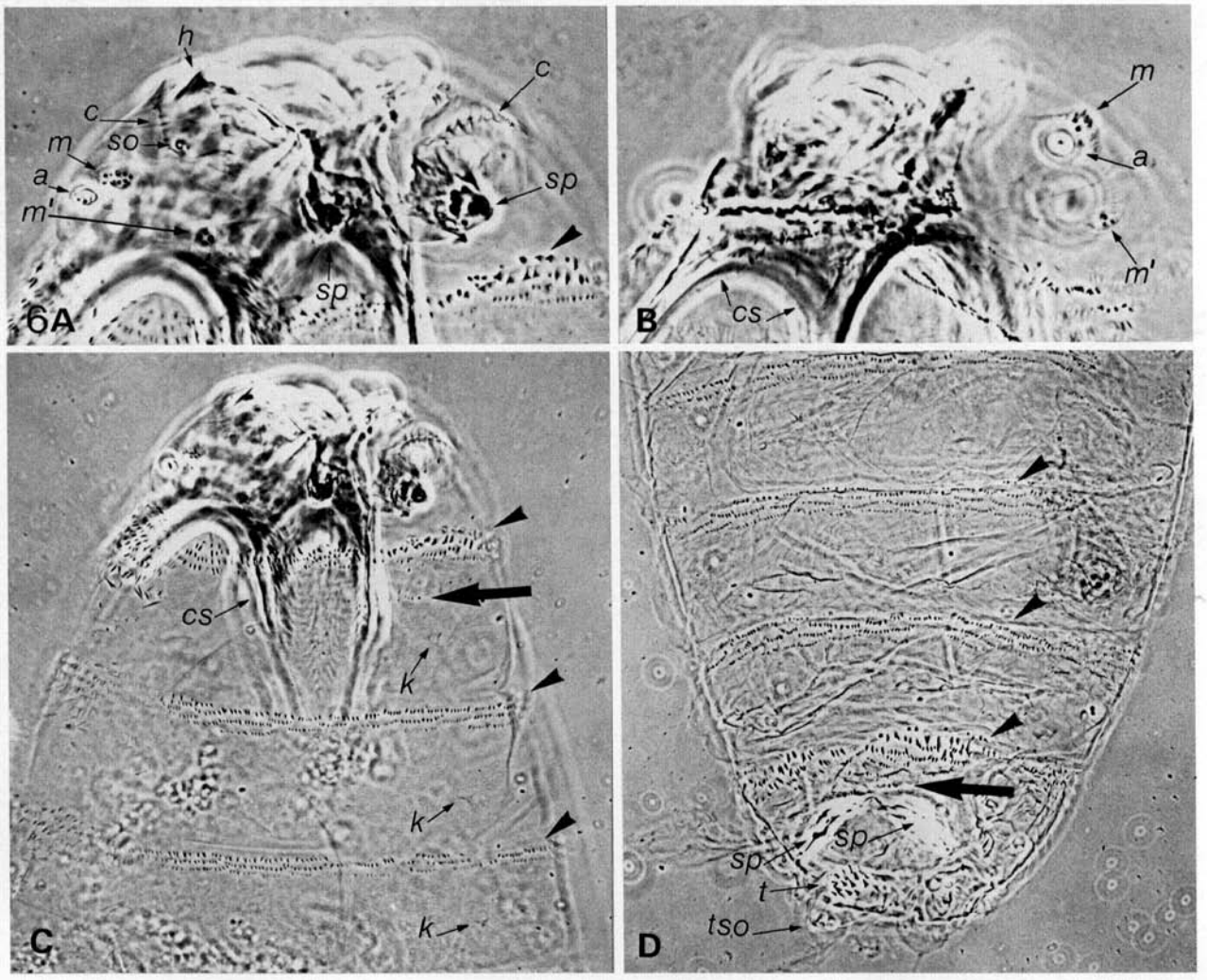
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esc$^-$ embryos carrying only one copy of the *Pc*$^+$ gene show a more complete transformation of all the segments towards the eighth abdominal segment than that of standard *esc*$^-$ embryos, approaching the phenotype of *esc*$^-$ embryos carrying four copies of the entire *bithorax*-complex (see above). The phenotype of *esc*$^-$ embryos which are homozygous for an apparent null mutation of the *Pc* locus, Pc^2, show a more complete transformation similar to, if not more extreme than, that of *esc*$^-$ embryos carrying four copies of the *bithorax*-complex. In contrast, *esc*$^-$ embryos carrying four copies of the *Pc*$^+$ gene appear indistinguishable from standard *esc*$^-$ embryos. Thus, the *esc*$^-$ phenotype depends on the number of copies of the *Pc*$^+$ gene only within a defined range. As the number of genes decreases from two to one to zero, the transformation of all segments to eighth abdominal segments becomes more complete; however, adding extra copies seems to have no effect.

Indirect evidence for the role of the esc$^+$ *gene product in ensuring the selective expression of homeotic genes outside of the* bithorax*-complex*

esc$^+$ embryos homozygous for *Df(3R)P9* show a homeotic transformation of the metathorax and first seven abdominal segments into mesothoracic segments, and of the eighth abdominal segment into a segment resembling in part an intermediate between the meso- and prothoracic segments (Lewis, 1978; Struhl, 1981*a*; Fig. 5). The eighth abdominal segment also carries bilaterally symmetric

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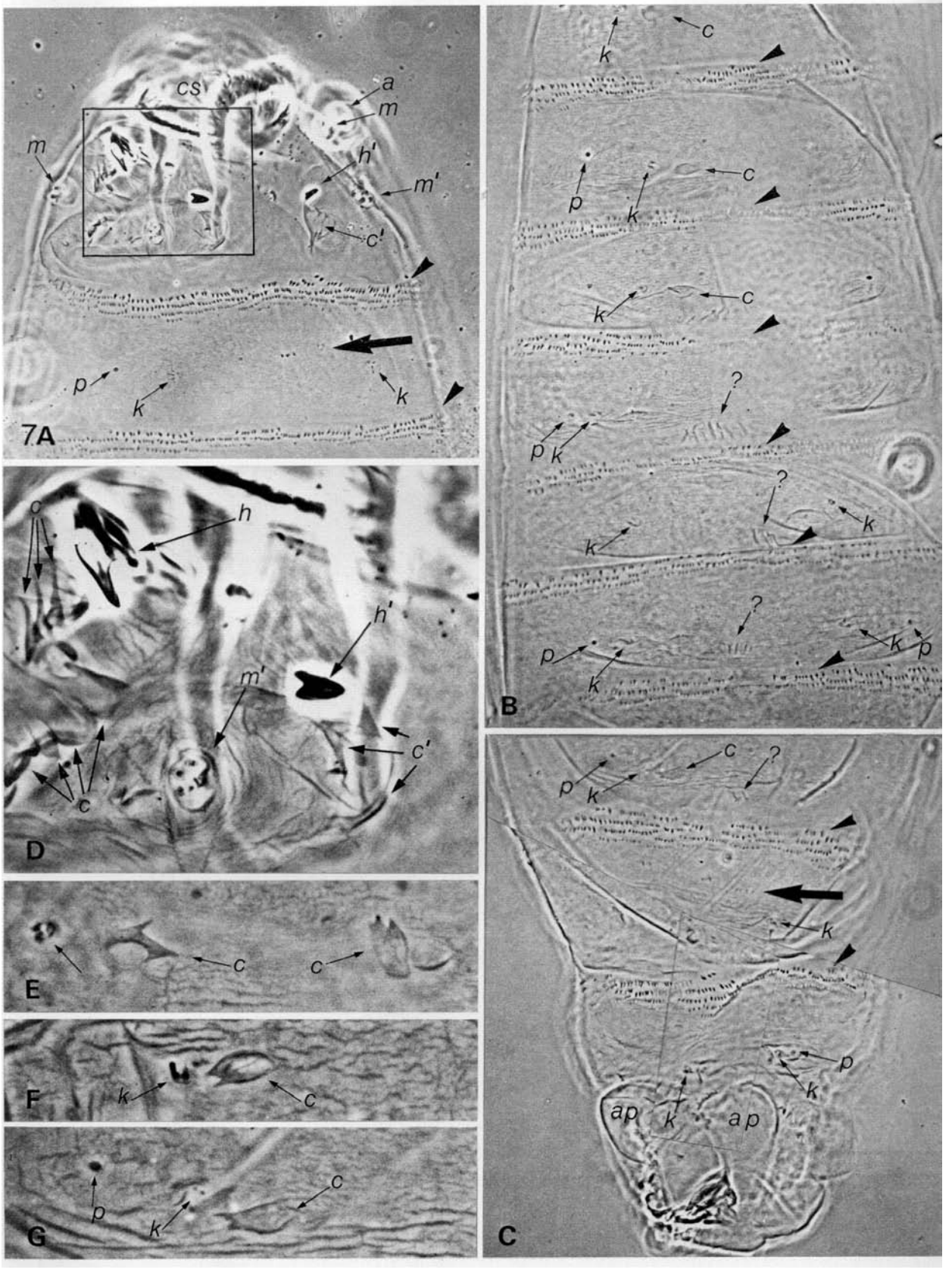
Fig. 6. *Scr*$^-$ embryos lacking the entire *bithorax*-complex. A) ventrolateral aspect of the head; a black arrow head marks the anterior margin of the prothorax. The labium is absent and appears to be replaced by bilaterally symmetric regions of sclerotized cuticle (*sp*). The cirri (*c*) and mouth hooks (*h*) remain intact, as does the cephalopharyngeal skeleton (*cs*). It is also possible to see the sense organ (*so*) normally associated with the cirri. Note the presence of the normal antennal (*a*) and maxillary (*m*) sense organs as well as an extra maxillary sense organ (*m'*) just anterior to the prothorax. B) Dorsolateral aspect of the head of the embryo in A. Again, note the presence of a normal antennal (*a*) and maxillary (*m*) sense organ, as well as an extra maxillary sense organ (*m'*) lying between the normal sense organ and the prothorax. C) Ventral aspect of the thorax of the embryo in A and B. Black arrow heads mark the anterior margins of the pro-, meso- and metathorax. Both bands of hairs carried by the prothorax bear fewer rows of hairs than in the normal prothorax (e.g., compare with Figs 5A and 5B); consequently the hair pattern of this segment appears intermediate between that of the pro- and mesothorax (the posteriorly situated band is indicated by a large arrow). All three thoracic segments bear Keilin's organs (*k*) and ventral and dorsal pits (not shown). D) Posterior abdominal segments; black arrow heads mark the anterior margins of the sixth, seventh, and eighth segments. These segments appear identical to their counterparts in *Scr*$^-$ embryos lacking the entire *bithorax*-complex (Fig. 5B); i.e., the sixth and seventh segments develop like the normal mesothorax, and the eighth segment like an intermediate between the normal pro- and mesothorax. Note the presence of bilaterally symmetric plates of sclerotized cuticle (*sp*) at the posterior margin of the eighth segment, as well as the presence of the tuft (*t*), and a terminal sense organ (*tso*); the remaining terminal sense organ as well as the anal plate are out of the plane of focus. Magnification: A, B = ×500; C, D, = ×330.

plates of sclerotized cuticle which have been interpreted as rudimentary mouth hooks (Lewis, 1978; Fig. 5). If the *esc*$^+$ gene product were required for ensuring the correct expression of only the *bithorax*-complex genes, embryos lacking the entire *bithorax*-complex would be expected to develop identically whether or not they carried the *esc*$^+$ gene product. However, *esc*$^-$; *Df(3R)P9* embryos differ in phenotype from *Df(3R)P9* embryos in that they show a transformation of the thoracic and abdominal segments as well as a head segment into a thoracic-like segment which appears to be an intermediate between the meso- and prothorax (Struhl, 1981*a*; Fig. 5). Also, these embryos lack the bilaterally symmetric plates of sclerotized cuticle present in the eighth abdominal segment of *Df(3R)P9* embryos. Assuming that *Df(3R)P9* does indeed remove the entire *bithorax*-complex, this second phenotype can be interpreted as a consequence of inappropriate expression of homeotic genes other than those of the *bithorax*-complex (Struhl, 1981*a*). To test whether the phenotype of *esc*$^-$; *Df(3R)P9* embryos reflects the phenotype of *esc*$^-$ embryos lacking the entire *bithorax*-complex, the phenotype of *esc*$^-$; *Df(3R)P115* embryos has been examined (*Df(3R)P115* deletes the salivary chromosome bands 89B-89E5,6 (Lewis, personal communication, as quoted in Morata & Garcia-Bellido, 1976) and hence, unquestionably removes the *bithorax*-complex in its entirety). As in *esc*$^-$; *Df(3R)P9* embryos, these embryos show a transformation of all thoracic and abdominal segments into intermediates between the meso- and prothorax, and lack the plates of sclerotized cuticle in the eighth abdominal segment. In addition, in favourable preparations it is possible to detect transformation of a head segment into a thoracic segment as in *esc*$^-$; *Df(3R)P9* embryos. Thus, the phenotype of *esc*$^-$; *Df(3R)P115* embryos provides further, though indirect, evidence that the *esc*$^+$ gene product is required for the correct expression of homeotic genes outside the *bithorax*-complex.

Role of the esc$^+$ *gene product in ensuring the selective expression of the Scr*$^+$ *gene*

The phenotype of embryos homozygous for *Sex combs reduced* (*Scr*) mutations has been described by Wakimoto & Kaufman (1981). Embryos homozygous for apparent null mutations of the *Scr*$^+$ gene show a partial transformation of the prothoracic segment into the mesothorax, as well as a disruption of the larval mouthparts (e.g., Fig. 6). To their description, it should be added that the disruption of the larval mouthparts is associated with absence of the labium and presence of a second pair of maxillary sense organs lying between the normal mouthparts and the prothorax (Fig. 6). Finally, the mesothorax, metathorax, and abdominal segments appear normal. Thus, the *Scr*$^+$ gene appears to be required in the prothorax to specify pro- and opposed to mesothoracic development, and in the labial segment in order to specify labial as opposed to maxillary development; however, it does not appear to be required in more posterior segments.

esc$^-$ embryos which lack the *Scr*$^+$ gene appear identical to standard *esc*$^-$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embryos except in the following respect. In standard *esc*$^-$ embryos, the remnants of the labial pits are found associated with a small tuft of ventral hairs, and anterior to these structures, a square band of three to five rows of ventral hairs is formed (e.g., Fig. 2). In *esc*$^-$; *Scr*$^-$ embryos, the labial pits are absent and replaced by a second pair of maxillary sense organs. In addition, the small tuft of ventral hairs lying between this second set of maxillary sense organs is enlarged to form a square band of three to five rows of ventral hairs. This phenotype suggests that the small tuft of hairs formed in standard *esc*$^-$ embryos corresponds to the labial segment, that the square band of hairs immediately anterior corresponds to the maxillary segment, and that in *esc*$^-$; *Scr*$^-$ embryos, the labial segment develops like the maxillary segment of standard *esc*$^-$ embryos.

One method to examine whether or not the *esc*$^+$ gene product acts to ensure the correct spatial expression of the *Scr*$^+$ gene is to identify segments in which the *Scr*$^+$ gene is normally not required, and then to test whether absence of the *esc*$^+$ gene product leads to inappropriate activity of the *Scr*$^+$ gene in these segments. As discussed above, the *Scr*$^+$ does not appear to be required in the meso- or

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Fig. 7. *esc*$^-$ embryos lacking both the *Scr*$^+$ gene and the entire *bithorax*-complex. A) Ventral aspect of the head and prothorax. Black arrow heads mark the anterior margins of the pro- and mesothorax. The head bears the cephalopharyngeal skeleton (*cs*), maxillary (*m*) and antennal (*a*; out of the plane of focus) sense organs, mouth hooks (*h*), and cirri (*c*), as well as a second set of mouth hooks (*h'*), cirri (*c'*), and maxillary (*m'*) sense organs in place of the labium (see also D). Note that the pattern of hairs of the anterior band of the prothorax closely resembles that of the anterior band of the normal mesothorax rather than the intermediate pro- to mesothoracic pattern found in the prothorax of *esc*$^+$ embryos similarly deficient for the *Scr*$^+$ gene and the *bithorax*-complex (see Fig. 6C). Similarly, the posteriorly situated band (large arrow), consists of only a few hairs. Keilin's organs (*k*) and ventral pits (*p*) are indicated when in the plane of focus. B) Ventral aspect of the thorax and anterior abdomen of a second *esc*$^-$ embryo lacking both the *Scr*$^+$ gene and the *bithorax*-complex. Black arrow heads mark the anterior margins of the meso- and metathorax, and the first four abdominal segments. Note that all segments bear an anterior band of hairs closely resembling that found on the normal mesothorax, and also that few if any of the segments carry a second band of hairs (compare with Fig. 5D). Note also that some segments bear novel structures (*c*) resembling rudimentary cirri (see E, F and G) as well as regions of vertically ridged cuticle (*?*) near the posterior margin. C) The posterior abdominal segments of the embryo in B. Black arrow heads mark the anterior margins of the seventh and eighth abdominal segments. The sixth segment bears rudimentary cirri as well as ridged cuticle, and the seventh segment bears a patch of a few hairs (large arrow) posterior to the anterior band. Note that the eighth segment bears a band of anterior hairs which appear mesothoracic, but lacks the posteriorly situated band found in *esc*$^-$ embryos carrying the *Scr*$^+$ gene, but lacking the *bithorax*-complex (Fig. 5E). Note also that the bilaterally symmetric plates of sclerotized cuticle found in corresponding *esc*$^+$ embryos (Fig. 6D) are absent. The anal plates (*ap*) remain intact as does the tuft and terminal sense organs (not in the plane of focus). D) Region of the head boxed in A; sensory organs labelled as in A. E) Rudimentary cirri in the prothorax of the embryo in B. F) Rudimentary cirri formed in the third abdominal segment of another *esc*$^-$; *Scr*$^-$ *Df(3R)P9* embryo. G) Rudimentary cirri formed in the sixth abdominal segment of the embryo in C. Magnifications: A, B, C, =×350; D =×1000; E, F, G =×800.

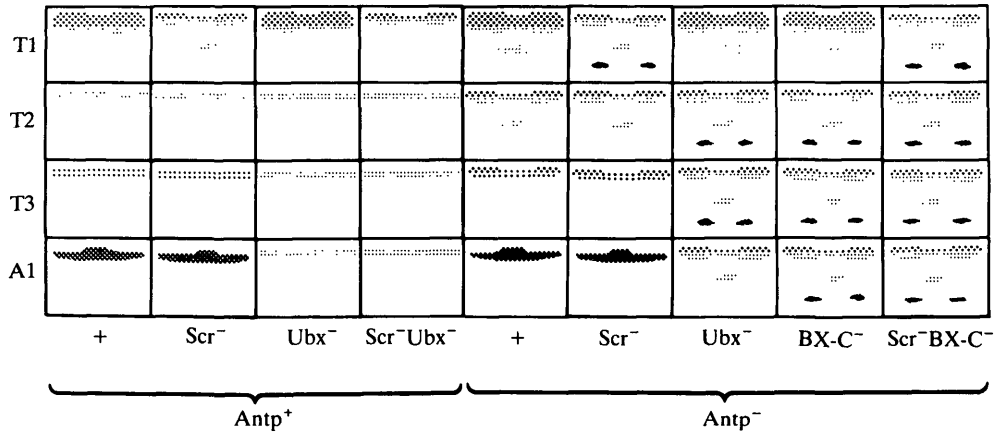

Fig. 8. Summary of the phenotypes of *Antp*$^+$ and *Antp*$^-$ embryos lacking the *Scr*$^+$ gene, the *bithorax*-complex (*BX-C*), or both. The segmental phenotypes of each mutant genotype are displayed as a matrix in which the three thoracic segments (*T1*, *T2*, and *T3*), and the first abdominal segment (*A1*) are rows, and the mutant genotypes are columns. For simplicity, only the pattern of ventral hairs and, when present, regions of sclerotized cuticle are shown. The left-most column depicts the wild-type phenotype (see description in Figs 1A, C). In *Antp*$^+$ embryos, absence of the *Scr*$^+$ gene alters the phenotype of only the prothorax, partially transforming it towards the mesothorax, whereas absence of the *Ubx*$^+$ gene, or the entire *bithorax*-complex alters the phenotypes of only the metathorax and first abdominal segment, transforming both into mesothoracic segments. Absence of both the *Scr*$^+$ gene and the *bithorax*-complex results in an additive phenotype. In *Antp*$^-$ embryos that are otherwise wild type, only the meso- and metathorax appear to be affected, both appearing to be transformed towards the prothoracic segment. This phenotype indicates that the *Antp*$^+$ gene is normally required in the meso- and metathorax. *Scr*$^-$ *Antp*$^-$ embryos differ from *Scr*$^-$ embryos in two respects. First, the prothorax bears bilaterally symmetric regions of sclerotized cuticle indicating that in the absence of the *Scr*$^+$ gene, the particular phenotype of this segment depends on the presence or absence of *Antp*$^+$ gene. Assuming that absence of the *Scr*$^+$ gene in the prothorax does not in itself cause inappropriate expression of the *Antp*$^+$ gene, this result suggests that the *Antp*$^+$ gene is normally active in the prothorax. Second, the meso- and metathorax appear partially transformed towards the prothorax, just as they do in *Antp*$^-$ embryos. Hence, this aspect of the *Antp*$^-$ phenotype does not depend on the presence of the *Scr*$^+$ gene. *Antp*$^-$ *Ubx*$^-$ embryos differ in two respects from *Ubx*$^-$ embryos. First, the pattern of ventral hairs in the meso- and metathorax, as well as the first abdominal segment, appears partially prothoracic (as in the mesothorax of *Antp*$^-$ embryos). Assuming that absence of the *Ubx*$^+$ gene does not in itself alter the pattern of expression of the *Antp*$^+$ gene, this result indicates that the *Antp*$^+$ gene is normally active in the first abdominal segment (as well as in the meso- and metathorax). Second, bilaterally symmetric regions of sclerotized cuticle are found in the meso- and metathorax, whereas they are normally not found in these segments in either *Antp*$^-$ or *Ubx*$^-$ embryos. That this particular phenotype depends in the metathorax on the presence or absence of the *Ubx*$^+$ gene suggests that the *Ubx*$^+$ gene is normally active in this segment. *Antp*$^-$ embryos lacking the entire *bithorax*-complex differ from *Antp*$^+$ embryos similarly deficient for the *bithorax*-complex in that the meso- and metathorax, as well as the first seven abdominal segments now appear like the mesothorax of *Antp*$^-$ *Ubx*$^-$ embryos. Again, assuming that absence of the *bithorax*-complex genes does not affect the normal pattern of expression of the *Antp*$^+$ gene, this result indicates that the *Antp*$^+$ gene is normally active in the first

metathorax, or in the abdominal segments. However, in both esc^+ and esc^- embryos, some or all of the *bithorax*-complex genes are active in these segments, thereby posing the possibility that activity of the *bithorax*-complex genes might obscure a requirement for the Scr^+ gene. Consequently, it is necessary to test for the presence or absence of the Scr^+ gene activity in embryos lacking the *bithorax*-complex.

In Scr^- embryos lacking the entire *bithorax*-complex (i.e., homozygous for *Df(3R)P9*); referred to subsequently as $BX\text{-}C^-$), the meso- and metathorax, as well as the first seven abdominal segments, all bear mesothoracic patterns of ventral hairs just as they do in $BX\text{-}C^-$ embryos (Figs. 5, 6). In addition, the eighth abdominal segment appears identical in embryos of both genotypes (Figs 5, 6). Hence, even in the absence of the *bithorax*-complex, these segments appear to develop identically whether or not the Scr^+ gene is present[1]. In contrast, the phenotype of these same segments in esc^-; $BX\text{-}C^-$ embryos clearly depends on whether or not the Scr^+ gene is present. Two differences are conspicuous. First, in esc^-; $BX\text{-}C^-$ embryos, a second band of fine hairs normally found only on the prothorax is present in all of the transformed segments of the thorax and abdomen (Fig. 5). However, in esc^-: Scr^- $BX\text{-}C^-$ embryos, this second band of hairs is reduced or eliminated, so that the thoracic and abdominal segments appear predominantly mesothoracic in character (Fig. 7). Second, rudimentary mouthpart structures, the cirri, and possibly other mouthpart structures, are occasionally found in the thoracic and abdominal segments of esc^-; Scr^- $BX\text{-}C^-$ embryos (Fig. 7), but were never observed in esc^-; $BX\text{-}C^-$ embryos.

Thus, the particular homeotic phenotype observed in the thoracic and

[1] It should be noted that in the absence of at least one gene of the *bithorax*-complex (Ubx^+), the determined state of imaginal cells giving rise to posterior portions of the adult meso- and metathorax can depend on the presence or absence of the Scr^+ gene (Struhl, 1982). However, this abnormal requirement for the Scr^+ gene does not provide evidence that the Scr^+ gene is active in those embryonic cells giving rise to the meso- or metathoracic segments of $BX\text{-}C^-$ larvae.

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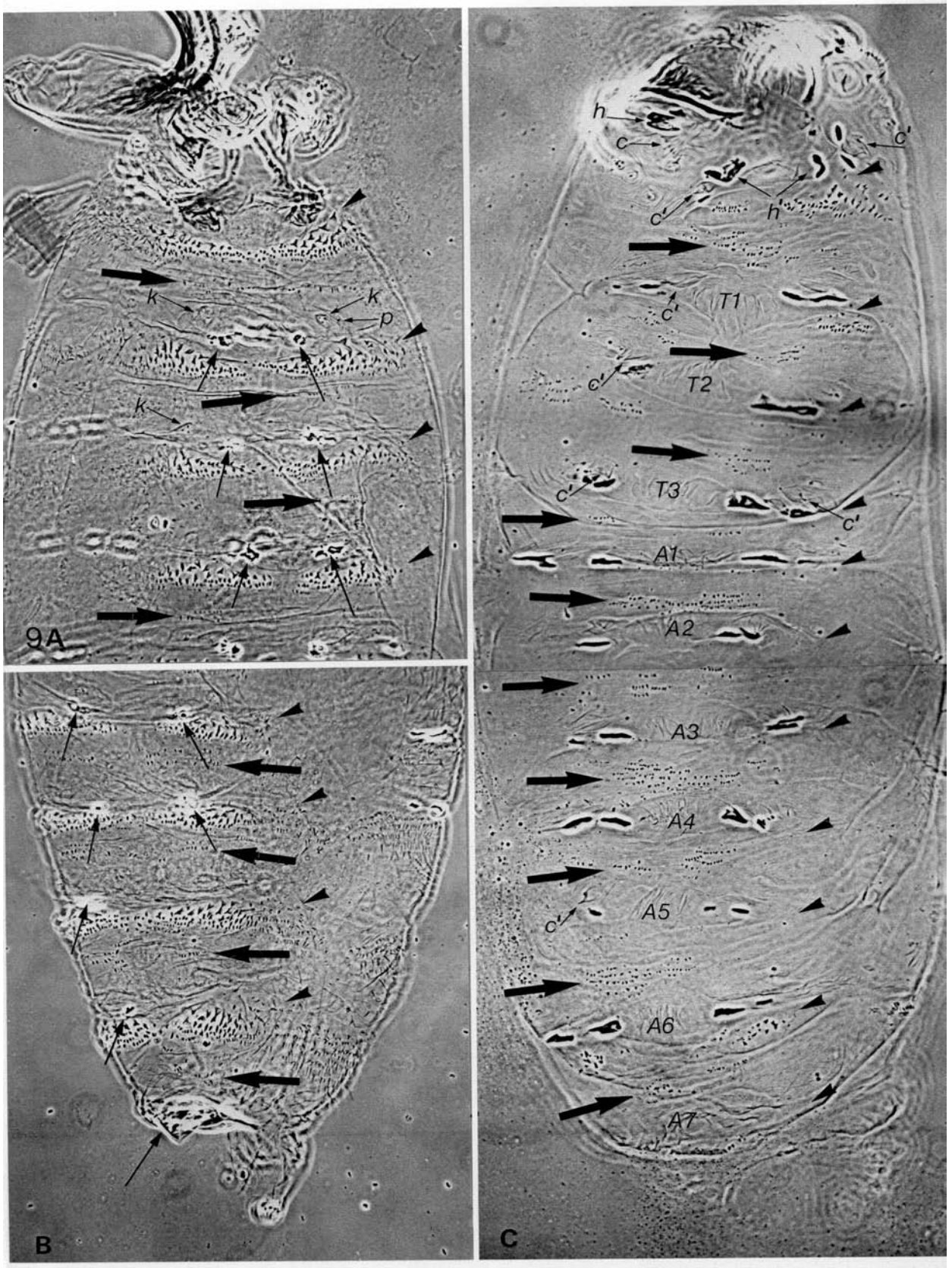
seven abdominal segments (as well as the meso- and metathorax). In contrast, the eighth abdominal segment of $Antp^-$ $BX\text{-}C^-$ embryos appears similar to the eighth abdominal segment of $BX\text{-}C^-$ embryos (see Fig. 9) suggesting that the $Antp^+$ gene is normally not active in this segment. Finally, it is curious to note that the first abdominal segment of $Antp^-$ $BX\text{-}C^-$ embryos bears regions of sclerotized cuticle (as in the meso- and metathorax) whereas $Antp^-$ Ubx^- embryos do not. This aspect of the phenotype suggests that Ubx^- mutations do not eliminate a *bithorax*-complex function which is normally active in the first abdominal segment. Taken together, all these results can be interpreted as evidence that the $Antp^+$ gene is normally active in all three thoracic segments, and the first seven abdominal segments, but is not active in the eighth abdominal segment. This conclusion is clearly illustrated by the result that the thoracic and first seven abdominal segments of $Antp^+$ embryos lacking both the Scr^+ and the entire *bithorax*-complex all differ from their counterparts in $Antp^-$ embryos similarly deficient for these other genes (right-most column), whereas the eighth abdominal segment does not (Figs 6D and 9B).

abdominal segments of esc^-; $BX\text{-}C^-$ embryos depends on the presence of the Scr^+ gene; however, no such dependence is observed in esc^+ embryos similarly deficient for the *bithorax*-complex. These results suggest that absence of the esc^+ gene product leads to inappropriate activity of the Scr^+ gene in the thoracic and abdominal segments in question, and hence to a novel function of the gene in these segments. It should be noted that inappropriate expression of the Scr^+ gene in these segments leads to a meso- to prothoracic transformation, as well as an apparent suppression of latent maxillary cirri. Both of these effects are consistent with the normal roles attributed to selective activity of the Scr^+ gene in the prothoracic and labial segments.

Role of the esc$^+$ *gene product in ensuring the selective expression of the* Antp$^+$ *gene*

The approach with the *Antennapedia*$^+$ (*Antp*$^+$) gene is the same as in the preceding section dealing with the Scr^+ gene; that is, to identify a segment in

Fig. 9. esc^+ and esc^- embryos lacking the Scr^+ and $Antp^+$ genes, and the entire *bithorax*-complex. A) Ventral aspect of the head and thorax of an esc^+ embryo lacking the Scr^+ and $Antp^+$ genes and the entire *bithorax*-complex; black arrow heads mark the anterior margins of the three thoracic and first abdominal segments. Note that each of these segments bears a pattern of ventral hairs similar to that of the prothorax of Scr^- embryos (as in Fig. 6C): that is, two bands of hairs similar to those normally found in the prothorax, but each having fewer rows of hairs (the posteriorly situated band of hairs is marked with large arrows). Note also that each segment bears bilaterally symmetric patches of sclerotized cuticle (slender arrows) which are highly refractile. Keilin's organs (*k*) and ventral pits (*p*) are indicated when in focus. B) Ventral aspect of the posterior abdominal segments of the embryos in A. Black arrow heads mark the anterior margins of the fifth, sixth, seventh, and eighth abdominal segments (labelled as in A). Note that all of these segments appear similar to each other and to the thoracic and first abdominal segments shown in A, the only exception being the eighth abdominal segment in which more prominent plates of sclerotized cuticle are formed at the posterior margin. Note also that the eighth abdominal segment appears similar to that of both Scr^+ and Scr^- embryos lacking *bithorax*-complex (Figs 5B and 6D). C) Ventral surface of an esc^- embryo lacking the Scr^+ and $Antp^+$ genes as well as the entire *bithorax*-complex. Black arrow heads mark the anterior margins of the three thoracic and first seven abdominal segments. The thoracic and first six abdominal segments each bear bilaterally symmetric plates of sclerotized cuticle close to the posterior margin of the segment (note that each black arrow head lies immediately posterior to a plate of sclerotized cuticle formed in the adjacent anterior segment). These sclerotized cuticular plates resemble the normal mouth hooks (*h*) as well as the rudimentary mouth hooks (*h'*) which appear in the labial segment (as in esc^- embryos lacking both the Scr^+ gene and the *bithorax*-complex (Fig. 7A)). Note also that like both the normal mouth hooks and the rudimentary mouth hooks formed in the labial segment, the sclerotized cuticular plates in the thoracic and abdominal segments are sometimes associated with rudimentary cirri (*c'*; indicated when present and in the plane of focus). Each of the thoracic and first seven abdominal segments also bears a region of vertically ridged cuticle lying between the plates of sclerotized cuticle; these structures are labelled *T1, T2, ...A6,* and *A7* according to the segment bearing them. Finally, note that each thoracic and abdominal segment also bears a posteriorly situated band of fine hairs (large arrows), and in some cases, a few incomplete rows of thicker hairs at the anterior margin. Magnifications: A, B = ×275; C = ×300.


9A
k
k
k
p
B
h
c
c'
h
c'
c'
T1
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T2
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T3
c'
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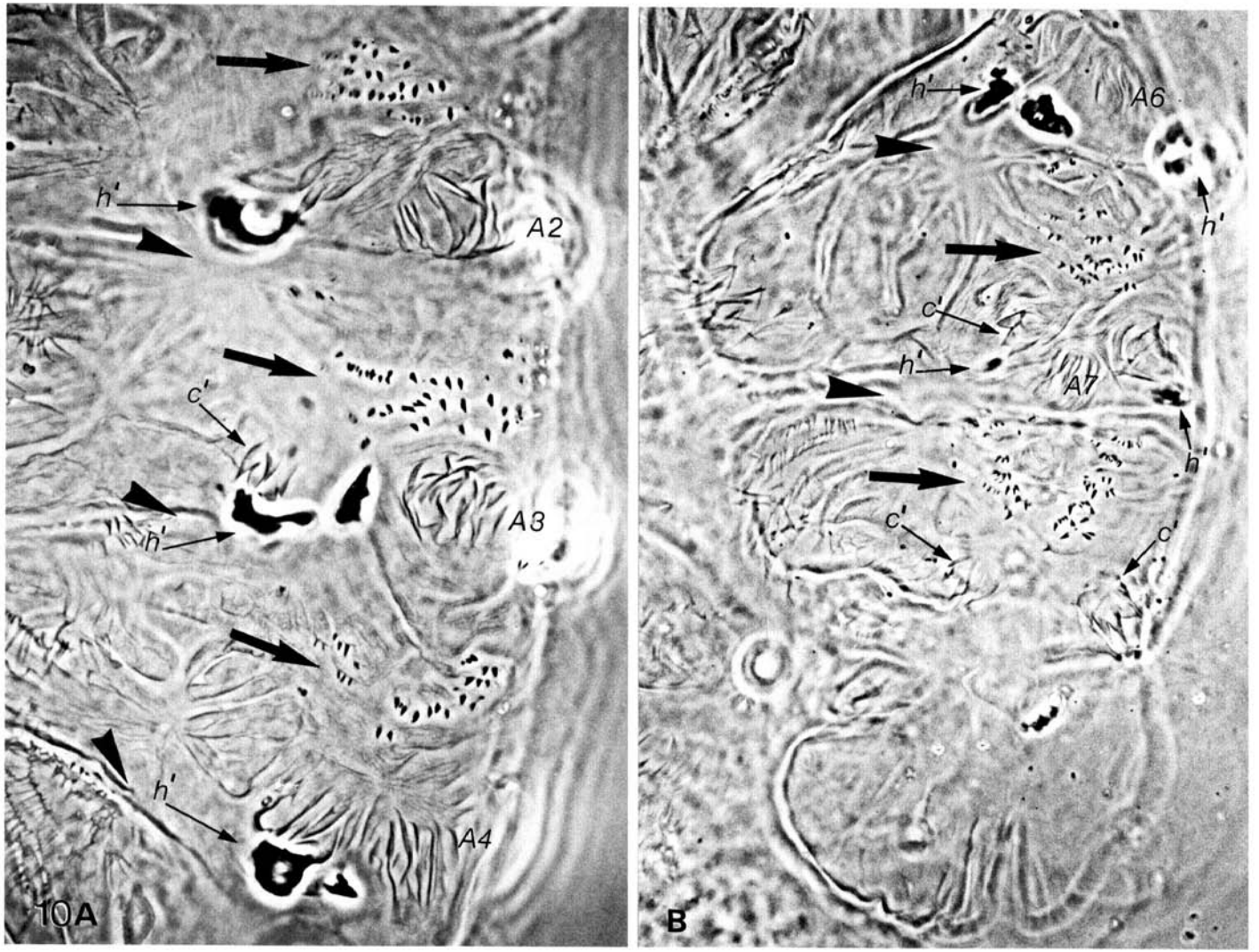
A1
A2
A3
A4
c'
A5
A6
A7
C


h'
A2
c'
A3
h'
h'
A4
10A
h'
A6
h'
c'
h'
A7
h'
c'
c'
B

which the *Antp*$^+$ gene is normally not required, and to test whether absence of the *esc*$^+$ gene product leads to a novel function of the *Antp*$^+$ gene in this segment.

The phenotype of *esc*$^+$ embryos homozygous for *Antp*$^-$ mutations has been described by Wakimoto & Kaufman (1981). They report that embryos homozygous for apparent null mutations of this locus develop into first instar larvae in which both the meso- and metathorax appear partially transformed into prothoracic segments. Since only the meso- and metathoracic segments of these embryos appear abnormal, it seems superficially that the *Antp*$^+$ gene is normally required in only these segments.

To examine the possibility that the *Antp*$^+$ gene is normally required not only in the meso- and metathorax, but also in other segments, the phenotypes of *Antp*$^-$ embryos lacking part or all of the *bithorax*-complex, the *Scr*$^+$ gene, or both the *Scr*$^+$ gene and the entire *bithorax* complex have been examined. The results obtained are described in Fig. 8 and can be summarized as follows. In the absence of both the *Scr*$^+$ gene and the entire *bithorax*-complex, the phenotypes of all three thoracic segments as well as the first seven abdominal segments appear to depend on the presence of the *Antp*$^+$ gene; however, the phenotype of the eighth abdominal segment does not. This result can be interpreted as evidence that the *Antp*$^+$ gene is normally active in the thoracic and first seven abdominal segments, but inactive in the eighth abdominal segment. Consequently, if loss of the *esc*$^+$ gene product leads to inappropriate expression of the *Antp*$^+$ gene in segments in which it is normally inactive, one might expect to see a novel function of the *Antp*$^+$ gene in the eighth abdominal segment.

esc$^-$; *Antp*$^-$ embryos appear identical in phenotype to standard *esc*$^-$ embryos, thereby providing no evidence that the *esc*$^+$ gene product is required for the correct expression of the *Antp*$^+$ gene. However, the results of deleting the *Scr*$^+$ gene as well as the *bithorax*-complex in *Antp*$^-$ embryos indicate that gene activity at either locus might obscure a requirement for the *Antp*$^+$ gene. Hence, to test whether or not the *esc*$^+$ gene product is required for the correct expression of the *Antp*$^+$ gene, it is necessary to perform the test in the absence of both the *Scr*$^+$ gene and the entire *bithorax*-complex.

Fig. 10. Ventrolateral aspects of the abdominal segments of *esc*$^-$ embryos lacking the *Antp*$^+$ and *Scr*$^+$ genes as well as the *bithorax*-complex. A) The second, third, and fourth segments. Black arrow heads mark the anterior margins of the third, fourth and fifth segments. Note the presence of sclerotized cuticular plates (*h'*) in each segment as well as posteriorly situated bands of fine hairs (large arrows), and regions of vertically ridged cuticle (labelled *A2*, *A3*, and *A4*). Note also the presence of rudimentary cirri (*c'*) associated with the sclerotized plates in segment three. B) The seventh and eighth segments. Black arrow heads mark the anterior margins of the seventh and eighth segments. The sixth and seventh segments bear plates of sclerotized cuticle (*h'*) and vertically ridged cuticle (*A6, A7*), but the eighth segment does not. However, the seventh and eighth segments both bear a posteriorly situated band of fine hairs (large arrows) as well as rudimentary cirri. Magnifications; A, B, =×650.

The phenotypes of all of the thoracic and first eight abdominal segments of *esc*$^-$; *Scr*$^-$ *BX-C*$^-$ embryos clearly depend on whether or not the *Antp*$^+$ gene is present. First, none of the thoracic or abdominal segments of *esc*$^-$; *Scr*$^-$ *BX-C*$^-$ embryos show bilaterally symmetric regions of sclerotized cuticle (Fig. 7); however, the thoracic and first seven abdominal segments of *esc*$^-$; *Scr*$^-$ *Antp*$^-$ *BX-C*$^-$ embryos bear such sclerotized regions (Figs 9, 10). Also, in many cases, these regions resemble mouth hooks of the wild-type larva and are often associated with other rudimentary mouthpart structures (e.g., rudimentary cirri and a ridged cuticle resembling a portion of the normal cephalopharyngeal skeleton (Figs 9, 10)). Curiously, the eighth abdominal segment of such embryos does not bear bilaterally symmetric plates of sclerotized cuticle, though rudimentary cirri are often formed (Fig. 10). Second, in *esc*$^-$; *Scr*$^-$ *BX-C*$^-$ embryos, the pattern of ventral hairs in each segment (including the eighth abdominal segment) appears to be primarily mesothoracic in character (Fig. 7); however, in *esc*$^-$; *Scr*$^-$ *Antp*$^-$ *BX-C*$^-$ embryos, the pattern of ventral hairs appears to be partially prothoracic in character, most notably, a square band of hairs characteristic of the second (posterior) band of hairs of the normal prothorax, appears in each segment of the thorax and abdomen (Figs 9, 10). Thus, the phenotype of the eighth abdominal segment depends on the presence or absence of the *Antp*$^+$ gene in *esc*$^-$; *Scr*$^-$ *BX-C*$^-$ embryos; however, no such dependence is observed in *esc*$^+$; *Scr*$^-$ *BX-C*$^-$ embryos. These results can be interpreted as evidence that in the absence of the *esc*$^+$ gene product, the *Antp*$^+$ gene functions inappropriately in the eighth abdominal segment.

Role of the esc$^+$ *gene product in ensuring the selective expression of homeotic genes other than the* bithorax*-complex genes, and the* Antp$^+$ *and* Scr$^+$ *genes*

If the early role of the *esc*$^+$ gene product was restricted to ensuring the correct expression of only the genes of the *bithorax*-complex, and the *Antp*$^+$ and *Scr*$^+$ loci, then one would predict that the presence or absence of the *esc*$^+$ gene product would be irrelevant in embryos lacking all of these homeotic genes. However, *esc*$^-$ embryos lacking all of these genes differ in at least two respects from corresponding *esc*$^+$ embryos (Figs 9, 10). First, all of the thoracic and first seven abdominal segments, as well as the labial segment, bear rudimentary head structures such as the cirri, not found on corresponding *esc*$^+$ embryos. In addition, the paired regions of sclerotized cuticle present in *esc*$^-$ embryos now approach the appearance of mouth hooks. Second, as in *esc*$^-$; *Df(3R)P9* embryos, a segment of the head (possibly the antennal segment) may be transformed into a thoracic-like segment, although this transformation is difficult to ascertain with certainty. Equivalent results were also obtained when a small deficiency deleting both the *Antp*$^+$ and *Scr*$^+$ gene, *Df(3R)4Scb* (Jurgens, unpublished), was used instead of the *Antp*$^{Ns+RC3}$ and *Scr*^{13A} mutations; however, the phenotypes observed are complicated by the absence of the *R14* gene (Lewis *et al.* 1980*a*,*b*; Wakimoto & Kaufman, 1981) which results in pairwise fusion of the segments.

Thus, the phenotypes of *esc*$^-$ and *esc*$^+$ embryos lacking the *Antp*$^+$, *Scr*$^+$, and

all of the *bithorax*-complex genes are not identical, suggesting the possibility that other, as yet unidentified homeotic genes (perhaps genes involved in the determination of head segments) may also require the esc^+ gene product for their correct expression.

DISCUSSION

The process of segmental determination requires the selective activation and continuous activity of small numbers of homeotic genes such as the *bithorax*-complex genes in particular segments. However, the mechanism by which the correct combinations of active and inactive homeotic genes are initially established in each segmental primordium is mysterious. In a previous study (Struhl, 1981*a*), I described the existence of a gene product which plays a critical role in this process. Specifically, I showed that the product of the esc^+ gene is required early in development for ensuring the correct pattern of expression of most, or all, of the *bithorax*-complex genes. Here, I examine whether the esc^+ gene product has a more general role in ensuring the correct expression of all segment-specific homeotic genes. To this end, I have described the phenotype of embryos lacking the esc^+ gene product, and then asked to what extent this phenotype depends on the presence of other homeotic genes. I discuss first the dependence of the esc^- phenotype on the number of copies of the *bithorax*-complex and Pc^+ genes, second, the evidence that the esc^+ gene product is necessary for initiating the correct patterns of expression of homeotic genes other than those of the *bithorax*-complex (e.g., the Scr^+ and $Antp^+$ genes), and third, a tentative outline of the roles of the products of all these genes in the process of segmental determination.

Dependence of the esc$^-$ *phenotype on the number of copies of* bithorax-*complex and* Pc$^+$ *genes*

bithorax-*complex*

In embryos lacking the esc^+ gene product, the extent to which most segments appear to be transformed towards the eighth abdominal segment increases as the number of doses of the *bithorax*-complex rises from one to two to four. Moreover, a similar dose dependence is observed when only fragments of the *bithorax*-complex are used. Thus, the extent of the esc^- phenotype appears to be directly proportional to the number of copies of the *bithorax*-complex whether above or below the normal two copies.

One explanation for this dose dependence is as follows. Previous studies of esc^- embryos lacking part or all of the *bithorax*-complex have established that absence of the esc^+ gene product causes many, if not all, of the *bithorax*-complex genes to become active inappropriately in segments in which they are normally inactive (Struhl, 1981*a*). However, these studies do not establish that the *bithorax*-complex genes are fully active in these segments, but only that they are

at least partially active. Indeed, as described here in detail, most segments of *esc*$^-$ embryos do not form perfect replicas of the eighth abdominal segment as might be expected if all of the *bithorax*-complex gene were fully expressed in all segments, but rather, approach the pattern of this segment only to varying degrees. It is therefore possible that in the absence of the *esc*$^+$ gene product, the *bithorax*-complex genes are only partially derepressed in segments in which they are normally not active. Under these conditions, the degree to which anterior segments are transformed into eighth abdominal segments might be directly proportional to the amounts of *bithorax*-complex gene products synthesized in each segment, and hence, proportional to the number of copies of the *bithorax*-complex present. Interestingly, the eighth abdominal segment of *esc*$^-$ embryos appears indistinguishable from that of wild-type embryos suggesting that all the *bithorax*-complex genes are expressed at their normal levels in this segment.

A second explanation which should be considered is that other homeotic genes outside of the *bithorax*-complex become active inappropriately in *esc*$^-$ embryos, and hence modify the transformed phenotype resulting from inappropriate expression of the *bithorax*-complex genes. If, for example, all segment determining genes become active in all segments, they may specify a novel segment type with characteristics influenced primarily, but not completely, by the *bithorax*-complex genes. Consequently, the degree to which the genes of the *bithorax*-complex influence the determined state of the segment may be directly proportional to the abundance of their gene products relative to the products of the other homeotic genes. This second explanation, though it may in part explain the observed dosage dependence, cannot explain the varying extent to which different segments in *esc*$^-$ embryos are transformed towards the eighth abdominal segment. This segment-specific variation suggests that all homeotic genes are not expressed equally in all segments of *esc*$^-$ embryos, and hence, as proposed above, that some are only partially derepressed in segments where they are normally inactive.

Pc$^+$ *gene*

The dependence of the *esc*$^-$ phenotype on the number of copies of the *Pc*$^+$ gene contrasts with the results obtained with the *bithorax*-complex in two respects. First, the mutant phenotype becomes progressively more extreme as the number of copies of the *Pc*$^+$ gene falls from two to one to zero, and hence is inversely proportional to gene dosage. Second, *esc*$^-$ embryos carrying four copies of the *Pc*$^+$ gene appear indistinguishable from embryos carrying two copies; that is, dose dependence is only observed when the number of copies is less than or equal to the diploid complement of two. To interpret these results, it is helpful to compare them one at a time with the results of altering the number of *Pc*$^+$ genes in otherwise wild-type flies. First, a minimum of two copies of the *Pc*$^+$ gene are required for normal development. When only one copy is present, segments of the adult fly are transformed towards more posterior segments in a fashion suggesting that some of the *bithorax*-complex genes are partially derepressed in more anterior segments

(Lewis, 1978; Capdevila & Garcia-Bellido, 1981; Duncan & Lewis, 1982). When no copies are present, most segments of the embryo are transformed towards the eighth abdominal segment, a phenotype which appears to result from indiscriminate expression of the *bithorax*-complex genes in most or all segments (Lewis, 1978; Duncan & Lewis, 1982). Thus, in otherwise wild-type animals, the *bithorax*-complex genes become progressively derepressed as the number of Pc^+ genes falls from two to one to zero. Returning to esc^- animals, the results of lowering the number of Pc^+ genes appears to follow the same pattern; that is, as the number of Pc^+ genes falls from two to one to zero, the transformation of most segments towards the eighth abdominal segments appears to become more extreme, suggesting that the extent to which the *bithorax*-complex genes are inappropriately expressed increases. Second, raising the number of copies of the Pc^+ gene from two to four in otherwise wild-type animals seems to have no effect (Duncan & Lewis, 1982). Assuming that the amount of Pc^+ gene product is directly proportional to the number of copies of the Pc^+ gene, this result indicates that superabundance of the Pc^+ gene product does not in itself cause incorrect expression of the *bithorax*-complex genes. Again, returning to esc^- embryos, the same result is observed; that is the mutant phenotype, and hence presumably the extent to which the *bithorax*-complex genes are incorrectly expressed, appears unaffected when the number of Pc^+ genes is increased from two to four. Thus, in both esc^+ and esc^- animals, the effects of altering the dosage of the Pc^+ gene on the expression of the *bithorax*-complex genes appears to be the same. These results suggest that altering the levels of the esc^+ and Pc^+ gene products have additive and independent effects on the expression of the *bithorax*-complex genes, and hence, that these gene products have independent roles. This conclusion is not surprising given the different temporal requirements, and hence different presumed roles, of the two gene products (Struhl, 1981*a*; Struhl & Brower, 1982).

Evidence that the esc$^+$ *gene product is required for ensuring the selective expression of the* Scr$^+$ *and* Antp$^+$ *genes, as well as at least one other homeotic gene outside of the* bithorax*-complex*

Indirect evidence that the esc^+ gene product is required for the correct expression of homeotic genes outside of the *bithorax*-complex comes from studies of the phenotype of esc^- embryos lacking the entire *bithorax*-complex (Struhl, 1981*a*; here). In these embryos, most segments develop differently from their counterparts in esc^+ embryos similarly deficient for the *bithorax*-complex (see Fig. 11), suggesting that absence of the esc^+ gene product causes inappropriate expression of other homeotic genes in most or all of these segments. To test this interpretation, I have examined whether the esc^+ gene product is required for the correct expression of particular homeotic genes outside of the *bithorax*-complex. The general approach has been as follows. First, identify those segments in which a given homeotic gene is normally not required. Then test whether absence of the esc^+ gene product causes a homeotic phenotype

in these segments which can be attributed to adventitious function of the gene in question. Such experiments have been performed for two genes of the *Antennapedia*-complex, Scr^+ and $Antp^+$. The pertinent results are summarized in Fig. 11 and discussed below.

Scr^+

The requirement for the esc^+ gene product is clearest in the case of the Scr^+ gene. Consequently, a thorough examination of the evidence in this case provides the best opportunity for assessing the validity of the experimental method and of the resulting conclusions. Both the embryonic (Wakimoto & Kaufman, 1981; here) and adult (Kaufman, Lewis & Wakimoto, 1980; Lewis *et al.* 1980*a*,*b*; Struhl, 1982) phenotypes of *Scr* mutations indicate that the wild-type gene product is normally required in the labial and prothoracic segments to specify their correct segmental states. However, no requirement for the Scr^+ gene has been detected in the meso- or metathorax, or in the abdominal segments, even when other homeotic genes which normally function in these segments (the

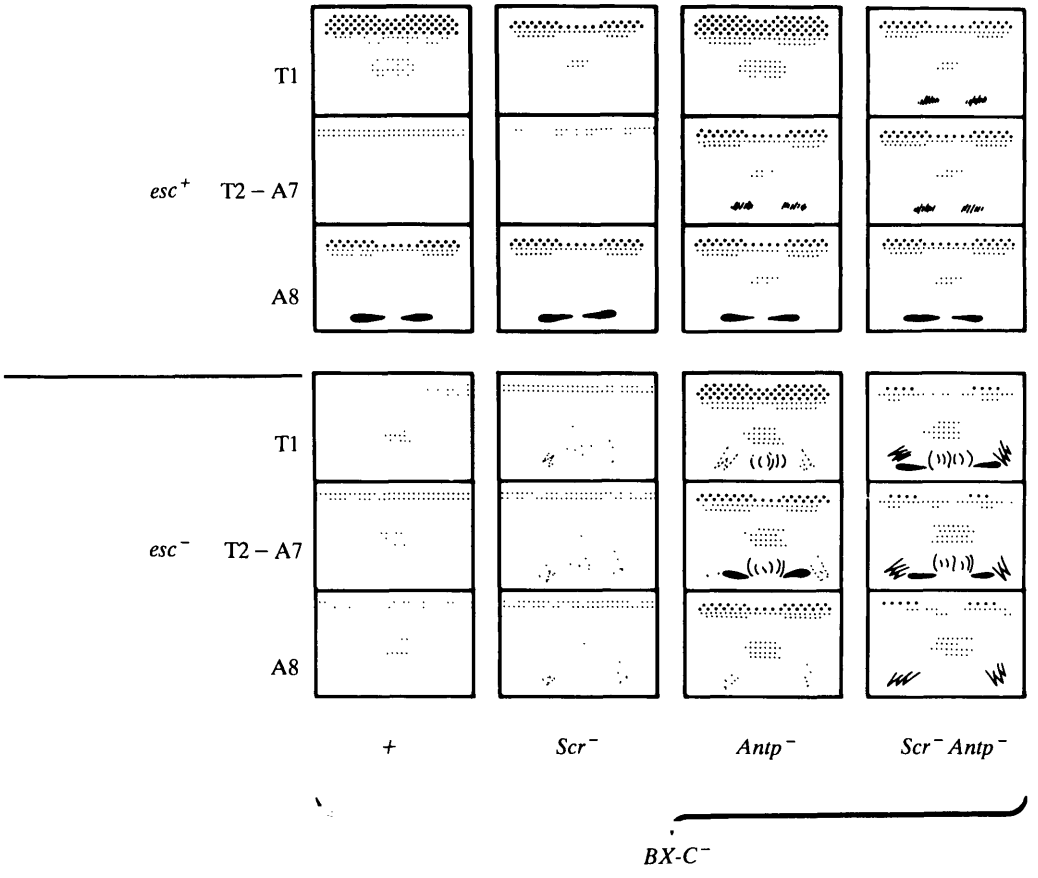


Fig. 11

bithorax-complex genes and the $Antp^+$ gene) are eliminated. In contrast, absence of the esc^+ gene product results in a detectable function of the Scr^+ gene in these segments. The simplest interpretation of this result is that the Scr^+ gene is normally active only in the labial and prothoracic segments, but that it becomes active inappropriately in all of the thoracic and abdominal segments when the esc^+ gene product is absent.

One criticism of this interpretation is that it depends on the assumption that absence of a detectable requirement for the Scr^+ gene in a given segment indicates that the Scr^+ gene is not active in that segment. Yet, absence of a detectable requirement is a negative result which may be misleading. For example, the number of cuticular structures which allow segmental status to be assessed in embryos is sufficiently small that some changes in segmental state caused by the absence of the Scr^+ gene might go undetected. Alternatively, it is possible that the normal activity of the Scr^+ gene in these segments might be masked or superfluous owing to the activity of other, as yet unidentified, homeotic genes. This criticism is countered by the nature of the novel function of the Scr^+ gene which arises in the absence of the esc^+ gene product. Normally, in $BX\text{-}C^-$ embryos, most of the thoracic and abdominal segments appear identical to the normal mesothorax. In esc^-; $BX\text{-}C^-$ embryos, the determined state of these segments now appears to be shifted towards the prothorax; however, if the Scr^+

Fig. 11. Summary of the evidence that the esc^+ gene product is required to prevent indiscriminate expression of the Scr^+ and $Antp^+$ genes, and possibly other homeotic genes involved in the determination of the head segments. The segmental phenotypes of the different mutant genotypes are displayed in an upper (esc^+) and lower (esc^-) matrix in which the prothorax (T1), all segments between the prothorax and eighth abdominal segment (T2–A7), and the eighth abdominal segment (A8) are rows, and the mutant genotypes, columns. All of the phenotypes diagrammed in the first (+), second (Scr^-) and fourth (Scr^- $Antp^-$) columns of both matrices are shown in detail in Figs 5, 6, 7, 9 and 10. For simplicity, only ventral hair patterns, and rudimentary mouthpart structures (bilaterally symmetric regions of sclerotized cuticle (Figs 8, 9), cirri (Figs 7, 9, 10), mouth hooks (Figs 9, 10), and vertically ridged cuticle resembling part of the cephalopharyngeal skeleton (Figs 7, 9, 10)) are illustrated. As shown in Figs 5, 6, 7, 9, and 10, the phenotypes are somewhat variable; the diagrams in this figure are intended to illustrate the average phenotype observed. Occasional as opposed to frequent appearance of cirri and vertically ridged cuticle is indicated by drawing these structures with dotted rather than continuous lines. The upper panel summarizes the evidence that the Scr^+ gene is normally not required in the meso-, and metathorax as well as all of the abdominal segments (i.e., absence of the Scr^+ gene does not alter the phenotype of any of these segments even when the $Antp^+$ gene and the entire *bithorax*-complex are removed) and the evidence that the $Antp^+$ gene is not required in the eighth abdominal segment (i.e., the phenotype of the eighth abdominal segment does not depend on the presence or absence of the $Antp^+$ gene, even when both the Scr^+ gene and the *bithorax*-complex genes are absent). The lower panel summarizes the evidence that adventitious functions of these genes are found in these segments in the absence of the esc^+ gene product (i.e., in each segment in which these genes do not appear to be required in esc^+ embryos, the phenotype in esc^- embryos now depends on whether they are present or absent).

gene is also absent, it is shifted back towards the mesothorax. Thus, in *esc*$^-$; *BX-C*$^-$ embryos, the *Scr*$^+$ gene functions abnormally to shift any segment which would otherwise have been a mesothoracic segment towards being a prothoracic segment. This abnormal function exactly parallels the normal function of the *Scr*$^+$ gene in the prothorax of both wild-type and *BX-C*$^-$ embryos, and hence, can be attributed to adventitious expression of the normal gene function in the wrong segments. Thus, the similarity between the normal and adventitious functions of the *Scr*$^+$ gene observed in *esc*$^+$ and *esc*$^-$ embryos provides positive evidence that this function is normally absent in most of the thoracic and abdominal segments.

A second criticism is that a novel function of the *Scr*$^+$ gene is observed only when the *bithorax*-complex genes are eliminated. Thus, strictly speaking, the results indicate only that the *esc*$^+$ gene product is required for the correct expression of the *Scr*$^+$ gene in the absence of the entire *bithorax*-complex. In principle, one could argue that absence of the *bithorax*-complex creates an artefactual situation in which the *esc*$^+$ gene product is required for the correct expression of the *Scr*$^+$ gene, whereas normally, no such requirement exists. However, a corollary of this argument is that activity of the *bithorax*-complex normally ensures the correct expression of the *Scr*$^+$ gene. Because the *esc*$^+$ gene product is required for the correct expression of the *bithorax*-complex, this argument leads ultimately to the conclusion that the correct pattern of expression of *Scr*$^+$ gene would still depend, albeit indirectly, on the function of the *esc*$^+$ gene product.

Antp$^+$

The evidence that the *esc*$^+$ gene product is required for the selective expression of the *Antp*$^+$ gene function is as follows. Based on the embryonic phenotypes of *Antp*$^-$ mutations alone, and together with *Scr*$^-$ mutations, or various deficiencies of the *bithorax*-complex, the *Antp*$^+$ gene appears normally to be required in all of the thoracic segments and in the first seven abdominal segments, but not in the eighth abdominal segment. However in *esc*$^-$ embryos lacking both the *Scr*$^+$ gene and the entire *bithorax*-complex, the homeotic phenotype of the eighth abdominal segment now depends on the presence or absence of the *Antp*$^+$ gene. Subject to the reservations discussed above in the case of the *Scr*$^+$ gene, these results suggest that the *Antp*$^+$ gene function is normally not active in the eighth abdominal segment, but that it is expressed adventitiously in this segment if the *esc*$^+$ gene product is absent.

Other genes

The evidence that the *esc*$^+$ gene product is required for the correct expression of at least one other homeotic gene outside of the *bithorax*-complex, and the *Antp* and *Scr* loci is identical to that initially provided for genes outside of the *bithorax*-complex. That is, in the absence of both the *Scr*$^+$ and *Antp*$^+$ genes, as

well as the entire *bithorax*-complex, the homeotic phenotype still depends on the presence or absence of the esc^+ gene product. This result suggests that at least one other homeotic gene also depends on the esc^+ gene product for its correct expression. It is interesting that the effect of inappropriately expressing this gene (or genes) appears to be an enhancement of mouthpart structures appearing in most segments of Scr^- $Antp^-$ embryos lacking the *bithorax*-complex. This phenotype suggests the possibility that the additional homeotic gene, or genes, may be involved normally in the specification of head segments.

Tentative outline of the temporal and spatial requirements for the esc^+, Pc^+, bithorax-*complex,* Scr^+, *and* $Antp^+$ *gene products in the process of segmental determination*

Fig. 12 summarizes a tentative outline of the temporal and spatial requirements for the homeotic genes discussed in this paper, and is based on embryonic phenotypes described here, and elsewhere (Lewis, 1978; Struhl, 1981*a*, Wakimoto & Kaufman, 1981; Duncan & Lewis, 1982). In this outline, the process of segmental determination is interpreted in terms of the compartment hypothesis (Garcia-Bellido, 1975; Crick & Lawrence, 1975; Lawrence, 1981). That is, during or soon after the cellular blastoderm stage, the embryo is subdivided into a series of adjacent primordia of similar size. In response to their relative position in the anteroposterior axis, cells in each primordium initiate a particular combination of active and inactive homeotic genes ('selector' genes in Garcia-Bellido's terminology). During subsequent development, the correct combinations of active and inactive selector genes are maintained continuously in the descendant cells of each segmental polyclone, thereby specifying the appropriate developmental pathway. It should be noted that this outline makes no allowance for the possibility that the products of some segmental selector genes may regulate the expression of other such genes, either in a quantitative or qualitative fashion. Such regulatory interactions may well occur (Struhl, 1982); however, they cannot be readily assayed until molecular probes for the expression of these genes are available. Consequently, the outline represents a formal description of the genetic results in which it is assumed that cross regulation does not occur between different members of the segmental selector genes except in those few cases where such interactions are supported by independent genetic evidence (Struhl, 1982). Similarly, this model makes no allowance for the possibility that the levels and kinds of gene products expressed by some segmental selector genes may vary between segments in which these genes are normally active, or during the development of a single segment. Again, such variations in function in time and space may well occur (e.g., Morata & Kerridge, 1981; Kerridge & Morata, 1982; Struhl, 1981*b*, 1982) but they cannot be described in detail until molecular probes are available. Bearing these constraints in mind, the particular roles of the esc^+, Pc^+, and segmental selector genes can be interpreted according to this model as follows.

esc

As shown here and elsewhere (Struhl, 1981*a*), the *esc*$^+$ gene product appears to be required early in development to ensure the selective expression of all of the segment-specific homeotic genes which have been tested. In the absence of the *esc*$^+$ gene product, *all* of these genes appear to be expressed indiscriminately in most segments of the body. Finally, loss of the *esc*$^+$ gene product after a discrete early period of embryogenesis appears to have relatively little effect on segmental determination (Struhl & Brower, 1982). Taken together, these results suggest that the *esc*$^+$ gene product is part of a negative regulatory mechanism

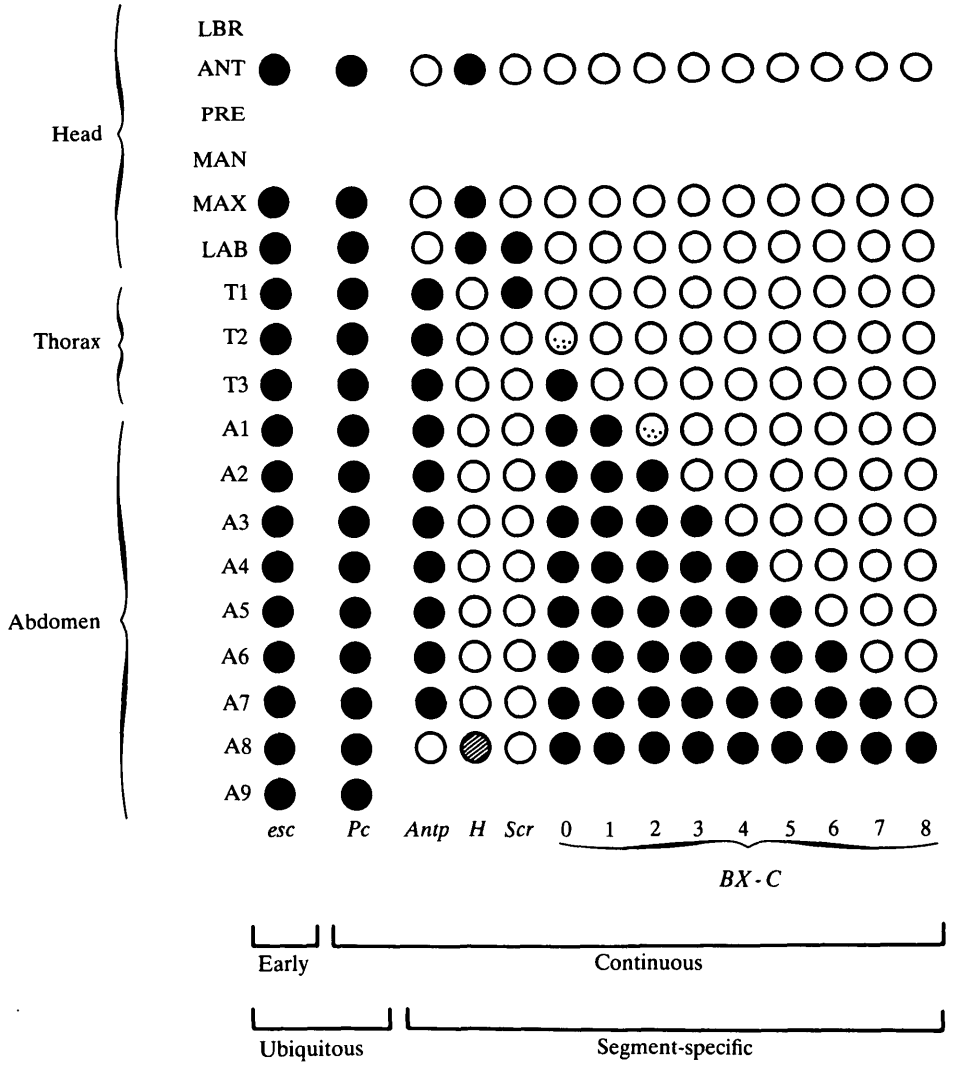


Fig. 12

which keeps or turns off particular combinations of selector genes when the segmental polyclones are first established. For example, it could be part of a mechanism which measures the amount of a graded positional cue, and then dictates which segmental selector genes are to be inactivated, or not activated, accordingly. Alternatively, it may be involved in a subsequent step in which selector genes not to be expressed are labelled, or otherwise regulated, such that they remain inactive in all the descendant cells (see Struhl & Brower, 1982). Whatever the exact nature of the early role of the esc^+ gene product, it is important to stress that it is part of the initial process by which the position of an embryonic cell is translated into a heritable commitment to develop as part of a particular segment.

Fig. 12. Tentative outline of the temporal and spatial requirements for the esc^+, Pc^+, *bithorax*-complex (*BX-C*), Scr^+ and $Antp^+$ gene products in the process of segmental determination. The normal realms of action of the products of all of these genes (see text) are diagrammed as a matrix in which the segments are rows, and the genes, columns. Note that each segment requires a unique combination of active (closed circles) and inactive (open circles) gene products. Hence, the determined state of each segment may be said to be specified by a unique combinatorial code word of on and off genes. Note also that the esc^+ and Pc^+ gene products appear to be required in most, or all, segments, and that they are required at different times. As described in the text, the products of both of these genes are required for the correct, as opposed to indiscriminate, expression of the segment-specific homeotic genes. However, their different temporal requirements, as well as their functional independence, suggest that they have separate roles in initiating and maintaining the correct combinations of active and inactive segmental selector genes in each segment. The head is thought to be composed initially of six embryonic segments (Anderson, 1972; Rempel, 1975; Struhl, 1981*c*) designated the labral (*LBR*), antennal (*ANT*), premandibular (*PRE*), mandibular (*MAN*), maxillary (*MAX*), and labial (*LAB*) segments. Little is known about genes controlling segmental specification in the labral, premandibular, and mandibular segments. The presence of an extra transformed abdominal segment posterior to the eighth abdominal segment in esc^- embryos indicates the presence of a latent ninth abdominal segment in the normal embryo, though little is known about the genes specifying its determined state. In addition to the esc^+, Pc^+, *bithorax*-complex, Scr^+ and $Antp^+$ genes, a set of hypothetical genes involved in specifying segmental determination in the head are indicated by *H*. Aside from *a priori* considerations, the existence of such genes is suggested by the phenotype of esc^- embryos lacking the Scr^+, $Antp^+$ and *bithorax*-complex genes (see text). The possibility that some or all of these head-determining genes may normally be active in the eighth abdominal segment (indicated by a light shaded circle) is suggested by the hypothesis that the expression of these genes is controlled by the presence or absence of $Antp^+$ gene function. However, it is possible that other genes regulate the expression of these head-determining genes in this segment such that they remain inactive even though the $Antp^+$ gene is also normally inactive. The *bithorax*-complex has been subdivided into nine genetic elements normally required in some but not other segments of the thorax and abdomen (see text). Evidence presented in Fig. 8 suggests the possibility that genes *0* and *2* may function to some extent in the mesothorax and first abdominal segment respectively (indicated by lightly shaded circles). Finally, it should be stressed that this is a simplified outline. It is likely that other segment-specific homeotic genes remain to be identified, and that the rules underlying the genetic specification of each segment are considerably more complex than indicated.

Pc

As in the case of the *esc*$^+$ gene product, the product of the *Pc*$^+$ gene appears to be required in most or all segments to ensure the correct expression of the *bithorax*-complex genes, and possibly other homeotic genes (Lewis, 1978; Capdevila Garcia-Bellido, 1981; Duncan & Lewis, 1982). Moreover, in the absence of the *Pc*$^+$ gene product, selective expression of these homeotic genes yields to indiscriminate expression. However, absence of the *Pc*$^+$ gene product during larval development appears to have the same effect as absence of the product during embryonic development; namely, the transformation of most segments towards a terminal abdominal segment (Struhl, 1981*a;* Duncan & Lewis, 1982). Thus, unlike the product of the *esc*$^+$ gene, the *Pc*$^+$ gene appears to play the same role in preventing indiscriminate expression of the segmental selector genes throughout development. These results can be interpreted as evidence that the *Pc*$^+$ gene product is part of the mechanism which allows the correct pattern of homeotic gene expression to be inherited each time a cell divides. For example, just as the *esc*$^+$ gene product may be required initially to label segmental selector genes which are not to be expressed, the *Pc*$^+$ gene product may be required to propagate this label each time the genome replicates, or to prevent the expression of segmental selector genes bearing such labels. Alternatively, stable expression of the correct segmental selector genes may not be controlled by a mechanism involving *cis*-acting labels, but rather by some form of *trans*-acting regulation (e.g., once active, the products of the segmental selector genes might act as positive regulators of their own expression, creating a stable feedback loop). Accordingly, the *Pc*$^+$ gene product may act to ensure that whatever control circuits are set up early in embryonogenesis remain stable during subsequent development. In either case, absence of the *Pc*$^+$ gene product any time during development would result in indiscriminate expression of all segmental selector genes. It should be noted that this view of the role of the *Pc*$^+$ gene is at odds with the proposition (Lewis, 1978; Duncan & Lewis, 1982) that the *Pc*$^+$ gene product is distributed in a graded fashion both within segments and between segments, and that it directly controls the spatial pattern of expression of the *bithorax*-complex genes by binding with differential affinities to their promoters and thereby repressing their expression to different degrees. One weakness of this rather elaborate model is illustrated by experiments in which the number of copies of the *Pc*$^+$ gene is raised or lowered. As discussed above, altering the number of copies of the *Pc*$^+$ gene, whether in *esc*$^+$ or *esc*$^-$ animals, appears to affect the expression of the *bithorax*-complex when the number falls beneath two, but not when it is raised to four. Assuming that the amount of the *Pc*$^+$ gene product is directly proportional to the number of copies of the gene, this result indicates that the expression of the *bithorax*-complex is not rigidly controlled by the amount of *Pc*$^+$ gene product present. It therefore seems reasonable to adopt a simpler view of the *Pc*$^+$ gene product as an essential component in the process

of regulation for which there is an equal, minimum requirement in all segments.

bithorax-*complex*

Figure 12 presents a simplified view of the *bithorax*-complex drawn principally from the work of Lewis (Lewis, 1963, 1964, 1978, 1981, 1982; Duncan & Lewis, 1982). The complex comprises a series of genetic units each required in particular segments of the thorax and abdomen. With the exception of an isolated early function (Morata & Kerridge, 1981; Kerridge & Morata, 1982; here, see Fig. 8), none of the *bithorax*-complex genes appear to act in the mesothorax, suggesting that this segment represents a ground state (Lewis, 1978; Struhl, 1982). In more posterior thoracic and abdominal segments, the selective activities of particular combinations of *bithorax*-complex genes appear to specify the unique determined states of each segment. Thus, in the metathorax, the activity of gene '0' specifies meta- as opposed to mesothoracic development, in the first abdominal segment, the additional activity of gene '1' specifies first abdominal, as opposed to metathoracic development, and so forth till the eighth abdominal segment. Studies at both the genetic (Lewis, 1963; 1964, 1978, 1981, 1982) and molecular levels (Bender *et al.* 1983) indicate that the situation is more complex than indicated by this simplistic model. However, the model may well be correct in broad outline.

Scr

As described above, the Scr^+ gene appears to act only in the prothoracic and labial segments, specifying their particular determined states as opposed to mesothoracic and maxillary states respectively (Kaufman *et al.* 1980; Lewis *et al.* 1980*a,b*; Wakimoto & Kaufman, 1981; Struhl, 1982). The role of the Scr^+ gene in the prothorax may be seen simply as an analogue of the role of the *bithorax*-complex genes in the metathoracic and abdominal segments – namely, to specify a particular segmental type instead of the ground state, mesothorax (Struhl, 1982). However, it should be noted that even apparent null alleles of the Scr^+ locus do not cause a clean transformation of the pro- to mesothorax in the embryo, but instead, transform the prothorax only partially towards the mesothorax (Wakimoto & Kaufman, 1981; this paper). This phenotype suggests the possibility that specification of the larval prothorax as distinct from the mesothorax may require the activities of other, as yet unidentified homeotic genes. The role of the Scr^+ gene in the labial segment is also somewhat difficult to interpret. One possibility is that a set of presently unidentified genes specifies the unique developmental paths followed by the head segments in a fashion comparable to that of the *bithorax*-complex genes in the thorax and abdomen. Just as the Scr^+ gene appears to raise the prothorax from an underlying, ground state towards the prothoracic level, it may similarly raise the labial segment from an underlying maxillary state. Presumably, the different responses of the labial and prothoracic segments to the loss of the Scr^+ gene reflects the selective activity

of at least one head-determining gene in the labial, but not the prothoracic, segment.

Antp

The role of the *Antp*$^+$ gene is uncertain principally because the phenotype of *Antp*$^-$ embryos cannot be easily interpreted. As described above (see also Fig. 8), the phenotypes of *Antp*$^-$ embryos lacking the *Scr*$^+$ gene, the entire *bithorax*-complex, or both, can be interpreted as evidence that the *Antp*$^+$ gene is normally required in all three thoracic segments as well as the first seven abdominal segments. Absence of the *Antp*$^+$ gene causes the transformation of any segment that would otherwise develop as a mesothoracic segment into a segment of novel character bearing in part a prothoracic pattern of ventral hairs and bilaterally symmetric regions of sclerotized cuticle (Wakimoto & Kaufman, 1981; here). This phenotype has been interpreted as a partial transformation of the meso- towards the prothorax, and hence, as evidence that the *Antp*$^+$ and *Scr*$^+$ genes have mutually exclusive functions and realms of activity, the *Antp*$^+$ gene specifying meso- as opposed to prothoracic development, and vica versa, the *Scr*$^+$ gene specifying pro- as opposed to mesothoracic development, (Wakimoto & Kaufman, 1981). However, there are several problems with this interpretation. First, it predicts that the *Antp*$^+$ gene should not be active in the prothorax; yet, the phenotypes of *Scr*$^-$ and *Scr*$^-$ *Antp*$^-$ embryos suggest that the *Antp*$^+$ gene is normally active in the prothorax (see Fig. 8). Second, paired regions of sclerotized cuticle are not normally found in the prothorax of wild-type embryos; hence, their presence in the transformed mesothorax of *Antp*$^-$ *BX-C*$^-$ embryos cannot be satisfactorily explained as a transformation of the mesothorax to prothorax. Third, if the *Scr*$^+$ gene functions in an analogous fashion to the *bithorax*-complex genes in specifying one segmental state (prothorax) as opposed to the ground state (mesothorax), it should not be possible to obtain prothoracic development without the *Scr*$^+$ gene. However, the apparent meso- to prothoracic transformation caused by absence of the *Antp*$^+$ gene appears identical whether or not the *Scr*$^+$ gene is present. Finally, as described below, absence of the *Antp*$^+$ gene in cells giving rise to the adult mesothorax does not result in meso- to prothoracic transformations, but rather in a transformation of some portions of the mesothorax into corresponding portions of the antenna. Thus, the interpretation of Wakimoto & Kaufman (1981) does not satisfactorily explain several aspects of both the embryonic and adult mutant phenotypes.

An alternative interpretation of the role of the *Antp*$^+$ gene has been proposed on the basis of the adult phenotypes resulting from absence of the *Antp*$^+$ gene (Struhl, 1981*b*; 1982). Absence of the *Antp*$^+$ gene results in the transformation of some portions of the second leg to corresponding portions of the antenna. Conversely, dominant mutations of the *Antp*$^+$ gene cause the reverse transformation of antenna to second leg (Lindsley & Grell, 1968; Kaufman *et al.*, 1980). These results suggest that the *Antp*$^+$ gene acts in the mesothorax to

prevent inappropriate expression of genes specifying antennal development, but not in the antenna itself, where these 'head-determining' genes are normally required (Struhl, 1981*b*). Absence of the *Antp*$^+$ gene in the pro- and metathorax can result in abnormal development of the first and third legs indicating that the *Antp*$^+$ gene is normally required in these segments. As in *Antp*$^-$ embryos which also lack the *Scr*$^+$ and *bithorax*-complex genes, the ventral portions of all three adult thoracic segments develop alike when the *Antp*$^+$, *Scr*$^+$, and *Ultrabithorax*$^+$ (*Ubx*$^+$) genes are removed together (i.e., all develop as second legs in which some portions are transformed into antenna). These results have led to the hypothesis that the *Antp*$^+$ gene has the same role in all three thoracic segments in preventing the inappropriate expression of homeotic genes involved in specifying the head segments (Struhl, 1982). This interpretation is consistent with some aspects of the embryonic phenotype, notably (i) the requirement for the *Antp*$^+$ gene in all three thoracic segments, (ii) the appearance of paired regions of sclerotized cuticle in the thoracic and abdominal segments of *Scr*$^-$ *Antp*$^-$ embryos lacking the *bithorax*-complex (these structures can be interpreted as rudimentary mouthpart structures resulting from inappropriate expression of one or more head determining genes) and (iii) the enhancement of mouthpart structures in these segments caused by removing the *Antp*$^+$ gene from *esc*$^-$; *Scr*$^-$ *BX-C*$^-$ embryos. However, it does not provide a satisfactory explanation for why the mesothorax appears partially transformed towards the prothorax in *Antp*$^-$ embryos, or for why the reverse transformation of the prothorax towards the mesothorax is found in *esc*$^-$; *BX-C*$^-$ embryos, but not in *esc*$^-$; *Antp*$^-$ *BX-C*$^-$ embryos (see Fig. 11). One major difficulty in interpreting these 'prothoracic' phenotypes is that, as described above, absence of the *Scr*$^+$ gene does not completely transform the prothorax of the embryo into the mesothorax. Although there are many explanations for this incomplete transformation, one possibility is that the genetic specification of the embryonic pro- and mesothorax differs from that of their adult counterparts. Hence, it is possible that the *Antp*$^+$ gene has an additional early role in this particular process which is distinct from its postulated role in regulating the expression of head-determining genes. However, whatever this role is, it cannot simply be to act in the mesothorax to specify meso- as opposed to prothoracic development. Thus, a consistent and satisfactory interpretation of the role of the *Antp*$^+$ gene may require more information about the roles and regulation of genes controlling the difference between the meso- and prothorax of the embryo.

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REFERENCES

BENDER, W., AKAM, M., KARCH, F., BEACHY, P. A., PEIFER, M., SPIERER, P., LEWIS, E. B., and HOGNESS, D. S. (1983). Molecular genetics of the bithorax complex of *D. melanogaster*. *Science*, in press.

CAPDEVILA, M. P., & GARCIA-BELLIDO, A. (1981). Genes involved in the activation of the bithorax complex of *Drosophila*. *Wilhelm Roux's Arch. devl Biol.* **190**, 339–350.

CRICK, F. H. C., & LAWRENCE, P. A. (1975). Compartments and polyclones in insect development. *Science* **189**, 340–347.

DENELL, R. E., HUMMELS, K. R., WAKIMOTO, B. T., & KAUFMAN, T. C. (1981). Developmental studies of lethality associated with the *Antennapedia* gene complex in *Drosophila melanogaster*. *Devl Biol.* **81**, 43–50.

DUNCAN, I. M. (1982). Polycomblike: a gene that appears to be required for the normal expression of the bithorax and Antennapedia complexes of *Drosophila melanogaster*. *Genetics*, **102**, 49–70.

DUNCAN, I., & LEWIS, E. B. (1982). Genetic control of body segment differentiation in *Drosophila*. In *'Developmental Order: Its Origin and Regulation'* S. Subtelny, ed.), pp 533–554. New York: Liss.

GARCIA-BELLIDO, A. (1975). Genetic contol of wing disc development in *Drosophila*. In *'Cell Patterning'*, (S. Brenner, ed.), *Ciba Foundation Symposium* **29**, pp. 161–182. Amsterdam, Oxford, New York: Associated Scientific Publishers.

GARCIA-BELLIDO, A. (1977). Homeotic and atavic mutations in insects. *Amer. Zool.*, **17**, 613–629.

HERTH, W., & SANDER, K. (1973). Mode and timing of body pattern formation (regionalization) in the early embryonic development of Cyclorrhapic Dipterns (*Protophormia, Drosophila*). *Wilhelm Roux Arch. entw Mech. Org.* **172**, 1–27.

INGHAM, P. W. (1981). *trithorax*: a new homeotic mutation of *Drosophila melanogaster*. II. The role of trx^+ after embryogenesis. *Wilhelm Roux' Archiv. devl Biol.* **190**, 365–369.

INGHAM, P. W., & WHITTLE, R. (1980). *trithorax*: a new homeotic mutation of *Drosophila melanogaster* causing transformations of abdominal and thoracic imaginal segments. I. Putative role during embryongenesis. *Molec. gen. Genet.*, **179**, 607–614.

KAUFMAN, T. C., LEWIS, R., & WAKIMOTO, B. (1980). Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homeotic gene complex in polytene chromosome interval 84A-B. *Genetics*, **94**, 115–133.

KERRIDGE, S., & MORATA, G. (1982). Developmental affects of some newly induced *Ultrabithorax* alleles of *Drosophila*. *J. Embryol. exp. Morph.* **68**, 211–234.

LAWRENCE, P. A. (1973). A clonal analysis of segment development in *Oncopeltus* (Hemiptera). *J. Embryol. exp. Morph.*, **30**, 681–699.

LAWRENCE, P. A. (1981). The cellular basis of segmentation in insects. *Cell* **26**, 3–10.

LEWIS, E. B. (1963). Genes and developmental pathways. *Amer. Zool.* **3**, 33–56.

LEWIS, E. B. (1964). Genetic control and regulation of developmental pathways. In *'The Role of Chromosomes in Development'* (M. Locke, ed.), pp. 231–252. New York: Academic Press.

LEWIS, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565–570.

LEWIS, E. B. (1980). New mutants: report of E. B. Lewis. *Drosoph. Inf. Serv.* **55**, 207–208.

LEWIS, E. B. (1981). Developmental genetics of the bithorax complex in Drosophila. In *'Developmental Biology Using Purified Genes'*. ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. XXIII (eds D. D. Brown, and C. F. Fox), pp. 269–288. New York: Academic Press.

LEWIS, E. B. (1982). Control of body segment differentiation in Drosophila by the bithorax gene complex. In *'Embryonic Development: Genes and Cells.'* (ed. M. Burger), New York: Alan Liss, Inc.

LEWIS, R. A., KAUFMAN, T. C., DENNELL, R. E., & TALLERICO, P. (1980*a*). Genetic analysis of the *Antennapedia* gene complex (*ANT-C*) and adjacent chromosomal regions of *Drosophila melanogaster*. I. Polytene chromosome segments 84B-84D. *Genetics* **95**, 367–381.

LEWIS, R. A. WAKIMOTO, B. T., DENELL., R. E., & KAUFMAN, T. C. (1980*b*). Genetic analysis of the *Antennapedia* gene complex (*ANT-C*) and adjacent chromosomal regions of *Drosophila melanogaster*. II. Polytene chromosome segments 84A-84B1,2.

LINDSLEY, D. L. & GRELL, E. L. (1968). Genetic variations of *Drosophila melanogaster*. *Carnegie Inst. Wash., Publ. No.* **627**.

LOHS-SCHARDIN, M., CREMER, C. & NÜSSLEIN-VOLHARD, C. (1979). A fate map of the larval epidermis of *Drosophila melanogaster*: localized defects following irradiation of the blastoderm with an ultraviolet laser microbeam. *Devl Biol.* **73**, 239–255.

MORATA, G. & GARCIA-BELLIDO, A. (1976). Developmental analysis of some mutants of the *bithorax* system of *Drosophila*. *Wilhelm Roux' Arch. devl Biol.* **179**, 125–143.

MORATA, G. & KERRIDGE, S. (1981). Sequential functions of the bithorax complex of *Drosophila*. *Nature* **290**, 778–781.

SCHOELLER, J. (1964). Recherches descriptives et expérimentales sur la céphalogenèse de *Calliphora erythrocephala* (Meigen), au cours de developpements embryogennaire et post-embryogennaire. *Arch. Zool. exp. Gen.* **103**, 1–216.

SLIFER, E. H. (1942). A mutant stock of *Drosophila* with extra sex-combs. *J. exp. Zool.* **30**, 31–40.

STRUHL, G. (1981*a*). A gene product required for correct initiation of segmental determination in *Drosophila*. *Nature* **293**, 36–41.

STRUHL, G. (1981*b*). A homeotic mutation transforming leg to antenna in *Drosophila*. *Nature* **292**, 635–638.

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

STRUHL, G. (1982). Genes controlling segmental specification in the *Drosophila* thorax. *Proc. natn. Acad. Sci., U.S.A.* **79**, 7380–7384.

STRUHL, G. & BROWER, D. (1982). Early role of the esc^+ gene product in the determination of segments in *Drosophila*. *Cell* **31**, 285–292.

SZABAD, J., SCHUPBACH, T. & WIESCHAUS, E. (1979). Cell lineage and development in the larval epidermis of *Drosophila melanogaster*. *Devl Biol.* **73**, 256–271.

TOKUNAGA, C. & STERN, C. (1965). The developmental autonomy of extra sex combs in *Drosophila melanogaster*. *Devl Biol.* **11**, 50–81.

TURNER, F. R. S. & MAHOWALD, A. P. (1979). Scanning electron microscopy of *Drosophila melanogaster* embryogenesis: III. Formation of the head and caudal segments. *Devl Biol.* **68**, 96–109.

VAN DER MEER, J. M. (1977). Optically clean and permanent whole mount preparation for phase-contrast microscopy of cuticular structures of insect larvae. *Drosoph. Inf. Serv.* **52**, 160.

WAKIMOTO, B. T. & 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.

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