Deletion analysis of the *achaete-scute* locus of *Drosophila melanogaster*

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The *achaete-scute* gene complex (AS-C) is involved in the development of the central and peripheral (sensory chaetae, sensilla) nervous system. To assess the contribution of the different parts of the complex in the generation of the adult chaetae pattern, we have determined the phenotypes and molecular positions of the breakpoints of 74 terminal deficiencies of the X chromosome. According to these and previous data, the AS-C is organized, distally to proximally, as follows: the *achaete* region, with most of its DNA (10 kb) located upstream from the putative *achaete* (T5) gene; an intermediate region, approximately 18 kb long, whose deletion only weakly affects the scute function; and the *scute* region, with most of the DNA critical for its function extending 4–5 kb upstream and 50 kb downstream of the putative *scute* (T4) gene. The DNA extending far upstream of the T5 gene and downstream of the T4 gene may provide chromatin conformations adequate for efficient expression of these genes. However, in the case of the T4 gene, the available data suggest the presence of a small number of elements, scattered in the long downstream region, that would respond to topological cues and *cis*-activate this gene in specific anatomical regions.

[Key Words: Terminal deficiencies; *achaete-scute* complex; *Drosophila*; chaetae pattern; *cis*-control]

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The epidermis of insects contains innervated sensory organs (chaetae, sensilla) distributed according to species-specific patterns. In *Drosophila*, differentiation of these organs depends, both in the larva and the adult, on the activity of the *achaete-scute* complex (AS-C), which is located at the tip of the X chromosome (region 1B1-4). Its genetic analysis (García-Bellido 1979) has allowed its subdivision into *achaete* (*ac*), *scute* (*sc*) α , *lethal of scute* (*l'sc*), and *sc* β regions (distally to proximally). Recently, the existence of an additional region, proximal to *sc* β and named *sc* γ , has been proposed (Dambly-Chaudière and Ghysen 1987; Jiménez and Campos-Ortega 1987).

In the adult, *ac* mutations remove mostly hairs (microchaetae) and *sc* mutations affect mostly bristles (macrochaetae). *ac* and *sc* mutations complement each other, but pairs of *ac* or *sc* mutations complement only partially (García-Bellido 1979). In mitotic recombination clones, deletion of the whole AS-C prevents, with a few exceptions, differentiation of all chaetae and sensilla (García-Bellido and Santamaria 1978). On the other hand, deletion of the *l'sc* region causes the death of developing cell precursors of the embryonic central nervous system (Jiménez and Campos-Ortega 1979, 1987). Other partial deletions of the complex remove different subsets of larval sensory organs (Dambly-Chaudière and Ghysen 1987). Taken together, these results indicate that the AS-C is involved in the development of both peripheral and central nervous systems. This conclusion is further strengthened by the expres-

sion of the AS-C genes in the neurogenic region of *Drosophila* embryos (Cabrera et al. 1987; Romani et al. 1987).

The cloning of the AS-C DNA has provided a molecular correlate to the genetic subdivision of the complex (Campuzano et al. 1985). The AS-C occupies at least 90 kb of DNA located proximally and adjacent to the *yellow* (*y*) locus (Fig. 1). The few previously characterized *ac* mutations (3) map within 5 kb in the distal part of the complex. An RNA (T5) transcribed from this region is thought to be involved in the *ac* function. Most of the many *sc* mutations analyzed (23) are associated with lesions scattered over the proximal 50 kb of the complex. This DNA spans the proximal *sc* α , *l'sc*, and *sc* β regions (distally to proximally). At least three RNAs are transcribed from these regions. One (T4 RNA) is transcribed from the proximal *sc* α region and is thought to be most important for the *sc* function. Another (T3 RNA) is involved in the *l'sc* function (F. González, S. Romani, M. Ruiz-Gómez, F. Jiménez, and J. Modolell, in prep.). The putative product encoded by the third one (T2 RNA) and its spatial distribution indicate that, most likely, this RNA is irrelevant for nervous system development (F. González, S. Romani, and J. Modolell, unpubl.). The structural genes of the AS-C RNAs are separated by long stretches of DNA. Most *sc* mutations map within presumably nontranscribed DNA. Long-range *cis*-perturbations of the expression of the T4 RNA (and perhaps of other transcripts) by the DNA lesions have been invoked to explain the mutant phenotypes (Campuzano et al. 1985). The *sc* γ region, located proximally

Figure 1. Location of breakpoints of *Df(1)RT* chromosomes. DNA present in each deficiency is represented by a continuous line under the *achaete-scute* system of coordinates (Campuzano et al. 1985). Dashed lines represent the uncertainty in the location of the breakpoints. On top of the figure, a simplified physical map of the AS-C is represented, indicating its major subdivisions. Location of breakpoints of several inversions, *T(1;2)sc¹⁹*, *sc¹* (a gypsy insertion), and the *sc⁶* deletion are indicated above the coordinate line or on the simplified map of the AS-C. Thick horizontal arrows indicate the regions where transcripts T2–T6 arise. Location of the T6 (*yellow*) gene is according to Chia et al. (1986) and to our unpublished observations. Several embryonic transcripts arising from the region comprised between the T5 and T6 genes (Campuzano et al. 1985; Chia et al. 1986) and another transcript arising from the *scute* γ region are being characterized in our laboratory and have not been indicated. Transcription of the T4 gene starts approximately 160 bp to the right of an *XhoI* site at coordinate 33.4 (Villares and Cabrera 1987). The most proximal deficiency (214) has the breakpoint within 0.7 kb to the right of this *XhoI* site. All the RT deficiencies mapped are *y*[−].

from the *sc¹⁹* breakpoint (Fig. 1), is important for the development of the larval sensory organs (Dambly-Chaudière and Ghysen 1987), but appears to have little bearing on the development of the adult chaetae pattern (García-Bellido 1979; Campuzano et al. 1985; our unpubl. data).

In this work, we have carried out a deletion analysis of the distal part of the AS-C, with the aim of defining the role(s) of its long untranscribed regions. More specifically, we wanted to address the question as to how the topological distribution of the *ac* and *sc* functions is encoded in the AS-C DNA. The results indicate that part of

the untranscribed regions may have a structural role in providing favorable chromatin conformations for T4 and T5 gene expressions. Moreover, they suggest the presence in regions downstream and far removed from the T4 gene of elements controlling its spatial expression.

Results

Molecular mapping of RT deficiencies

A set of 74 terminal deficiencies of the X chromosome (*Df(1)RT*; Mason et al. 1984, 1986) whose phenotypes indicated that they had breakpoints within the *y* locus

or the distal part of the AS-C have been used in this work. Their breakpoints, determined by Southern blot analyses, are evenly distributed over 40 kb of DNA, from the *y* locus to the T4 gene (Fig. 1 and Table 1). The most proximal deficiency (214) lacks, at least, the majority of the T4 gene 5'-flanking region or, at most, all the 5' region and part of the T4 transcribed sequences. Two deficiencies (696 and 623) had more than one breakpoint within the AS-C.

Phenotypes of the RT deficiencies

The molecular map obtained indicates that the RT deficiencies should be useful for studying the contribution of the distal part of the AS-C in the generation of the chaetae pattern. Accordingly, we examined the phenotypes of females heterozygous for *Df(1)RTs* and intercalary deficiencies of the AS-C. In general, RT deficiencies with closely mapping breakpoints had similar phenotypes, suggesting that the probable heterogeneity of the foreign DNA sequences distal to the RT breakpoint did not affect greatly the *ac* and/or *sc* phenotypes associated with the RT chromosomes.

The *ac* phenotypes. We used combinations of the *Df(1)RTs* with the *Df(1)sc¹⁹*, which removes most of the AS-C, or the *In(1)y^{3PLsc^{8R}}*, which is deficient for *y* and *ac* but is almost *sc⁺* (García-Bellido 1979; Campuzano et al. 1985). *ac* phenotypes were determined by counting the number of microchaetae on the notum and by scoring for the presence of *ac*-sensitive macrochaetae (AVT, PDC, ADC, and PSA, García-Bellido 1979; for macrochaeta nomenclature, see Fig. 4). Figure 2 shows phenotypes of RT deficiencies with breakpoints located in the *y* gene and the most distal part of the AS-C. The number of microchaetae was a sensitive indicator of amount of *ac* function. With only a few exceptions, loss of microchaetae correlated linearly with the proximity of the breakpoints to the T5 gene structural sequences. Breakpoints 10 kb upstream from it, still within the *y* transcribed region, already showed microchaetae suppression. The *ac*-sensitive macrochaetae were also affected by breakpoints far removed from the T5 gene (legend to Fig. 2; Fig. 3). In general, the loss of macrochaetae was greater for the deficiencies that mapped closer to the T5 gene, an exception being the AVT macrochaeta that exhibited an erratic pattern of suppression (Fig. 3). Breakpoints in the immediate vicinity of the T5 gene (*RT626*), or those that removed this gene, caused maximal macrochaetae suppression.

RT deficiencies 242 and 691 were exceptions to the correlation between location of breakpoint and *ac* phenotype. They mapped closely, the second one having a breakpoint less than 0.9 kb upstream from the T5 transcribed sequences. Both had relatively weak *ac* phenotypes.

The wild-type heminotum has approximately 135 microchaetae. On its medial region, microchaetae are arranged in four or five longitudinal rows, whereas on the lateral part, the distribution is more irregular, with some

Table 1. Localization within the AS-C of breakpoints of RT deficiencies

<i>Df(1)RT</i>	Localization on the DNA ^a
394, 473, 653	H (72.3)–B (71.6)
276, 741	Pv (71.5)–C (69.5)
627	C (69.5)–Pv(68.7)
733	R (68.6)–Pv(67.8)
81	Pv (67.8)–P (67.7)
94	P (67.7)–G (67.2)
303, 628, 689	G (67.2)–A (66.3)
497	G (65.6)–P (65.3)
625	P (65.3)–G (64.8)
488	X (64.2)–P (63.2)
589	R (62.9)–X (62.4)
195, 633, 659	X (62.4)–G (61.8)
242, 331	Hc(61.2)–Hc(60.3)
739	Hc(60.3)–S (59.9)
696	P (60.2)–S (59.9)
	B (53.2)–S (52.4)
	B (47.9)–R (45.8)
691	S (59.9)–R (59.7)
626	R (59.7)–B (58.9)
398, 558	B (58.9)–P (58.2)
293	R (57.6)–R (56.6)
212	H (56.8)–S (56.3)
97, 655	B (55.8)–R (54.3)
95, 503, 624	R (54.3)–B (53.2)
84, 86, 701	B (53.2)–S (52.4)
216, 356, 520, 590	S (52.4)–B (50.6)
90	S (52.4)–G (50.2)
92, 438	B (50.6)–P (49.4)
83	G (50.2)–P (49.4)
93, 637, 730	P (49.4)–B (47.9)
336, 351, 742	B (47.9)–H (46.8)
85, 89, 288, 643	R (46.0)–H (45.4)
165, 630, 685	H (45.4)–H (43.9)
623	H (45.4)–G (44.4)
	Pv (36.7)–B (34.4)
304	G (44.4)–H (43.9)
524	G (42.3)–B (42.1)
103, 252	B (42.1)–B (41.5)
29, 551, 629	G (42.3)–H (39.5)
618	H (39.5)–H (37.1)
233, 748	X (38.4)–Pv(36.3)
631, 650	H (37.1)–P (35.5)
343	Pv (36.3)–B (33.8)
150	B (33.8)–O (33.4)
214	O (33.4)–G (32.7)

^aLocalization, given according to the coordinates of the physical map of the AS-C (Campuzano et al. 1985), was determined in November 1986 for the first 11 deficiencies (not including RT303), in March 1986 for RT233, 748, 343, 150, and 214, and in January–June 1985 for the remaining ones. Restriction site nomenclature is as follows: (A) *Ava*I; (B) *Bam*HI; (C) *Cl*aI; (G) *Bg*III; (H) *Hind*III; (Hc) *Hinc*II; (O) *Xho*I; (P) *Pst*I; (Pv) *Pvu*II; (R) *Eco*RI; (S) *S*all; (X) *Xba*I.

short transversal rows in the vicinity of the intrascutellar suture (Fig. 4). The RT deficiencies showed that this pattern undergoes two types of modifications with decreasing *ac* function (Fig. 4). The first is a general decrease in the density of microchaetae. The second is a progressive reduction in the area of the notum bearing

Figure 2. Amounts of T5 RNA in 0- to 1-day-old pupae in several combinations *Df(1)RT/In(1)y^{3PLsc}sc^{8R}* (Δ) and number of microchaetae on the notum of combinations *Df(1)RT/In(1)y^{3PLsc}sc^{8R}* ($\circ$) and *Df(1)RT/Df(1)sc¹⁹* ($\bullet$). One hundred percent represents: the amount of T5 RNA found in 0- to 1-day-old heterozygous Oregon R/*In(1)y^{3PLsc}sc^{8R}* control females; the number of microchaetae on the heminotum of females of genotypes wild-type Canton S/*In(1)y^{3PLsc}sc^{8R}* (120.1 ± 5.6 , not including the humeral microchaetae) and wild-type Vallecas/*Df(1)sc¹⁹* (123.4 ± 3.7 , including the humerals). Canton S/*In(1)y^{3PLsc}sc^{8R}* females had 129 ± 6 microchaetae, including the humerals. Homozygous Oregon R, Canton S, and Vallecas females had 135 ± 2 , including the humerals (9 ± 1). Standard deviations for the number of microchaetae in the *Df(1)RT/In(1)y^{3PLsc}sc^{8R}* and *Df(1)RT/Df(1)sc¹⁹* ranged from 5.3 to 12.6 and 2.3 to 16.8, the most common values being between 6 and 8, and 4 and 8, respectively. Dorsocentral macrochaetae were affected in the combinations of *In(1)y^{3PLsc}sc^{8R}* with the RT733 (18% of heminotums), 81 (33%), 94 (44%), 628 (58%), 689 (35%), 303 (53%), and all the remaining more proximal deficiencies. PSA macrochaeta was affected in RT303 and all the more proximal ones. Positions of several deficiency breakpoints do not coincide with those shown in Fig. 1 and Table 1, since phenotypes were determined and the breakpoint positions reexamined ~ 1.5 yr after the original localization. Determination of the phenotypes represented by filled symbols was performed 5–7 months before the determination of the breakpoints; therefore, their position on the DNA coordinate is only approximate. Positions of T6 and T5 transcribed sequences are indicated. When two or three deficiency breakpoints share the same location, the microchaetae symbol with the largest ordinate corresponds to the top RT identifying number, the second symbol to the second number, and so on. The homozygous *In(1)y^{3PLsc}sc^{8R}*, although deficient for *ac*, differentiates 40% as many microchaetae as the wild type (Fig. 6). Originally this deficiency was an extreme *ac* mutant. The accumulation of modifiers has led to a partial replacement of the missing *ac* function, probably by the *sc* function. This may explain that RT deficiencies lacking most or all of the *ac* function show more moderate *ac* phenotypes in combination with the *In(1)y^{3PLsc}sc^{8R}* than with the *Df(1)sc¹⁹*.

microchaetae. Thus, the weakest mutations affect only the area surrounding the DC macrochaetae at the posterior third of the notum. The next area affected is the anterior and central part of the notum. In more extreme mutations, microchaetae appear only in the central area of each heminotum.

The *sc* phenotypes. These phenotypes were determined for all the *Df(1)RTs* except the 11 more distal ones by scoring for the presence of macrochaetae on the head and notum. When two or more deficiencies had their breakpoints within the same restriction fragment and had similar phenotypes, only the phenotype of the most viable combination will be shown. Phenotypes were determined in *Df(1)RT/Df(1)sc¹⁹* combinations (Fig. 3) or, if these were lethal, in combinations with *In(1)sc^{8Lsc}sc^{4R}* or *In(1)sc^{8Lsc}sc^{9R}* (Fig. 5). It should be stressed that *In(1)sc^{8Lsc}sc^{9R}*, although a strong *sc* mutant, still has some *sc* function. For the sake of comparison, the phenotypes of some RT deficiencies that were viable in combination with *Df(1)sc¹⁹* or *In(1)sc^{8Lsc}sc^{4R}* are also shown over the *In(1)sc^{8Lsc}sc^{4R}* or *In(1)sc^{8Lsc}sc^{9R}*.

Deficiencies mapping most distally caused, at the most, extremely weak *sc* phenotypes (Fig. 3). Similar phenotypes were observed with the control combination $+/Df(1)sc¹⁹$ (not shown). The majority of more proximal deficiencies (mapping between coordinates 55 and 38) showed significant but weak *sc* phenotypes (Figs. 3 and 5); those with breakpoints within 4–5 kb from the T4 gene transcriptional start caused intermediate or strong *sc* phenotypes. Surprisingly, most phenotypes of combinations with the *In(1)sc^{8Lsc}sc^{4R}* were stronger than those

with the *Df(1)sc¹⁹* (RT630, 304, 524, 629, 618, and 631). This effect was especially noticeable with the ANP, PS, and PNP macrochaetae. It suggests that the phenotypes of the RT deficiencies can be modified by the homolog chromosome, even if this is *sc⁻*, or by the genetic background.

The *In(1)sc^{8Lsc}sc^{4R}* has an apparently intact *ac* region that does not fully support development of *ac*-sensitive macrochaetae (AVT, PDC, ADC, and PSA), probably due to variegation of the *sc⁸* breakpoint (Fig. 5). RT deletions deficient for the *ac* region partially or completely rescued the AVT and A/PDC macrochaetae, suggesting that their development can be mediated by both *ac* and *sc* functions. This interpretation is reinforced by the partial removal of A/PDC macrochaetae in combinations of *In(1)sc^{8Lsc}sc^{9R}* with *Df(1)RTs* that are relatively strong *sc*, but not with those that are weak *sc* (Fig. 5).

Abundance of T5 RNA in *Df(1)RT* pupae

The T5 gene has been directly implicated in the *ac* function (Campuzano et al. 1985, 1986). As a measure of its activity in RT deficiencies, we have determined the abundance of T5 RNA in 0- to 1-day-old pupae of combinations *Df(1)RT/In(1)y^{3PLsc}sc^{8R}*. (This stage of development coincides with a maximum of T5 RNA accumulation.) Figure 2 shows that RT deficiencies with *ac* phenotypes have decreased abundances of T5 RNA and that there is a positive correlation between abundance of T5 RNA and number of microchaetae on the notum.

Figure 3. Phenotypes of the *Df(1)RT/Df(1)sc¹⁹* combinations. The standard nomenclature for each macrochaeta is used (see Fig. 4). (Mc) microchaetae. The bars indicate that the corresponding macrochaeta is absent in <10% (|), 10–39% (||), 40–69% (|||), or 70–100% (||||) of the heminotums or half heads. Twelve macrochaetae are grouped in six pairs (A/PDC, HU, A/PPA, A/MOR, SP, and SC). For these macrochaetae 100% represent the complete absence of both members of the pair. For Mc, the same symbols represent percent of suppressed microchaetae. Some combinations had poor viability. In these cases, the number of flies examined, if less than 12, is indicated on top of the corresponding phenotype. *Df(1)RTs* are ordered according to the position of their breakpoints on the DNA (Fig. 1). The phenotype of *RT150* was obtained from dead pharate adults removed from the puparium. *Df(1)RT685*, not shown, has a breakpoint at coordinate 45, and, remarkably, a very weak *ac* phenotype and many extrachaetae in ectopic positions (Hairy-wing phenotype, not shown). The analysis of this deficiency will be presented elsewhere. Approximate locations of *T5* and *T4* genes and *In(1)sc⁸* breakpoint are indicated.

Interactions between *Df(1)RT* and *h¹* or *emc^{pel}*

It has been shown that loss-of-function mutations at the *hairy* (*h*) and *extramacrochaetae* (*emc*) loci promote an excess of function of the AS-C, with the generation of supernumerary chaetae on ectopic positions (Moscoso del Prado and García-Bellido 1984a,b). To identify AS-C regions important for these interactions, we examined the effects of homozygous *h¹* and *emc^{pel}* mutations on the phenotypes of *Df(1)RTs/Df(1)y^{3PLsc^{8R}}*. The analysis was restricted to the *ac* region (Fig. 6). *h¹* did not significantly affect the phenotype of the control *Df(1)y^{3PLsc^{8R}}* (see also Moscoso del Prado and García-Bellido 1984a), but partially rescued the *ac* phenotype of most RT defi-

ciencies. *emc^{pel}* had a larger rescuing effect, which was more pronounced, in absolute number of microchaetae, when some *ac* function remained; rescuing included the

Figure 4. Chaetae positions on the heminotum of females heterozygous for the *In(1)y^{3PLsc^{8R}}* and a wild type (Canton S) or a *Df(1)RT* chromosome. Small dots and empty circles represent positions of micro- and macrochaetae, respectively. Patterns have been drawn after photographs of a typical individual of the indicated genotype. The central longitudinal rows are more apparent on the fly due to the anterior-posterior tilt of microchaetae. Macrochaeta nomenclature, as described in Lindsley and Grell (1968) or García-Bellido (1979), is indicated. The breakpoint position for *RT497* is that indicated in Fig. 2; that for *RT739* was to the right of the *T5* gene, making this deficiency null for *ac* but *sc⁺*. *RT214* is a strong *sc*.

Figure 5. Phenotypes of the combinations *Df(1)RT/In(1)sc^{8L}sc^{4R}* (left panel) and *Df(1)RT/In(1)sc^{8L}sc^{9R}* (right panel). Symbols and nomenclature are as in Fig. 2. (C) Phenotype of pharate male flies *In(1)sc^{8L}sc^{4R}* dissected from the puparium. Deficiencies RT89, 103, 150, 214, 233, 252, 288, 336, 343, 551, and 748 were lethal in combination with *Df(1)sc¹⁹*. The lethality was not due to the lack of *l'sc* function; all were viable over the *In(1)sc^{8L}sc^{9R}* (*l'sc⁻*). Deficiencies 233, 748, and 214 were lethal in combination with the *In(1)sc^{8L}sc^{4R}*.

control *ac⁻* deficiency. Thus, the *ac* region distal from the breakpoint of *RT691* can be deleted without impairing the *h¹* and *emc^{pel}* effects. (We interpret the small rescue by *h¹* and *emc^{pel}* in the absence of *ac* function, *RT589* and *701*, as mediated by the *sc* function.) Interestingly, in the combinations of *RT691* and *242* with *emc^{pel}*, the distribution of microchaetae on the notum was very similar to that of the deficiencies with the weakest *ac* phenotypes or even to that of the wild type (not shown). This indicates that, under these conditions, most of the region upstream from the *T5* gene is unnecessary for the generation of the microchaetae pattern.

Discussion

The achaete region

One of the goals of this work has been to define molecularly the extent of the *ac* and distal *sc a* regions. We have assumed that they extend as far as the locations of breakpoints with *ac* or *sc* phenotypes. Evidently, the regions thus defined may not be confined to the DNA con-

Figure 6. Phenotypes of the combinations *Df(1)RT/In(1)y^{3PL}sc^{8R}*, *Df(1)RT/In(1)y^{3PL}sc^{8R};h¹/h¹*, and *Df(1)RT/In(1)y^{3PL}sc^{8R};emc^{pel}/emc^{pel}*. One hundred percent represents the number of microchaetae of the wild-type heminotum (135). Positions of RT breakpoints are as indicated (see legend to Fig. 2). (C) Phenotypes of the control homozygous *In(1)y^{3PL}sc^{8R}* females in combinations with wild type, *h¹* and *emc^{pel}* alleles.

taining specific regulatory sequences for the ac and sc functions. They may also comprise stretches of chromatin whose structure is a prerequisite for wild-type function but are not regulatory in a strict sense. For example, deficiencies with breakpoints within the *y* gene have weak ac phenotypes (Fig. 2; *RT627*, *733*, and *81*). Thus, in this broad sense, the distal limit of the ac region appears to be within the *y* gene. [The proximal limit should be contained within the *Dp(1;Y)sc⁸* since this duplication complements the ac phenotypes of RT deficiencies mapping at both sides of the *sc⁸* breakpoint (Ruiz-Gómez 1986).]

In general, the ac phenotypes of the RT deficiencies are stronger the more ac DNA they eliminate. *RT242* and *691* are exceptions: they have ac phenotypes weaker than those of deficiencies with neighboring breakpoints. Possibly, the adjacent foreign DNA somehow enhances the ac function. However, the close proximity of the *242* and *691* breakpoints suggests alternative explanations. For instance, the deletions may have eliminated distal DNA sequences that depress ac function; or the chromatin structure of the remaining ac region is more favorable for ac function than that adopted by more complete but still truncated ac regions.

Role of the ac upstream region

We have proposed that the T5 gene is directly implicated in the ac function (Campuzano et al. 1985). Consistent with this proposal, the amounts of T5 RNA are decreased in *Df(1)RTs* and correlate roughly with the number of microchaetae on the notum (Fig. 2). Thus, the interference of *Df(1)RTs* with ac function is probably mediated by a decreased expression of the T5 gene. On the other hand, the gradual decrease of ac function with increasing removal of the DNA comprised between 10 and 2 kb upstream from the T5 gene suggests that most of this DNA is somehow relevant for the ac function, the deletion analysis having failed to single out one or a few subregions as especially important. However, the very weak ac phenotypes of the combinations *Df(1)RT242* or *691/In(1)y^{3PL}sc^{8R};emc^{pel}* indicate that under some conditions, most or all of this DNA is dispensable. Only 0.9 kb of 5' sequences flanking the T5 gene (*RT691*) are sufficient to implement an almost wild-type microchaetae pattern. Thus, most DNA elements controlling the temporal and spatial expression of the T5 gene are probably located within this short 5' region or proximally from it. The long region further upstream may provide a chromatin conformation adequate for efficient transcription. A relatively homogeneous distribution of T5 product might lead to a regular pattern of microchaetae by means of cell interactions (Ghysen and Richelle 1979; García-Bellido 1981). Decreased transcription reveals inhomogeneities in the notum since there is a preferential loss of microchaetae from certain areas (Fig. 4). Thus, the microchaetae pattern probably results from a combination of local requirements for T5 product and differential activity of the T5 gene, refined by cell interactions. It should be

pointed out that a set of overlapping RNAs, found only in embryos, is transcribed from the region between the T5 and T6 (*y*) genes (Campuzano et al. 1985; Chia et al. 1986; L. Balcells, R. Villares, and J. Modolell, unpubl.). Their spatial distribution seems confined to yolk nuclei/cells (J. Garrell and J. Modolell, unpubl.). These and other properties (our unpubl. results) argue against a role in promoting chaetae development.

The scute region

The phenotypes of RT deficiencies indicate that the sc region extends approximately 22 kb to the left of the T4 structural gene, a gene thought to be most important for the sc function (Campuzano et al. 1985; Villares and Cabrera 1987). Breakpoints in the distal 18 kb of this region cause weak sc phenotypes at most, and those mapping within 4–5 kb of the T4 gene cause moderate or strong sc phenotypes. Thus, the sc region distal to the T4 gene may be divisible into two subregions according to the phenotype strength of their respective deletions.

Within the distal 18-kb subregion, there is no correlation between the weak and erratic sc phenotypes and the proximity of the breakpoints to the T4 gene (Fig. 3 and Fig. 5, left panel). Probably the proximity of the breakpoint and/or the telomeric region causes an insufficiency of the sc function. Alternatively, although more unlikely, an element involved in the sc function is located at the distal end of the subregion. Whatever the explanation, this subregion seems relatively unimportant for the sc function.

The proximal subregion, whose deletion causes moderate or strong sc phenotypes, comprises the 4–5 kb immediately upstream from the T4 gene. This contrasts with the long (50 kb) downstream sc region defined by most of the previously described sc mutations (Campuzano et al. 1985). A property of these mutations is that the extent of their effect can be measured in the same series of macrochaetae positions affected. That is, macrochaetae can be seriated according to the sensitivity of each chaeta to the sc mutations (García-Bellido 1979). In Figures 3 and 5, macrochaetae are listed following this order of sensitivity, the SC being the most sensitive. Clearly, the sc phenotypes of the RT deficiencies do not fit that series. The HU, PS, and PNP are most sensitive and SC and OC are very resistant to the RT deletions, whereas the opposite occurs with the downstream mapping mutations. Thus, upstream and downstream modifications of the sc DNA affect the spatial distribution of the sc function differently. Two alternatives, or a combination of both, seem the most likely explanations.

Roles of the regions upstream and downstream of the T4 gene

The first alternative assumes that, by analogy with many other genes, the sequences controlling the temporal and spatial expression of the T4 gene are located in the neighborhood of the transcriptional start. Perturbations of the chromatin conformation by the far down-

stream lesions would decrease T4 gene expression. Since there is a positive correlation between the strength of the *sc* phenotype and the proximity to the T4 gene of the DNA lesion (Campuzano et al. 1985), it is assumed that the closer the lesion, the stronger its interference would be. Assuming also that chaeta precursor sites differ in the amounts of T4 gene product required for chaeta differentiation (García-Bellido and Santamaría 1978), a quantitative decrease in T4 product could explain the gradation of *sc* phenotypes. Due to their proximity the upstream RT breakpoints would interfere more directly with the spatial controlling elements and affect the distribution, as well as the amounts, of T4 product. This could explain their phenotypes not fitting the series of chaeta positions.

The second alternative assumes that *cis*-acting, site-specific elements located both 5' and 3' from the T4 gene control its spatial expression. The elements would respond to topological cues and activate the T4 gene by a structural modification or via a product. The downstream mapping *sc* mutations suggest locations for the 3' elements. These mutations can be grouped in four clusters defined by similarity of phenotypes and physical proximity of the lesions (see Fig. 2 of Campuzano et al. 1985). Examples of mutations of each cluster are *In(1)sc^d*, *T(1;3)sc^{KA8}*, *scⁱ*, and *T(1;2)scⁱ⁹* (Fig. 1). There would be at least one regulatory element in the DNA corresponding to each cluster, probably in the part most distant from the T4 gene. By interrupting the physical (chromosomal rearrangements) or functional (actively transcribing insertions like *gypsy*) continuity of the *sc* DNA, the lesions would impair the *cis*-activity of the regulatory element and decrease or eliminate T4 gene transcription in the region(s) of the imaginal disk containing the precursor cells of the affected chaeta(e). The gradation of phenotypes would be due to the progressive removal of consecutive regulatory elements.

This alternative predicts that an internal deletion in the downstream region should only affect the chaetae controlled by the deleted element(s). This is the case for the *sc⁶* deletion (Fig. 1). Its phenotype is similar to that of mutations within the same cluster (i.e., *scⁱ*), the most striking difference being that *sc⁶* does not affect the SC macrochaetae. Mutations in the rightmost cluster only affect these macrochaetae (i.e., *T(1;2)scⁱ⁹*), suggesting that this cluster contains a scutellar-specific element whose action would not be impaired by the deletion. Unfortunately, no other conveniently located deletions are available to check the generality of the prediction and to help locate the putative control elements.

The following observations also support the second alternative. The *Hw^{ac}* mutation causes overexpression of the T4 gene and development of many supernumerary macrochaetae; however, such overexpression does not rescue the simultaneous *sc* phenotype of this mutation due to a breakpoint located between the *sc* α and *sc* β regions (L. Balcells and J. Modolell, unpubl.; García-Alonso and García-Bellido 1986). Similarly, the excess of function of the AS-C in *emc* mutants does not rescue the macrochaetae suppressed by *sc* mutations (Moscoso del

Prado and García-Bellido 1984a,b; García-Alonso and García-Bellido 1986). Thus, lesions in the downstream region cause local, site-specific depletions of *sc* function that are not compensated by its general overexpression.

Regardless of their physical localization, the spatial controlling sequences might only specify transcription in relatively large but distinct areas of the imaginal disks. Interactions between cells could later refine the pattern of expression (Wigglesworth 1940; Ghysen and Richelle 1979; Moscoso del Prado and García-Bellido 1984b). Evidently, as discussed for the *ac* pattern, different requirements for amounts of T4 product for chaeta development in distinct areas might also be involved in defining the *sc*-controlled macrochaetae pattern.

Materials and methods

Drosophila stocks

Seventy-four stocks carrying the *Df(1)RTs* were a gift of Dr. J. Mason. They were kept as *Df(1)RT/y²scⁱY/CDX y f*. Other stocks used here, described in Lindsley and Grell (1968), are as follows: *In(1)y^{3PL}sc^{8R}*, *In(1)sc^{8L}sc^{4R} v f/In(1)dl-49 y Hw m² g⁴*, *In(1)sc^{8L}sc^{9R}sn³ w²/In(1)dl-49 y Hw m² g⁴*, *Df(1)scⁱ⁹ f³⁶/FM6*, and *y;mwh h¹. emc^{pe1}* is a spontaneous, homozygous viable mutation of the *extramacrochaetae* locus (Moscoso del Prado and García-Bellido 1984a).

Phenotype scoring

Unless otherwise indicated, phenotypes were determined in 12–20 female flies of the indicated phenotypes grown at 25°C. Presence of macrochaetae on the head and notum was scored. Microchaetae on the notum were counted. Averages and standard deviations were calculated. Since it has been shown (H. Biessmann and L. Mason, in prep.), and we have confirmed, that most RT deficiencies are unstable and gradually lose sequences adjacent to the telomer, phenotypes were determined simultaneously with the position of the breakpoints, except where indicated.

Molecular mapping of breakpoints

DNA of adult flies heterozygous for an RT deficiency and a partial intercalary deletion of the AS-C [*In(1)y^{3PL}sc^{8R}* or *In(1)sc^{8L}sc^{4R}*, Fig. 1] was extracted and analyzed in Southern blots using as probes fragments of cloned AS-C DNA covering the intercalary deletion. Five RT deficiencies with breakpoints between the *sc⁸* and *sc¹⁸* breakpoints were sublethal (RT748, 343, and 150) or lethal (233 and 214) over the *In(1)sc^{8L}sc^{4R}*. They were finely mapped using the DNA of heterozygous females *Df(1)RT/ y² Hw^{Ua}*. The *Hw^{Ua}* allele has a *cop* element inserted within the transcribed sequences of the T4 gene (Campuzano et al. 1986). Therefore, the restriction fragments comprising its insertion point (coordinate 32.4, Fig. 1) are modified in size and, in the Southern blot analyses, could easily be distinguished from the fragments of the RT chromosomes.

RNA preparations

Third instar female larvae of the indicated genotypes were selected from cultures and allowed to pupate overnight. RNA was extracted in guanidinium isothiocyanate and was purified as previously described (Maniatis et al. 1982; Campuzano et al.

1986). Quantitation of T5 RNA in total RNA was carried out by an RNase protection method (Campuzano et al. 1986).

Other methods

Drosophila DNA preparations and genomic Southern blot analyses were performed as described in Carramolino et al. (1982) and Maniatis et al. (1982), respectively.

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