A study of shaggy reveals spatial domains of expression of achaete–scute alleles on the thorax of Drosophila

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Summary

A study of shaggy mutant clones on the notum reveals that a greater number of cells are diverted into the bristle pathway of differentiation and fewer cells remain to produce the epidermis. shaggy clones differentiate supernumerary microchaetae and macrochaetae but these are found in the correct spatial locations, e.g. clusters of macrochaetae are formed round the position of the extant macrochaetae. The shaggy mutant phenotype requires the functioning of the genes of the achaete–scute (AS-C) complex but a dosage study shows that it is unlikely that the AS-C is overexpressed in shaggy cells. Data are presented that argue, also, for a correct spatial expression of the AS-C in shaggy mutants. A study of clones doubly mutant for shaggy and different achaete and scute alleles is consistent with the hypothesis that the clusters of macrochaetae formed by shaggy represent the restricted spatial domains of expression of the AS-C. The results can be reconciled with the known role for the AS-C, in determining which bristle types differentiate where, and a role for shaggy in the cell interactions, within domains of the AS-C expression, leading to the definition of only one bristle mother cell.

Key words: Drosophila, development, pattern, mutant, scute, shaggy.

Introduction

A rather precise pattern of sensory bristles develops on the notum of Drosophila. Exactly eleven large bristles, or macrochaetae, each of which has been named, are found at defined positions on each heminotum. They are thought to develop as a response of the cells to their position within the field of positional information. The small bristles, or microchaetae, on the other hand, are indistinguishable from one another and their number varies slightly from fly to fly. They are, however, regularly spaced out and the spacing is thought to result from a phenomenon of lateral inhibition (Wigglesworth, 1940; Richelle & Ghysen, 1979; Held & Bryant, 1984).

The development of both macro- and microchaetae is known to be under the control of genes of the achaete–scute complex (AS-C). achaete is required mainly for the formation of the microchaetae, whereas scute is required mainly for the formation of the macrochaetae (Garcia-Bellido, 1979). The functions of these two genes, however, overlap considerably and the full pattern requires both. Different alleles of these genes result in the loss of specific bristles and there exist also hypermorphic alleles, Hairy wing, that result in overexpression and cause the production of supernumerary bristles (Campuzano et al. 1986; García-Alonso & Garcia-Bellido, 1986; Balcells et al. 1988). Two other, unlinked, genes, extra macrochaetae and hairy, are thought to act as repressors of scute and achaete, and loss of function of either of these genes also results in the production of either supernumerary macro- or microchaetae (Moscoso del Prado & Garcia-Bellido, 1984). It is thus thought that the AS-C is involved in the decision to differentiate bristles in defined places (Garcia-Bellido & Santamaria, 1978). The spatial restriction of the AS-C expression would thus be the main factor that controls the basic pattern of sensory bristles [as well as other sensory organs and also some central neurones (Dambly-Chaudière & Ghysen, 1987; Garcia-Bellido & Santamaria, 1978; Cabrera et al. 1987; Romani et al. 1987)].

A molecular analysis of the AS-C (Carramolino et al. 1982) led to the description of several transcripts only two of which correspond to achaete and scute, respectively (Campuzano et al. 1985). Many achaete and scute alleles are rearrangements with break points scattered throughout the complex and mapping some distance away from the coding regions. A comparison of the position on the map of a number of terminal deficiencies and the mutant phenotype led Ruiz-Gomez & Modolell (1987) to suggest that the scute transcript is regulated by cis-acting site-specific elements that respond to topological cues. Therefore, different control sites would activate scute in each precise area where a bristle will form. Presumably this area will involve a
were induced in flies of the following genotypes: (3) Df(1)sc19, y~ sgg f36a/+; mwh jv/+ 
(4) sc1 sgg f36a/y sc1 
v 
(5) sc1 sgg f36a/y 
(6) y ac1 sgg w f36a/ac2; sc1/+ 
(7) y ac1 sgg w f36a 
(8) y sgg f36a/+; emc1/TM2, emc 
(9) y sgg f36a/+; emc1/+ 
(10) y sgg f36a/+; h+/- 
(11) y sgg f36a/+; h+/h+ 
(12) y sgg f36a/+; h+/h+ 
(13) Df(1)sc19, y~ sgg f36a/+; Dp(1; 2)sc19, y~/+ 
(14) y sgg/+; CyO/+ 
(15) y sgg f36a/+; CyO/+ 
(16) y sgg f36a/+; Dp(1; 2)sc19, y~/+ 
(17) Dp(3; Y; 1)M2, emc y sgg w v/+ 
(18) sgg f36a/+ 

Flies of genotypes (8), (9), and (10) were obtained from the cross Q y sgg f36a/FM6; TM2, emc+/+ x sgg+/+; TM2 carries a weak allele of emc that is viable over emc' (Moscoso del Prado & Garcia-Bellido, 1984). emc1/TM2 flies therefore have an emc phenotype. Flies of genotype (9) can be distinguished from those of genotype (10) by virtue of the syngergism between sgg and emc (see Results); they invariably display at least one extra macrochaete on the thorax. Flies of genotypes (11) and (12) were obtained from the cross Q FM6/y sgg f36, h+/+ x sgg+/h+. Flies of genotypes (13) and (14) were obtained from Q Df(1)sc19, y~ sgg f36a/FM6 and y sgg/FM6 that were both crossed to Dp(1; 2)sc19, y~/CyO males and placed in the same bottles. Flies of genotypes (15) and (16) were obtained from Q y sgg f36a/FM6 x C y Dp(1; 2)sc19, y~/CyO. Flies of genotypes (17) and (18) were obtained from Q FM7/Dp(3; Y; 1)M2, emc y sgg w v and Q sgg f36a/FM6 that were both crossed to Ore R males and grown in the same bottles.

Materials and methods

Flies were raised on standard medium and maintained at 25°C.

Lethal mutations at the zw3 locus were described by Judd et al. (1972) and map to 3B1. We have called this gene l(1)zw3<sup>v</sup> (shaggy) (Simpson et al. 1988). Throughout this report we have used the amorphic allele sgg<sup>b12</sup> (isolated by P. Ripoll), which we will refer to simply as sgg. In one single experiment, another allele, sgg<sup>b15</sup> (caused by an inversion, Judd et al. 1972), was also used and this is specified in the text (Table 5). For a description of other mutations and rearrangements see Lindsley & Grell (1968) and Lindsley & Zimm (1985), DIS vols 62, 64 and 65.

Clonal analysis

Clones mutant for sgg were produced in various genetic backgrounds by X-ray-induced mitotic recombination. Unless otherwise specified in the text, 24 h egg collections were made and flies were irradiated between 48 and 72 h AEL. The clones resulting from mitotic recombination were marked with yellow (y), forked (f<sup>36a</sup>), javelin (jv), multiple wing hair (mwh) or stubbly chaete (sc1). Flies were irradiated with 1000 R of X-rays (100 kV, 4 mA given for 3 min 18 s, 1.5 mm aluminium filter, Philips MG102 constant potential X-ray system, beryllium window). Thoraces were heated in 10% KOH and mounted between coverslips in Euparal. Clones were drawn onto standard diagrams of the notum. Clones were induced in flies of the following genotypes:

(1) sgg f<sup>36a</sup>/y 
(2) y sgg f<sup>36a</sup>/+

Results and discussion

shaggy mutant clones reveal a precise pattern of bristles on the notum

Clones of cells mutant for shaggy (sgg) were produced on the dorsal thorax by mitotic recombination. These produce dense clusters of extra bristles (see Figs 1, 2). A study of sgg f<sup>36a</sup> clones and y control clones issued
Expression of scute alleles in shaggy

Fig. 1. (A) Standard diagram of the wild-type heminotum of Drosophila showing the positions of macrochaetae, large circles, and microchaetae, small circles. The macrochaetae are named as follows: DC, dorsocentral; Scu, scutellar; PA, postalar; SA, supraalar; NP, notopleural; PST, presutural. (B) The distribution of macrochaetae, large dots, observed in a study of 100 y sgg f36a clones induced in y sgg f36a/+ flies. The flies were irradiated between 48 and 72 h AEL, the average clone size was 16.4 ± 2.0 bristles, the largest clone comprised 69 bristles. Clones are a mixture of micro- and macrochaetae, only the macrochaetae are shown. These can be seen to be clustered around the positions of the extant macrochaetae. More bristles are found in the positions of the dorsocentral and scutellar bristles than in the positions of the other macrochaetae. It is thought that some clones in these regions may be lost, see text. Examination of a much larger sample of clones, however, shows that macrochaetae also arise in clusters around the postalar, supraalar, notopleural and presutural bristles (results not shown). (C) The distribution of microchaetae, small dots, observed from a study of the same 100 y sgg f36a clones as in B. 15 clones are shown. The bristles, which develop at a high density, can be seen to differentiate in areas normally covered by microchaetae. No microchaetae are found on the scutellum or in the vicinity of the posterior postalar bristle.

Table 1. Size of twin clones resulting from irradiation-induced mitotic recombination at 72 ± 2 h AEL in sgg f36a/y flies

<table>
<thead>
<tr>
<th></th>
<th>Average number of bristles ± s.e.</th>
<th>Number of clones</th>
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<tbody>
<tr>
<td>sgg f36a</td>
<td>7.9 ± 0.5*</td>
<td>61</td>
</tr>
<tr>
<td>y</td>
<td>4.2 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

* P<0.01 when compared with controls.

Table 2. The spacing between bristles of y sgg f36a clones induced in y sgg f36a/+ flies measured by counting the number of intervening trichomes. As a control, spacing was measured between wild-type bristles in the same position as the clone on the other hemithorax

<table>
<thead>
<tr>
<th></th>
<th>Control wild-type bristles</th>
<th>Number of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>sgg</td>
<td>2.7 ± 0.23*</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>4.9 ± 0.12</td>
<td></td>
</tr>
</tbody>
</table>

* P<0.01 when compared with controls.

from the same recombination event (twin spots) at 72 h AEL shows that about twice as many bristles are made by the mutant cells (Table 1). The surface area occupied by the sgg clones is not enlarged, however. Rather, the spacing between the bristles is reduced and fewer trichome-producing epidermal cells are present (Table 2). Therefore a greater number of cells are diverted into the pathway for sensory bristles, and fewer cells are left to make the epidermis. In the area surrounding the sgg clones, as on the remainder of the thorax, the pattern of bristles remains normal. Control clones produced marked bristles in normal positions and caused no disruption of the pattern.

Clones of sgg cells produced earlier than 72 h AEL show a tendency to round up and contract. Sometimes vesicles of cuticle bearing marked bristles are found inside the thoraces that carry sgg clones. This is perhaps due to different cell affinities such as those described for some areas of the wing (Ripoll et al. 1988). Differential cell affinities may also be the reason why many sgg clones are found in the centre of the thorax and fewer clones are found in the region of the postalar, supraalar and notopleural bristles (see Fig. 1B). Cell proliferation is probably unaffected in sgg mutant cells: a study of twin spots on the wing blade where accurate cell measurements can be made revealed clones of expected sizes (Simpson et al. 1988).

Both extra microchaetae and macrochaetae are formed in shaggy clones. They develop, however, at fairly precise locations. The positions of micro- and macrochaetae from 100 y sgg f36a clones were drawn onto standard diagrams. On areas of the notum where microchaetae normally differentiate, sgg clones too produce microchaetae (Figs 1C, 2B). Macrochaetae, therefore, although formed at a greater density, develop in those parts of the thorax that are normally covered with them. Microchaetae are not formed in ectopic positions, e.g. no microchaetae differentiate on the scutellum. Therefore, the basic spatial distribution is unaltered. In the regions surrounding the positions of the extant macrochaetae sgg clones produce both macrochaetae and microchaetae. Macrochaetae, however,
are only found clustered around the positions of the extant macrochaetae (Figs 1B, 2A), they do not differentiate elsewhere. This is particularly clear for the dorsocentral and the scutellar bristles, to which we will confine the rest of our analysis. Therefore as with the microchaetae, the basic spatial distribution of macrochaetae is unaltered in the sgg mutant. These observations therefore suggest that, although extra bristles are formed by sgg cells, the basic spatial organization that dictates which type of bristle is to be placed where remains unaltered. It has in fact been shown that sgg cells on the wing blade know where they are and respond correctly to positional cues (Simpson et al. 1988).
The shaggy phenotype requires the function of achaete–scute but is not attributable to an overexpression of achaete–scute. The decisions governing which type of bristles are to be placed where on the notum are thought to fall under the control of the genes of the achaete–scute complex (AS–C). In the absence of the AS–C no bristles form (Garcia-Bellido & Santamaria, 1978). The Hairy wing (Hw) alleles of the AS–C represent a gain of function. They cause an overexpression of either achaete (ac) or scute (sc) and lead to the production of extra macro- or macrochaetae (Balcells et al. 1988). Their phenotype superficially, therefore, resembles that of sgg. (There are, however, important differences that will be discussed later). It was therefore of interest to determine whether or not the AS–C is expressed normally in sgg mutant cells or whether the sgg phenotype could be attributed to an overexpression of the AS–C. To address the question of whether the AS–C is required for the phenotype of sgg, clones doubly mutant for sgg and a deletion of the AS–C were made. Clones were induced in y Df(1)sc19, sgg f36a+/+; mwh jv/+; flies [Df(1)sc19 was also used and gave identical results to those with Df(1)sc19, data not shown]. In the first experiment, 50 thoraces from a late irradiation (72 ± 4 h AEL) were mounted and scored for the presence of clones. A total of 29 mwh jv clones were observed together with 25 naked patches presumed to be AS–C– sgg clones. In order to establish that this result pertains to all bristles of the notum, a further 34 naked patches, from flies irradiated between 48 and 72 h AEL, were selected under the dissecting microscope. Such presumed clones covered most of the area of the notum and no y f36a bristles were ever seen. The AS–C is therefore epistatic over sgg for the thoracic phenotype and the supernumerary bristles seen in sgg clearly require the wild-type achaete and scute functions. In order to test the hypothesis that ac and sc might be overexpressed in sgg mutant cells, a gene dosage study was performed. Flies carrying only one dose of sgg and one, two, three or four copies of the AS–C were constructed and found to display a wild-type appearance (results not shown). Furthermore, flies heterozygous for the AS–C and carrying one, two or three copies of sgg were also wild-type (results not shown). Finally the phenotype of Hw, a gain-of-function allele of ac, remains unaltered in the presence of one, two or three doses of sgg (results not shown). As a further test, clones mutant for sgg were made in flies bearing one, two or three copies of the AS–C and their phenotype was found to be unchanged (Table 3). From these observations, we conclude that sgg plays no regulatory role concerning the expression of the AS–C. It therefore seems unlikely that the sgg phenotype is due to a derepression of the AS–C.

Not all of the bristles found in the adult fly require the function of the AS–C. For example, many of the bristles of the wing margin, medial and dorsal triple row bristles and bristles of the double row differentiate in marginal clones that are AS–C– (Garcia-Bellido & Santamaria, 1978). shaggy, on the other hand, affects all bristle types found on the fly body including those of the wing margin, such that sgg clones on the wing blade transform epidermal hairs into marginal bristles (Simpson et al. 1988). AS–C– clones on the wing blade (away from the wing margin) differentiate trichomes. It was therefore possible to test the epistatic relationship between sgg and AS–C for this part of the body. Doubly mutant AS–C– sgg clones on the wing blade have a sgg phenotype, that is they differentiate bristles (results not shown). Therefore the AS–C appears to be epistatic over sgg only in those regions of the body in which the AS–C is required. In other words, the sgg phenotype does not result from a derepression of ac or sc such that these genes are ectopically expressed. These results argue again that the spatial expression of AS–C elements is not altered in the sgg mutant, and this would therefore explain why on the notum, although there are more bristles than is usual in sgg clones, the bristles are nevertheless formed in the correct general positions. Shaggy is perhaps therefore involved in some other aspect of bristle differentiation, and plays a different role from that of proneural genes such as the AS–C for the notum and other, perhaps related, genes (Villares & Cabrera, 1987; Alonso & Cabrera, 1988) for the wing that govern the spatial distribution of morphologically distinct bristles.

The shaggy pattern reveals the spatial domains of expression of different scute alleles

Stern (1954a,b) made a study of clones mutant for ac which removes the dorso-central bristles. Such clones behave autonomously. When the mosaic border line runs through the dorso-central region, however, occasionally a bristle will form at some distance from the normal site. This locally restricted nonautonomy led Stern to suggest that the macrochaete position is first defined as a region and later narrowed down to a single cell. Our observations on sgg clones are consistent with this idea; the clusters of extra macrochaetae seen in sgg suggest the presence of a small region around each extant bristle, the limits of which specify position for that bristle. This supports the notion that determination of a bristle results from a collective decision of a group

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Number of doses AS–C</th>
<th>Number of bristles per clone ± S.E.</th>
<th>Number of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>(13)</td>
<td>1</td>
<td>18.6 ± 2.0</td>
<td>38</td>
</tr>
<tr>
<td>(14)</td>
<td>2</td>
<td>17.3 ± 2.5</td>
<td>49</td>
</tr>
<tr>
<td>(15)</td>
<td>2</td>
<td>18.9 ± 2.8</td>
<td>34</td>
</tr>
<tr>
<td>(16)</td>
<td>3</td>
<td>23.2 ± 2.0</td>
<td>52</td>
</tr>
</tbody>
</table>

* Flies of genotypes (13) and (14) were grown together in the same bottles and flies of genotype (15) and (16) were siblings, see Materials and methods.
of cells in a defined area of the epidermis. Such a group of cells can be called an equivalence group (Palka, 1986). It is then thought that cell interactions between members of the group will lead to the determination of only one bristle mother cell which then inhibits its neighbours from also becoming bristles by a mechanism of lateral inhibition (Wigglesworth, 1940). A similar mechanism appears to govern the segregation of neuroblasts in the embryo (Doe & Goodman, 1985).

A number of ac and sc alleles have been described that each remove a specific bristle or a subset of bristles on the thorax. Many of these alleles are due to chromosomal rearrangements with break points at some distance from the coding regions (Campuzano et al. 1985). The sc break points are thought to affect cis-acting site-specific control elements that govern the expression of the sc transcript (Ruiz-Gomez & Modolell, 1987). This would mean that each control site specifically activates sc in the area in which the bristle under its control is destined to be produced. It is unlikely that this activation of sc occurs precisely in the one cell that will become the bristle. Rather sc would be activated in all the cells of the equivalence group that is responsible for that bristle. In other words, the size of the equivalence group would correspond to the number of cells expressing sc. It has in fact been observed that sc transcripts are found in groups of cells in the areas of the imaginal disc where each bristle will later form (Romani, Campuzano, Macagno & Modolell, in preparation). It has also been observed in the embryo that sc transcripts are found in groups of cells from which one will segregate as a neural precursor (Cabrera et al. 1987).

If sc is thus expressed in small regions over the thorax and if, as we have argued, this regional control is unaltered in sgg, then the clustering of macrochaetae around the position of the extant bristles seen in sgg clones would represent the spatial domains of expression of sc. In order to explore this possibility, we have made clones simultaneously mutant for sgg and specific ac or sc alleles. We chose sc', which removes the two scutellar bristles and the anterior notopleural, and ac' and ac, which remove the posterior dorsocentral bristle.

Fig. 3. (A) The distribution of macrochaetae (closed circles) from 90 sgg clones marked with f36a and induced in sc1 sgg f36a/y sc1 v flies. Flies were irradiated between 48 and 72 h AEL and the average clone size was 15-7 ± 2-6 (n = 54). In sc1 flies, in which the scutellar bristles are absent, the sgg clones, which are also mutant for sc', fail to differentiate bristles anywhere on the scutellum unlike the sgg clones induced in sc1 flies in Fig. 1B. Elsewhere the clones show the typical sgg phenotype. (B) The distribution of macrochaetae from 89 sgg clones marked with y f36a and induced in y ac1 sgg w f36a/ac1, stc/+ flies. Flies were irradiated between 48 and 72 h AEL and the average clone size was 15-1 ± 2-1 (n = 58). Few clones produce macrochaetae in the region of the posterior dorsocentral bristle when compared with those differentiated by sgg clones in an ac+ background (Fig. 1B). The presence of occasional sgg bristles at or close to the site of the posterior dorsocentral bristle can be attributed to the hypomorphic nature of the ac1 and ac mutations; such bristles are often intermediate in size, see text.

shaggy clones in sc+ flies can differentiate a cluster of macrochaetae on the scutellum as in Fig. 2A. The largest clone seen on the scutellum comprised 7 macrochaetae. In the first experiment, sgg clones were induced in animals mutant for ac1. (sc sgg f36a/y sc1 v flies were irradiated.) The distribution of macrochaetae is shown in Fig. 3A. No sgg f36a macrochaetae were found on the scutellum. sgg f36a macrochaetae were still found clustered in the region of the dorsocentral bristles. In the second experiment, sc1 sgg clones were induced in otherwise sc+ flies (sc1 sgg f36a/y flies were irradiated). Fifteen sc1 sgg f36a clones were observed on the nonscutellar regions of the thorax; they had a sgg phenotype. Twelve normal control y clones were also seen. Four y clones were found on the scutellum. Six cases of naked patches on the scutellum were observed, four of which removed one scutellar bristle while the two others removed both. One of these naked patches was associated with a y twin spot and one with a sgg f36a clone on the scutum (Fig. 2D). They thus presumably correspond to the sc sgg clones. sc' is therefore only epistatic over sgg in that region of the thorax where the mutant phenotype is seen. Elsewhere the AS–C is functioning normally and the sgg mutant phenotype is expressed. Therefore, the domain of expression of scute for the scutellar bristles, the spatial control of which is abolished in the sc1 allele, extends only over the scutellum, but covers an area larger than that occupied by each of the two normal scutellar bristles.

sgg clones were also made in flies mutant for ac1/ac2. In such flies, the posterior dorsocentral bristles are lacking. ac1 and ac2 are hypomorphic mutations, however, and occasionally a posterior dorsocentral bristle will arise; very often this bristle is considerably smaller than normal. (y ac1 sgg w f36a/ac1, stc/+ flies were irradiated.) The distribution of macrochaetae found in a study of 58 sgg clones is presented in Fig. 3B. Bristles are found around the positions of all macrochaetae
Expression of scute alleles in shaggy 63

Table 4. Numbers of macrochaetae and microchaetae found in sgg clones produced in flies carrying <1, 1, 2 or 4 copies of emc* by irradiation-induced mitotic recombination at 48–72 h AEL

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Dosage emc</th>
<th>Number of bristles per clone ± S.E.</th>
<th>Number of macrochaetae per clone</th>
<th>Number of microchaetae per clone</th>
<th>Number of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>(8)</td>
<td>&lt;1</td>
<td>21.4 ± 2.7</td>
<td>1.44</td>
<td>20.0</td>
<td>27</td>
</tr>
<tr>
<td>(9)</td>
<td>1</td>
<td>13.7 ± 2.0</td>
<td>0.95</td>
<td>12.2</td>
<td>40</td>
</tr>
<tr>
<td>(10)</td>
<td>2</td>
<td>16.4 ± 2.1</td>
<td>0.5</td>
<td>14.8</td>
<td>36</td>
</tr>
<tr>
<td>(18)</td>
<td>2</td>
<td>12.1 ± 2.2</td>
<td>0.45</td>
<td>11.7</td>
<td>49</td>
</tr>
<tr>
<td>(17)</td>
<td>4</td>
<td>11.0 ± 2.3</td>
<td>0.22</td>
<td>10.8</td>
<td>46</td>
</tr>
</tbody>
</table>

* Flies of genotypes (8), (9) and (10) were siblings. Flies of genotypes (17) and (18) were grown together in the same bottles.

(8) y sgg f300/+; emc'/TM2, emc
(9) y sgg f300/+; emc'/+
(10) y sgg f300/+; d/y
(17) Dp[3; Y; 1]M2 emc* y sgg w v/++; +/++
(18) sgg f300/+; +/++

Fig. 4. Distribution of macrochaetae differentiated by sgg clones in which the cells were also carrying a variable number of copies of emc*. The flies of the genotypes used in A, B and C were siblings. All flies were irradiated between 48 and 72 h AEL. Data pertaining to numbers of clones observed and clone sizes can be obtained from Table 4. (A) Flies were of the genotype y sgg f300/+; emc'/TM2, emc and show an emc mutant phenotype; they carry less than one dose of emc*. Macrochaetae are also seen on the anterior half of the thorax in an area that only bears microchaetae in emc+ flies. (B) Flies were of the genotype y sgg f300/+; emc'/+ and therefore only carry one copy of emc+. Consistent with experiments revealing the synergism that exists between sgg and emc, in these flies, too, supernumerary macrochaetae are observed on the anterior thorax. (C) Control flies that were of the genotype y sgg f300/+; +/++; +/+ and were therefore diploid for emc*. Macrochaetae are only found clustered around the positions of the extant bristles as in Fig. 1B. (D) Flies were of the genotype Dp[3; Y; 1]M2 y emc* sgg w v/++; +/+ and therefore the y sgg clones in this experiment carry 4 copies of emc*. Fewer macrochaetae were formed by these clones, see Table 4, they are restricted to the dorsocentral and the scutellar bristle clusters. Control flies of the genotype sgg f300/+; +/+ and therefore diploid for emc* were grown in the same bottles as those carrying the duplication. The distribution of macrochaetae in these sgg clones was similar to that of Figs 1B and 4C, not shown.

except the posterior dorsocentrals where only one sgg bristle was observed. Interestingly this bristle was also intermediate in size. One control stc bristle was also found in this position from observations of 26 clones. ac1 sgg clones were also produced in flies that were otherwise ac+ (y ac1 sgg w f300 flies were irradiated). The distribution of macrochaetae from 54 sgg clones was very similar to that of the preceding experiment (not shown). Amongst these flies eleven cases of a missing posterior dorsocentral bristle were recorded, five of which were associated with either y microchaetae or with f300 control bristles. Seven cases of f300 posterior dorsocentral bristles were found. We have shown, therefore, that the cluster of macrochaetae produced by sgg cells in the region around the posterior dorsocentral bristle is removed when these cells are also mutant for
These flies, posterior dorsocentral bristles were 1111; posterior dorsocentral bristle is missing. Occasionally in small domains on the thoracic epidermis, domains that the idea that the AS-C transcripts are expressed in cross $\text{Hw}^{10}$, these flies. Flies were irradiated between 48 and 72 h AEL. (A) 28 clones from $y$ sgg $^{f_{36a}}$; $h^{+}$; $h^{+}$ flies are shown from a study totalling 68 clones. The clones were found to encompass ectopic microchaetae on the scutellum. (B) 21 clones from $y$ sgg $^{f_{36a}}$; $h^{+}$; $h^{+}$ sibling flies are represented from a total of 45 clones studied. The distribution of microchaetae resembles that seen in flies diploid for $h^{+}$ as in Fig. 1C.

Table 5. Numbers of macrochaetae found on the thorax (including the humerus) of flies heterozygous for both sgg and emc

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of macrochaetae per heminotum ± S.E.</th>
<th>Number of flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{FM6}^{+}$; +; emc$^{1}$/+</td>
<td>13-4 ± 0-16</td>
<td>18</td>
</tr>
<tr>
<td>sgg$^{D127}$/+; +; $\text{Sb}^{f}$/+</td>
<td>13-0 ± 0-2</td>
<td>25</td>
</tr>
<tr>
<td>sgg$^{D127}$/+; +; emc$^{1}$/+</td>
<td>14-8 ± 0-2*</td>
<td>32</td>
</tr>
<tr>
<td>sgg$^{D127}$/+; +; emc$^{act}$/+</td>
<td>14-0 ± 0-18*</td>
<td>25</td>
</tr>
<tr>
<td>sgg$^{D127}$/+; +; emc$^{act}$/+</td>
<td>14-6 ± 0-2*</td>
<td>25</td>
</tr>
<tr>
<td>sgg$^{D127}$/+; +; emc$^{act}$/+</td>
<td>14-0 ± 0-16*</td>
<td>25</td>
</tr>
</tbody>
</table>

Flies of the first three genotypes were siblings obtained from the cross $Q\text{FMC}^{+}$sgg$^{D127}$ × $Q$ emc$^{1}$/Sb. *P < 0.1 when compared with sgg$^{D127}$/+; $\text{Sb}^{f}$/+ flies. † $\text{FM6}$ actually carries a rearrangement causing a slight $\text{Hw}$ effect. This presumably accounts for the slight increase in bristle number in these flies.

**ac**$^{1}$ (or $ac^{1}$/ac$^{3}$) even though in ac flies only the single posterior dorsocentral bristle is missing. Occasionally in these flies, posterior dorsocentral bristles were observed at some distance from the normal site (one case of a y bristle, two of wild-type bristles and one case of a $f_{36a}$ bristle; see also Stern, 1954a, b).

These results, therefore, provide further evidence for the idea that the AS-C transcripts are expressed in small domains on the thoracic epidermis, domains that cover an area considerably greater than the single cell that will finally produce a bristle. In the sgg mutant, the process that normally leads to the singling out of only one bristle mother cell is somehow defective such that a group of bristles are formed in each domain. Therefore sgg permits us to visualize the extent of the domains, within which the AS-C transcripts are effective.

Unlike shaggy, hairy and extra macrochaetae may disrupt the spatial expression ofachaete and scute

The genes hairy ($h$) and extramacrochaetae ($emc$) are thought to act as repressors of ac and sgg on the notum (Moscoso del Prado & Garcia-Bellido, 1984). The phenotype of emc is somewhat similar to that of sgg: mutant clones of lethal alleles produce extra macro- and microchaetae (Garcia-Alonso & Garcia-Bellido, 1988). Viable alleles of emc result in the formation of extra macrochaetae (Moscoso del Prado & Garcia-Bellido, 1984). Flies mutant for $h$ carry extra microchaetae. Similarly the gain-of-function $\text{Hw}$ alleles also cause additional bristles. $\text{Hw}^{10}$, which causes an overproduction of ac and sgg, leads to the differentiation of extra

### Table 6. Numbers of macrochaetae found on the thorax of emc$^{1}$/TM2, emc flies bearing one, two or three doses of sgg

<table>
<thead>
<tr>
<th>Genotype†</th>
<th>Number of doses sgg*</th>
<th>Number of macrochaetae per heminotum ± S.E.</th>
<th>Number of flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>(19)</td>
<td>1</td>
<td>21-1 ± 0-3</td>
<td>26</td>
</tr>
<tr>
<td>(20)</td>
<td>2</td>
<td>20-0 ± 0-15*</td>
<td>40</td>
</tr>
<tr>
<td>(21)</td>
<td>3</td>
<td>18-5 ± 0-2</td>
<td>31</td>
</tr>
</tbody>
</table>

* $P < 0.01$.
† Flies of genotypes (19), (20) and (21) were siblings, see materials and methods.

<table>
<thead>
<tr>
<th>Genotype†</th>
<th>Number of doses sgg*</th>
<th>Number of macrochaetae per heminotum ± S.E.</th>
<th>Number of flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>(19)</td>
<td>1</td>
<td>13-0 ± 0-1</td>
<td>23</td>
</tr>
<tr>
<td>(20)</td>
<td>2</td>
<td>15-2 ± 0-2</td>
<td>24</td>
</tr>
<tr>
<td>(21)</td>
<td>3</td>
<td>18-2 ± 0-1</td>
<td>25</td>
</tr>
</tbody>
</table>

† Flies of genotypes (22) and (23) were siblings. Flies of genotypes (24), (25) and (26) were siblings, see materials and methods.

Table 7. The numbers of macrochaetae found on the thorax (including the humerus) of flies heterozygous for both sgg and emc and bearing one, two or three doses of AS-C

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Number of copies AS-C</th>
<th>Number of macrochaetae per heminotum ± S.E.</th>
<th>Number of flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>(22)</td>
<td>1</td>
<td>13-0 ± 0-1</td>
<td>23</td>
</tr>
<tr>
<td>(23)</td>
<td>2</td>
<td>15-2 ± 0-2</td>
<td>24</td>
</tr>
<tr>
<td>(24)</td>
<td>3</td>
<td>18-2 ± 0-1</td>
<td>25</td>
</tr>
<tr>
<td>(25)</td>
<td>3</td>
<td>14-8 ± 0-2</td>
<td>25</td>
</tr>
<tr>
<td>(26)</td>
<td>3</td>
<td>14-8 ± 0-2</td>
<td>25</td>
</tr>
</tbody>
</table>

* Flies of genotypes (22) and (23) were siblings. Flies of genotypes (24), (25) and (26) were siblings, see materials and methods.
† This genotype was included as emc interacts by itself with AS-C.

**ac**$^{1}$ (or $ac^{1}$/ac$^{3}$) even though in ac flies only the single posterior dorsocentral bristle is missing. Occasionally in these flies, posterior dorsocentral bristles were observed at some distance from the normal site (one case of a y bristle, two of wild-type bristles and one case of a $f_{36a}$ bristle; see also Stern, 1954a, b).

These results, therefore, provide further evidence for the idea that the AS-C transcripts are expressed in small domains on the thoracic epidermis, domains that cover an area considerably greater than the single cell that will finally produce a bristle. In the sgg mutant, the process that normally leads to the singling out of only one bristle mother cell is somehow defective such that a group of bristles are formed in each domain. Therefore sgg permits us to visualize the extent of the domains, within which the AS-C transcripts are effective.

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Expression of scute alleles in shaggy

micro- and macrochaetae, whereas $H_w^d$, which causes an overproduction of ac, leads to the development of extra microchaetae (Garcia-Alonso & Garcia-Bellido, 1986; Campuzano et al. 1986; Balcels et al. 1988). The phenotypes of all these mutants, however, differ fundamentally from that of sgg in that the supernumerary macrochaetae or microchaetae are found not only in the regions of notum where they would normally develop but also in ectopic positions. For example, in $H_w^d$ flies microchaetae are found on the wing blade and, in $h$ flies, microchaetae develop on the scutellum. Similarly in the case of $emc$, macrochaetae are found in more anterior positions on the thorax with a new distribution, the pattern of which is reminiscent of other dipteran in that the supernumerary phenotypes of all these mutants, however, differ fundamentally.

but also in ectopic positions. For example, in $H_w^d$ regions of notum where they would normally develop ectopic locations (Balcells et al. 1986; Ghysen & Dambly-Chaudière, 1988). A recent study reveals a normal spatial expression of ac and sc transcripts in $emc$ and $h$ wing discs so these genes may affect a very late expression of the AS–C (Romani, Campuzano, Macagno & Modolell, in preparation).

shaggy acts synergistically with extra macrochaetae

Because of the similarity of their phenotypes, we examined the relationship between sgg and $emc$ and $h$. A dosage study revealed no relationship between sgg and $h$ (results not shown). On the other hand, sgg and $emc$ were found to act synergistically: the double heterozygotes display additional macrochaetae on the thorax (Table 5). The phenotype of $emc$ was in fact found to vary with the number of doses of sgg: the number of additional macrochaetae on the thorax of $emc^+ / TM2$, $emc$ flies decreases with extra doses and increases with fewer doses of sgg (Table 6). Similarly the phenotype of clones mutant for sgg is also altered in flies carrying one, two or four doses of $emc^+$. Although the overall clone size does not vary, a greater number of macrochaetae are found in sgg clones in which the dosage of $emc^+$ is reduced, whereas fewer macrochaetae were formed by sgg clones that also carried four copies of $emc^+$. Furthermore, the spatial distribution of macrochaetae differentiated by the sgg clones is not only altered in flies that were mutant for $emc$, but also in those that were heterozygous for $emc$ (Fig. 4B). Therefore to some extent, through their respective roles on bristle differentiation, sgg and $emc$ can compensate for one another.

The synergism between sgg and $emc$ appears to be due to overexpression of the AS–C since it is suppressed by haploidy of the AS–C and enhanced by triploidy of the AS–C (Table 7).

Conclusions

We have shown that the production of supernumerary bristles caused by the sgg mutant does not appear to result from a spatial derepression of the genes of the AS–C. This is in opposition to the mutants $h$ and $emc$ for which a gene dosage study revealed regulatory roles on ac and sc (Moscoso del Prado & Garcia-Bellido, 1984). The results are consistent with the idea that, whereas the AS–C specifies the general position of each bristle through small regionally restricted zones of expression, sgg acts through an independent cellular phenomenon that is concerned with cell interactions leading to the sponsorship of only one bristle mother cell within each zone. Under this hypothesis, therefore, each cluster of macrochaetae produced by sgg mutant cells reflects the normal geographical extent of the zones of expression of the AS–C transcripts.
The following picture for the respective roles of the AS-C, emc, h and sgg is consistent with published data and with that presented here. The AS-C products are expressed in small discrete regions over the epidermis of the thorax as a result of the repressing effects of emc and h. These zones of expression lead to the formation of equivalence groups within which sgg and probably other genes such as those of the neurogenic class (Campos-Ortega, 1985; Dietrich & Campos-Ortega, 1984) play a role in the cell–cell interactions that lead to the definition of one bristle mother cell. This cell would then prevent other cells of the group from becoming bristles and the range of lateral inhibition would extend over the radius of each equivalence group. When the amount of emc is reduced, the regional control of the AS-C is no longer so effective and the sc products appear in larger areas and also in some ectopic locations. Consequently the equivalence group becomes larger than the area over which the mechanism of lateral inhibition can function and extra bristles appear. The synergism between sgg and emc can therefore be explained as follows. A single dose of emc leads to a relaxation of the regional control of the AS-C but in general the effect is insufficient to lead to an abnormal pattern and the mechanism of lateral inhibition is still effective. When, however, the amount of sgg is simultaneously reduced in these cells, the cell interactions leading to the definition of only one bristle cell are also less effective and as a result of these two effects extra bristles are formed. Lowering the number of copies of the AS-C in such flies doubly heterozygous for emc and sgg, compensates for the less effective repression by emc and restores the normal bristle pattern.

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References


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