The Homeotic Gene *Sex combs reduced* of *Drosophila melanogaster* Is Differentially Regulated in the Embryonic and Imaginal Stages of Development

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ABSTRACT

The *Sex combs reduced* (*Scr*) locus is unique among the genes contained within the Antennapedia complex (ANT-C) of *Drosophila melanogaster* in that it directs functions that are required for both cephalic and thoracic development in the embryo and the adult. Antibodies raised against protein encoded by *Scr* were used to follow the distribution of this gene product in embryos and imaginal discs of third instar larvae. Analysis of *Scr* protein accumulation in embryos hemizygous for breakpoint lesions mapping throughout the locus has allowed us to determine that sequences required for establishment of the *Scr* embryonic pattern are contained within a region of DNA that overlaps with the identified upstream regulatory region of the segmentation gene *fushi tarazu* (*ftz*). Gain-of-function mutations in *Scr* result in the presence of ectopic sex comb teeth on the first tarsal segment of mesothoracic and metathoracic legs of adult males. Heterozygous combinations of gain-of-function alleles with a wild-type *Scr* gene exhibit no evidence of ectopic protein localization in the second and third thoracic segments of embryos. However, mesothoracic and metathoracic leg imaginal discs can be shown to accumulate ectopically expressed *Scr* protein, implying a differential regulation of the *Scr* gene during these two periods of development. Additionally, we have found that the spatial pattern of *Scr* gene expression in imaginal tissues involved in the development of the adult thorax is governed in part by synapsis of homologous chromosomes in this region of the ANT-C. However, those imaginal discs that arise anteriorly to the prothorax do not appear to be sensitive to this form of gene regulation. Finally, we have demonstrated that the extent of *Scr* expression is influenced by mutations at the *Polycomb* (*Pc*) locus but not by mutant alleles of the *zeste* (*z*) gene. Taken together, our data suggests that *Scr* gene expression is differentially regulated both temporally and spatially in a manner that is sensitive to the structure of the locus.

THE morphogenetic pathways that determine body segment identity in *Drosophila melanogaster* involve intricate networks of gene action and interaction (AKAM 1987). The diversification of these segments is specified by the precise temporal and spatial expression of homeotic genes in the Antennapedia (ANT-C) and Bithorax (BX-C) complexes (LEWIS 1978; KAUFMAN, LEWIS and WAKIMOTO, 1980; LEWIS *et al.* 1980a,b; GEHRING and HIROMI 1986; KAUFMAN, SEEGER and OLSEN 1990). Three levels of homeotic gene control have been reported in the embryo. These are regulation by segment enumeration genes (DUNCAN 1986; INGHAM, ISH-HOROWICZ and HOWARD 1986; INGHAM and MARTINEZ-ARIAS 1986; WHITE and LEHMANN 1986; HARDING and LEVINE 1988; JACK, REGULSKI and MCGINNIS 1988), cross-regulatory interactions among genes within the ANT-C and BX-C (HAFEN, LEVINE and GEHRING 1984; HARDING *et al.* 1985; STRUHL and WHITE 1985), the significance of which remains questionable (GONZALES-REYES *et al.* 1990), and *trans*-regulatory interactions by the *Polycomb*-group and *trithorax*-group genes (JÜRGENS 1985; WEDEEN, HARDING and LEVINE 1986; DURA *et al.* 1988; KENNISON and TAMKUN 1988; SHEARN 1989; ZINK and PARO 1989; PHILLIPS and SHEARN 1990). The combinatorial action of homeotic gene control at these three levels ensures that a unique set of genes is expressed in each embryonic segment (SCOTT AND O'FARRELL 1986).

Several of the homeotic genes, including all of those resident in the two complexes, are known to contain a homeobox (MCGINNIS *et al.* 1984a,b; SCOTT and WEINER 1984). Sequence comparisons have revealed a high degree of similarity between the homeobox-containing genes of Drosophila and the mammalian transcription factors *Pit-1*, *OCT-1* and *OCT-2* (reviewed in SCOTT, TAMKUN and HARTZELL 1989), which have been demonstrated to function as DNA-binding proteins (BODNER *et al.* 1988; INGRAHAM *et al.* 1988; KO *et al.* 1988). Furthermore, structural examination of the Drosophila *Antennapedia* homeodomain by nuclear magnetic resonance spectroscopy has shown that the homeodomain contains a helix-turn-helix motif and subsequent functional analysis

has demonstrated that it confers a sequence-specific DNA-binding function (MÜLLER *et al.* 1988; OTTING *et al.* 1988). Taken together, the functional and molecular analyses have led to the conclusion that the homeotic genes of the ANT-C and BX-C control the expression of a battery of essential yet unidentified genes, the products of which further serve to convey identity to each segment.

One of these homeotic genes, *Sex combs reduced* (*Scr*), is located in the ANT-C. The expression pattern of *Scr* transcripts and protein in wild-type embryos and imaginal discs has been previously described (MARTINEZ-ARIAS *et al.* 1987; MAHAFFEY and KAUFMAN 1987; RILEY, CARROLL and SCOTT 1987; GLICKSMAN and BROWER 1988; LEMOTTE *et al.* 1989; MAHAFFEY, DIEDERICH and KAUFMAN 1989). Phenotypically, loss of Scr^+ function in embryos results in oppositely oriented prothoracic to mesothoracic and labial to maxillary transformations (WAKIMOTO, TURNER and KAUFMAN 1984; SATO, HAYES and DENELL 1985; PATTATUCCI, OTTESON and KAUFMAN 1991). Data from clonal analysis and viable allelic combinations reveal that Scr^+ function is similarly required for the correct development of the adult prothoracic and labial derivatives (WAKIMOTO and KAUFMAN 1981; PATTATUCCI, OTTESON and KAUFMAN 1991). This makes *Scr* unique among the ANT-C genes in that its function is required for both proper head and thoracic development. However, little is known about its regulation for any period of development. A major question yet to be answered for *Scr* is how this homeotic gene product is involved in conferring two completely different cellular identities in two discrete regions of the organism.

In the accompanying analysis, genetic lesions representing a graded phenotypic series that define a 75-kilobase region of DNA necessary for proper function of the *Scr* locus are characterized. An interesting paradox introduced by this study is that dominant gain-of-function lesions, when heterozygous with a wild-type *Scr* allele, result in an adult transformation of second and third thoracic segments to a first thoracic segment identity. However, these same lesions show no evidence of this conversion in embryonic cuticle preparations, but instead exhibit a moderate to strong loss of function phenotype (*i.e.*, labial to maxillary and prothorax to mesothorax). This raises the possibility that the regulation of the *Scr* locus may be dissimilar during the embryonic and larval periods of development. A comparable example of differential regulation at the *Ultrabithorax* locus has recently been reported (LITTLE, BYRD and BROWER 1990).

Although analysis of transcript accumulation for homeotic genes in both the ANT-C and BX-C has indicated that they are expressed throughout development, little is known about their postembryonic regulation or how the products of homeotic genes confer specific cellular identities in the adult. To address whether differential regulation of *Scr* takes place as development proceeds, we have investigated the expression pattern of *Scr* protein in embryos and imaginal discs of various allelic combinations at the *Scr* locus. The results of this analysis strongly support the hypothesis that control of *Scr* gene expression is qualitatively different in larval relative to embryonic development, and describes a novel regulatory mechanism governing larval expression of the *Scr* gene.

TABLE 1

Mutant alleles analyzed in this study

Mutant chromosome	Cytology	Source
T(2;3)Antp^{17},red e	T(2;3)84B2; 25F	A
Df(3R)BD5,e ca	Df(3R)84A1,2; B1,2	B
T(2;3)bw^{VDe3},Ubx bxd	T(2;3)59D; 81F	C
In(3R)Pc^3	In(3R)85CE; 88EF	D
T(2;3)pg^{map3}	T(2;3)84A4,5; 26DF	E
z^a	Normal	C
z^1	Normal	C

Descriptions of other alleles studied are presented in a companion study (PATTATUCCI, OTTESON and KAUFMAN 1991). Allele designations are as they will appear in *The Genome of Drosophila melanogaster* (LINDSLEY and ZIMM 1991). A, ABBOTT and KAUFMAN (1986); B, R. J. DIEDERICH and T. C. KAUFMAN (unpublished results); C, LINDSLEY and GRELL (1968); D, E. B. LEWIS (1980); E, M. A. PULTZ and T. C. KAUFMAN (unpublished results).

MATERIALS AND METHODS

Fly stocks: Stocks were grown on standard corn meal agar medium at 22°. In all experiments, wild-type third chromosomes were derived from the *Oregon-R* strain (represented as "+" in text and figures). Mutant alleles were maintained over the *TM6B* balancer chromosome (CRAYMER 1984), which is Scr^+ and carries the dominant larval marker *Tubby* (*Tb*).

Embryo staining: Embryos were collected and stained with polyclonal anti-*Scr* (MAHAFFEY and KAUFMAN 1987) according to the procedure of MAHAFFEY and KAUFMAN (1987). Embryos described in RESULTS were obtained from virgin Scr^{GOF}/*TM6B* females (where *GOF* indicates one of the eight gain-of-function alleles examined) mated to *Df(3R)BD5/TM6B* males, a deletion for the ANT-C (Table 1). The morphological aberrations that characterize *TM6B* homozygotes were known to us from a variety of other crosses using this balancer. However, to assure the proper of assignment genotype for each of the remaining three classes of embryonic phenotypes that were observed among stage 12 (CAMPOS-ORTEGA and HARTENSTEIN 1985) embryos produced by Scr^{GOF}/*TM6B* ♀ × *Df(3R)BD5/TM6B* ♂, examination of stained embryos from two additional groups of matings was necessary. The phenotypes of Scr^{GOF}/*TM6B* embryos were identified from the crosses Scr^{GOF}/*TM6B* ♀ × Scr^{GOF}/*TM6B* ♂ as the phenotypic class from each mating accounting for approximately 50% of the sample. Stained embryos from the *Df(3R)BD5/TM6B* stock were examined to determine the *Scr* expression pattern in *Df(3R)BD5/TM6B* heterozygotes. For all four nontranslocation *Scr* alleles (see below), the one remaining genotypic class, Scr^{GOF}/*Df(3R)BD5*, was primarily identified by general disorgani-

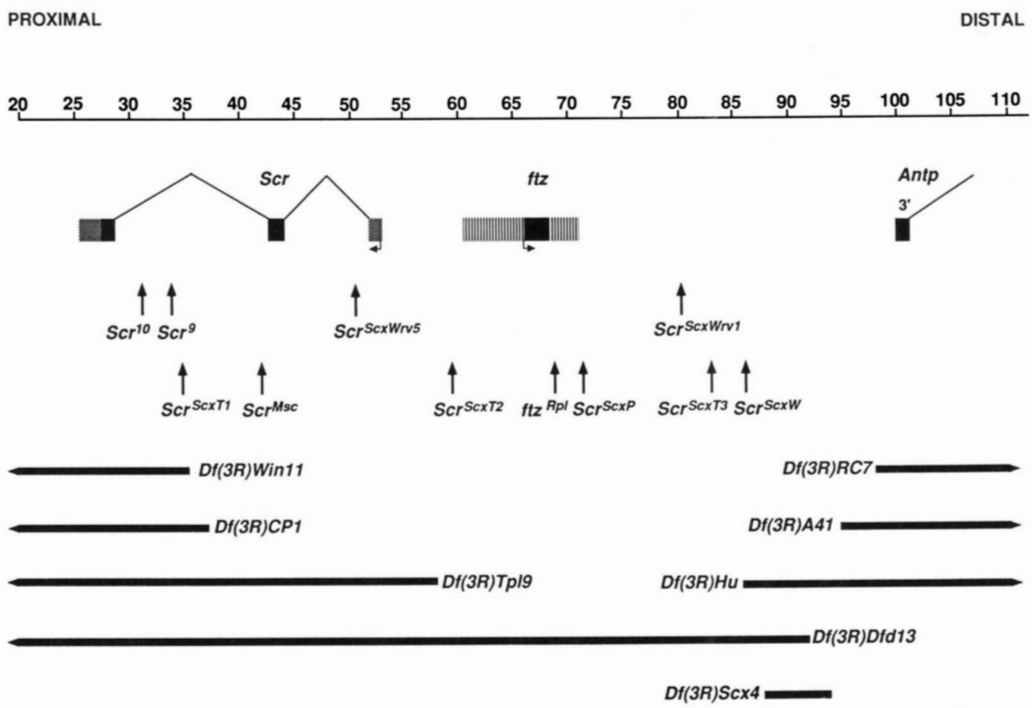


FIGURE 1.—*Scr-ftz* Molecular map. The coordinates (in kilobases) are based on a zero point defined by SCOTT *et al.* (1983) in the *Deformed* (*Dfd*) locus. The known *Scr* transcription unit consists of three exons–solid blocks indicate coding exons and hatched blocks represent noncoding exons or portions of exons. The *ftz* locus consists of two exons indicated by a solid block in this figure, with flanking 5′ and 3′ regulatory sequences symbolized by vertical lines. The 3′ most exon if *Antennapedia* (*Antp*) is shown below coordinate +100. Solid bars represent the positions of deficiency breakpoints and sequences absent within this stretch of DNA. Solid vertical arrows depict the positions of rearrangement breakpoints. The top row are chromosomal rearrangements that exhibit a standard loss of *Scr* function phenotype both in embryos and adults (Scr^{9} and Scr^{10}) or are revertants of the Scr^{ScxW} chromosome ($Scr^{ScxWrv5}$, $Scr^{ScxWrv1}$). The bottom row shows a series of chromosomal rearrangements that exhibit gain-of-function phenotypes of varying degrees in adults (Scr^{ScxT1}, Scr^{Msc}, Scr^{ScxT2}, ftz^{Rpl}, Scr^{ScxP}, Scr^{ScxT3}, Scr^{ScxW}).

zation of and problems with segment movements in the head.

Four of the Scr^{GOF} mutations used in these experiments are associated with translocations (Table 1, Figure 1). Thus, in addition to the four genotypic classes described above, segmental aneuploid embryos were also produced by crosses involving these rearrangements. Among embryos from all such cases, the classes described above were present, plus a class of embryos that exhibited weak, spotty staining in regions corresponding to the labial lobes and first thoracic ganglion. Additionally, Scr^{ScxT2}/*Df(3R)BD5* and ftz^{Rpl}/*Df(3R)BD5* were identified by their exhibiting a ftz phenotype (see Table 1). We were unable to distinguish Scx^{s}/*Df(3R)BD5* embryos from Scx^{s}/*TM6B*.

Imaginal disc preparation: Larvae carrying the desired combinations of mutant alleles were chosen by their Tb^{+} phenotype, then dissected according to the procedure of GLICKSMAN and BROWER (1988) with the following modifications. All dissections were performed in Tri-PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 0.2% Triton X-100, pH 7.5) on ice. After tearing the larvae in half and inverting the anterior portion, all gut tissue was removed from mutant larvae by cutting at the esophagus just anterior to the proventriculus with a pair of dissecting forceps. *Oregon-R* larvae were dissected in the same manner except that the proventriculus was left attached to the esophagus and only the posterior midgut removed. This excess tissue served as a marker that allowed both mutant and *Oregon-R* dissected larvae to be processed in the same tube, thus providing an internal control for all experiments.

Imaginal disc staining: After several animals (usually five of each genotype) had been dissected and placed in the Tri-PBS, the original solution was removed and replaced with 280 μl of PBS, 120 μl of 10% paraformaldehyde (Fisher) in PBS, and 500 μl heptane (Aldrich). This mixture was shaken by hand for 30–45 seconds and the solution drawn off. The dissected larvae were then rocked for a period of twenty minutes in a solution consisting of 520 μl of PBS, 240 μl of 10% paraformaldehyde in PBS, and 40 μl DMSO (Sigma, ACS). After removing the previous solution, two quick washes with absolute methanol were performed, followed by rocking for 30 min in 980 μl absolute methanol and 20 μl hydrogen peroxide (Fisher). The methanol/hydrogen peroxide solution was then drawn off and replaced with 990 μl Tri-PBS and 10 μl of 10% bovine serum albumin (BSA) fraction V (Sigma). Four consecutive 10-min washes were performed in this solution followed by rocking for 30 min in 452 μl Tri-PBS, 5 μl of 10% BSA, and 40 μl normal goat sera (GIBCO). Three microliters of rabbit polyclonal antibody against *Scr* protein (MAHAFFEY and KAUFMAN 1987) were added to the solution and the mixture rocked at room temperature overnight.

Following antibody incubation, five washes were performed in 990 μl Tri-PBS and 10 μl of 10% BSA, increasing the duration of each wash by 5 min (5′-10′-15′-20′-25′). The dissected larvae were then rocked for 30 min in a blocking solution consisting of 445 μl Tri-PBS, 5 μl of 10% BSA, and 40 μl of normal goat sera. After blocking, 10 μl of Fab′ goat anti-rabbit horseradish peroxidase conjugate (Protos Immunoresearch) was added and the solution

rocked for 90 min. Two washes lasting 10 min each were then performed in 990 μl Tri-PBS and 10 μl of 10% BSA, followed by three subsequent washes in Tri-PBS alone. To stain, the dissected larvae were rocked for five minutes in a solution consisting of 450 μl Tri-PBS and 50 μl of diaminobenzidine tetrahydrochloride (Sigma) 5 mgs/ml in 0.1 M Tris pH 7.5; 5 μl of 0.3% hydrogen peroxide were then added and the solution rocked until sufficient color had developed in the imaginal discs. Five quick washes in PBS were subsequently performed to stop the reaction and clear any residual chromogen. Imaginal discs were dissected using tungsten needles and mounted in Aqua-Polymount (Polysciences). Preparations were photographed under Nomarski optics on a Zeiss Photoscope III using Kodak VRG 100 color print film.

RESULTS

Analysis of *Scr* expression in mutant embryos: To determine if the second and third thoracic to first thoracic transformation observed in adults heterozygous for *Scr* gain-of-function alleles could be attributed to misexpression of *Scr* protein early in development, embryos that were approximately stage 12 in development and heterozygous for an *Scr* gain-of-function allele over the *TM6B* balancer were examined. *Scr* protein has been previously reported to accumulate in the first thoracic segment and the labial lobes of wild-type embryos at this stage (MAHAFFEY and KAUFMAN 1987; RILEY, CARROLL and SCOTT 1987; LEMOTTE *et al.* 1989; MAHAFFEY, DIEDERICH and KAUFMAN 1989). Figure 1 presents a group of breakpoint mutations that define a stretch of DNA spanning 75 kilobases that have been genetically defined to be required for *Scr* gene expression (see Pattatucci, Otteson and KAUFMAN 1991). Dominant gain-of-function lesions are all associated with chromosomal rearrangements, and their corresponding breakpoints are located throughout the *Scr* locus.

When examining *Scr* protein expression in Scr^{GOF}/*TM6B* embryos, no appreciable difference from the wild-type pattern is observed (Figure 2, A, C, E and G). Indeed, certain of the mutations (Scr^{Msc} and Scr^{ScxT1}) appear to accumulate less *Scr* protein in the first thoracic and labial segments when compared to wild-type or other mutant embryos (*e.g.*, Figure 2, G compared with A). Ectopic *Scr* protein was not detected in the second or third thoracic segments for the genotypes tested at any point in embryogenesis. We conclude therefore that if the misregulation of the *Scr* locus resulting in the defects observed in adults is associated with an ectopic expression pattern in T2 and T3, this effect is not initiated during embryogenesis, but likely takes place at a later period in development.

Because the *TM6B* chromosome is Scr^{+}, it was not possible to determine the degree to which the balancer contributed to the observed *Scr* expression pattern relative to the gain-of-function chromosome. Therefore, it was of interest to examine the pattern of *Scr* protein accumulation in embryos hemizygous for these gain-of-function alleles by placing them in combination with *Df(3R)BD5*, which removes the entire *Scr* region.

Embryos hemizygous for the Scr^{ScxT1} allele over *Df(3R)BD5* display no detectable *Scr* protein at any point in embryogenesis (data not shown). This is consistent with the expectation that Scr^{ScxT1} is a null allele, based on its association with a breakpoint that disrupts the *Scr* transcription unit in the second intron (Figure 1). Moving distally along the chromosome, the next gain-of-function allele is Scr^{Msc}, with its breakpoint positioned just 3′ of the second exon. Hemizygous embryos of this genotype show no evidence of *Scr* expression during early and mid-embryogenesis (Figure 2H). However, late in embryogenesis weak *Scr* protein accumulation is observed in the region of the first thoracic ganglion of the CNS (data not shown). One explanation for this unexpected result is that the Scr^{Msc} inversion fuses a novel promoter in frame with the third *Scr* exon enabling a truncated protein to be made that is recognized by our polyclonally derived *Scr* antisera. The validity of this hypothesis awaits further molecular testing.

Embryos hemizygous for the Scr^{ScxT2} and ftz^{Rpl} alleles can be identified unequivocally by their ftz phenotype. The staining profile observed for Scr^{ScxT2} is similar to that observed for Scr^{Msc} (Figure 2F). Like Scr^{Msc}, late stage embryos that are hemizygous for Scr^{ScxT2} have a weak band of *Scr* staining in the region corresponding to the first thoracic ganglion of the CNS (data not shown). The fact that two separate lesions, one broken in the *Scr* transcription unit (Scr^{Msc}) and the other broken approximately 6 kb 5′ of the transcription start site (Scr^{ScxT2}), exhibit the same late embryonic phenotypes (with respect to *Scr* protein accumulation) suggests that sequences important for directing *Scr* gene expression during later periods of embryogenesis in the CNS are located 3′ of the Scr^{Msc} breakpoint.

Unlike the more proximal rearrangement breakpoints, embryos hemizygous for the ftz^{Rpl} lesion display a pattern of *Scr* protein expression similar to that manifested in wild-type embryos (Figure 2D). The heaviest accumulation of *Scr* protein is at the anterior border of the first thoracic segment in both genotypes, although staining throughout the rest of the first thoracic segment is stronger in wild-type than for ftz^{Rpl} animals. Embryos hemizygous for the Scr^{ScxW} lesion show an *Scr* staining profile that is ostensibly identical to wild type (Figure 2B). In addition, Scr^{ScxP}, Scr^{ScxT3}, and Scx^{S} hemizygotes show *Scr* protein localization to areas of the embryo that are analogous to that seen with Scr^{ScxW} (data not shown). These observations demonstrate that a proximal to distal polarity exists at the *Scr* locus, with successively more of the wild-type embryonic *Scr* expression pattern associated with

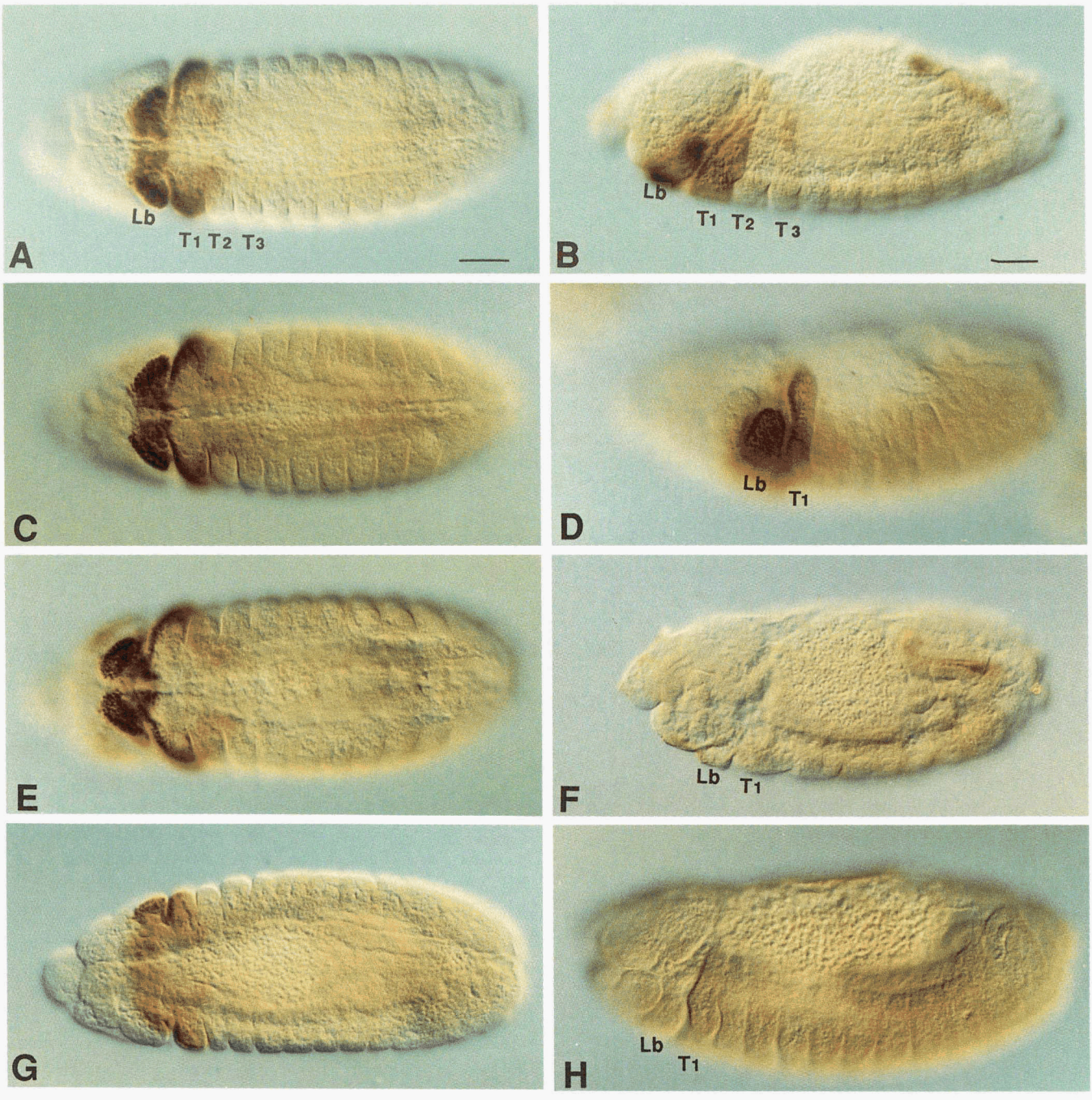


FIGURE 2.—Embryos stained with anti-*Scr*. Ventral and lateral views of embryos at approximately stage 12 of development. A, Scr^{ScxW}/*TM6B*. B, Scr^{ScxW}/*Df(3R)BD5*. C, ftz^{Rpl}/*TM6B*. D, ftz^{Rpl}/*Df(3R)BD5*. E, Scr^{ScxT2}/*TM6B*. F, Scr^{ScxT2}/*Df(3R)BD5*. G, Scr^{Msc}/*TM6B*. H, Scr^{Msc}/*Df(3R)BD5*. *Scr* transcripts and protein have been previously shown to accumulate in the anterior and posterior midgut (MARTINEZ-ARIAS *et al.* 1987; MAHAFFEY and KAUFMAN 1987). *Scr* protein accumulation in the midgut is seen in B and F. (Lb) labial lobes, (T1) prothoracic segment, (T2) mesothoracic segment, (T3) metathoracic segment. (Original magnification, ×250; bar = 4 μm.)

breakpoints that are located more 5′ from the identified start of transcription. Comparisons between embryos hemizygous for Scr^{ScxT2} and ftz^{Rpl}, the former exhibiting only weak *Scr* expression late in embryogenesis and the latter showing an *Scr* staining pattern that approximates the wild-type expression profile, have allowed us to conclude that sequences residing between these breakpoints are sufficient to direct the expression of the *Scr* transcription unit in the epidermis of the early embryo. However, we cannot rule out the possibility that sequences distal to the ftz^{Rpl} breakpoint also may be involved in and necessary for normal levels of *Scr* embryonic expression. Indeed the analysis of the phenotypic consequences of breakpoint mutations distal to *ftz* would argue that this is the case (PATTATUCCI, OTTESON and KAUFMAN 1991).

***Scr* expression in wild-type and mutant imaginal discs:** Temperature-sensitive studies suggest that the

most likely time for *Scr* misregulation resulting in the adult gain-of-function phenotype is during the late larval to pupal period, when the adult prothoracic identity is established (PATTATUCCI, OTTESON and KAUFMAN 1991). Therefore, we examined *Scr* expression in wild-type and mutant imaginal discs from late third instar larvae to determine if abnormalities in the *Scr* protein accumulation pattern were present.

The pattern of *Scr* protein expression observed in imaginal discs from wild-type third instar larvae has been previously described (GLICKSMAN and BROWER 1988) and is presented here for purposes of comparison. Briefly, strong *Scr* accumulation is seen in both the disc proper and epithelium of the labial and prothoracic leg discs. The prothoracic leg discs show the most intense level of staining in an area near the disc stalk that gives rise to proximal thoracic cuticle (BRYANT 1978). Additionally, strong *Scr* localization is detected in a sector of cells between the central knob and peripheral margin of the disc. The stained cells form a semicircle in the anterior half of this region of the disc. These cells have been fate-mapped as the progenitors of the adult anterior tarsus and tibia, including the sex combs of adult males (BRYANT 1978). *Scr* protein is also expressed in the adepithelial cells of the mesothoracic and metathoracic leg discs, but is not detectable in the wing (Figure 3) or haltere discs (data not shown). Finally, expression of *Scr* protein is observed in the dorsal prothoracic disc, in a discrete group of cells at the base of the antennal disc, in the polyploid nuclei of the salivary gland duct, and the diploid nuclei of the imaginal ring of the salivary gland (data not shown).

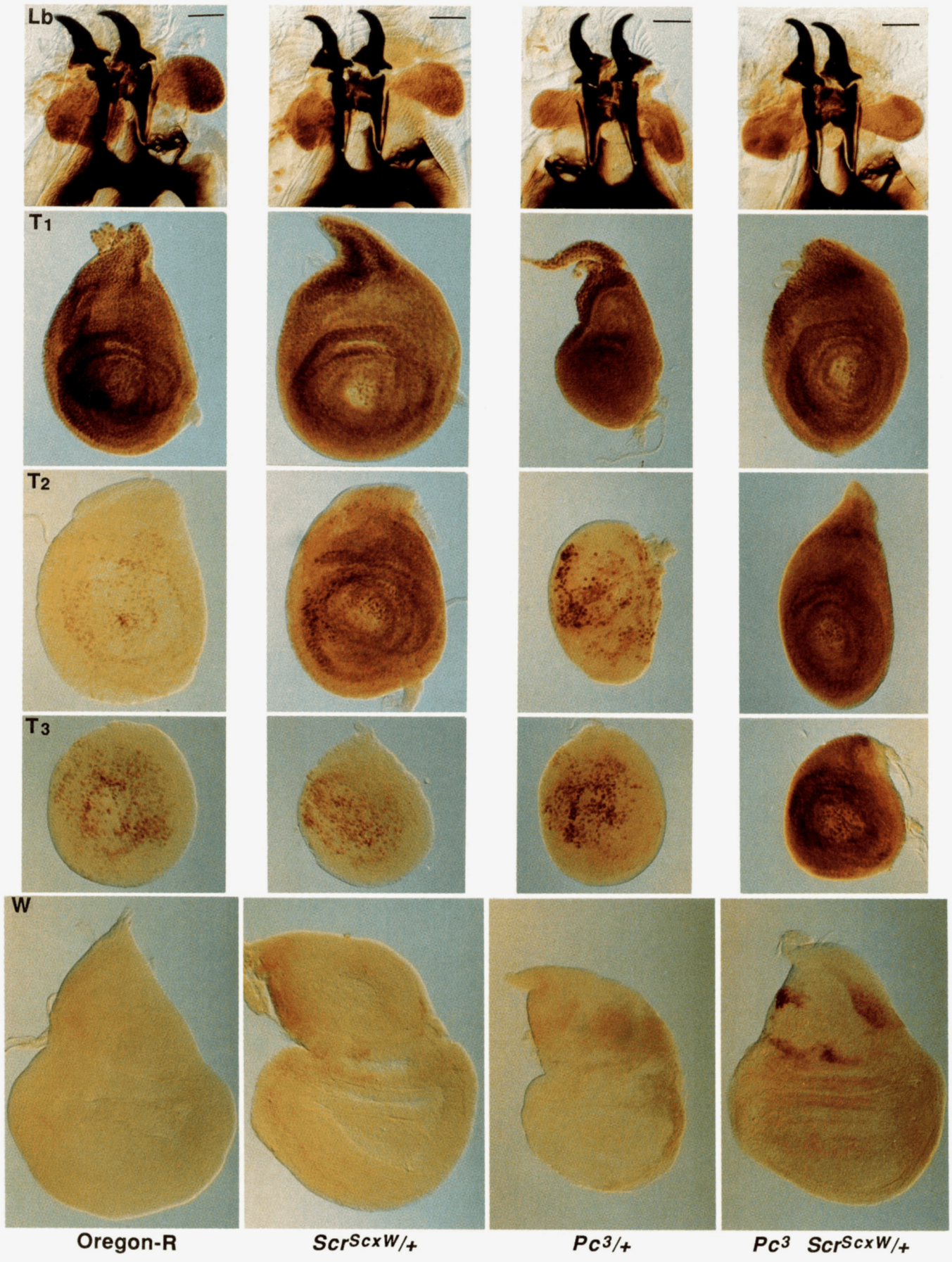
Scr protein accumulation is strikingly altered in mesothoracic leg discs of Scr^{ScxW}/+ larvae (Figure 3). In these animals it appears that most, if not all, of the cells in the disc proper express low levels of *Scr* protein. The pattern is reminiscent of the staining observed in prothoracic leg discs of wild-type animals, suggesting that the mesothoracic leg disc of Scr^{ScxW}/+ animals is being transformed toward a prothoracic identity. Furthermore, there is strong accumulation in the epithelial cells that will give rise to the sternopleurum, as well as the crescent of cells fate mapped to be progenitors of the anterior tarsus and tibia including the anlagen of the sex combs in the adult male. This pattern accounts for the Scr^{ScxW}/+ adult phenotype in which the sternopleural bristles are either reduced or missing and a nearly complete complement of sex combs exists on the mesothoracic legs of males. *Scr* expression in Scr^{ScxW}/+ metathoracic leg discs is variable, ranging from approximately wild-type (Figure 3) to a cluster of intensely staining cells in the region fate-mapped to give rise to sex combs (not shown). Wing discs exhibit very faint staining in distinct groups of cells that will give rise to the postnotum, postpleura and scutellum (BRYANT 1978). Consistent with the haplo-insufficiency of the *Scr* locus, staining is less intense in the labial and prothoracic leg discs when compared to their wild-type counterparts. Similar protein distribution profiles are observed for Scr^{ScxP}/+, Scx^{S}/+, and Scr^{Msc}/+ (data not shown).

***Scr* expression in a *Polycomb* mutant background:** The *Polycomb* (*Pc*) locus, which when mutated produces an adult extra sex combs phenotype similar to that seen in Scr^{GOF}/+ adults, has been implicated in the proper maintenance of homeotic gene function (LEWIS 1978; CAPDEVILA and GARCIA-BELLIDO 1981; STRUHL and AKAM 1985; WEDEEN, HARDING and LEVINE 1986). It was therefore of interest to examine the pattern of *Scr* expression in imaginal discs heterozygous for a mutant allele of the haploinsufficient *Pc* gene.

For this analysis, the Pc^{3} allele was employed (DENELL 1978; LEWIS 1978, 1980). This is thought to be the strongest *Pc* allele and is antimorphic in character. Both the mesothoracic and metathoracic leg discs of Pc^{3}/+ larvae tend to show more intense accumulation of *Scr* protein in adepithelial cells compared to wild-type controls (Figure 3). In addition, cells in the disc epithelium show strong staining, and these are in the region that will give rise to sex combs in adult males. Interestingly, few cells in the disc proper accumulate *Scr* protein, suggesting that only a weak, but specific posterior to anterior transformation is occurring. The intensity and number of ectopically expressing cells is highly variable for this genotype, and the example depicted in Figure 3 is at the more extreme end of the phenotypic spectrum. A corresponding variability is also observed when examining adult males, with phenotypes ranging from no evidence of extra sex combs to a few ectopic sex comb teeth on mesothoracic and metathoracic legs.

The double mutant combination of Pc^{3} Scr^{ScxW}/+ exhibits striking ectopic accumulation of *Scr* protein in mesothoracic and metathoracic leg discs. Transformation to a prothoracic identity is nearly complete for these discs. Moreover, strong ectopic *Scr* expression is seen in the wing disc localized to regions that will give rise to the postnotum, postpleura, scutellum, and prescutum, as well as the anterior and posterior

FIGURE 3.—Imaginal discs stained with anti-*Scr*. All imaginal discs are derived from third instar larvae. Note the faint localization of *Scr* protein along the medial edge of the Scr^{ScxW}/+ wing disc and the enhancement of this when *Polycomb* is present in the genotype, Pc^{3} Scr^{ScxW}/+. (Lb) labial discs, (T1) prothoracic leg disc, (T2) mesothoracic leg disc, (T3) metathoracic leg disc, (W) wing disc. (Original magnification, ×125; bar = 8 μm.)


Lb
T1
T2
T3
W
Oregon-R
Scr^ScxW/+
Pc^3/+
Pc^3 Scr^ScxW/+

notal wing processes (BRYANT 1978). Consistent with the apparent additive effects of this genotype, adult males have a nearly complete set of sex combs on all six legs, lack sternopleural bristles entirely, and have held-out wings (PATTATUCCI, OTTESON and KAUFMAN 1991).

***Cis-trans* test with Scr^{ScxW}:** Because embryos hemizygous for Scr^{ScxW} show a pattern of *Scr* expression similar to that observed in wild-type embryos, it was of interest to examine the analogous situation in larvae. In this case, it was not possible to obtain third instar larvae that were hemizygous over *Df(3R)BD5* due to the fact that the distal breakpoint of the Scr^{ScxW} inversion makes these animals $Antp^{-}$ (Table 1). Therefore, a point null lesion, Scr^{4}, that produces no detectable *Scr* protein as a homozygote was employed. This lesion was tested both in *trans* with Scr^{ScxW}, as well as recombined in *cis* on the Scr^{ScxW} chromosome and examined over a wild-type homolog (see PATTATUCCI, OTTESON and KAUFMAN 1991). A remarkable difference in the *Scr* staining profile is observed depending on whether the Scr^{4} lesion is in *trans* or in *cis* with Scr^{ScxW}. In the case of the *trans* arrangement, ectopic expression of *Scr* protein in wing, metathoracic and mesothoracic leg discs appears to be completely suppressed (Figure 4). This suggests that the ectopic accumulation of *Scr* protein in $Scr^{ScxW}/+$ imaginal discs cannot be accounted for by the misexpression of the *cis*-coupled structural gene. In contrast, when the Scr^{4} lesion is in a *cis* configuration with respect to Scr^{ScxW}, ectopic *Scr* protein is detected in the mesothoracic and metathoracic leg discs (Figure 4). The pattern is similar but not identical to $Scr^{ScxW}/+$ (compare Figures 3 and 4). However, comparisons of prothoracic leg discs from both Scr^{ScxW}/Scr^{4} and Scr^{4} $Scr^{ScxW}/+$ to $+/+$ prothoracic leg discs suggest that the differences in staining pattern are associated with lower levels of protein accumulation in Scr^{4} $Scr^{ScxW}/+$ discs and probably reflect the haploinsufficiency of the *Scr* locus. These results imply that the ectopic accumulation of *Scr* protein identified in wing, metathoracic and mesothoracic leg discs of $Scr^{ScxW}/+$ larvae occurs as a result of the misexpression of the wild-type structural gene in *trans* to the Scr^{ScxW} gain-of-function lesion.

Strict *cis-trans* tests have not been performed for Scr^{ScxP} and Scx^{S} due to difficulty in obtaining recombinants that place the Scr^{4} lesion in *cis* configuration to these gain-of-function lesions. Nevertheless, *trans* combinations of Scr^{4} with either Scr^{ScxP} or Scx^{S} display a failure of ectopic expression of *Scr* protein similar to that seen with Scr^{ScxW}/Scr^{4} (Figure 4). Additionally, a second point null mutation, Scr^{2}, was tested in *trans* combinations with each of these gain-of-function lesions and gave identical results to those described for Scr^{4} (data not shown). Combinations of either Scr^{4} or Scr^{2} with Scr^{Msc} cannot be tested for *cis-trans* effects due to embryonic lethality of these genotypes.

The proposed *trans*-activation described above appears to be associated with two components. First, the fact that all *Scr* gain-of-function lesions recovered to date are associated with rearrangements suggests that a rearranged chromosome is required. Second, a number of rearrangements associated with the *Scr* locus have been recovered that do not result in a gain-of-function phenotype (see legend to Figure 1), suggesting that either the juxtaposition of novel sequences within or near the *Scr* locus, or the removal of specific *cis*-acting regulatory elements is also required for *trans*-activation.

Knowing that ectopic *Scr* expression in $Scr^{ScxW}/+$ animals is derived primarily if not exclusively from the wild-type homolog lead us to question the origin of *Pc*-enhanced Scr^{ScxW}-driven ectopic expression of *Scr* protein. Thus, the *cis-trans* test described previously was repeated in the presence of Pc^{3}. Interestingly, misexpression of *Scr* protein is now observed in both *cis* and *trans* combinations of Scr^{4} with Scr^{ScxW} in a Pc^{3} background (Figure 4). The ectopic expression is not as extensive as that observed for Pc^{3} $Scr^{ScxW}/+$, but this again is likely due to the haplo-insufficiency of the *Scr* locus. While Pc^{3} enhances both *cis* and *trans* combinations, ectopic expression of *Scr* is more extensive in genotypes having Scr^{4} and Scr^{ScxW} in *trans*. Enhancement of ectopic *Scr* protein accumulation in Scr^{4} Scr^{ScxW}/Pc^{3} can be accounted for by the additive effects of *trans*-activation by Scr^{ScxW} and *cis*-misregulation of the companion homolog by Pc^{3}. However, our results demonstrate that the Scr^{ScxW} lesion is incapable of ectopically activating its *cis*-coupled structural gene by itself. Thus, ectopic *Scr* protein accumulation in Scr^{4}/Pc^{3} Scr^{ScxW} must be accounted for by the effects of the Pc^{3} mutation alone. In this configuration, ectopic *Scr* expression, which is more extensive than that observed for $Pc^{3}/+$, could be due to the absence of a *Polycomb* function which normally prevents ectopic activation of the *cis*-coupled *Scr* structural gene by Scr^{ScxW}. We conclude, therefore, that *Polycomb* regulates primarily the *cis* functions of *Scr* gene activity as seen in this assay, and plays little or no role in the *trans* regulatory system previously described. Figure 5 presents a model illustrating the above results.

Imaginal disc phenotype for Scr^{ScxT1}: The Scr^{ScxT1} allele (Table 1) was originally recovered as a weak *Scr* gain-of-function lesion (S. Y. K. TIONG, personal communication). However, at present, adult males heterozygous for $Scr^{ScxT1}/+$ exhibit a standard loss of *Scr* function phenotype consisting of reduced sex combs on the prothoracic legs with no evidence of ectopic sex comb teeth. A likely explanation for this change is that, at the time this mutant was recovered, the observed phenotype resulted from a fortuitous con-

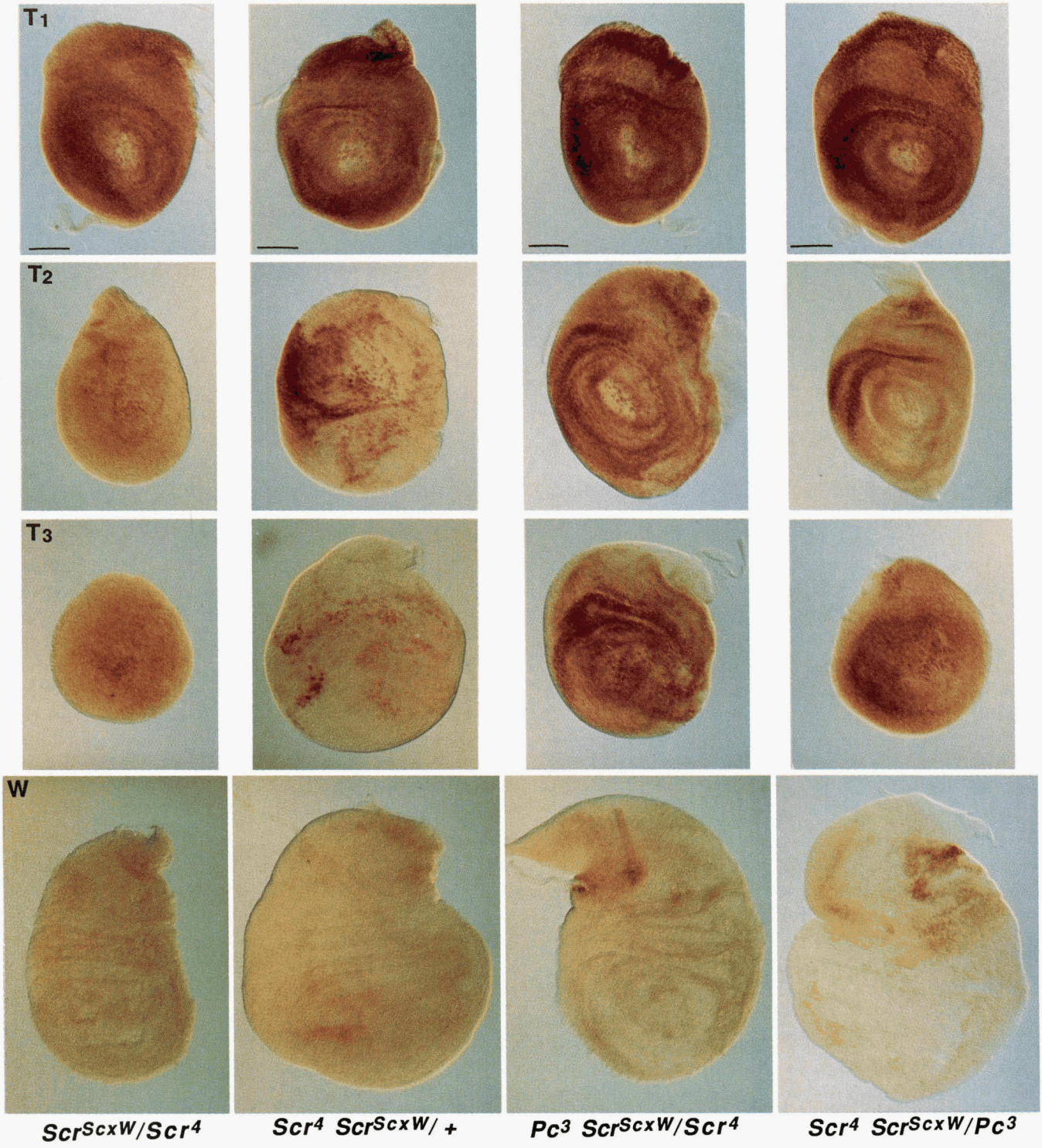


FIGURE 4.—*Cis-trans* test. Imaginal discs are from third instar larvae and stained with anti-*Scr*. Note that the proportional difference in ectopic protein accumulation is greater between rows 1 and 3 (*cis* test) compared to rows 2 and 4 (*trans* test). Original magnification, ×125; bar = 8 μm.)

junction of two separate mutations. The combination consisting of one lesion in *Scr* and a second lesion located elsewhere in one of the many loci known to confer an extra sex combs phenotype (JÜRGENS 1985). If correct, it is likely that the second lesion was lost over time as the stock has been maintained.

Imaginal disc staining of Scr^{ScxT1}/+ reveals a pattern of *Scr* accumulation that is similar to wild-type, but is significantly reduced in intensity (compare Figures 3 and 7). This imaginal disc phenotype is virtually identical to that observed for larvae heterozygous for either the Scr^4 or Scr^2 null mutations and a wild-type chromosome (data not shown). Thus, in our hands, Scr^{ScxT1}, Scr^4 and Scr^2 are all apparent null mutations

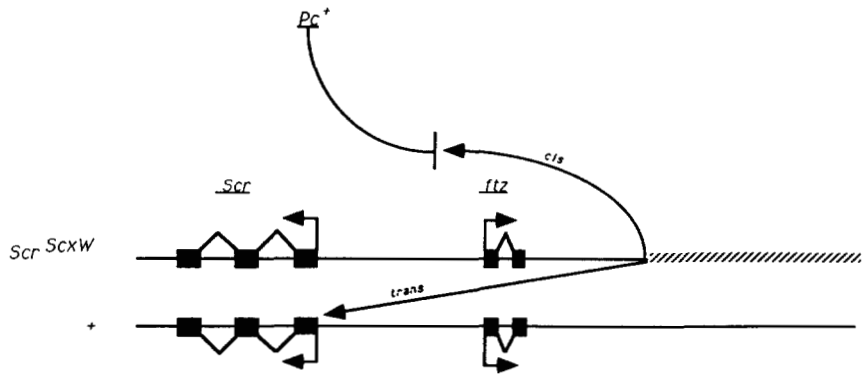


FIGURE 5.—Model depicting the Scr^{ScxW} gain-of-function phenotype. The Scr^{ScxW} lesion appears to exert its effect exclusively on the *Scr* structural gene in *trans* when in combination with a wild-type homolog. Gain-of-function effects on the *cis*-coupled structural gene are blocked by the presence of Pc^{+} gene product. Novel *Antp* sequences from the region just 5′ of the P2 promoter that have been juxtaposed to *Scr* sequences by this chromosomal inversion are indicated by a stippled bar.

at the *Scr* locus. Therefore, it might be expected that Scr^{ScxT1} would behave analogously to Scr^{4} in heterozygous combinations with Scr^{ScxW} and Pc^{3} Scr^{ScxW}. However, when heterozygous with Pc^{3}, Scr^{ScxW}, and Pc^{3} Scr^{ScxW} chromosomes, Scr^{ScxT1} is associated with an increasingly more extensive pattern of ectopic *Scr* protein accumulation (Figure 6). Scr^{ScxT1}/Pc^{3} exhibits a disc staining phenotype that is more pronounced than either $Pc^{3}/+$ or $Scr^{ScxW}/+$ and extra sex combs are observed on all six legs of adult males of this genotype. Scr^{ScxT1}/Scr^{ScxW} imaginal discs show a pattern of *Scr* protein accumulation that is similar in distribution and intensity to that observed for Pc^{3} $Scr^{ScxW}/+$ (compare Figures 3 and 7). Both mesothoracic and metathoracic leg discs appear to be almost completely transformed to a prothoracic identity. Pc^{3} Scr^{ScxW}/Scr^{ScxT1} exhibits the most severe effects on *Scr* gene regulation. The wing, metathoracic and mesothoracic leg discs show extensive and intensive *Scr* ectopic protein accumulation. Additionally, the metathoracic leg disc and wing disc characteristically exhibit mirror image duplications, possibly indicative of the occurrence of cell death. Animals of this genotype die shortly after pupariation and fail to eclose. Four other *Scr* breakpoint alleles–Scr^{Msc}, Scr^{ScxT2}, ftz^{Rpl} and Scr^{ScxT3}–all show loss-of-function phenotypes of varying degrees over a wild-type chromosome and behave in a similar fashion to Scr^{ScxT1} when in combination with Pc^{3}, Scr^{ScxW} and Pc^{3} Scr^{ScxW} (data not shown).

We conclude that these rearrangement lesions, three of which display null *Scr* phenotypes (Scr^{ScxT1}, Scr^{ScxT2} and Scr^{Msc}) and therefore are incapable of being *trans*-activated by Scr^{ScxW}, can nonetheless have drastic effects on *Scr* gene regulation specifically by affecting expression of their *trans* homolog. Each of these rearrangement lesions could remove and/or interfere with a regulatory function that normally prevents Scr^{ScxW} from ectopically activating its *cis*-coupled structural gene. The inclusion of the Pc^{3} allele into these mutant genotypes apparently causes complete or nearly complete misregulation of the *Scr* locus in the leg and wing imaginal discs.

Tests to determine if gene regulation at *Scr* is synapsis-sensitive: Since the only apparent difference between Scr^{ScxT1} and Scr^{4} is the rearrangement associated with the former, Scr^{ScxT1} might have its effect on *Scr* gene expression by impeding normal synapsis of homologous chromosomes in this region. According to this hypothesis, a component of *Scr* negative regulation in imaginal discs associated with development of the thorax would be the requirement for proper synapsis of homologous chromosomes in this region of the genome. It is noteworthy that the other alleles that behave in a phenotypically similar manner to Scr^{ScxT1} (Scr^{Msc}, Scr^{ScxT2}, ftz^{Rpl} and Scr^{ScxT3}), are also rearrangement mutations in *Scr*. We tested seven other rearrangements with breakpoints in the *Scr* locus. Scr^{9}, Scr^{10}, *Df(3R)CP1*, *Df(3R)Tpl9* are associated with breakpoints within the *Scr* structural gene and display a standard loss of function phenotype in adults (see Figure 1). Each of these lesions exhibited varying degrees of enhanced ectopic *Scr* protein accumulation when tested in combination with Pc^{3} and Scr^{ScxW} (data not shown). Three other rearrangements, *Df(3R)Hu*, *Df(3R)A41*, and *Df(3R)RC7* are associated with breakpoints in the *Scr* regulatory region 3′ of *ftz* (see Figure 1). Each of these deficiencies removes *Antennapedia* sequences and therefore cannot be tested in combination with Scr^{ScxW}, which has its distal breakpoint in *Antp* (Table 1). However, these deficiencies do show slightly enhanced ectopic *Scr* expression when heterozygous with Pc^{3}, Scr^{ScxP}, or Scx^{S}.

If *Scr* gene expression is in some manner sensitive to the proper synapsis of homologous chromosomes in this region of the ANT-C, then rearrangements with breakpoints that are between the centromere and the *Scr* gene but not in *Scr per se* should cause an enhancement of *Scr* ectopic protein accumulation when heterozygous with Scr^{ScxW}. Conversely, rearrangements with breakpoints that are distal to the *Scr* locus should have little or no effect on the pattern. This is based upon the proposal that synapsis of homologous chromosomes occurs in a bidirectional manner with origins from both proximal and distal associations (ROBERTS 1970, 1972). Thus, rearrangements with breakpoints proximal to a gene

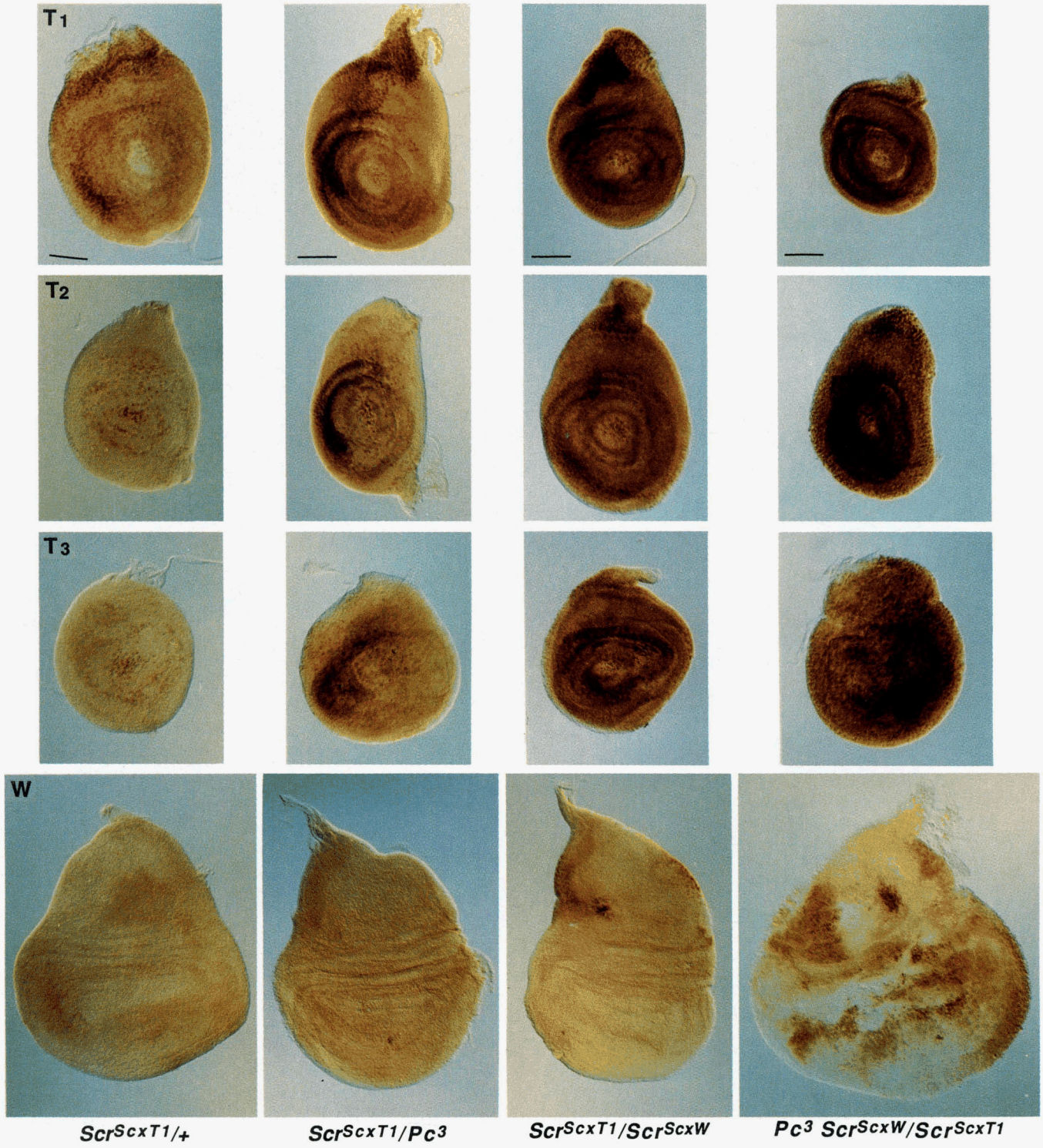


FIGURE 6.—Heteroallelic combinations with Scr^{ScxT1}. Imaginal discs are from third instar larvae and stained with anti-*Scr*. Note the mirror image duplication in the region of the disc stalk for T3 of Pc^3 Scr^{ScxW}/Scr^{ScxT1}. (Original magnification, ×125; bar = 8 μm.)

such as *Scr*, which is located near the base of the right arm of chromosome three, are predicted to have their maximum effect on synapsis originating from proximal associations while only having minor influences on chromosomal pairing with origins from distal associations, and the reciprocal situation should be true for breakpoints located distal to the gene. Figure 7 shows the results of such an analysis. $T(2;3)bw^{VDe3}$ is a rearrangement that translocates virtually all of the right arm of the third chromosome from polytene band 81F to the tip of the right arm of the second chromosome. Based upon interactions with Cbx^1 Ubx^1 (E. B. LEWIS, personal communication), this rearrangement is expected to significantly reduce ho-

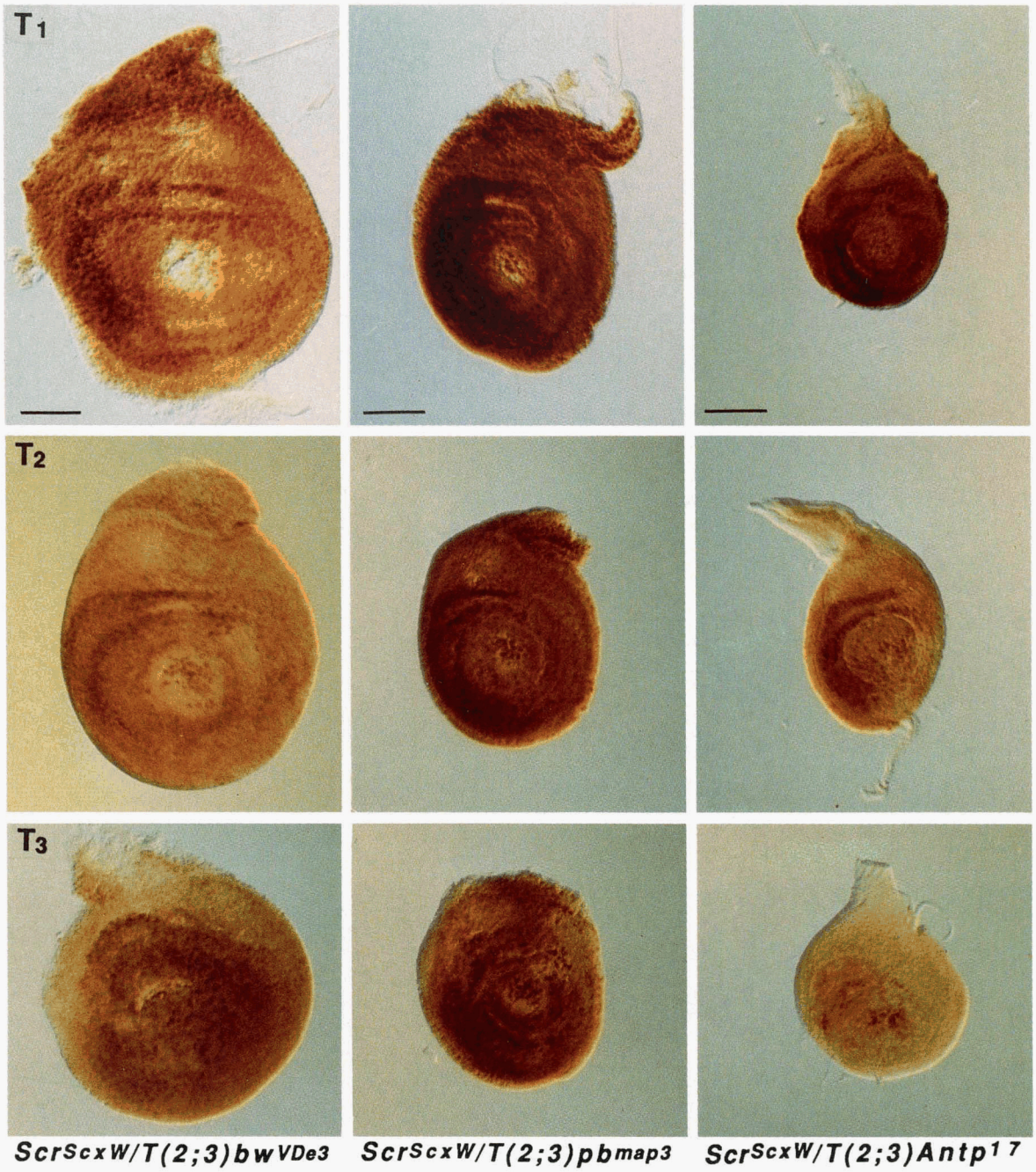


FIGURE 7.—Tests with breakpoints outside the *Scr* locus. Imaginal discs are from third instar larvae and stained with anti-*Scr*. Note that ectopic accumulation of *Scr* protein is significantly increased for breakpoints located proximal to the *Scr* locus, $T(2;3)bw^{VDe3}$ and $T(2;3)pb^{map3}$ relative to Scr^{ScxW} over a wild-type homolog (Figure 3), whereas no appreciable difference is seen for the breakpoint located distal to the *Scr* locus, $T(2;3)Antp^{17}$. (Original magnification, ×125; bar = 8 μm.)

mologous chromosome pairing in the proximal region of the right arm of the third chromosome, where the *Scr* gene is located. Heterozygous combinations of $T(2;3)bw^{VDe3}$ with Scr^{ScxW} show enhanced ectopic *Scr* protein accumulation over that observed for $Scr^{ScxW}/+$ (compare Figures 3 and 7). This is most clearly seen when comparing the metathoracic leg discs. A second rearrangement, $T(2;3)pb^{map3}$, which has its breakpoint much closer to the *Scr* locus exhibits a more pronounced pattern of ectopic *Scr* expression in combination with Scr^{ScxW} than $T(2;3)bw^{VDe3}$. This is consistent with the proximal to distal polarity already observed among breakpoint lesions within the *Scr* locus. Finally, $T(2;3)Antp^{17}/Scr^{ScxW}$ shows a pattern of *Scr* protein accumulation that is ostensibly the same as that observed for $Scr^{ScxW}/+$ (compare Figures 3 and 7). This is consistent with the notion that rearrangements broken distally to the *Scr* locus should not markedly affect the pairing of homologous chromosomes compared to those broken proximally. The introduction of Pc^3

into these genetic backgrounds significantly enhances *Scr* ectopic protein expression in a similar fashion to that previously observed for Scr^{ScxT1} with the exception that $T(2;3)Antp^{17}/Pc^3\ Scr^{ScxW}$ shows no detectable enhancement above that seen in $Pc^3\ Scr^{ScxW}/+$ animals. In addition, heteroallelic combinations of the double mutant chromosome $Scr^4\ Scr^{ScxW}$ with $T(2;3)bw^{VDe3}$ and $T(2;3)pb^{map3}$ have revealed that the extra sex combs phenotype is suppressed in males of these genotypes. This is to be expected due to the fact that $T(2;3)bw^{VDe3}$ and $T(2;3)pb^{map3}$ will remove the wild-type *Scr* structural gene in *trans* from the proximity of the Scr^{ScxW} gain-of-function lesion and therefore prevent *trans*-activation of the normal *Scr* locus. The $Scr^4\ Scr^{ScxW}$ homolog is of course incapable of producing *Scr* protein and its pattern of expression is irrelevant to the phenotype of the adults.

Tests with alleles at the *zeste* locus: Previous studies have demonstrated that the expression of certain BX-C, *decapentaplegic* (*dpp*) and *white* (*w*) genotypes is synapsis-sensitive. If heterozygosity for a chromosomal rearrangement prevents two copies of these loci from properly synapsing in somatic tissues, gene expression is altered (JACK and JUDD 1979; GELBART and WU 1982). Mutations in the *zeste* gene have been shown to modify the expression of these gene complexes (reviewed in WU and GOLDBERG 1989). For example, z^a alleles alter certain *bithorax* mutant phenotypes in a manner that is similar to that observed with synapsis-disrupting rearrangements (KAUFMAN, TASAKA, and SUZUKI 1973; BABU and BHAT 1981; GELBART and WU 1982). Additionally, both z^a and z^1 mutant alleles can interfere with complementation between several alleles at the *dpp* locus (GELBART and WU 1982; WU 1984). These results are consistent with the general participation of *zeste* in the expression of at least a subset of the genes that exhibit synapsis-sensitive gene regulation. It was therefore important to test the heteroallelic combinations at the *Scr* locus that appear to show synapsis dependence in a *zeste* mutant background. Experiments with z^a or z^1 (Table 1) combined with $Scr^{ScxW}/+$, $Scr^4\ Scr^{ScxW}/+$ and Pc^3 showed no apparent influence of these *z* alleles on *Scr* gene regulation. There was neither an enhancement nor a suppression of extra sex combs on adult males for any of the genotypes tested.

Curiously, in all *Scr* heteroallelic combinations examined, imaginal discs anterior to the prothoracic leg disc exhibited no significant alteration in the distribution of *Scr* protein, although reductions in staining intensity were observed when compared to wild-type controls (Figure 3). This suggests that control of *Scr* gene expression in imaginal discs that give rise to head structures is not sensitive to the regulatory phenomena observed for the imaginal discs that are involved in specifying thoracic structures.

DISCUSSION

***Scr* is differentially regulated temporally and spatially:** Our analysis indicates that the *Scr* locus is differentially regulated both temporally and spatially in a manner that is dependent on the structure of the locus. Differences in the temporal regulation of *Scr* are demonstrated by the fact that embryos heterozygous for dominant gain-of-function *Scr* lesions and a wild-type chromosome show virtually no spatiotemporal alteration in the wild-type *Scr* protein expression pattern, but manifest conspicuous ectopic accumulation of *Scr* protein in imaginal discs during larval development. These stage-dependent differences in ectopic *Scr* expression imply that the mechanisms of gene regulation at the *Scr* locus, and possibly some of the *trans*-acting gene products involved in this process, differ during these two periods.

Spatial differential regulation of *Scr* is exhibited during the larval to adult period of development. This aspect of *Scr* gene regulation is revealed by the observation that the gain-of-function lesions mentioned above exhibit ectopic *Scr* protein accumulation that is restricted to imaginal discs associated with development of the thorax, whereas imaginal discs that give rise to head structures remain unaffected. At present, mutations in the *Scr* locus have not been recovered that reveal the existence of differential spatial regulation in the embryo.

Finally, the fact that *Scr* gene regulation is sensitive to the structural integrity of the locus is demonstrated by the fact that a cytologically normal null mutation at *Scr* exhibits different phenotypes when in combination with an *Scr* gain-of-function lesion depending on whether it is located on the *trans* homolog or recombined in a *cis* configuration with respect to the gain-of-function mutation. Cytologically normal null mutations in *trans* suppress the gain-of-function phenotype while a *cis* configuration has no noticeable affect except to decrease the amount of available *Scr* protein.

Sequences important for *Scr* embryonic expression lie between the Scr^{ScxT2} and ftz^{Rpl} breakpoints: Observations on the pattern of *Scr* protein accumulation in embryogenesis reveals a proximal to distal differential in defects associated with genotypes that are hemizygous for *Scr* gain-of-function lesions. That is, those breakpoints that are proximal to *ftz* (Scr^{ScxT1}, Scr^{Msc} and Scr^{ScxT2}) either do not produce detectable *Scr* protein or do so abnormally, while those that are within or are distal to *ftz* (ftz^{Rpl}, Scr^{ScxP}, Scr^{ScxT3}, and Scr^{ScxW}) exhibit near normal or apparently wild-type patterns of *Scr* expression respectively. These observations suggest that DNA sequences located within the region defined by the Scr^{ScxT2} and ftz^{Rpl} breakpoints are necessary and sufficient to produce a pattern of *Scr* protein accumulation similar to that observed in

wild-type embryos. However, genetic analysis has indicated that a number of lesions that map distal to the ftz^{Rpl} breakpoint have discernable phenotypic effects on *Scr* function during embryonic and imaginal development (PATTATUCCI, OTTESON and KAUFMAN 1991). Additionally there may be sequences 3′ of the Scr^{Msc} breakpoint that act to direct *Scr* gene expression during late embryogenesis in the CNS. One way to rationalize these observations is to conclude that sequences within the interval defined by the Scr^{ScxT2}-ftz^{Rpl} breakpoints may function in the establishment of the *Scr* expression pattern, while those that are outside might participate in the more subtle aspects of refinement and maintenance of this established pattern. The actual explanation however awaits further genetic and molecular dissection of this large "regulatory" interval.

***Scr* regulation during the larval period involves complex interactions:** It would appear that regulation of *Scr* expression in the imaginal discs that give rise to thoracic structures involves both positive and negative interactions in *cis* as well as negative control in *trans*. However, an alternate regulatory program seems to be operational in those imaginal discs that will form adult head structures. This is evidenced by the fact that heteroallelic combinations that exhibit noticeable ectopic *Scr* protein accumulation in imaginal discs associated with the development of the thorax have virtually no influence on *Scr* expression in those discs related to head development. The only recognizable effect observed for imaginal discs giving rise to adult head structures in the heteroallelic combinations examined is a general decrease in the staining intensity for those genotypes in which the *Scr* locus has been interrupted. For example, $Scr^{ScxW}/+$, which disrupts the *Scr* locus, shows a decrease in staining intensity in the labial imaginal discs compared to wild-type controls (Figure 3). We conclude from these data that the differential regulation of the *Scr* locus occurs minimally at three hierarchical levels: two of which are operational during the larval to adult period of development and one occurring throughout embryogenesis.

Scr gene control in the larval to adult period of development appears to involve at least two different but possibly overlapping classes of genes. Because our analysis has revealed that imaginal discs that are anterior to the prothoracic leg disc exhibit no significant alteration in *Scr* gene expression in the heteroallelic combinations tested, we propose that regulation of *Scr* is of a qualitatively different nature in these discs when compared to that which occurs in the leg, wing and haltere discs. From these data, we predict that the gene products involved in regulating *Scr* in anterior imaginal tissues are probably not the same as those controlling *Scr* expression in the more posterior imaginal discs.

The genes involved in modulating *Scr* gene expression in the head are at the present time unknown. However, a number of loci have been implicated in regulating *Scr* gene expression in the thorax by virtue of their mutant alleles either enhancing or suppressing extra sex comb phenotypes. Members of the *trithorax*-group genes are good candidates for regulators of *Scr* in the thorax because of their implicated role in the positive control of homeotic genes. For example, the homeotic transformations arising from mutations in *trithorax*, *ash-1* and *ash-2* (discs *a*bsent, *s*mall or *h*omeotic) are comparable to those caused by loss of function mutations in genes of both the ANT-C and BX-C, most notably the reduced sex comb phenotype characteristic of prothoracic to mesothoracic transformations seen in *Scr* loss of function mutants (SHEARN, HERSPERGER and HERSPERGER 1987; SHEARN 1989). Furthermore, the fact that the extra sex combs phenotype conferred by the Scr^{Msc} gain-of-function lesion is in part suppressed in a *trithorax* mutant background suggests that the wild-type *trithorax* gene product is involved in positively regulating *Scr* (J. KENNISON, personal communication).

The second group of *Scr* regulator genes in the thorax seem to be in some way involved in the negative control of *Scr* gene function. The *Polycomb* gene product apparently is a member of this class. Preliminary studies of imaginal phenotypes have indicated that other genes in the *Polycomb*-group affect *Scr* gene expression in a similar fashion to that observed for *Polycomb* (A. M. PATTATUCCI and T. C. KAUFMAN, manuscript in preparation).

The Scr^{ScxW} gain-of-function lesion appears to exert its effect on *Scr* gene expression exclusively through the wild-type structural gene in *trans*. This is demonstrated by the fact that Scr^{ScxW}, when placed in a *trans* configuration with the Scr^{4} null lesion, shows an accumulation pattern of *Scr* protein that is similar to +/+ (although reduced in intensity), whereas Scr^{4} $Scr^{ScxW}/+$ exhibits ectopic *Scr* expression in the mesothoracic and metathoracic leg discs. Our interpretation of this result is that negative regulation of *Scr* gene expression is in some way interrupted by the Scr^{ScxW} lesion allowing ectopic activation of the *Scr* gene in imaginal discs that will give rise to posterior thoracic structures. However, the disruption of negative regulation of *Scr* seems to occur only in *trans*, while *cis* negative regulation appears to remain intact. This is shown by the Scr^{ScxW}/Scr^{4} genotype, which has the potential to express *Scr* protein only from the structural gene in *cis* to the gain-of-function lesion, and does not do so ectopically. Thus, the Scr^{ScxW} homolog remains sensitive to the negative regulation which normally prevents posterior ectopic expression.

However, negative regulation in *cis* can be interrupted by combining with Scr^{ScxW} either a mutation in the *Polycomb* gene or a rearrangement with a breakpoint located between the centromere and the *Scr* locus. These combinations of lesions result in enhancement of ectopic protein accumulation, apparently caused by the ectopic activation of both the *cis* and *trans Scr* structural genes.

***Polycomb* regulates *Scr* primarily through *cis* interactions:** Phenotypically, it would appear that mutations in the *Polycomb* gene and rearrangement breakpoints between the centromere and the *Scr* locus are interchangeable and analogous [see Pc^3 Scr^{ScxW}/+ (Figure 3) and Scr^{ScxW}/Scr^{ScxT1} (Figure 6)]. If rearrangements between the centromere and the *Scr* locus and mutations in the *Polycomb* gene do affect the same aspect of *Scr* gene regulation, then one would expect these two genotypes to show similar patterns of *Scr* ectopic protein accumulation. However, comparisons between Pc^3/+ (Figure 3) with Scr^{ScxT1}/+ (Figure 6) indicate that this is clearly not the case. The fact that Pc^3/Scr^{ScxT1} is significantly enhanced compared to Pc^3/+ suggests that Scr^{ScxT1} is interfering with a separate regulatory component over and above that affected by Pc^3. This is further substantiated by the fact that Scr^9 and Scr^{10}, which are rearrangements that are similar both in type and breakpoint position to Scr^{ScxT1}, do not exhibit significantly enhanced phenotypes relative to Pc^3/+ controls (see Table 5 in Pattatucci, Otteson and KAUFMAN 1991).

The greatly enhanced ectopic *Scr* protein accumulation seen for Pc^3/Scr^{ScxT1} also supports the hypothesis that the *Polycomb* gene product is involved primarily in the *cis*-regulation of *Scr*. This can be inferred from the result demonstrating that the gain-of-function phenotype observed for Scr^4 Scr^{ScxW}/+ is suppressed by introducing $T(2:3)bw^{VDe3}$ into the genotype, presumably by removing the wild-type structural gene in *trans* from the proximity of the Scr^{ScxW} lesion. In the same way, Scr^{ScxT1} should also sufficiently disrupt synapsis so that *trans*-activation would not be possible. Additionally, the fact that Scr^{ScxT1} is a null lesion that produces no detectable protein means that the ectopic expression must have its origin from the Scr^+ gene on the companion homolog. Therefore, we conclude that the enhanced ectopic expression of *Scr* seen with Pc^3/Scr^{ScxT1} is most likely the result of *cis*-misregulation.

***Scr* gene regulation is synapsis-sensitive:** In addition to temporal and spatial differential regulation at the *Scr* locus, our analysis has revealed a structural component that appears to be in some manner pairing-sensitive. Somatic chromosome synapsis between homologs is well documented in dipterans (METZ 1916). This is best reflected in the polytene chromosomes, where homologous chromosomes are endoreplicated, as well as being aligned and paired in exact register. METZ (1916) concluded from his analysis that the paired association of chromosomes in somatic cells is not due to random assortment, but is highly selective and dependent upon a qualitative similarity between associating homologs. EPHRUSSI and SUTTON (1944) presented a comprehensive discussion relating somatic chromosome synapsis to gene expression. They noted that certain genes exhibiting position effect variegation could be altered in their manner of phenotypic expression when a second rearrangement with a breakpoint near the locus under investigation was introduced into the genotype. They concluded that position effect is a consequence of chromosome synapsis that results in a modification of structural integrity near the affected loci. Lewis (1954, 1955) extended these previous observations and experimentally verified a relationship between somatic chromosome synapsis and gene expression at the BX-C. WU (1984) has used the term *proximity modulated gene expression* to describe the phenomenon in which expression of a gene is altered depending on its relative distance from its sister gene on the homologous chromosome. Instances of proximity modulated gene expression have been reported at several loci–most notably *cubitus interruptus* (EPHRUSSI and SUTTON 1944), *Ultrabithorax* (LEWIS 1954), *white* (JACK and JUDD 1979),*decapentaplegic* (GELBART 1982), *engrailed* (GUBB 1985), *brown* (HENIKOFF and DRESSEN 1989), *light* (WAKIMOTO and HEARN 1990) and *yellow* (GEYER, GREEN and CORCES 1990). For example, allelic complementation is observed in genotypes in which somatic chromosome pairing of certain *bithorax* alleles occurs normally. However, genotypes with a chromosomal rearrangement that interferes with proper synapsis of homologs in the region between the centromere and the *bithorax* locus suppress this allelic complementation. E. B. LEWIS (1954) termed this phenomenon of synapsis-dependent gene expression *transvection*. Additionally, null alleles at the *zeste* locus appear to be able to effect a change in phenotype reminiscent of that observed for synapsis-disrupting rearrangements at the *bithorax* locus as well as inhibit transvection (KAUFMAN, TASAKA and SUZUKI 1973; GELBART and WU, 1982).

All gain-of-function alleles at *Scr* recovered to date are associated with chromosomal rearrangements. These rearrangements have the potential to disrupt homologous chromosome synapsis in the ANT-C region of the genome, remove important *Scr* regulatory sequences, and/or juxtapose novel regulatory sequences capable of directing *Scr* expression ectopically. The combination of both a rearranged chromosome and the juxtaposition or removal of important regulatory sequences seems to be a prerequisite for producing the *Scr* gain-of-function phenotype. This is most clearly evidenced by the fact that cytolog-

ically normal gain-of-function alleles at *Scr* have not been recovered and only a subset of the rearrangements in or near the *Scr* locus exhibit a gain-of-function phenotype.

From these observations, we conclude that the primary component altered by the rearrangement on the gain-of-function chromosome is the juxtaposition of permissive sequences capable of allowing *Scr* gene expression ectopically in *trans*. As a secondary consequence, these gain-of-function rearrangements interfere with the ability of the homologous chromosomes to properly synapse. The net result is the disruption of an important aspect of negative regulation at *Scr*. However, disruption of synapsis alone is not sufficient to confer a gain-of-function phenotype. This is most clearly demonstrated by the fact that rearrangements with breakpoints either between the centromere and the *Scr* locus or within the *Scr* locus itself that interfere with normal chromosome synapsis do not by themselves exhibit gain-of-function phenotypes (A. M. PATTATUCCI and T. C. KAUFMAN, unpublished observations).

Rearrangements between the centromere and the *Scr* gene, when in conjunction with rearrangements that confer an *Scr* gain-of-function phenotype, probably exert their effects on *Scr* gene expression by virtue of their potential to further hinder chromosome pairing in this region of the genome. However, this effect is only detectable in the presence of juxtaposed permissive sequences or in genetic backgrounds such as *Polycomb*, where negative regulation at *Scr* has already been compromised. They also have the added feature of creating an environment favorable for the novel sequences juxtaposed by the gain-of-function rearrangement to direct the ectopic expression of the *Scr* structural gene in *cis* as well as in *trans*.

The remarkable similarity between *Scr* heteroallelic interactions in *cis vs. trans* and those described for Cbx^1 Ubx^1 (LEWIS 1955), have lead us to conclude that the *Scr* locus also exhibits transvection. We find it noteworthy that *Ubx*, *dpp* and *Scr*, all exhibit haploinsufficient phenotypes and are very large genes with extensive regulatory regions. It is notable that, in the case of *Scr*, its extensive regulatory region is split by *ftz*, another developmentally important gene. Studies examining *ftz* sequences have identified specific DNA fragments at the bases of chromatin loops which have been designated SARS–for scaffold attached regions (MIRKOVITCH, GASSER and LAEMMLI 1987). SARS contain a large number of sequences related to the *topoisomerase II* consensus cleavage site (GASSER and LAEMMLI 1986). They tend to be located close to the promoters of potential highly active genes and are associated with their upstream regulatory sequences. Although yet to be demonstrated, the specific positioning of SARS close to active genes such as *ftz* has been proposed to serve to bring distant regulatory sequences close together for the purpose of creating functional complexes for the regulation of transcription (GASSER and LAEMMLI 1987). An intriguing possibility is that the SAR sequences located 5′ and 3′ to the *ftz* locus not only are important for *ftz* expression, but are also in some manner significant for *Scr* regulation. Interestingly, clusters of sequences similar to *topoisomerase II* cleavage sites have recently been identified in the upstream regulatory regions of the *labial* gene of the ANT-C (S. CHOUINARD, personal communication).

The idea that chromatin architecture might be in some way linked to gene expression at *Scr* could lead to the proposal that the locus is regulated in a synapsis-*dependent* manner. However, this is clearly not the case. As noted above, rearrangement heterozygotes that disrupt homologous chromosome synapsis in the region of the ANT-C exhibit no visible phenotypic abnormalities in adults, nor do they show any abnormalities in staining patterns of imaginal discs. Therefore, we favor a model in which proper gene regulation at *Scr* is not *dependent* on the homologous chromosomes being synapsed but instead proper regulation is *pairing-sensitive* such that gene regulation is more efficient when chromosomes are synapsed. Our results with Scr^4 Scr^{ScxW}/Pc^3 and Scr^4/Pc^3 Scr^{ScxW} support this model. Scr^4 Scr^{ScxW}/Pc^3 shows only minor enhancement of ectopically expressed protein compared to Scr^4/Pc^3 Scr^{ScxW}. The most parsimonious explanation for this minor enhancement observed for Scr^4 Scr^{ScxW}/Pc^3 is that it is the result of the additive effects of *trans*-activation by Scr^{ScxW} and *cis*-misexpression by *Polycomb* on the companion homolog. On the other hand, our data demonstrate that Scr^{ScxW} is incapable of ectopically activating its own *cis*-coupled structural gene. Therefore, the striking enhancement of ectopically expressed *Scr* protein in Scr^4/Pc^3 Scr^{ScxW} must be accounted for solely by the Pc^3 mutation. We propose that the Pc^3 mutation results in the absence of a function that normally prevents ectopic activation of the *cis*-coupled structural gene by Scr^{ScxW}. Thus, in wild-type animals, the *Polycomb* gene product probably serves to block *cis*-activation of the *Scr* gene by certain positive regulator proteins.

A number of loci have been identified that have been implicated in the control of *Scr* gene expression. Unfortunately, very little molecular data is available for any of these genes. However, it could be that the products of one or a set of these genes will turn out to perform standard *cis*-regulatory functions that have evolved to work at peak efficiency as a consequence of normal synapsis of homologous chromosomes. Nevertheless, tests with Pc^3/Scr^{ScxT1} and Scr^{ScxT1}/Pc^3 Scr^{ScxW} have revealed that a powerful *trans*-regulatory component does exist at *Scr*; the phenotype of the

latter is significantly enhanced over that of the former or Pc^3 $Scr^{Scx^W}/+$. This is most easily explained in light of a disruption in pairing sensitivity brought about by the $Scr^{Scx^{T1}}$ rearrangement. Therefore, it might be expected that at least a subset of these loci will in some manner be involved in either mediating synapsis or conferring chromatin architectural functions.

RANDAZZO, CRIBBS and KAUFMAN (1991) have proposed the *prison hypothesis* to account for the large size of the homeotic genes in the ANT-C and BX-C and the fact that these complexes, as well as the linear order of the genes within the complexes, have been essentially preserved for over five hundred million years of evolutionary time. They suggest that the promoters of these genes are exquisitely sensitive to position effect and that selection against the ectopic expression of these powerful developmental proteins has maintained these genes in stable complexes. Our demonstration that *Scr* expression is proximity modulated is consistent with the view of gene complex members as highly specialized, relatively inflexible, regulatory units. As is the case with *ftz* and *Scr*, many genes contained in large complexes such as ANT-C, BX-C and *dpp* may have overlapping promoter and regulatory elements. With this in mind, the *prison hypothesis* suggests an enticing link between the linear organization of these gene complexes and three-dimensional chromatin structure. Although yet to be demonstrated, it may be that the fine-tuned regulation of genes within these complexes requires a higher order chromatin architecture that could be mediated both by multimeric proteins and DNA scaffolding. Any significant alteration in the arrangement of overlapping regulatory elements along the DNA would result in potentially lethal misregulation of one or more genes in the complex, thus preventing such new arrangements from entering the gene pool. In this sense, these genes would indeed be *prisoners* of the complexes in which they are found.

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