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Competence to develop sensory organs is temporally and spatially regulated in *Drosophila* epidermal primordia

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The *Drosophila* adult cuticle displays a stereotyped pattern of sensory organs (SOs). Its deployment requires the expression of the *achaete* (*ac*) and *scute* (*sc*) genes. Their products confer to cells of epidermal primordia (imaginal discs and histoblasts) the ability to become SO precursors (SOPs). In imaginal discs, *ac* and *sc* expression is spatially restricted to cell clusters within which one or a few cells become SOP(s). With the help of ubiquitous *sc* expression provided at different developmental times by a heat shock-*sc* (*HSSC*) chimeric gene, we have analyzed the response of epidermal primordia to the proneural action of the *sc* product, and have tested whether the patterned distribution of *ac/sc* products is necessary to position SOs correctly within the epidermis. Each primordium responds to *HSSC* expression by developing SOs only during a characteristic developmental period. In the absence of the endogenous *ac* and *sc* genes, most SOs induced by *HSSC* are of the correct type and are located in wild type positions. These results indicate that the capacity of primordia to respond to *sc* is temporally and spatially regulated, that specification of the type of SO does not depend on *ac/sc*, and that SO positioning utilizes topological information independent of the spatially restricted distribution of *ac/sc* products.

Key words: heat-shock/pattern formation/*scute* gene/sensory organ

Introduction

The adult *Drosophila melanogaster* carries on its cuticle several thousands of sensory organs (SOs) arranged in very reproducible patterns. SOs can be either large or small bristles (macro- or microchaetae) or sensilla of other types. Each SO develops from a single SO precursor (SOP) cell that undergoes two differential divisions (Hartenstein and Posakony, 1989). One pair of sister cells gives rise to the external part of the SO (basal cell and either a bristle shaft or another type of process). The other pair of cells differentiates into a sensory neuron and an accessory cell. Some SOs, like the macrochaetae of the head and notum, occupy extremely reproducible positions, so that each SO is uniquely identified. Other SOs, like the microchaetae of the notum or the macro- and microchaetae of the tergites are arranged in rows or are more or less evenly spaced within well defined areas. Their number, within an area is relatively constant. Some regions of the fly, like the posterior compartment of the wing and the thoracic and abdominal pleura, are normally devoid of SOs. A standing question in developmental biology is how the positional specification of these SOs is accomplished.

The *achaete* (*ac*) and *scute* (*sc*) genes, two members of *achaete–scute* complex (AS-C), are intimately involved in the generation of that pattern of SOs (García-Bellido, 1979; Campuzano *et al.*, 1985). They are thought to act in SO determination by conferring to ectodermal cells the ability to become SOPs (for reviews see Ghysen and Dambly-Chaudière, 1988, 1989). It has been shown for the primordium of the notum, wing and thoracic pleura (the imaginal wing disc) that *ac* and *sc* are expressed in distinct clusters of cells arranged in a disposition that prefigures the distribution of SOPs (Romani *et al.*, 1989). Hypomorphic *sc* mutations alter this pattern of expression at sites consistent with the observed loss of SOs from allele-specific positions (Romani *et al.*, 1989; P.Cubas and S.Campuzano, unpublished). Moreover, ectopic *ac* or *sc* expression caused by *Hairy-wing* (*Hw*) mutations promotes development of extra SOs in ectopic positions (Campuzano *et al.*, 1986; Balcells *et al.*, 1988). Thus, it has been proposed that the spatially restricted expression of *ac* and *sc* delimits regions of epidermal anlagen where SOs arise (Romani *et al.*, 1989).

The discovery that the *ac* and *sc* proteins, as well as two other proteins encoded in the AS-C, contain the conserved basic region-helix-loop-helix (bHLH) motif (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; González *et al.*, 1989; Murre *et al.*, 1989a) has thrown some light on how these proteins may operate. The bHLH domain is found in a family of well characterized mammalian transcriptional regulators (Davis *et al.*, 1990; Murre *et al.*, 1989a). It allows these proteins to dimerize with themselves and/or other members of the family and interact with DNA (Murre *et al.*, 1989a; Lassar *et al.*, 1889; Davis *et al.*, 1990). So, by analogy, it is assumed that the *ac* and *sc* proteins are also transcriptional regulators that control the activity of genes involved in SO differentiation. In fact, it has been shown that one of the AS-C proteins (*lethal of sc*) and the product of the *Drosophila daughterless* (*da*) gene, another bHLH protein required for SO development (Caudy *et al.*, 1988a,b), can form heterodimers that bind *in vitro* to specific DNA sequences (Murre *et al.*, 1989b). This ability to form heterodimers may also account for the negative effect of *extramacrochaetae* (*emc*) and *hairy* (*h*) products (Moscoso de Prado and García-Bellido, 1984a; García-Alonso and García-Bellido, 1988) on the proneural activity of *ac* and *sc* proteins. Both *emc* and *h* contain the HLH motif but lack (*emc*) or have an altered basic region (*h*) (Rushlow *et al.*, 1989; Ellis *et al.*, 1990; Garrell and Modolell, 1990). So, it has been proposed that they would function by sequestering *ac* and *sc* proteins in complexes inefficient for DNA interaction. However, many questions remain about the participation of the *ac/sc* proteins in the mechanism of SO generation. For instance, when and for how long is their

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expression required; what is the role of these proteins in the positioning of SOs; do they specify the types of SOs; have different positions of the epidermal primordia different quantitative requirements of *ac/sc* products to develop SOs?

To address these questions, we have examined the effect on SO development of the generalized, temporally controlled expression of a *sc* gene fused to an *hsp70* promoter. We have determined the ability of its expression to interfere with normal patterning, by generating additional SOs, and to replace the endogenous *ac/sc* expression in individuals carrying null alleles for both of these genes. The results show that each anlage only responds to the proneural effects of the *sc* protein during a characteristic developmental period. Within tissues, there are local heterogeneities which make some sites more responsive than others to the ubiquitous expression of *sc*. In general, regions of enhanced responsiveness are those where precursors of the wild type pattern arise. This indicates that positioning of SOPs involves topological cues additional to the patterned distribution of *ac/sc* products.

Results

By means of P-element mediated transformation (Rubin and Spradling, 1982), we obtained two *Drosophila* lines, named *HSSC-2* and *HSSC-3*, carrying in the second (39B) or third (74D) chromosome a cloned *sc* gene controlled by the *hsp70* promoter (Figure 1). From these lines, stocks containing two or four copies of the chimeric gene were prepared. Hybridizations *in situ* to sections of late third instar larvae, which carried one copy of each insertion and a deletion of the endogenous *sc* gene [$Df(1)sc^{8L}sc^{4R}$; Campuzano *et al.*, 1985], demonstrated that *HSSC* is induced and homogeneously expressed under heat-shock conditions (37°C, Figure 1A). Thirty min after heat treatment, most *sc* RNA had decayed in the imaginal discs. The remaining RNA was still homogeneously distributed (not shown).

We determined, in adult flies, the effect of this ectopic *sc* expression, administered at different developmental times and either in the presence or in the absence of the endogenous *ac* and *sc* genes, on the number and position of SOs. We used stocks carrying two copies of *HSSC* and an X chromosome with either a wild type AS-C or the $In(1)sc^{10.1}$ mutation. The $sc^{10.1}$ chromosome has a breakpoint near the transcriptional start of the *ac* gene and a nonsense mutation in the *sc* coding sequence (Campuzano *et al.*, 1985; Villares and Cabrera, 1987). As a consequence, $sc^{10.1}$ flies completely lack SOs on the notum. Most SOs in other parts of the body are also absent, excepting the middle and ventral rows of the triple row of chaetae on the anterior wing margin, whose development is not controlled by *ac/sc* (García-Bellido and Santamaría, 1978). The non-heat-shocked flies of the

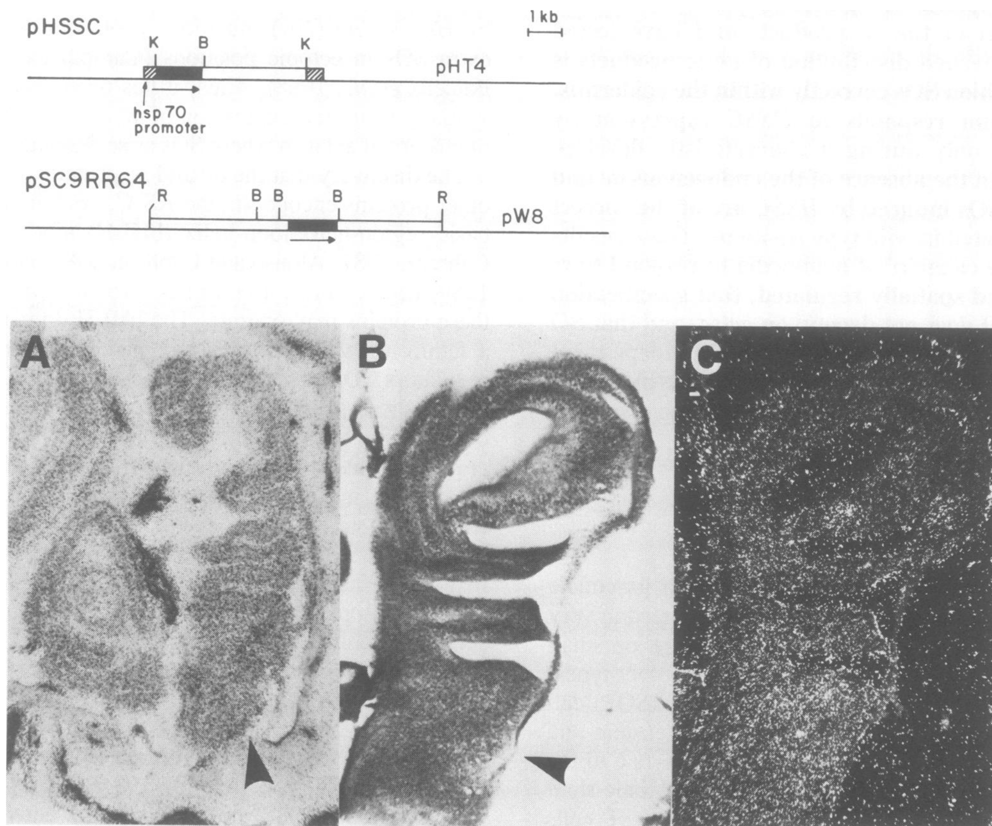

Fig. 1. Plasmids used for transformation (**top**) and generalized distribution of *sc* mRNA in wing imaginal discs from transgenic larvae obtained with the above plasmids (**bottom**). Thin lines, vector DNA; filled boxes, *sc* transcribed sequences; empty boxes, *sc* flanking sequences; stipled boxes, *hsp70* sequences. Restriction site nomenclature: B, *Bam*HI; K, *Kpn*I; R; *Eco*RI. For a description of pSC9RR64 see last section of Results. *In situ* hybridizations with a *sc* RNA probe were performed on late third instar larvae sections. (**A**) section approximately perpendicular to the main plane of the wing disc of an $In(1)sc^{8L}sc^{4R}$;*HSSC-2/+*;*HSSC-3/+* male larva dissected immediately after a heat shock (1 h at 37°C). (**B**) and (**C**) clear and dark field images of a section approximately parallel to the main plane of the wing disc of an $In(1)sc^{8L}sc^{4R}$;*SC9RR64/+* male larva. Arrowheads point to the region of the presumptive notum. No increased abundance of *sc* mRNA in groups of cells was observed in these or other sections, except on the $In(1)sc^{8L}sc^{4R}$;*SC9RR64/+* presumptive wing region, where four clusters were apparent (part of one is visible in C).

above described genotypes were phenotypically identical to the control flies without *HSSC* insertions. This indicates that a basal transcription of *HSSC*, if it occurs, is insufficient to promote development of SOs. Similarly, a 1 h heat shock at 37°C, administered at any time during the larval and pupal stages, did not have an effect. In contrast, *HSSC* expression driven by three 1 h heat shocks (37°C), separated by 2 h intervals at 25°C, generated extra SOs in the AS-C$^+$ background or partially rescued the absence of SOs in the $sc^{10.1}$ flies. In both backgrounds, heat shocks were only effective when started between approximately 16 h before puparium formation (BPF) and 32 h after puparium formation (APF). Within this interval, different regions of the body showed characteristic periods of competence to develop SOs in response to *HSSC* expression (Figure 2). Moreover, for a given region, the period of competence to develop macrochaetae was always earlier than that of microchaetae. Note also that for each region and type of SO examined, competence periods were similar in wild type and $sc^{10.1}$ backgrounds. In the following paragraphs, we summarize the phenotypes observed in different regions of the fly.

Notum

Heat-shocked AS-C$^+$;*HSSC* flies developed extra macrochaetae on the notum. Throughout the sensitive period, they tended to appear close, but in variable positions, around all extant macrochaetae (Figure 3B). They were most abundant in the dorsocentral (DC) and presutural (PS) regions. Around the time of maximal effect, ectopic macro- and mesochaetae appeared in positions far removed from

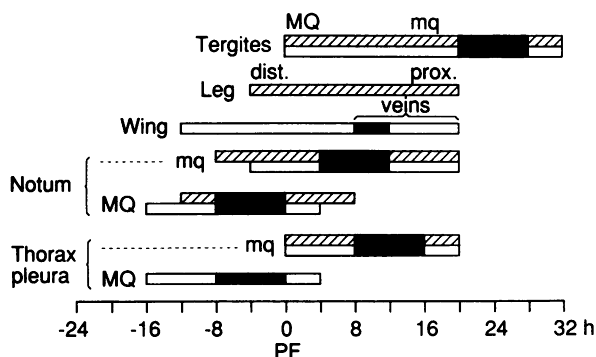

Fig. 2. Competence periods for SO development in response to heat shock in *HSSC* (empty boxes) and $sc^{10.1}$;*HSSC* (stipled boxes) flies. A schedule of three 1 h heat shocks at 37°C, separated by 2 h intervals at 25°C were administered to larvae or pupae of all developmental ages. *HSSC* expression, induced by heat treatment, promoted development of SOs only when started at the ages covered by the bars. Analyzed regions of the adult cuticle are indicated. MQ, macrochaetae; mq, microchaetae; PF, puparium formation. Filled boxes indicate times when maximum number of SOs developed as a result of the *HSSC* induction. Approximate values for some of these maxima were: in *HSSC* flies, thoracic pleura 10 MQ and 80 mq, heminotum 14 extra MQ and 50 extra mq; in $sc^{10.1}$;*HSSC* flies, thoracic pleura 15 mq, heminotum 40 mq, tergites 60 mq. Extra SOs on the wing were chaetae of different types and sensilla campaniformia. *HSSC* expression between 8 and 20 h APF induced differentiation of extra wing veins. Although only forelegs were analyzed in detail, general conclusions (text and legend to Figure 6) were essentially the same for the remaining legs. Chaetae were recovered mostly on the distal segments in the early part of the competent period and more proximally at later times.

the normal macrochaetae, mostly on lines extending anteriorly from the DC chaetae position and towards the midline from the PS chaeta, and in the supraalar region (Figure 4A). This pattern is strongly reminiscent of that of *emc* mutants (García-Alonso and García-Bellido, 1986). $sc^{10.1}$;*HSSC* individuals partially recovered notum macrochaetae. Remarkably, most of these had normal morphology and occupied positions undistinguishable from those of the wild type pattern (Figure 3C, see below). The DCs, PS and PSA were the most frequently recovered macrochaetae. Between 4 and 8 h APF, only the PS macrochaeta (and occasionally the PSA) could be rescued, but, at this time and in AS-C$^+$;*HSSC* individuals, no extra macrochaetae appeared in these positions. This might indicate an inhibition by the extant chaeta of the development of the extra chaeta.

Some regions of the notum also displayed enhanced sensitivities and preferential times to develop microchaetae. Thus, in $sc^{10.1}$;*HSSC* flies and early in the competence period, microchaetae appeared on two lines extending anteriorly from the DC bristles and on the presutural region; later they extended posteriorly towards the central and lateral regions. At the time of maximum rescue (4 − 12 h APF), they developed over most of their normal area (Figure 3G). In *HSSC* flies, maximum numbers of extra microchaetae (approximately 50 per heminotum) developed at the same time; they were located over most of the normal area and, ectopically, at both sides of the scutum-scutellar suture and other regions (Figure 3F).

Thoracic pleura

Thoracic pleura are normally devoid of SOs. *HSSC* expression caused development of ectopic SOs in both *HSSC* and $sc^{10.1}$;*HSSC* flies. In the first instance, macrochaetae appeared early in the competence period (Figure 4B), followed by both macro- and microchaetae, and then exclusively microchaetae in the later part of the period (Figure 4C). Both types of SOs appeared most abundantly in the preepisternum and anepisternum, as they also do in *Hw, h* and *emc* mutants (Moscoso del Prado and García-Bellido, 1984a,b; García-Alonso and García-Bellido, 1986, 1988; Balcells *et al.*, 1988). In $sc^{10.1}$;*HSSC* flies, only microchaetae were obtained. They appeared in small numbers and in the same positions as in *HSSC* flies.

Wings

Due to their generally crumpled state, wings were not examined in $sc^{10.1}$;*HSSC* flies. In *HSSC* individuals and at the beginning of the competence period (12 − 8 h BPF), extra SOs appeared almost exclusively on the veins and radius and were preferentially sensilla campaniformia. Later (4 h BPF to 4 h APF), the frequency of microchaetae increased very much, especially on vein 2. At the time of maximal response (8 − 12 h APF) the numbers of both types of SOs were very large and also occurred in intervein spaces (Figure 5A). Microchaetae were the most abundant in many regions, while sensilla campaniformia appeared preferentially on the proximal part of veins 3 and 4, the anterior crossvein and the wing base (Figure 5B,C). Occasionally, structures intermediate between these types of SOs were present, as described for *Hw* and *h* mutants (Lees and Waddington, 1942). At this and later times, extra veins and disruption of the posterior crossvein were apparent.

Legs

Although only forelegs were examined in detail, the general features were similar for the remaining legs. In *sc*$^{10.1}$;*HSSC* flies, recovery of chaetae went from distal to proximal during the competence period. Thus, flies heat-shocked at the beginning of the period had almost exclusively the sex combs and tarsal microchaetae (Figure 6B). Subsequently, microchaetae appeared preferentially on the tibia (Figure 6C), and later on the femur, being then less abundant in the more distal segments. Only weak recovery occurred in the femur. Chemosensory (recurved) chaetae appeared earlier (Figure 6B) than the mechano-receptor (straight) chaetae (Figure 6C). Although within any segment the number of chaetae recovered was smaller than that of the wild type pattern (Figure 6D), the disposition and type of chaetae were always as in the wild type. In *HSSC* heat-shocked individuals, extrachaetae appeared close to extant chaetae in the same distal to proximal sequence (not shown).

Abdomen

sc$^{10.1}$;*HSSC* flies heat-shocked early in the sensitive period, rescued mainly macrochaetae, which were correctly positioned on the posterior border of the tergites (Figure 7A). Later, only microchaetae developed, reaching numbers similar to those of the wild type (20 to 28 h APF). Combining early and late heat shocks, patterns resembling those of the wild type could be obtained (Figure 7C). However, a small number of ectopic microchaetae developed on the abdominal pleura, close to the border with the tergites (not shown). Similar ectopic chaetae have been observed in *Hw*^{1} mutants (A.García-Bellido, personal communication). In *HSSC* individuals, the density of chaetae was increased and many microchaetae, specially those nearer to the posterior border of the tergite, were converted into mesochaetae (not shown). Near the time of maximal phenotype in tergites, large numbers of microchaetae developed on the abdominal pleura (Figure 7D).

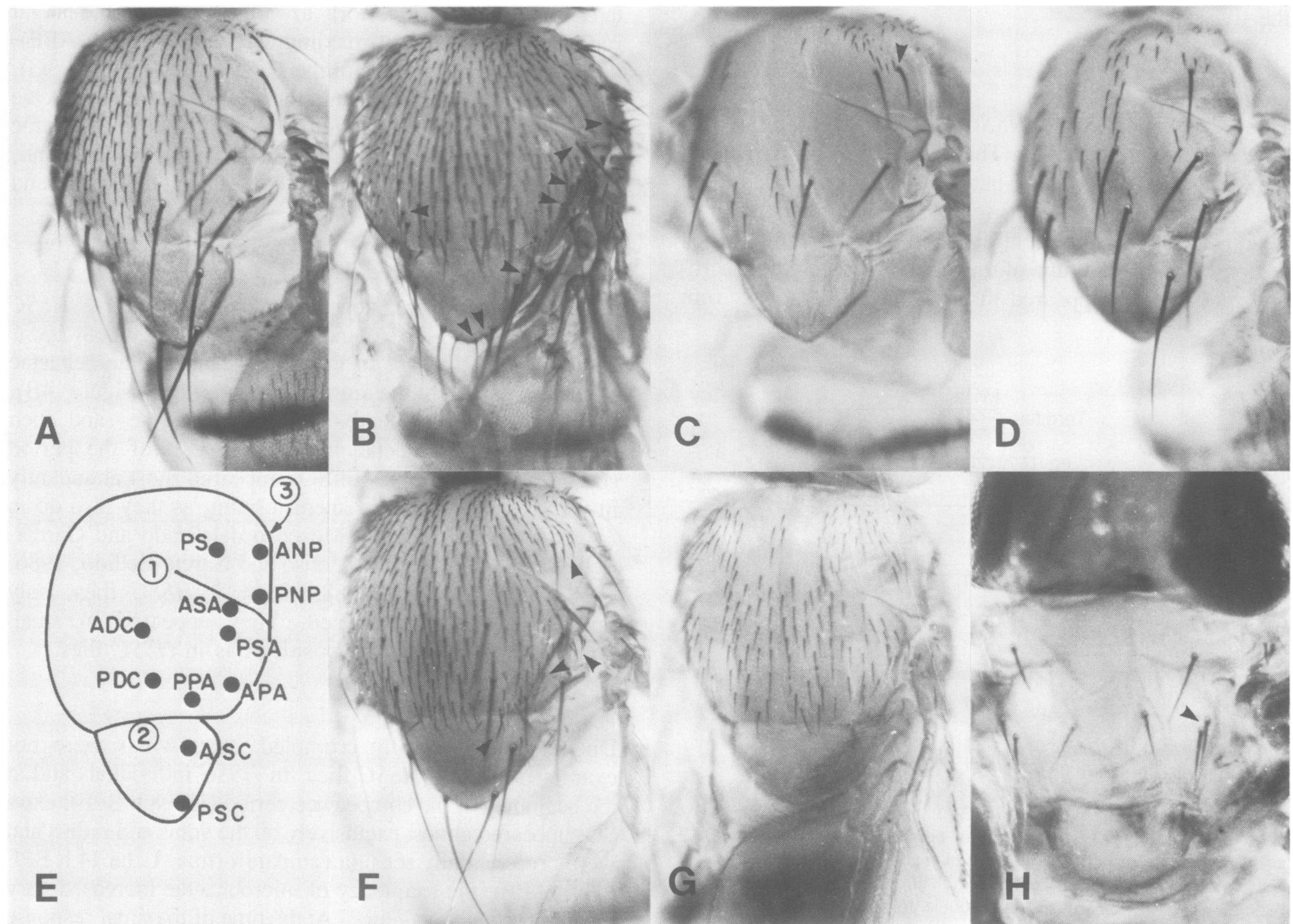

Fig. 3. Notum chaetae of wild type and transformant flies. (**A**) wild type Oregon R. A drawing of the macrochaetae positions of this fly with the corresponding chaeta nomenclature and an indication of the sutures taken as reference to measure the chaeta positions given in Table I is shown in (**E**) 1, intrascutal suture; 2, scutum-scutellar suture; 3, scutum-pleural suture. (**B**) AS-C^{+} fly with 2 copies of the *HSSC* gene, heat-treated at 4–0 h BPF. Some extra macrochaetae have been arrowed. Note that they appear close to extant macrochaetae and that the density of microchaetae is increased. (**C**) *sc*$^{10.1}$;*HSSC-2/+*;*HSSC-3/+* fly, with heat-treatment started at 12–8 h BPF. Arrowhead points to a misplaced chaeta between the PS and ANP positions. (**D**) notum of a *sc*$^{10.1}$;*SC9RR64/SC9RR64* fly. Note macrochaetae developing in correct positions. (**F**) AS-C^{+} fly with 2 copies of the *HSSC* gene, heat-shocked at 8–12 h APF. Density of microchaetae is increased in normal areas; areas containing ectopic microchaetae are indicated. (**G**) *sc*$^{10.1}$;*HSSC-2/+*;*HSSC-3/+* fly, with heat-treatment started at 4–8 h APF. (**H**) *In(1)y*^{3PL}*sc*^{4R};*HSSC-2/+*;*HSSC-3/+* pharate adult dissected from puparium, with heat-treatment started at 4–0 h BPF. Arrowhead points to a duplicated PSA.

Positional specification of notum macrochaetae

Most chaetae recovered in heat-shocked $sc^{10.1}$;*HSSC* flies occupied positions very similar to those of the wild type pattern. This was specially striking in the case of the notum macrochaetae, since the expression of the *ac* and *sc* genes, restricted to cell clusters of the notum anlage, might provide information for specifying the positions of the chaetae mother cells (Romani *et al.*, 1989) and, therefore, of the resulting SOs. The even distribution of *sc* mRNA resulting from the heat shock should not provide such spatial information. We assessed the position of the rescued chaetae by measuring their distances to fixed landmarks (Table I). Distances were generally not significantly different from those of the wild type. The ADC macrochaeta seemed posteriorly displaced. However, the ADC was generally unaccompanied by the PDC, and in mutants lacking the PDC, the ADC is similarly displaced (Stern, 1954).

The *sc* gene of the $sc^{10.1}$ mutant is transcribed with a pattern similar to that of the wild type gene (Romani *et al.*, 1989). It should give rise to a truncated protein (Villares and Cabrera, 1987) that, although inactive by itself, it might conceivably complement the heat shock-induced *sc* protein and provide increased *sc* activity in cell clusters topologically coincident with those of the wild type. This could explain the correct position of the heat shock-induced macrochaetae. This possibility was ruled out by repeating the heat shock experiment with a stock carrying two copies of the chimeric *sc* gene and the $In(1)y^{3PL}sc^{4R}$, which carries a deletion of both *ac* and *sc* (Campuzano *et al.*, 1985). Only a few $In(1)y^{3PL}sc^{4R}$;*HSSC* males reached the pharate adult stage. They were dissected out of the puparium, and although their wrinkles prevented measurement, macrochaetae positions were clearly similar to those of the wild type (Figure 3H).

We confirmed these results with transformant flies carrying, in the second chromosome (23B), a *sc* gene that lacked most of its controlling sequences (Ruiz-Gómez and Modolell, 1987) (pSC9RR64, Figure 1). This gene is homogeneously, albeit weakly, expressed in the notum anlage of the late third instar larva (Figure 1B,C). Similarly to the $sc^{10.1}$;*HSSC* transformant, and in either the $sc^{10.1}$ or $In(1)y^{3PL}sc^{4R}$ backgrounds, it promoted the generation of macro- and microchaetae. One copy of pSC9RR64 only rescued the PSA, while two copies allowed development, although at low frequency in some positions, of most notum macrochaetae in correct positions (Table 1 and Figure 3D). Taken together, the above results indicate that SOP positioning can be accomplished in the absence of the spatial information that may be provided by the patterned distribution of the *ac*/*sc* products. Thus, epidermal primordia should contain topological information relevant for SO positioning and independent of the AS-C system.

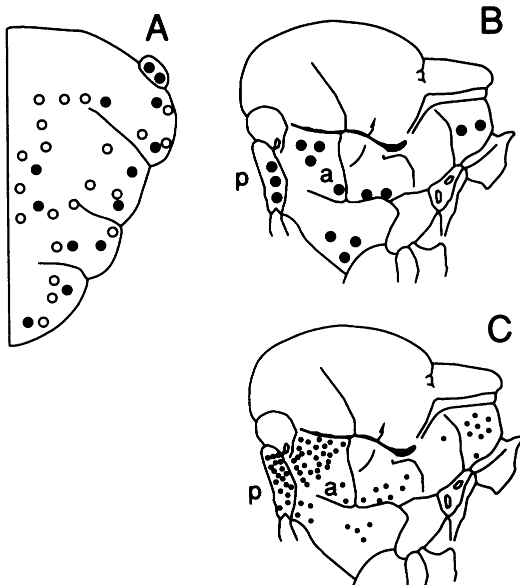

Fig. 4. Notum macrochaetae (**A**) and thoracic pleura macro- (**B**) and microchaetae (**C**) in AS-C^{+}flies with two copies of the *HSSC* gene. Heat-shocks were started at 8–4 h BPF (A,B) or 8–12 h APF (C). Macro- and microchaetae are represented by large and small dots, respectively. In A, filled dots: extant macrochaetae; open dots, extra macrochaetae. In the thoracic pleura, chaetae were most abundant in the preepisternum (p) and anepisternum (a).

Discussion

By means of the transient expressions of a *sc* gene driven by an *hsp70* promoter, we have shown that the competence of epidermal primordia to respond to the proneural effects of the *sc* product is temporally restricted to characteristic developmental periods and it is preferentially localized at specific sites. For the sake of clarity, these two aspects of competence will be separately discussed.

Periods of responsiveness to the sc product

Developmental analyses indicate that determination of SOs in different parts of the body occurs at different times (García-Bellido and Santamaría, 1978). Thus, notum macrochaetae are the earliest to be determined, followed by notum microchaetae, tergite macrochaetae and tergite microchaetae, respectively. The notum microchaetae depend mostly on *ac* for development, while the other chaetae depend principally on *sc* (García-Bellido, 1979). A possible explanation for this temporal sequence of events could be the sequential activation of these genes in the corresponding primordia, namely, the wing disc and the abdominal histoblasts. However, in these primordia *HSSC* expression can give rise to both macro- and microchaetae, the type of SO depending on the developmental time the *sc* product is administered. We have also found that, for a given segment of the leg, chemosensory chaetae are induced earlier than mechano-receptor chaetae. Thus, the developmental age of the target tissue is critical for its cells both to respond to the *sc* product and to decide the class of SO into which they develop. Our results further reveal that competence to respond appears at different times in different regions of a primordium. For instance, depending on the time the *sc* product is provided, microchaetae develop preferentially in different areas of the notum, legs or abdomen. Finally, there is for each primordium a characteristic developmental time when response, as measured by the number of developing SOs, is maximal. Taken together, these results indicate that within each primordium competence to respond the *sc* product is temporally and spatially regulated.

To understand the significance of competence periods, we have looked for correspondences between these periods and the times when SOPs are first recognizable and divide. *Drosophila* transformant lines which express the bacterial

LacZ gene in SOPs and their descendants (A37 and A101.IF3; Ghysen and O'Kane, 1989), have revealed that macrochaetae precursors become sequentially recognizable by these most early markers in a period of time that goes from more than 20 h BPF to 5 h APF, and notum microchaetae precursors appear from 10 to 16 h APF (F.Huang, S.Campuzano and P.Cubas, personal communication). These developmental times are almost entirely comprised within the respective competence periods. It is also known that the precursors of the notum macro- and microchaetae and the tergite microchaetae undergo their first differential division at approximately the time of puparium formation, 15 h APF and 24–30 h APF, respectively (Hartenstein and Posakony, 1989; S.Campuzano and P.Cubas, unpublished). These events coincide with the later part of the respective competence periods, at the time of maximum response. The second differential division approximately coincides with the end of the corresponding competence periods since it occurs several hours after the first one (Poodry, 1975; Hartenstein and Posakony, 1989). Moreover, Poodry (1975) has also shown that the direction in which the microchaetae of the leg become radio-insensitive (presumably at the time they undergo the second differential division) is generally from distal to proximal, and that in the tergites macrochaetae become insensitive before microchaetae. So, the competence periods to respond to *sc* and the acquisition of radio-insensitivity occur in the same spatial and temporal sequences. From these observations we infer that our competence periods reveal a critical cellular state that appears to exist from several hours before SOPs become recognizable up to the moment they undergo terminal divisions. However, clonal analyses indicate that, in the notum anlage, lineage separation between epidermal and SO precursors occurs as early as 40 h BPF, suggesting that the first determinative events towards SOP commitment occur at approximately this time (García-Bellido and Merriam, 1971). At 40 h BPF, *sc* expression is already detectable in the notum primordia (Romani *et al.*, 1989; S.Campuzano and P.Cubas, unpublished), but pulses of *HSSC* expression have no phenotypic effect. This suggests that, although at these early developmental times the *sc* product may start cells into the SOP developmental pathway, its presence is necessary until later times for maintaining them into the pathway and achieving irreversible commitment.

The SOPs for the sensilla campaniformia on vein 3 and part of the notum macrochaetae are determined earlier than the time of the maximal response to *HSSC* for their corresponding regions. Indeed, at this time, most of the normal sensilla campaniformia on that vein are already

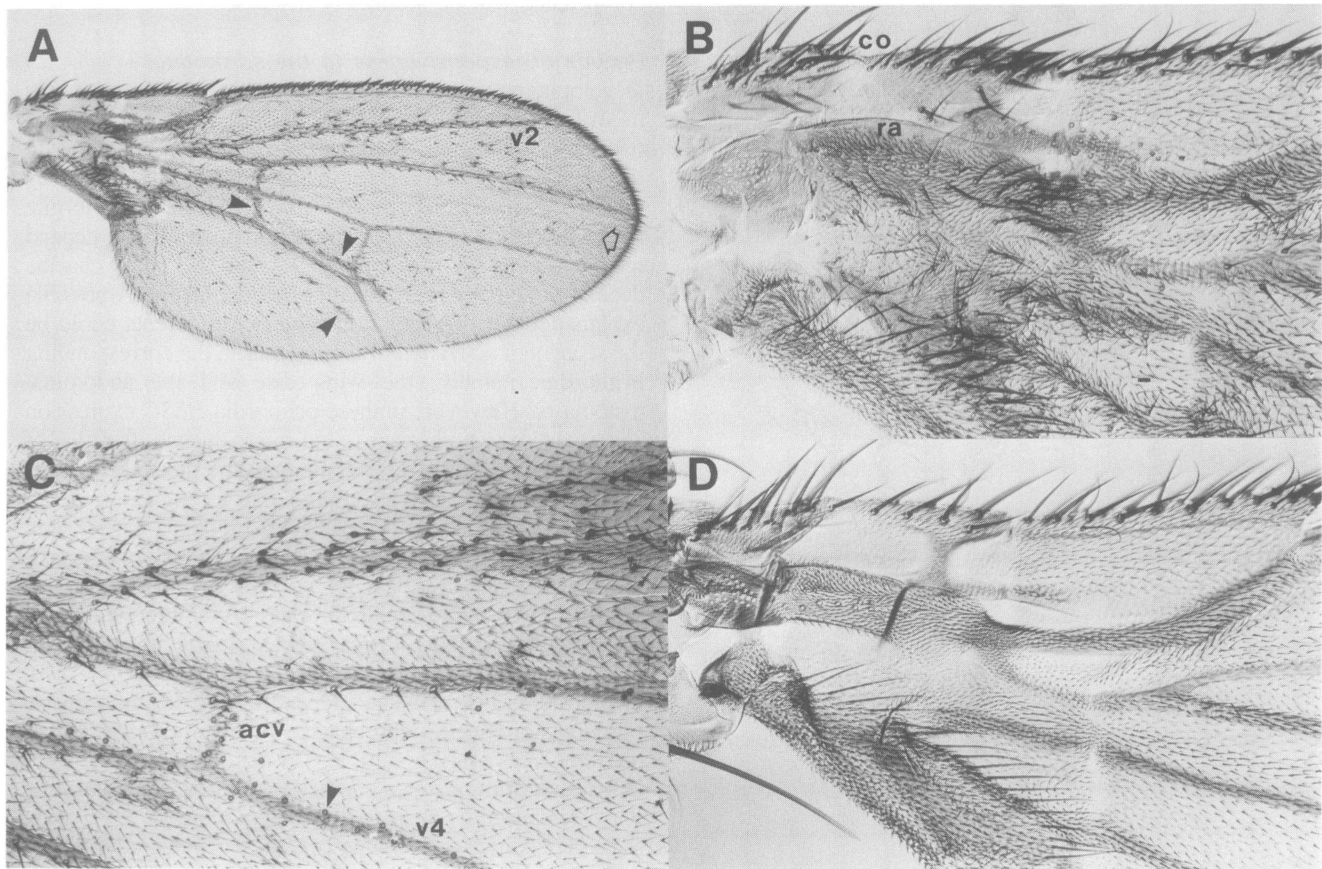

Fig. 5. Wings of flies carrying 2 copies of the *HSSC* gene, with heat-treatment started 8–12 h APF. (**A**) Overall view of the wing. Ectopic SOs, principally microchaetae, accumulate on veins, specially on vein 2 (v2). This phenotype is reminiscent of that observed in *hairy* mutants. Ectopic SOs appear less frequently in the intervein spaces, especially between veins 3 and 4 (arrowed). Note the formation of extra veins (some are pointed at by arrowheads). (**C**) 4× higher magnification of the central area of a similar wing showing preferential accumulation of sensilla campaniformia (an example is pointed at) on the anterior crossvein (acv) and vein 4 (v4). (**B**) and (**D**) wing base of the wing shown in A and of a wild type wing, respectively. Magnification is the same as in C. D is shown for the sake of comparison with B. Note the very large amount of ectopic sensilla campaniformia and chaetae in B. They are most abundant on the radius (ra) and veins. Number of bristles on the costa (co) is also increased.

differentiating their neurons (Murray *et al.*, 1984) and part of the notum macrochaetae precursors have already initiated their differential divisions (F.Huang, S.Campuzano and P.Cubas, unpublished). Perhaps, the strategy of determining SOPs at times earlier than those of maximal response (as determined by means of *HSSC* expression) facilitates controlling the number of SOs in the wild type. This suggests that, in such regions, endogenous *sc* expression is repressed or its product is made ineffectual at the time of maximal sensitivity. Interestingly, this time coincides with the intervals when heat-shock induced ectopic expression of *h* suppresses h^- microchaetae (Rushlow *et al.*, 1989) and when *HSSC* causes phenotypes reminiscent of *emc* and *h* mutations. This indicates that *emc* and *h* proteins (Rushlow *et al.*, 1989; Garrell and Modolell, 1990; Ellis *et al.*, 1990) help prevent development of excess SOs when tissues are specially sensitive to the effects of the *ac/sc* proteins. These times are also those when removal of *Notch* and *shibire* functions causes development of many extra chaetae on the head and notum (Shellenbarger and Mohler, 1978; Poodry *et al.*, 1973), suggesting that mechanisms of cell communication (lateral inhibition) are also important at these times to prevent appearance of excess SOs.

Replacement of ac by sc

Loss of function mutations indicate that notum microchaetae and the DC and PSA macrochaetae depend on *ac* for development, while the other macrochaetae depend on *sc* (García-Bellido, 1979). In $sc^{10.1}$;*HSSC* heat-shocked flies, the *sc* product replaces the missing *ac* function in the development of these *ac*-dependent chaetae. A similar replacement has been previously found in the Hw^{685} mutant (Balcells *et al.*, 1988). Morever, the *sc* product is able, depending on the anlage and the period of expression, to generate different kinds of SOs. Thus, we conclude that the type of SO is specified by genes other than *ac*, *sc* or their combinations.

Positional specification of SOs

In the absence of the *sc* and *ac* endogenous genes, the homogeneous distribution of *sc* product, provided by either *HSSC* or a *sc* gene devoid of most of its *cis*-controlling sequences, promotes development of SOs in correct positions in most regions of the fly. One of the clearest examples is the set of rescued notum macrochaetae, whose positions are in most cases remarkably similar or identical to those of the wild type. This result indicates that the wild type, patterned

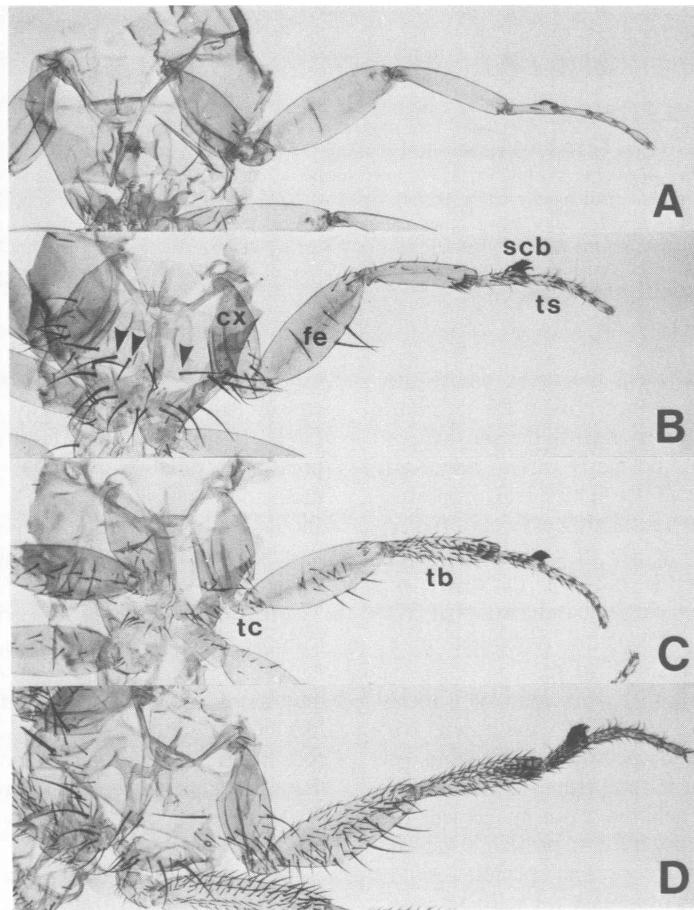

Fig. 6. Foreleg and part of the thoracic pleura of males of the following genotypes: $sc^{10.1}$ (**A**), $sc^{10.1}$;*HSSC-2*/+;*HSSC-3*/+ heat-treated at either 4–0 h BPF (**B**) or 4–8 h APF (**C**), and wild type Oregon R (**D**). $sc^{10.1}$ legs are almost completely devoid of SOs (A). Early *HSSC* expression rescues chaetae on the distal part of the legs, mostly on tarsum (ts) and the sex comb (scb) (B). Note that expression at this time (4–0 h BPF) rescues many chemoreceptor (recurved) chaetae and also macrochaetae on the femur (fe), coxa (cx), sterum and sternopleura, and induces development of some ectopic macrochaetae (examples are arrowed; compare B and D). Maximal rescue on tibia (tb) is shown in C. At this time (4–8 h APF) most recovered SOs are mechano-receptor (straight) chaetae, which also appear on trocanter (tc) and coxa. Recovery of chaetae on femur was always low (compare C and D).

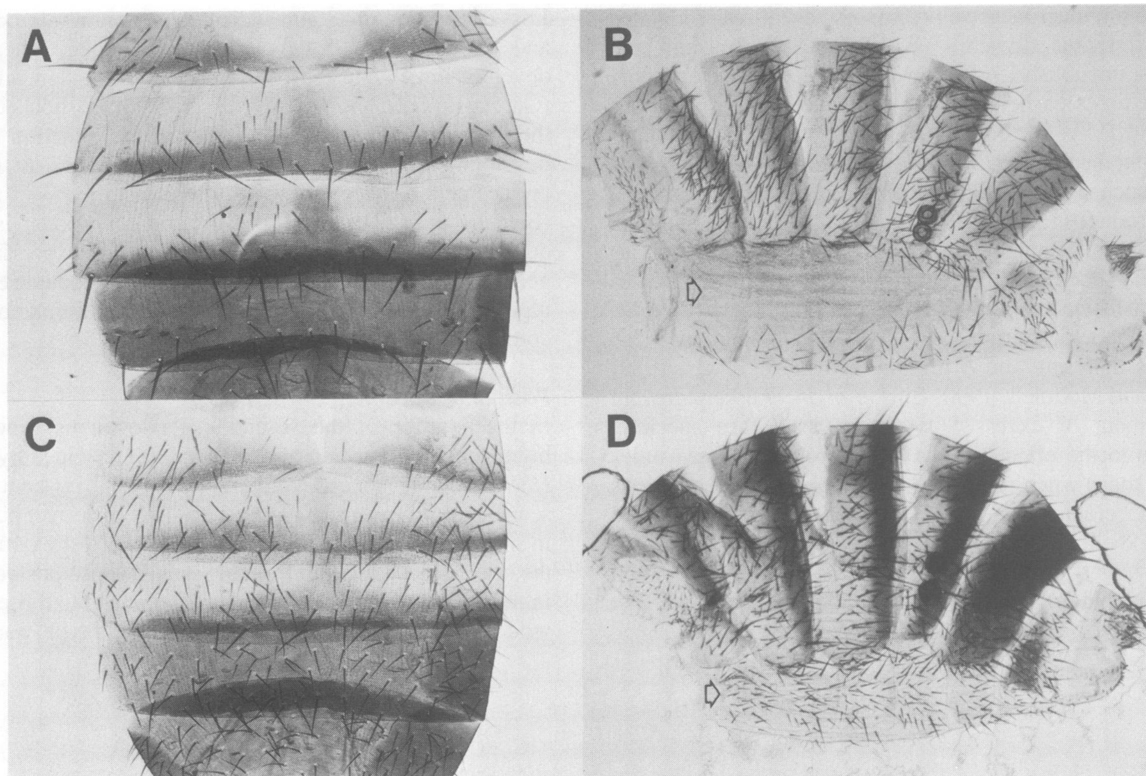

Fig. 7. Abdominal cuticle of $sc^{10.1}$;*HSSC-2*/+;*HSSC-3*/+ males heat-shocked at either 4–8 h APF (**A**) or 0–4 and 24–28 h APF (**C**), and wild type (**B**) female, and female with two copies of *HSSC* gene heat-treated at 20–24 h APF (**D**). $sc^{10.1}$ flies have tergites almost completely devoid of chaetae. A heat-shock early in the competence period rescued most macrochaetae in correct positions (posterior border of tergites) and a small fraction of the microchaetae, which were located mainly in the posterior part of each tergite (A). Later, heat-shocks rescued microchaetae also in the anterior part of the tergites. Although late heat-shocks only rescued microchaetae, an early and a late heat-shock rescued both types of chaetae (C). B and D show abdominal cuticles flattened on the side, so that tergites are folded by approximately their midline and chaetae on both of their sides are shown superimposed. Note that in wild type flies (B) abdominal pleura (arrowed) lack chaetae, but develop many of them in response to *HSSC* expression in the presence of the endogenous *ac*/*sc* genes (D).

expression of *ac*/*sc*, which delimits the areas where the macrochaetae precursors arise, is not critical for defining their positions. Thus, cells at or very near to the sites where the macrochaetae precursors normally arise have an enhanced tendency to become SOPs. This local property, to preferentially respond to the neuralizing effects of the *sc* protein, may develop independently of this (or the *ac*) product or require its presence but not its restricted distribution. In either case, our finding indicates that SO positioning uses topological information embodied in a differential ability of cells to respond to *sc*.

Although the molecular basis that determine such local enhanced sensitivity to the *sc* protein are unknown, we propose that they may consist in specific combinations of molecules which would define a competent cellular state. Such combinations would be generated during growth of anlagen at specific times and cells. If the *sc* protein is indeed a transcription factor, some of the constituent proteins could be other transcription regulators necessary for *sc* to activate the SO developmental pathway and/or to repress those genes promoting the epidermal pathway. Most likely candidates are other HLH proteins, like *da*, *h*, *emc*, and *E(spl)* (Klämbt *et al.*, 1989), implicated in controlling neurogenesis and that may facilitate or antagonize *sc* interaction with DNA (see Introduction). The AS-C encoded *asense* protein (Alonso and Cabrera, 1988; González *et al.*, 1989) may also play a role, especially in the tergites and scutelar chaetae, since its absence causes the removal of many of these SOs (Lindsley and Grell, 1968). Participation of *lethal of sc*, the fourth HLH protein encoded in the AS-C, is also a possibility. Other molecules that should qualify the cellular response to *sc* should be those encoded by the so called neurogenic genes, like *Notch* and *Delta* (Artavanis-Tsakonas, 1988; Campos-Ortega, 1988, reviews; P.Simpson, J.F.de Celis and M.Marí-Beffa, personal communications) and *shaggy* (Simpson and Carteret, 1989), which are involved in cell to cell interactions and, more specifically, in lateral inhibition. Evidently, other proteins and regulative processes like the proliferative state of cells may also have an important role in establishing competence. Thus, it has been shown that microchaetae precursors undergo their first differential division within clusters of dividing cells (Hartenstein and Posakony, 1989).

In contrast to the present experiments in the $sc^{10.1}$ background, generalized expression of *sc* in the Hw^{49c} mutation induces large numbers of SOs in ectopic positions (García-Alonso and García-Bellido, 1986; Balcells *et al.*, 1988). These contrasting results can be explained by the low (*SC9RR64*) or transient (*HSSC*) expression of *sc* in our experiments, which would only allow SO development on sites of maximal sensitivity. On the other hand, the large number of SOs induced in normal and ectopic positions by

Table I. Position of notum macrochaetae in wild type and transformant male flies

Chaeta	y				x			
	WT	A	B	C	WT	A	B	C
PS	8.8 ± 0.1	8.9 ± 0.9 (60)	9.9 ± 1.0 (11)	10.3 ± 0.6 (4)	12.7 ± 0.6	11.9 ± 0.9	12.5 ± 1.1	10.8 ± 1.0
ANP	13.9 ± 0.8	16.4 ± 0.8 (2)	–	–	0.8 ± 0.3	0.4 ± 0.1	–	–
PNP	3.4 ± 0.7	2.6 (1)	–	–	1.9 ± 0.8	1.3	–	–
ASA	2.6 ± 0.7	2.6 ± 0.5 (2)	–	–	12.5 ± 0.6	9.6 ± 0.3	–	–
PSA	9.1 ± 0.9	10.3 ± 0.7 (17)	10.3 ± 0.8 (18)	9.7 ± 0.6 (4)	15.0 ± 1.0	13.0 ± 1.4	14.1 ± 1.9	13.6 ± 2.0
APA	19.8 ± 0.7	–	21.4 ± 1.8 (7)	19.9 ± 1.0 (4)	14.1 ± 1.1	–	13.8 ± 1.4	14.4 ± 2.1
PPA	4.3 ± 1.4	–	–	2.6 (1)	–	–	–	–
ADC	25.1 ± 1.4	23.4 ± 1.5 (10)	22.9 ± 4.2 (16)	17.6 ± 0.8 (2)	–	–	–	–
PDC	11.1 ± 0.8	12.7 ± 1.4 (5)	12.2 ± 2.7 (16)	6.9 ± 0.3 (4)	–	–	–	–
ASC	7.0 ± 1.1	–	6.5 ± 2.0 (3)	–	–	–	–	–
PSC	22.0 ± 1.1	–	–	–	–	–	–	–

Distance (in hundredths of a mm) from macrochaetae sockets to either the intrascutal suture (PS to APA) or the scutoscutellar suture (PPA to PSC) (y) or to the straight prescutum-pleural suture or its imaginary posterior prolongation (x) (see Figure 3) was determined from camera lucida drawings. Number of heminota examined were: wild type Oregon R, 20; heat shocked $sc^{10.1}$;*HSSC-2*/+;*HSSC-3*/+, 72 (**A**); $sc^{10.1}$;*SC9RR64*/*SC9RR64*, 18 (**B**); and *In(1)y*^{3PL}*sc*^{4R};*SC9RR64*/*SC9RR64*, 4 (**C**). Averages of measurements and standard deviations were calculated. Figures in parenthesis indicate number of chaetae recovered.

HSSC (or *SC9RR64*, our unpublished data) in a wild type background, alternatively suggests that development of ectopic chaetae requires the presence of the endogenous *sc* (and/or *ac*) gene. This might either provide a basal and lasting level of *sc* product, insufficient by itself to promote SO commitment but capable to supplement and make *HSSC* expression more effective; or be activated in ectopic regions by *HSSC* expression, thus providing increased amounts of *sc* product over longer periods of time. The failure of meeting these conditions may explain the rarity of ectopic SOs in *HSSC*, ac^-, sc^- flies. In any case, the phenotypes of *Hw* and heat-shocked AS-C$^+$;*HSSC* flies underline the necessity of spatially restricting *sc* expression in order to limit SO differentiation to specific regions. And taken together with the above results, these phenotypes indicate that, in wild type flies, the patterned expression of *ac*/*sc* does not define the precise positions of SOs; rather, it would have a permissive role and allow SOs to appear only within defined regions.

Materials and methods

Plasmid constructions

pHSSC was derived from transformation vector pHT4 (Schneuwly *et al.*, 1987). It contains the *hsp70* promoter, 95 base pairs (bp) of the *hsp70* leader, a polylinker fragment, and 14 bp of the *sc* leader followed by the entire *sc* coding and trailing sequences (Villares and Cabrera, 1987). The plasmid was constructed by introducing into the unique *Kpn*I cloning site of pHT4 a 3.7 kb *Alu*I–*Kpn*I fragment comprising the *sc* gene and 3′ flanking sequences.

pSC9RR64 was derived from the transformation vector pW8 (Klemenz *et al.*, 1987). It contains an 8.8 kb *Eco*RI fragment that comprises the *sc* gene and flanking sequences (Campuzano *et al.*, 1985). This fragment was inserted into the vector *Eco*RI cloning site.

Drosophila transformation

P-element mediated transformation was performed according to Rubin and Spradling (1982) using 0.5 mg/ml of either pHSSC or pSC9RR64 plasmid DNA, and 0.15 mg/ml of pπ25.7wc DNA to provide for transposase.

Heat-shock treatment

Flies were allowed to lay eggs in culture bottles for five days and the cultures were aged for approximately 2 more days. Pupae were then collected every 8 or 16 h. Their developmental ages were estimated as follows (Roberts, 1986). Pupae from a 16 h collection were immersed in water. Those that remained on top were assumed to be 8–16 h old. These were examined for the presence (12–16 h old) or absence (8–12 h old) of loose larval mandibles. Those that sunk, were separated into two groups: the brown older pupae (4–8 h old) and the whiter younger ones (0–4 h old). Pupae from an 8 h collection were similarly separated into these two groups (0–4 and 4–8 h old). Pupae were then allowed to age at 25°C to have groups representing all ages of the pupal stage. Just prior to heat exposure, any remaining pupae were removed from the primary culture bottles. Heat treatment was then simultaneously administered to larvae in the primary cultures and to the staged pupae. Unless otherwise indicated, it consisted of three 1 h exposures at 37°C, separated by 2 h intervals at 25°C. All animals were then returned to 25°C, pupae collection from the primary cultures was again started 1 h after the third heat exposure, and it was later repeated every 8 or 16 h. Ages of collected pupae were estimated as above. All pupae were allowed to develop and the chaetae pattern of the emerged or pharate adults was examined. Indicated developmental age of the animals corresponds to that at the start of the heat treatment.

Other procedures

Hybridizations *in situ* to tissue sections and polytene chromosomes were performed as described (Romani *et al.*, 1989; Modolell *et al.*, 1983).

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