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A unitary basis for different *Hairy-wing* mutations of *Drosophila melanogaster*

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Hairy-wing (_Hw_) mutations are caused by modifications of the _achaete–scute_ complex (AS–C) which promote development of extra sensory organs on the cuticle of _Drosophila melanogaster_. We show that the extreme Hw^{49c} allele contains an inversion with a breakpoint within the AS–C, while the weak Hw^{685} allele is associated with a terminal deletion of the X chromosome which removes the _achaete_ region of the AS–C. In both cases, foreign DNA in contact with the breakpoints presumably enhances expression of AS–C genes. Overexpression of _achaete_ (T5) or _scute_ α (T4) genes was previously found in _Hw_ mutants associated with insertions of transposable elements (Hw^1, Hw^{BS} and Hw^{Ua}, Campuzano _et al._, 1986). _In situ_ hybridizations to Hw^{49c} and Hw^1 larval sections show that the overexpression causes an abnormally generalized distribution of T4 and/or T5 transcripts in imaginal discs. Such distribution correlates with development of extra sensory organs in ectopic positions. We also show that in Hw^{685} a moderate overexpression of the T4 gene largely replaces the absence of the T5 gene in the development of the notum chaetae pattern. We propose that overexpression of T4/T5 genes in normal or ectopic positions is at the basis of the Hw effect.

Key words: achaete–scute complex/*Hairy-wing* mutations/pattern formation/sensory organs/gene expression

Introduction

One of the aims of developmental biology is to understand how the linear information encoded in DNA is translated into morphological patterns. The nervous system (NS) of *Drosophila melanogaster* provides an appropriate subject for this analysis. On the cuticle of the larva and the imago, sensory organs (chaetae and sensilla of other kinds) and their innervating neurons (peripheral NS) differentiate according to highly reproducible patterns; and in the neurogenic regions of the embryo and in the larval/pupal central NS, neuroblasts appear in stereotyped patterns (Campos-Ortega and Hartenstein, 1985; Truman and Bate, 1988).

The *achaete–scute* gene complex (AS–C) is a cluster of genes that plays a significant role in the generation of these patterns (García-Bellido, 1979). The AS–C comprises 90 kb of DNA and contains at least seven transcription units (Campuzano *et al.*, 1985; Alonso and Cabrera, 1988). Only four of them, the so called T5, T4, T3 and T1a (Figure 1), which encode proteins with extensive homologous domains (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; F.González and J. Modolell, unpublished results), seem responsible for the neurogenic functions of the complex. Each of these genes is located within one of the following, genetically defined regions of the AS–C: *achaete (ac)* (T5), *scute (sc)* α (T4), *lethal of sc (l'sc)* (T3) and *sc* γ (T1a) (Figure 1). Although a gene (T2) is found in the genetically defined *sc* β region, the nature of its protein product and its spatial pattern of expression discard a role in neurogenesis (F.González, S.Romani and J.Modolell, unpublished results). *sc* β probably contains sequences that control the spatial expression of the T4 gene (Ruiz-Gómez and Modolell, 1987).

The four genes of the AS–C are involved in similar genetic operations. Thus, recessive, loss of function *ac* and *sc* mutations, or the deletions of the *ac, sc* α, *sc* β or *sc* γ regions, cause the loss of different, although overlapping, subsets of sensory organs (García-Bellido, 1979; Dambly-Chaudière and Ghysen, 1987). Deletion of the *l'sc* region promotes degeneration and death of many neural precursor cells of the embryonic CNS (Jiménez and Campos-Ortega, 1979, 1987). Moreover, deletions of other regions of the AS–C synergistically enhance the effect of the *l'sc* deletion, suggesting that the four genes act in the CNS, but the T3 gene can substitute for the absence of the others (Jiménez and Campos-Ortega, 1987). Thus, each AS–C gene shows specific, but partially overlapping, topological domains of action, each gene being required for the differentiation of at least some neural cells. This interpretation is reinforced by the expression of the four genes, which occurs in the neurogenic regions of the embryo and shows distinct but partially overlapping patterns (Cabrera *et al.*, 1987; Romani *et al.*, 1987; Alonso and Cabrera, 1988; S.Romani and S.Campuzano, unpublished results). These results and earlier genetic and developmental studies (García-Bellido and Santamaría, 1978) suggest that the AS–C genes are involved in committing cells to the neural state and in promoting the early stages of neural cell differentiation (Romani *et al.*, 1987).

The role of the AS–C in neural commitment is further supported by the dominant *Hairy-wing (Hw)* mutations. These correspond to modifications of the AS–C and promote the development of supernumerary chaetae and sensilla in ectopic positions of the fly (Lindsley and Grell, 1968; García-Alonso and García-Bellido, 1986; Campuzano *et al.*, 1986). Remarkably, the mutations so far analysed at the molecular level (Hw^1, Hw^{BS}, Hw^{Ua}) are associated with insertions of the transposable elements *gypsy* or *copia* within the T5 or T4 structural genes (Campuzano *et al.*, 1986). The truncated genes give rise to truncated transcripts which encode proteins without the conserved domain at the carboxyl

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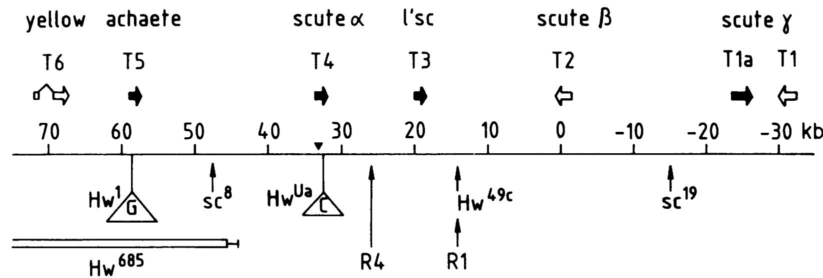

Fig. 1. Simplified physical map of the AS−C with indications of modifications found in *Hw* and other mutations. On top, the genetic subdivision of the region (García-Bellido, 1979; Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987) is indicated. Below them, thick horizontal arrows show the regions where transcripts T1 to T6 arise (Campuzano *et al.*, 1985; Chia *et al.*, 1986). Filled arrows indicate genes whose products share conserved domains (Villares and Cabrera, 1987; F.González and J.Modolell, unpublished results). Several embryonic transcripts arising from the region between the T5 and T6 genes have not been indicated. Characterization of T1a gene is in progress; its location may undergo slight modifications. T1a gene most likely corresponds to the T8 gene described by Alonso and Cabrera (1988). Thin vertical arrows, triangles and horizontal empty bar indicate position of breakpoints, insertion of transposable elements (G, *gypsy*; C, *copia*) and deleted DNA, respectively. R1 and R4 are abbreviations for $Hw^{49c + R1}$ and $Hw^{49c + R4}$. Arrowhead indicates position of an 8 bp deletion within the coding sequence of the T4 gene associated with $Hw^{49c + R5}$.

terminus (Villares, 1986). However, it has been proposed that the presence of truncated transcripts or proteins does not directly induce the Hw phenotype. This would depend on an increased abundance of the modified transcript, observed in whole organisms, which would give rise to increased amounts of truncated, but still functional, protein (Campuzano *et al.*, 1986).

To help elucidate the mechanism(s) by which excess function of the AS−C genes are generated, we have now molecularly characterized the modifications associated with other two *Hw* mutations. These are associated with chromosomal aberrations with breakpoints within the AS−C. The foreign DNA in contact with the AS−C seems to promote overexpression of AS−C genes. The results suggest a common basis for the generation of supernumerary sensory organs in *Hw* alleles.

Results

DNA lesions associated with Hw mutations

The molecular lesions associated with Hw^{49c}, three X-ray-induced revertants of Hw^{49c}, and Hw^{685} have been characterized.

Hw^{49c}. Southern blot analysis of the AS−C in this mutant showed only one modification that was located in a 1.3 kb *Bgl*II−*Sal*I fragment (coordinates 14.8−13.5 of the AS−C restriction map, Figures 1 and 2) (Carramolino, 1984). The modified region was isolated from a Hw^{49c} library. Two types of phages, containing foreign DNA and AS−C DNA from either side of the modification were obtained (Figure 2, phages λHw^{49c} 12 and 13). The foreign DNA was single copy, as judged from Southern blot analysis, and was located, in wild type chromosomes, in the middle of the early 2B ecdisone-induced puff, as determined by *in situ* hybridizations to salivary gland chromosomes (not shown). Comparing a restriction map of the 2B5 region (Chao and McGuild, 1986) with those of the inserts of the λHw^{49c} phages, it was found that the inserts could be aligned with the region between coordinates 111 and 122 of the 2B5 map (Figure 2). These data indicate that the modification is a chromosomal inversion with very little or no loss of DNA of either the AS−C or the 2B5 region. Positive hybridizations of our clones with those of the 2B5 regions (M.

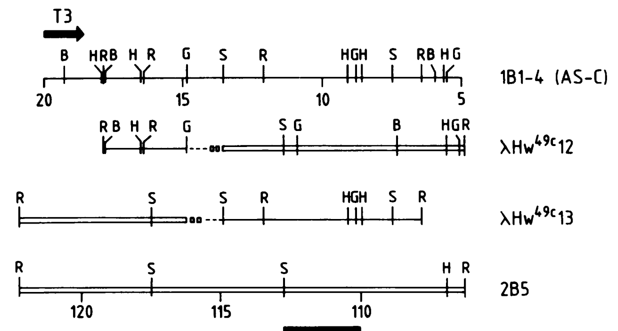

Fig. 2. Molecular maps of clones containing the break-points of $In(1)Hw^{49c}$. Representation of the λ vector arms has been omitted. Maps of the homologous regions in the AS−C (chromosomal region 1B1−4) and 2B5 are indicated for the sake of comparison. Coordinates for the AS−C are as in Figure 1 (Campuzano *et al.*, 1985); those for the 2B5 DNA are taken from Chao and McGuild (1986). Thick horizontal arrow or line below DNA lines represent transcribed regions. Restriction site nomenclature: *Bam*HI (B), *Bgl*II (G), *Eco*RI (R), *Hin*dIII (H), *Sal*I (S).

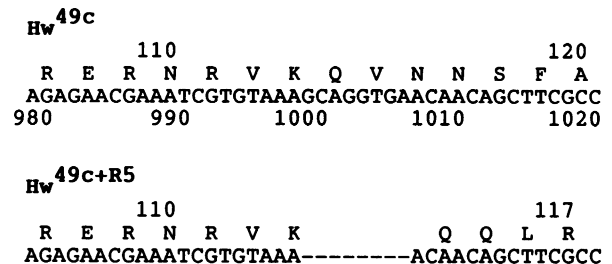

Fig. 3. DNA lesion located within the T4 gene coding sequence of $Hw^{49c + R5}$. Numerations for protein and DNA sequence are those published for Canton S T4 DNA (Villares and Cabrera, 1987). The 8 bp deletion shown is the only difference found between the T4 coding sequences of $Hw^{49c + R5}$ and the parental Hw^{49c}. Within this coding region, the Hw^{49c} sequence has been found to differ from the published Canton S sequence at the following positions: C669 is T and C1143 is G. The first modification is silent, but the second changes serine 161 into arginine. This modification was also found in a sequenced T4 cDNA (Villares and Cabrera, 1987). Due to an error, C1426 in Villares and Cabrera (1987) is actually a T in both Canton S and Hw^{49c} DNAs (Ll.Balcells and R.Villares, personal communication). This changes arginine 256 into cysteine.

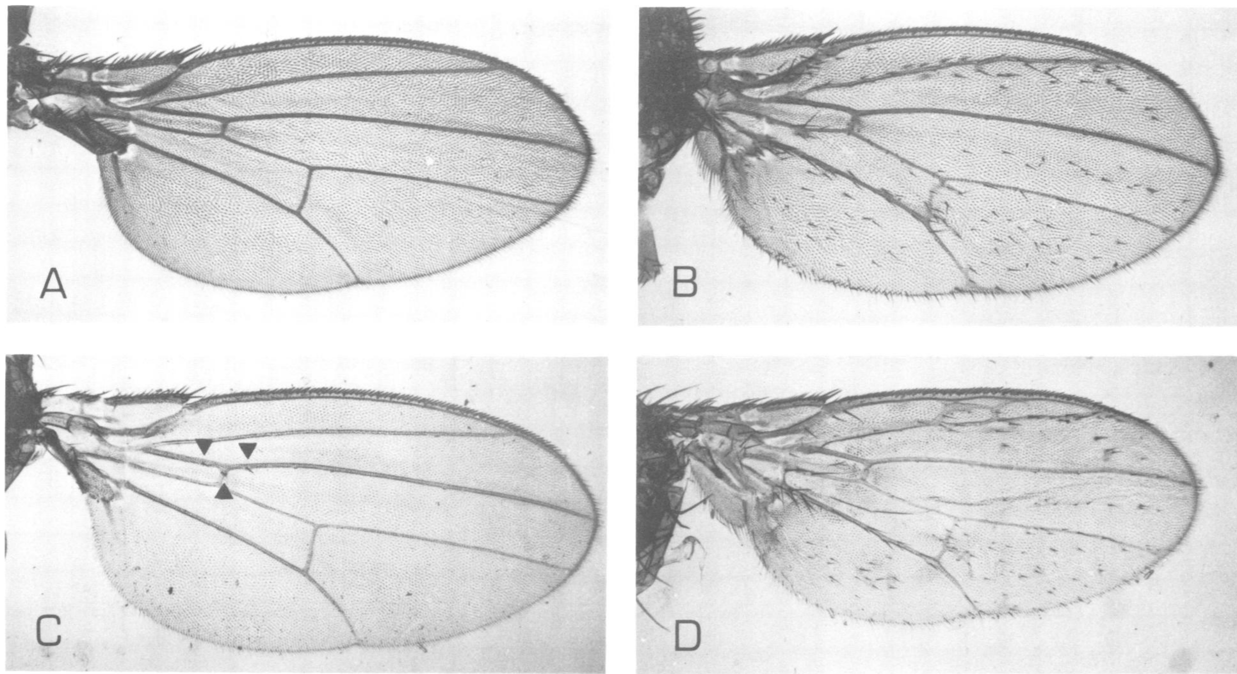

Fig. 4. Representative wings of **A**, wild type (Vallecas stock); **B**, Hw^{49c}; **C**, $Hw^{685}/Df(1)sc^{19}$; and **D**, Hw^{1} females. Hw^{49c} and Hw^{1} males have weaker phenotypes. Arrows in **C** point to the extra chaetae on vein 3. Note the numerous chaetae in the posterior half of the wing blade and on vein 2 of Hw^{1} and Hw^{49c} mutants.

Izquierdo, unpublished results) and cytological examination of HW^{49c} chromosomes confirmed this conclusion.

Hw^{49c+R4} and Hw^{49c+R1}. Southern blot analyses covering the whole AS−C showed alterations for the Hw^{49c+R4} and Hw^{49c+R1} revertants in the restriction fragments 1.8 kb *Ava*I (26.8)−*Bgl*II (25.0), which is located between the T3 and T4 genes (Figure 1), and 3.5 kb *Bgl*II (14.8)−*Sal*I (13.5), which contains the distal breakpoint of $In(1)Hw^{49c}$ (Figure 2), respectively. According to *in situ* hybridization data, the alterations correspond to translocations between the X and 2L or 3R chromosomes with breakpoints in 1B1−4 and 21A or 95A, respectively (not shown). Thus, both translocations separate the left part of the AS−C, containing the T4 and T5 genes, from the 2B5 region.

Hw^{49c+R5}. The extreme sc phenotype of this revertant (see below) suggested the presence of a modification in a region critical for the *sc* function. Since Southern blot analyses only detected the parental inversion, we cloned the T4 gene of this revertant and that of the parental stock, and sequenced the protein coding regions (Villares and Cabrera, 1987). The sequence of Hw^{49c+R5} shows an 8 bp deletion which creates a frameshift (Figure 3). The 345 amino acid-long T4 protein is replaced by a polypeptide that contains only the first 114 amino acids of the T4 protein, followed by 53 nonsense amino acids. This shortened polypeptide should most likely be inactive.

Hw^{685}. This mutation is associated with a terminal deletion of the X-chromosome [*Df(1)RT685*, Mason *et al.*, 1984, 1986] with a breakpoint near to that of the $In(1)sc^{8}$ (Figure 1; Ruiz-Gómez and Modolell, 1987). Hw^{685} is deficient for the whole *ac* region. It should be emphasized that out of 74 deficiencies analysed with breakpoints randomly distributed in the region between the T4 and T6 genes (Figure 1), only *Df(1)RT685* had a Hw phenotype (Ruiz-Gómez and Modolell, 1987).

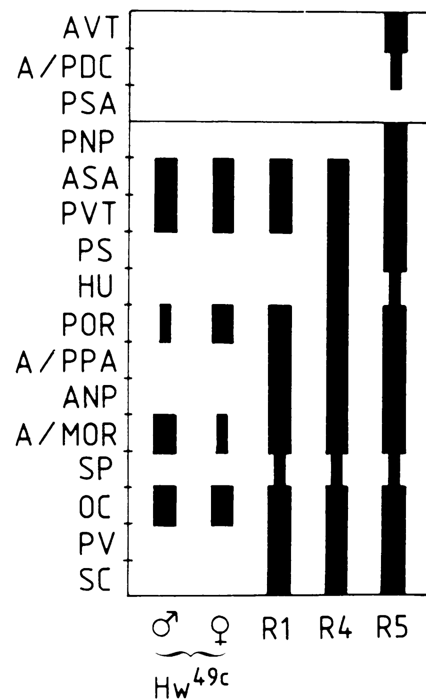

Fig. 5. Macrochaetae suppressed in Hw^{49c} mutants and in Hw^{49c+R1}, Hw^{49c+R4} and Hw^{49c+R5} males. The standard macrochaetae nomenclature (Lindsley and Grell, 1968; García-Bellido, 1979; Ruiz-Gómez and Modolell, 1987) is indicated in ordinates. Thick and thin bars indicate that the corresponding macrochaetae is absent in 50−100% and 10−49% of the heminota or half heads.

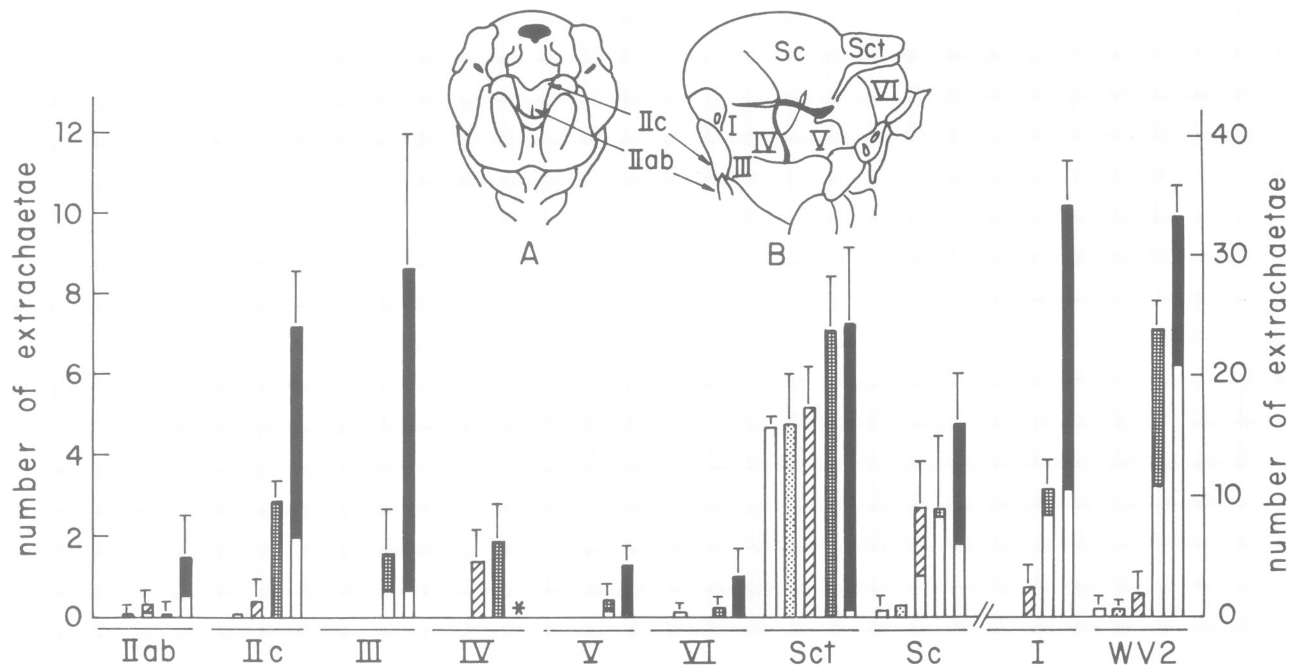

Fig. 6. Number of extra chaetae on different regions of the thorax and wing vein 2 (WV2) of flies heterozygous for *Df(1)RT685* and *Df(1)sc*[19] (□), wild type X chromosome (▩), *Hw*[Ua] (▨), *Hw*[1] (▦) and *Hw*[49c] (■). **A** and **B** are schematic ventral and lateral views of the thorax, respectively. Labelled regions are: I and IV, anepisternum; IIab and IIc, preepisternum1; III, preepisternum 2; V, anepimeron, VI, postnotum. Sc, scutum; Sct, scutellum (After García-Alonso and García-Bellido, 1986). Extra chaetae include micro- and macrochaetae, excepting on the scutum where only extra macrochaetae have been counted. Right ordinate applies only to region I and WV2. Extent of vertical lines on bars indicate standard deviations. Empty sections in bars corresponding to the combinations of *Hw*[685] with *Hw*[Ua], *Hw*[1] and *Hw*[49c] represent number of extra chaetae of these last three *Hw* alleles in combination with a wild type allele of the AS−C (García-Alonso and García-Bellido, 1986). An asterisk indicates that value of extra chaetae in position IV has been added to that of position I since the large number of chaetae in the combination *Hw*[685]/*Hw*[49c] made it difficult to unequivocally distinguish chaetae from each of these positions.

Phenotypes of Hw mutations

Hw[49c] is the strongest *Hw* mutation known. Numbers and positions of extra chaetae on many regions of the fly have been described (Lindsley and Grell, 1968; García-Alonso and García-Bellido, 1986; Figure 6). Figure 4B shows a *Hw*[49c] wing. Note the presence of many chaetae on wing vein 2 and the posterior half of the wing blade, regions normally devoid of sensory organs (Figure 4A). In addition, large numbers of other types of extra sensilla are also present (not shown). Despite its extreme Hw phenotype, *Hw*[49c] has on the notum a sc phenotype (Figure 5), presumably due to its breakpoint in the *sc* region.

The *Hw*[49c + R1] and *Hw*[49c + R4] revertants have strong sc phenotypes (Figure 5) similar to those of other *sc* mutations with neighbouring breakpoints, like *In(1)sc*[260−14] and *In(1)sc*[4] (see Figure 2 of Campuzano *et al.*, 1985). *Hw*[49c + R5] is an extreme *sc* (Figure 5). These revertants lack ectopic chaetae.

The patterns of extra chaetae generated by the *Hw* mutations so far described are allele-specific (García-Alonso and García-Bellido, 1986). *Hw*[685] is the only known *Hw* mutation to have a deletion in an important part of the AS−C. Therefore, its phenotype was examined in detail in combination with the *Df(1)sc*[19], an intercalary deletion for most of the AS−C, with a wild type AS−C and with other *Hw* alleles (Figure 6). *Hw*[685] is unique in generating lateral clusters of microchaetae on the scutellum. Even in the absence of another copy of the complex, it also promotes the differentiation of extra sensilla campaniformia on the dorsal wing radius (not shown), of microchaetae or other sensilla on wing vein 3 (Figure 4C) and occasional

Table I. Levels of T5, T4 and T3 transcripts in *Hw* mutants relative to the levels of these transcripts in *y* stocks

Experimental stock	T5		T4		T3
	Lc	EP	Lc	EP	Lc
I *y*	1.0	1.0	1.0	1.0	1.0[a]
Hw[49c]	2.3	1.4	3.9	1.7	1.7[a]
Hw[49c + R1]	0.59[a]	0.65[a]	0.50[a]	0.71	–
Hw[49c + R4]	0.77[a]	0.74[a]	0.75[a]	0.84	–
Hw[49c + R5]	1.7[a]	1.5[a]	3.0[a]	1.8	–
II *y/Df(1)sc*[19]	–	–	–	1.0	–
y/y	–	–	–	1.8	–
In(1)y[3PL]*sc*[8R]*/Df(1)sc*[19]	–	–	–	1.1	–
Df(1)RT685/Df(1)sc[19]	–	–	–	2.3	–
Df(1)RT623/Df(1)sc[19]	–	–	–	0.58	–

For each experiment and each developmental stage, levels of T5, T4 or T3 RNA in *y* or *y/Df(1)sc*[19] organisms have been arbitrarily set equal to 1.0. Abundances in organisms of other stocks are expressed relative to the control on top of each set of results. Quantitations in Experiment I were carried out in male individuals and, to homogenize the genomes of mutant and wild type individuals, one *Hw*[49c] male and one *y* female were crossed and the progeny was interbred for at least five generations before selecting males of the indicated genotype and stage to prepare RNA. Results are averages of at least two quantifications, except those marked ([a]) which correspond to a single determination. Abbreviations for developmental stages are: Lc, late third instar crawling larvae; EP, 0−1-day-old pupae. Note that besides being ac^-, the *RT623* deficiency is moderately sc (Ruiz-Gómez and Modolell, 1987); this may explain its reduced level of T4 RNA as compared with the wild type. Preliminary experiments carried out with a *Hw*[49c]*/Df(1)sc*[19] stock indicated that the developmental profiles of expression of T3, T4 and T5 genes were similar to those of the wild type. In similar experiments, involving RNA blots and λsc clones covering all the AS−C as probes, no RNAs different from those of the wild type were detected.

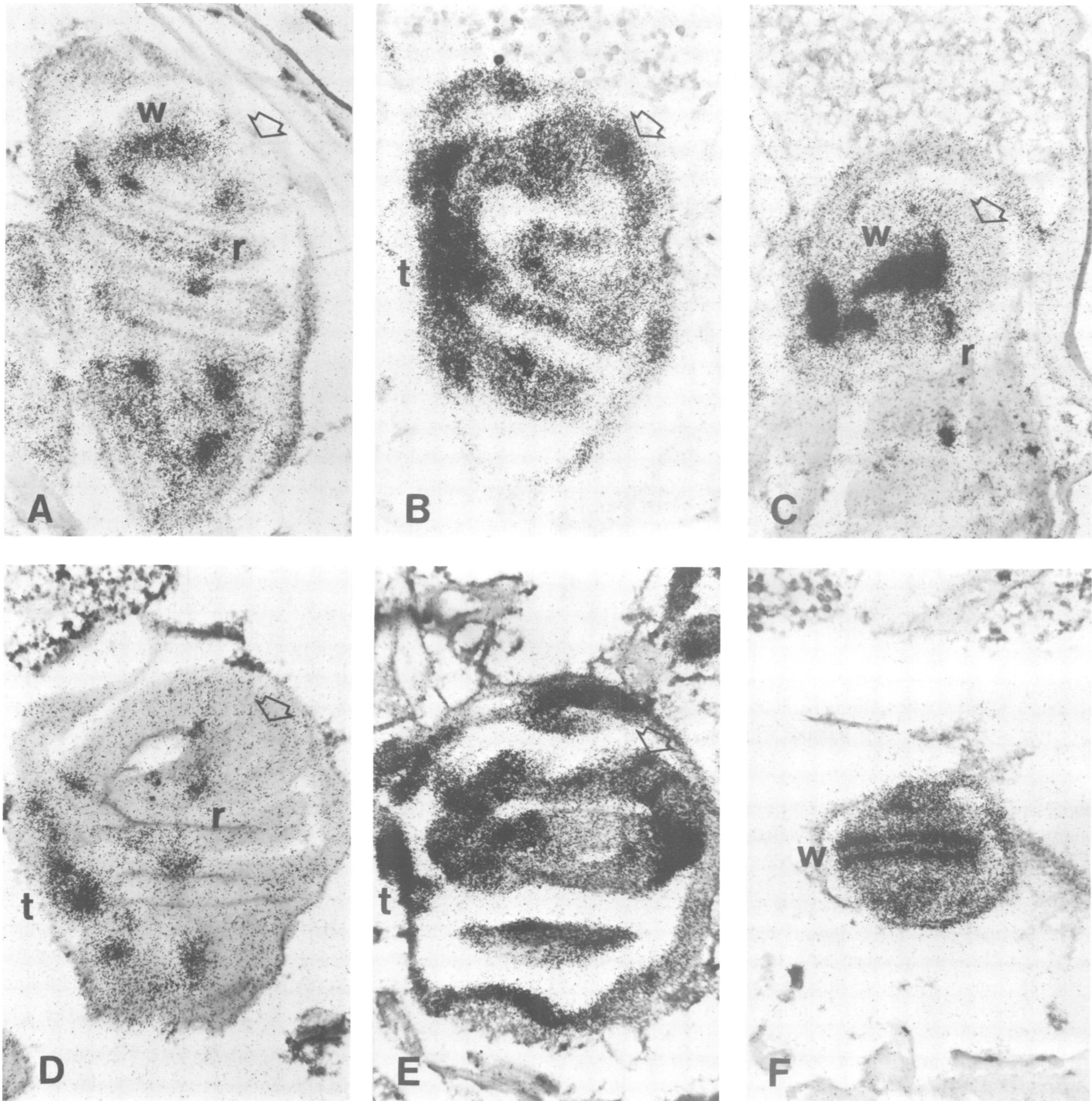

Fig. 7. Hybridizations *in situ* of T4 (**A–C**) and T5 (**D–F**) probes to third instar larvae sections. Panels display selected views showing sections through wing discs. All sections are approx. parallel to the main plane of the disc, but at different levels. All sections have the same orientation, so that anterior structures derive from the left part and dorsal structures from the bottom part of the discs. **A** and **D**, wild type discs. Patterns of expression of T4 and T5 genes are very complicated and no single disc section shows all the sites of expression. Some prominent sites, labelled with both probes, are the wing margin (w), the wing radius and vein 3 (r), and the tegula (t). Arrows point to the posterior region of the wing blade. For further description of sites on the fate map of the wing disc see Bryant (1975). **B**, Hw^{49c} disc. Note the generalized expression. **C**, section showing almost exclusively the wing blade region of a Hw^{685} disc. Pattern is similar to that of the wild type wing region in **A**. **E**, Hw^{1} disc. Note the generalized overexpression of the T5 gene. **F**, a more tangential section of the same disc as in **E** showing the anterior part of the wing blade region and the prominent label of the anterior wing margin.

microchaetae on wing vein 2 (Figure 6). The pattern of microchaetae on the notum is very similar to that of the wild type, although their number is slightly increased (132 $\pm$ 14 versus 120 $\pm$ 6 per heminotum, not including the humeral microchaetae, Ruiz-Gómez and Modolell, 1987). The increment is mostly confined to the posterior and lateral region of the notum, an area almost devoid of microchaetae in the wild type. Comparing the numbers of extra chaetae on different positions in the Hw^{685}, Hw^{Ua}, Hw^{1} and Hw^{49c} mutants with those generated by the combination of Hw^{685} with each of these alleles (Figure 6), it is observed that the combinations synergistically increase the number of chaetae in *all* the positions examined. Similarly to other *Hw* alleles, Hw^{685} also interacts synergistically with h^{1} or emc^{pel}(Ruiz-Gómez, 1986), mutations at the *hairy* and *extramacrochaetae* loci that promote the differentiation of extra chaetae on ectopic positions (Lindsley and Grell, 1968; Moscoso del Prado and García-Bellido, 1984a,b). Although Hw^{685} is deficient

for the *ac* region of the complex, it has a very weak ac phenotype, noticeable in the occasional absence of the posterior dorsocentral and supraalar macrochaetae, and the removal of a few *ac*-controlled sensilla campaniformia from the wing base (not shown).

Transcription of the AS–C in Hw mutants

In the previously characterized Hw^{1}, Hw^{BS} and Hw^{Ua} alleles, an increase in the abundance of either the T5 or T4 transcript seemed essential for the generation of the Hw phenotypes (Campuzano *et al.*, 1986). Thus, we have now examined whether a similar increase occurs in Hw^{49c} and Hw^{685} mutants (Table I). Determinations were carried out in late third instar crawling larvae and 0–1-day-old pupae, when T4 and T5 genes are maximally expressed (Campuzano *et al.*, 1985). For the sake of comparison, T3 gene expression has also been determined in Hw^{49c}. All these transcripts were significantly more abundant in Hw^{49c} than in wild type organisms, although the increase (2–4-fold) was largest for the T4 RNA. The increase was abolished in revertants $Hw^{49c\,+\,R1}$ and $Hw^{49c\,+\,R4}$, mutations which separated the left part of the AS–C from the 2B5 region; but it was not in $Hw^{49c\,+\,R5}$, the revertant with an 8 bp deletion within the T4 coding sequence.

Hw^{685} pupae also showed an increased level of T4 RNA, as compared with pupae carrying a wild type AS–C (*y* chromosome) or deletions deficient for the *ac* region, $In(1)y^{3PL}sc^{8R}$ and *Df(1)RT623*. However, note that the abundance of T4 RNA in females $Hw^{685}/Df(1)sc^{19}$ is only a little higher than that of the females with two doses of wild type AS–C.

Spatial expression of T4 and T5 genes in Hw alleles

To investigate further the relevance of the increased abundances of the T4 and/or T5 RNAs in *Hw* mutants for the generation of the Hw phenotypes, we examined their distribution in wing disc sections since this structure contains the cellular precursors of the notum, wing and mesothoracic pleura. It has been shown (S.Romani, S.Campuzano and J.Modolell, in preparation) that T4 and T5 RNAs are spatially restricted to groups of cells which comprise the areas of the disc where the cellular precursors of the sensory organs are found (Bryant, 1975). (T3 and T1a RNAs, corresponding to the *l'sc* and *sc* γ genes, are almost exclusively expressed in the CNS.) Figure 7 shows that, in contrast to the localized distributions observed in the wild type disc (panels A and D), in Hw^{49c} (panel B) or Hw^{1} (panel E) discs the T4 or T5 RNAs have increased abundances and generalized distributions. In Hw^{49c}, the T5 RNA is also generally expressed, although it is less abundant than the T4 RNA (not shown). Thus, the genes are expressed in areas in which they do not normally do so, like the posterior part of the wing blade. Figure 4B and D show that ectopic sensory organs are abundant in this region of the wing of Hw^{49c} or Hw^{1}. Note also that the distribution of label is not homogeneous and that distinctive sites of gene expression, like the presumptive anterior wing margin, are still clearly distinguishable (Figure 7F). Increased abundances and generalized distributions of transcripts also occur in other imaginal discs, but were not detected in the CNS (not shown). In Hw^{685} discs, no generalized expression of the T4 RNA was observed (Figure 7C), and within the limitations of the *in situ* hybridization technique, the spatial distribution of label was similar to that of the wild type. Nevertheless, the pattern observed is compatible with moderate increases of expression in the labelled regions of the wild type disc.

Discussion

Foreign DNA promotes overexpression of AS–C genes

In contrast to previously characterized *Hw* mutations associated with insertions of transposable elements within the *ac* (T5) or *sc* α (T4) genes (Campuzano *et al.*, 1986), the *Hw* alleles analysed in this work contain a euchromatic inversion (Hw^{49c}) and a terminal deletion of the X chromosome (Hw^{685}). Both mutations have breakpoints in nontranscribed regions of the AS–C, the second one being deficient for the *ac* region. In addition, Hw^{685} has at least several kb of DNA between the undeleted part of the complex and the end of the chromosome (M.Ruiz-Gómez, unpublished results). In both alleles, some special property of the DNA in contact with the AS–C, rather than the breakpoints themselves or their particular position within the complex, seems to induce overexpression of AS–C genes and the Hw phenotype. Thus, out of 74 terminal deletions of the X chromosome with breakpoints within the AS–C [seven of which had the breakpoint within 1.6 kb from that of *Df(1)RT685*] only this deficiency had a Hw phenotype (Ruiz-Gómez and Modolell, 1987); and all the known DNA lesions that map near (<4 kb) the Hw^{49c} breakpoint have sc but not Hw phenotypes [$In(1)sc^{260-14}$, $T(1;3)sc^{KA8}$ and sc^{e}, a gypsy insertion, Campuzano *et al.*, 1985 and our unpublished data]. Moreover, in Hw^{49c}, the separation of the AS–C region bearing the T4 and T5 genes from the foreign DNA ($Hw^{49c\,+\,R1}$ and $Hw^{49c\,+\,R4}$) abolishes the increased abundances of T4 and T5 RNA and the Hw phenotype. These data suggest that sequences in the foreign DNA may contain enhancers or modifiers of chromatin structure that facilitate transcription of the adjacent AS–C genes. Independent genetic evidence suggests that the 2B5 DNA in contact with the AS–C DNA bearing the T4 and T5 genes contains enhancer sequences (J.Sampedro and M.Izquierdo, submitted).

Previous work has shown that increased abundances of either T5 or T4 transcripts are also associated with the Hw^{1}, Hw^{BS} and Hw^{Ua} mutations and may be responsible for their phenotypes (Campuzano *et al.*, 1986). However, this interpretation is complicated by the shortening of the overly abundant transcripts and their encoding truncated, but presumably functional, protein products. In Hw^{49c} and Hw^{685}, the overexpressed transcripts seem intact; hence, increased amounts of AS–C products, and not their qualitative modifications, may be most relevant for the Hw effect.

A basis for the Hw effect

We have found that the two most extreme *Hw* mutants (Hw^{49c} and Hw^{1}), which show the highest overexpression of AS–C genes, have a generalized distribution of T4 and T5 transcripts in imaginal discs. In the wild type, these RNAs are confined to distinct regions of the discs, many of which coincide with areas where cellular precursors of the sensory organs are located (S.Romani, S.Campuzano, and J.Modolell, in preparation). The generalized expression

correlates with the appearance of extra sensory organs in regions normally devoid of them, like the posterior compartment of the wing. In contrast, Hw^{685}, a much weaker allele with only a small overexpression of the T4 gene (Table I), shows a spatial distribution of the T4 RNA similar, within the limitations of the *in situ* hybridization technique, to that of the wild type. Thus, we like to suggest that overexpression of T4 and/or T5 genes in allele-specific regions of the imaginal discs may be at the basis of the *Hw* effect. We further suggest that, in each *Hw* mutant, the specific distribution of T4/T5 RNAs is somehow related to the final characteristic pattern of sensory organs. However, this relationship is probably complicated by other processes that intervene in the generation of the pattern of sensory organs like *trans* interactions imposed by the *h* and *emc* genes (Moscoso del Prado and García-Bellido, 1984a; García-Alonso and García-Bellido, 1986; Figure 6), local differences in the responsiveness of cells to the presence of the AS−C products, and cell to cell interactions (Ghysen and Richelle, 1979) like lateral inhibition (Wigglesworth, 1940; Moscoso del Prado and García-Bellido, 1984b).

In the case of Hw^1, the gypsy insertion within the T5 gene seems to promote its generalized expression in discs, with maximal RNA abundance in many areas that seem to correspond to those of normal T5 gene expression. This, and the fact that in Hw^1 and in wild type larvae this gene is only expressed in the ectodermal cells of the discs, suggests that gypsy does not completely abolish the normal controls of the gene. Note that a possible increased stability of the truncated T5 RNA and the modification of the protein product may also contribute to the Hw phenotype (Campuzano *et al.*, 1986).

Hw^{49c}, the most extreme *Hw* allele known, also displays a sc phenotype, probably because of its AS−C breakpoint, which separates the T4 gene from putative *cis*-controlling elements harboured in its long (50 kb) downstream region (Ruiz-Gómez and Modolell, 1987). In contrast, Hw^{685}, a much weaker allele than Hw^{49c}, does not have a sc phenotype. Consistently with the above interpretation, the T4 regulatory regions are intact (Ruiz-Gómez and Modolell, 1987) and the T4 gene spatial pattern of expression seems little modified.

Hw phenotypes are also associated with some heterochromatic breakpoints with weak sc phenotypes [$In(1)sc^{V2}$ and $In(1)sc^8$] and partial duplications of the AS−C comprising either the *ac*, *sc* α, or *ac* and *sc* α regions [$In(1)sc^{8L}y^{3PR}$, $In(1)sc^{4L}sc^{8R}$ and $In(1)sc^{4L}y^{3PR}$, respectively; all breakpoints are heterochromatic]. (García-Bellido, 1979). Most of these rearrangements have variegating phenotypes. Although the expression of the AS−C has not been examined in these mutants, we suggest that overexpression of the T4/T5 gene(s) induced by position effects of the adjacent heterochromatin may be involved in generating the Hw phenotypes. It has also been proposed that unbalanced production of AS−C products caused by the partial duplications of the *ac* or *sc* α regions may be an important factor (García-Alonso and García-Bellido, 1986).

Replacement between T4 and T5 gene products

The development of the notum chaetae pattern normally requires both the T4 and T5 genes, since the absence of the first removes all macrochaetae, excepting the A/PDC and PSA, and that of the second removes 70−75% of the microchaetae and the A/PDC, PSA and AVT macrochaetae (García-Bellido, 1979; Ruiz-Gómez and Modolell, 1987). The absence of both T4 and T5 products (for instance, in the sc^{10-1} mutation, Villares and Cabrera, 1987) suppresses all chaetae from the notum and most chaetae and other sensilla from elsewhere on the fly (García-Bellido, 1979). Note that for the development of microchaetae, the T5 (*ac*) gene is sufficient, but that in its absence, the T4 gene promotes the development of up to 30% of microchaetae. [Replacement between different AS−C functions has also been observed for the development of the embryonic NS (Dambly-Chaudière and Ghysen, 1987, Jiménez and Campos-Ortega, 1987).] We now have found that in Hw^{685} the replacement can be carried out to a much greater extent, since the T4 gene alone (although moderately overexpressed) promotes an almost wild-type chaetae pattern on the notum. Evidently, the structural similarity between the T4 and T5 proteins (Villares and Cabrera, 1987) provides a molecular basis for this substitution. Moreover, in the wild type wing disc, T4 and T5 genes are expressed in very similar areas (S.Romani, S.Campuzano and J.Modolell, in preparation). Thus, to explain this replacement, we postulate that overexpression of the T4 gene only needs to occur in areas where it is normally expressed. (Note that such overexpression would be difficult to detect by *in situ* hybridization techniques.) On the other hand, the replacement is not complete. A group of four morphologically distinct, *ac*-dependent sensilla campaniformia on the medial dorsal wing radius are absent in Hw^{685}, even though there are extra sensilla of the sc-dependent type in the proximal, medial and distal radius, which suggests overexpression of the T4 gene in the presumptive region of the wing disc (our unpublished data). We have also shown that overexpression of the T5 gene seems incapable of replacing the absence of the T4 product ($Hw^{49c\ +\ R5}$). Thus, although the T4 and T5 genes seem involved in similar operations, they clearly have specific, albeit overlapping, domains of action. The possibility of replacement probably depends on the stringency of the requirement of specific cells for either the T4 or T5 product, the specific developmental time when the products are available, and their respective amounts within a given group of cells.

Materials and methods

Drosophila stocks

Df(1)RT685, also named Hw^{685} in this work, and *Df(1)RT623* (Mason *et al.*, 1984) are terminal deficiencies of the X chromosome. X-ray-induced revertants of Hw^{49c} were a gift from A.García-Bellido (García-Alonso and García-Bellido, 1986). emc^{pel} is a spontaneous, homozygous viable mutation at the *extramacrochaetae* locus (Moscoso del Prado and García-Bellido, 1984a). Other stocks used [$In(1)y^{3PL}sc^{8R}$, $Df(1)sc^{19}$ $f^{36}/TM6$, y^1, *y mwh* h^1 and Hw^{49c}] are described in Lindsley and Grell (1968).

Phenotype scoring

Phenotypes were determined with 12−20 individuals per genotype grown at 25°C.

Molecular mapping of mutations

DNA of adult males or females heterozygous for an RT deficiency and $Df(1)sc^{19}$ was extracted and the DNA lesions associated with the mutations were mapped by Southern genomic analyses and, in some cases, cloning of the affected regions. For all the mutations analysed, the modifications described were the only ones detected in the AS−C.

DNA sequencing

The coding region of the T4 gene in the Hw^{49c} and $Hw^{49c\ +\ R5}$ alleles was sequenced using the dideoxy chain termination method (Sanger *et al.*, 1977)

as modified by Garoff and Ansorge (1981). Subclones of the 1.8 kb *Bam*HI genomic fragments (coordinates 31.9 to 33.7 in Figure 1) were obtained in pEMBL (Dente *et al.*, 1983). Sequence was obtained using as primers four pentadecamer oligonucleotides with sequences corresponding to those starting in positions 620, 867, 1116 and 1360 of the T4 gene sequence (Villares and Cabrera, 1987).

Hybridizations in situ to larval sections

Crawling third instar larvae of the indicated genotype were collected, fixed in Carnoy's fixative after removing their posterior tips, dehydrated at room temperature and embedded in paraffin wax (Ingham *et al.*, 1985). Sections of 6 μm were cut and pretreated for hybridization as described (Romani *et al.*, 1987). T4 and T5 single stranded RNA probes (^{35}S-labelled, 1200 Ci/mmol) were synthesized on cDNA templates and hybridized to sections as described in Romani *et al.*, 1987. Exposures lasted 5–7 days.

Other methods

Phage and *Drosophila* DNA preparations, construction and screening of recombinant λ libraries, RNA preparations, RNA quantitations, Southern and RNA blot analyses and *in situ* hybridizations to salivary gland chromosomes were performed as described (Carramolino *et al.*, 1982; Maniatis *et al.*, 1982; Modolell *et al.*, 1983; Campuzano *et al.*, 1985, 1986).

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