Development 1993 Supplement, 29-39 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993



# A dual role for the protein kinase *shaggy* in the repression of *achaete-scute*

**Pat Simpson, Laurent Ruel, Pascal Heitzler and Marc Bourouis**

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 STRASBOURG Cédex, France

## SUMMARY

***achaete* and *scute* are expressed in a spatially restricted pattern and provide neural potential to cells. The domains of expression depend partly on *extramacrochaetae* whose product is itself spatially restricted and acts as a negative post-translational regulator of *achaete* and *scute*. The protein kinase shaggy also represses *achaete* and *scute* at many sites but may act via intermediate transcription factors. However *shaggy* and *extramacrochaetae* act synergistically and molecular studies suggest that they may be part of the same pathway. shaggy is functionally homologous to the mammalian glycogen synthase kinase-3 and analogy with the known physiology of this enzyme, suggests that this function of *shaggy* may result from the "constitutive" activity. At the site where a single neural precursor will develop, *achaete* and *scute* are initially expressed in a group of equivalent cells. The genes *Notch* and *Delta* are part of a lateral signal required to single out one precursor cell and to silence *achaete* and *scute* expression in the other cells. *shaggy* is required downstream of *Notch* for transduction of the inhibitory signal. This second role of *shaggy* may be due to modulation of enzymatic activity during signalling.**

Key words: *shaggy*, *extramacrochaetae*, gene regulation, bristle formation, *Drosophila*

## BRISTLE PATTERN AND THE EXPRESSION OF *achaete* AND *scute*

Sensory bristles of the peripheral nervous system arise in a precise pattern in adult *Drosophila* flies. On each hemithorax, small bristles or microchaetes are uniformly spaced over the scutum and eleven large bristles or macrochaetes occupy stereotyped positions (Fig. 1A). On the wing blade adjacent bristles are arranged at the margin in two or three rows. Bristle development is dependent on *achaete (ac)* and *scute* (*sc*), two genes of the *achaete-scute* complex (AS-C) (Garcia-Bellido, 1979; Ghysen and Dambly-Chaudière, 1988) and in their absence sensory organs are missing. Both genes encode transcriptional regulators of the neural fate and contain DNA-binding/dimerization domains of the basic helix-loop-helix type (b-HLH) (Villares and Cabrera, 1987; Murre et al., 1989a,b).

The wing and thorax arise from a single imaginal disc. In the thoracic epithelium *ac* and *sc* are expressed in a complex and dynamic pattern in "proneural clusters" of 20 to 30 cells at the sites of the future sensory organs (Romani et al., 1989; Cubas et al., 1991; Skeath and Carroll, 1991). Within each cluster a group of four, five or six cells, the "proneural field", accumulate *ac* and *sc* products to a higher level (Cubas and Modolell, 1992). All cells of the proneural field are neurally competent but a single sensory organ precursor (SOP) is selected and continues to accumulate *ac/sc* proteins to a high level, whereas in the remaining cells *ac/sc* expression ceases (Cubas and Modolell, 1992). From some proneural clusters two or three SOPs arise sequentially. The selection of a single SOP from the group of equivalent cells (Simpson and Carteret, 1990), and hence the spacing of bristles, is achieved by cell interactions during a process known as lateral inhibition (Simpson, 1990). All of the macrochaetes arise from these proneural clusters. After pupariation *ac* and *sc* are re-expressed to allow development of the microchaete precursors. In the wing epithelium *ac* and *sc* are expressed in two rows of cells along the prospective wing margin where the bristles arise and along the prospective third vein at the sites of other sensory organs (Cubas et al., 1991). Unlike those of the thorax, bristles along the wing margin are not spaced out from one another.

There are thus two separate issues relating to the development of the bristle pattern. How is the expression of *ac/sc* at specific sites controlled? What is the mechanism allowing a single cell to be chosen from a group of equivalent ones?

### The spatial regulation of *achaete* and *scute*

There is evidence for the existence of a complex array of *cis*-regulatory sequences within the AS-C that mediate expression of each gene at specific sites of the epithelium. Site-specific sequences responsible for *ac* and *sc* expression have been inferred from the study of mutants causing absence of one or a subset of sensory organs; they are mostly associated with chromosomal breakpoints in the vicinity of

the genes (Ruiz-Gomez and Modolell, 1987; Romani et al., 1989; Ruiz-Gomez and Ghysen, 1993). *achaete* and *sc* are activated independantly such that *ac* is expressed at certain sites on the notum and *sc* at others (Martinez and Modolell, 1991). Subsequently, however, they cross-activate one another, so that both are expressed at the locations of all future sensory organs (Martinez and Modolell, 1991; Skeath and Carroll, 1991). However both proteins appear to display equivalent properties.

Two genes, *extramacrochaetae (emc)* and *hairy (h)*, act as negative transregulators of *ac* and *sc*. In mutant *emc* flies supernumerary macrochaetes appear in new, ectopic, locations (Fig. 1B) whereas in flies mutant for *h*, additional microchaetes are observed in ectopic locations (Moscoso del Prado and Garcia-Bellido, 1984a,b; Garcia-Alonso and Garcia-Bellido, 1988). The ectopic bristles result from new, ectopic accumulations of *ac* and *sc* proteins, presumably caused by a lack of repression (Moscoso del Prado and Garcia-Bellido, 1984a; Cubas et al., 1991; 1992; Skeath and Caroll, 1991, 1992; Blair et al., 1992). *extramacrochaetae* and *h*, like *ac* and *sc*, encode an HLH motif (Ellis et al., 1990; Garrell and Modolell, 1990). It has been shown that *emc* competes with *daughterless/ac* and *daughterless/sc* heterodimers and thus interferes with their DNA-binding properties in a manner similar to the mammalian homologue *Id* that associates with *Myo D* (Van Doren et al.,1991; 1992; Benezra et al., 1990; Duncan et al., 1992). These observations reveal that *emc* may not repress the transcription of *ac* and *sc* directly but instead interferes with auto- and cross-regulation (Martinez and Modolell, 1991) and prevents local accumulation of *ac* and *sc* proteins (Van Doren et al. 1992). *extramacrochaetae* transcripts are heterogeneously expressed throughout the imaginal epithelium and high levels preferentially coincide with low levels of *ac* and *sc* and vice versa (Cubas and Modolell, 1992). Since the expression of *emc* is independant of *ac* and *sc*, *emc* may thus play a role in reducing *ac* and *sc* accumulation at non-proneural sites.

A uniform expression of *sc*, obtained through the use of a heat shock construct, resulted in the development of bristles at the correct sites in the absence of endogenous *ac/sc* activity (Rodrigues et al., 1990). This experiment suggests that the epithelium is differentially sensitive to the neuralising effects of these proteins. These differences could be due to differing amounts of *emc* protein and perhaps other proteins. Thus both spatial restriction of *ac/sc* expression and local differences between cells are important.

## Lateral inhibition

The selection of a single SOP from a competent group requires cell interactions (Doe and Goodman, 1985) and it is thought that the emerging SOP produces an inhibitory signal preventing the other cells of the group from realising their neural potential (Stern, 1954; Wigglesworth, 1940; Simpson, 1990). The products of the *Notch (N)* and *Delta (Dl)* genes (Lehmann et al., 1983; Campos-Ortega and Knust, 1990) may function as receptor and ligand respectively (Fehon et al., 1990; Rebay et al., 1991; Heitzler and Simpson, 1991), during transmission of the inhibitory signal.

## *shaggy* IS REQUIRED FOR THE REPRESSION OF *achaete-scute* OUTSIDE THE PRONEURAL SITES AS WELL AS FOR THE TRANSMISSION OF THE INHIBITORY SIGNAL

*shaggy (sgg)* encodes several cytosoluble protein kinases with predicted serine/threonine specificity (Bourouis et al., 1990, Ruel et al., 1993a; Siegfried et al., 1990) that are required for a number of different developmental processes (Bourouis et al., 1989; Perrimon and Smouse, 1989). Here we discuss the role of *sgg* in the regulation of *ac* and *sc*. Mutant *sgg* clones show a wild-type morphology on the wing margin and, in fact, this is the only place on the entire fly body where *sgg* is not required (unpublished observations). Elsewhere over the wing blade, mutant clones cause the appearance of adjacent ectopic bristles (Simpson et al., 1988; Fig. 1F) and it has been shown that *ac* is derepressed in these clones (Blair, 1992). On the thorax, absence of *sgg* does not lead to the development of ectopic bristles: bristles only arise at the usual wild-type positions, but there are more of them (Simpson and Carteret, 1989; Fig. 1D,E). Thus at the site of each macrochaete a group of about three macrochaetes develops, and microchaetes are more numerous and often adjacent. Hence, on the wing, *sgg* is required outside of the domains of *ac/sc* expression whereas on the thorax it is not. Furthermore, on the thorax, *sgg* is required within the domains of *ac/sc* expression whereas on the wing it is not.

In this paper we shall present arguments in favour of a dual role for *sgg* in the regulation of *ac/sc*. First, the gene may be part of a general repression mechanism preventing

**Fig. 1.** Mutant phenotypes. (A) Photograph of a wild-type thorax. Arrows point to the dorsocentral macrochaetes and arrowheads point to the scutellar macrochaetes. Note that the microchaetes are evenly spaced. (B) Photograph of a clone of cells on the thorax mutant for $emc^{1}$; the mutant bristles are marked with $forked^{36a}$ and have a curved appearance. Arrows point to two dorsocentral bristles found at wild-type positions. Additional ectopic macrochaetes have been formed. The microchaetes, as well as the ectopic macrochaetes are evenly spaced like wild-type microchaetes; they are never found to be adjacent. (C) Photograph of a clone of cells mutant for $Dl^{9P39}$ on the thorax; the bristles are not marked in this instance but mutant structures are apparent from their morphology. The microchaetes are adjacent to one another and are not separated by intervening epidermal hairs. This allele thus illustrates the result of a complete penetrance for the neurogenic phenotype: all cells within proneural areas adopt the neural fate. Bristles are, however, only formed at the correct sites, neither ectopic microchaetes nor ectopic macrochaetes are formed. (D) Photograph of a thorax of an animal homozygous for $sgg^{b12}$ that was rescued through embryogenesis by the use of a heat-shock construct expressing the SGG10 protein. Many more bristles than in the wild type are present and they are sometimes adjacent. (E) Photograph of a thoracic clone of cells mutant for $sgg^{D127}$, a protein null allele. The mutant bristles are marked with *yellow*. Note that they are densely packed and sometimes adjacent. As in *Dl* and *N* and unlike *emc*, bristles are only found at the proper sites: ectopic macrochaetes are not found. (F) Photograph of a clone of cells mutant for $sgg^{D127}$ on the wing blade. The mutant bristles are marked with *yellow*. In this case ectopic macrochaetes are formed (they can cover the entire wing blade) and like the wing marginal bristles they are always adjacent.

*ac/sc* expression. This is effective, for example, on the wing blade and explains the derepression of *ac/sc* there in the absence of *sgg* (Blair, 1992). We have been able to demonstrate that *sgg* also represses *ac/sc* in a similar fashion on the thorax, however, outside of the proneural sites this effect is masked by other, additional repression mechanisms. At the proneural sites, *sgg* represses both *ac* and *sc*. Since *ac* and *sc* are nevertheless expressed at high levels at these sites

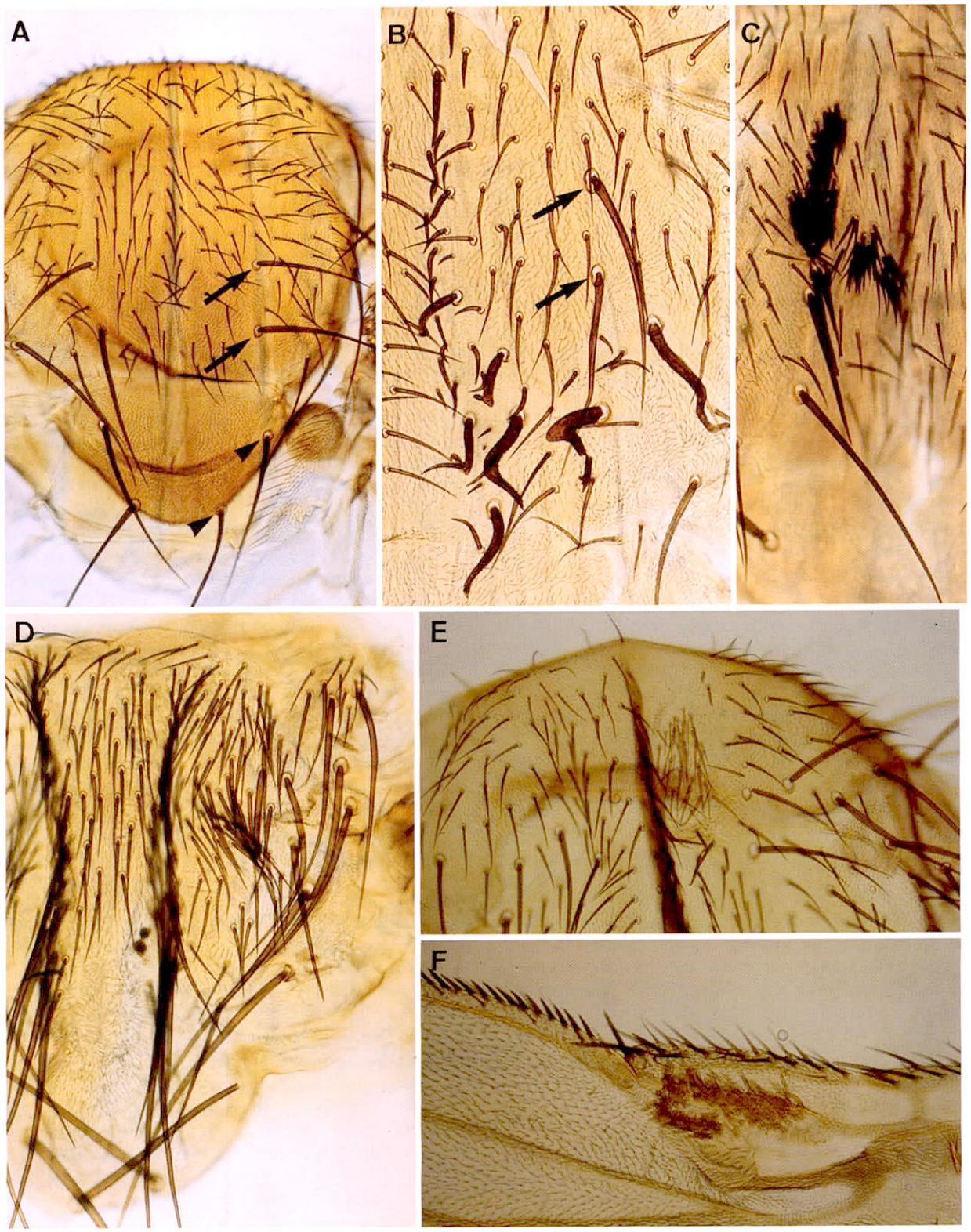


Fig. 1

a mechanism must exist to overcome or antagonize the effects of *sgg*. Second, genetic analyses reveal that *sgg* is also required downstream of *N* for lateral inhibition. In this case, when *sgg* is missing more than one cell per group adopts the neural fate, hence a cluster of macrochaetes develop at each site. This results from a failure to transduce the inhibitory signal. On the wing margin where the bristles are adjacent, lateral inhibition does not take place and hence there is no requirement there for *sgg*. Finally, it has been shown that *sgg* is a functional homologue of glycogen synthase kinase-3 (GSK-3) (Woodgett, 1991; Siegfried et al., 1992; de Groot et al., 1992; Ruel et al., 1993b). GSK-3 itself has been found to be phosphorylated and is active in resting cells, whereas a non-phosphorylated form of the protein is inactive (Hughes et al., 1993). Therefore it is possible that activity of the *sgg* protein in *Drosophila* may be regulated by phosphorylation or dephosphorylation at different times. For ease of description we shall refer to a "constitutive" role for *sgg* in the general repression of *ac* and *sc*, and to a second "induced" role after activation of *N* during signal transduction. We shall first discuss the "induced" role.

## A LATERAL SIGNAL, MEDIATED BY *Notch* AND *Delta*, ALLOWS THE SINGLING OUT OF ONE NEURAL PRECURSOR FROM A GROUP OF COMPETENT CELLS AND PREVENTS THE OTHER CELLS FROM BECOMING NEURAL

In the absence of either *N*, *Dl* or *sgg* neural hyperplasia occurs at the expense of epidermal cells (Lehmann et al., 1983; Bourouis et al., 1989). On the thorax of the adult fly this leads to the differentiation of a tuft of adjacent macrochaetes at the sites where in the wild type there is a single one, and a uniform field of microchaetes over the area where they are usually found in a spaced pattern (Simpson and Carteret, 1989; Heitzler and Simpson, 1991; see Fig. 1C). Supernumerary bristles are only found at wild-type positions, they do not arise at ectopic locations. The distribution of bristles corresponds to the areas of expression of the genes *ac* and *sc* (Simpson and Carteret, 1989, Cubas et al., 1991; Skeath and Carroll, 1991). On the notum, outside the domains of *ac/sc* expression, these mutants have no effect on the epithelium (Heitzler and Simpson, 1991).

Clones of cells triply mutant for *ac, sc* and *sgg,* or *ac, sc* and *N*, differentiate as epidermis (Simpson and Carteret, 1989; Heitzler and Simpson, 1991). This means that in the absence of *ac* and *sc*, cells do not have neural potential and the default state of the epithelium is then to develop as epidermis; this will happen whether or not *N* and *sgg* are present. Therefore *N* and *sgg* are not required for the differentiation of epidermal cells *per se*. On the contrary, cells expressing *ac* and *sc* do have neural potential and they will *all* develop as neural precursors in the absence of *N* and *Dl* and many do so in the absence of *sgg* (Cabrera, 1990; Heitzler and Simpson, 1991; Skeath and Carroll, 1992). Therefore these genes are required to single out spaced precursors and to prevent the other cells from realising their neural potential.

*Notch, Dl, sgg* and perhaps other genes of the neurogenic class may define a signalling pathway for lateral inhibition that would result in a repression of *ac* and *sc* expression. Cells mutant for *N* autonomously adopt the neural fate when adjacent to wild-type cells. They are thus insensitive to the inhibitory signal from their wild-type neighbours and this suggests that they are defective in the reception of the signal (Heitzler and Simpson, 1991). In contrast, cells mutant for *Dl* can be rescued by adjacent wild-type cells and will form epidermis; this suggests that the defect in these cells may reside rather in the signal itself. *Notch*, a phylogenetically conserved molecule, and *Dl* encode large transmembrane proteins with EGF-like motifs in the extracellular domains and *N* also carries a series of ankyrin repeats in the intracellular domain (Wharton et al., 1985; Kidd et al., 1986; Coffman et al., 1990; Ellisen et al., 1991; Weinmaster et al., 1991; Vässin et al., 1987; Kopczynski et al., 1988). A receptor-ligand relationship between these two gene products is consistent with the observation that *Dl*-expressing and *N*-expressing cells in culture bind together (Fehon et al., 1990; Rebay et al., 1991).

Mosaic analyses showed that from a group of cells expressing *ac* and *sc* any cell can become the precursor and that the cells form an equivalence group (Heitzler and Simpson, 1991; Simpson and Carteret, 1990). Therefore before a neural precursor can inhibit its neighbours, it must first be singled out. *N* and *Dl* are both expressed in all cells of the proneural clusters (Fehon et al., 1991; Kooh et al., 1993; Heitzler et al., 1993). We have shown that wild-type cells will adopt the epidermal fate if adjacent cells express a lower level of *N* activity than themselves, but produce neural precursors if adjacent cells express a higher level of *N* activity (Heitzler and Simpson, 1991). The opposite pertains for *Dl*. This shows there is competition between the cells and that the *N* and *Dl* proteins are required for the mechanism whereby cells choose between alternative fates. Cells with a reduced amount of *N* thus always inhibit their neighbours but in order to do so they require *Dl* (Heitzler and Simpson, 1993). This suggests that reception by N is negatively coupled to the signalling capacity of cells, via Dl, in a feedback loop (Fig. 2B). Thus a cell that finds itself with slightly less *N* product or slightly more *Dl* product would gain an early advantage, which would be reinforced by the feedback and then greatly amplified with time.

Within proneural clusters that give rise to only a single SOP, the SOP generally arises from a cell positioned near the centre where cells tend to have a higher level of *ac/sc* (Cubas et al., 1992). Mosaic analyses showed that the quantity of *ac* and *sc* products themselves can influence the choice of fate: cells with more *ac/sc* product are more likely to become neural (Cubas et al., 1991). This effect is mediated by the neurogenic genes: we have found that the expression of *Dl* and the signalling capacity of the cells is dependent on the genes of the AS-C (Heitzler et al., unpublished observations; Fig. 2B). Therefore the amount of *ac/sc* product may serve as an initial trigger and the role of *N* and *Dl* would be to ensure that only a single cell will ever become a precursor.

A group of dominant alleles of *N*, called *Abruptex (Ax)* (Welshons, 1971; Foster, 1975; Portin, 1975) cause the opposite phenotype to that of loss of function alleles and

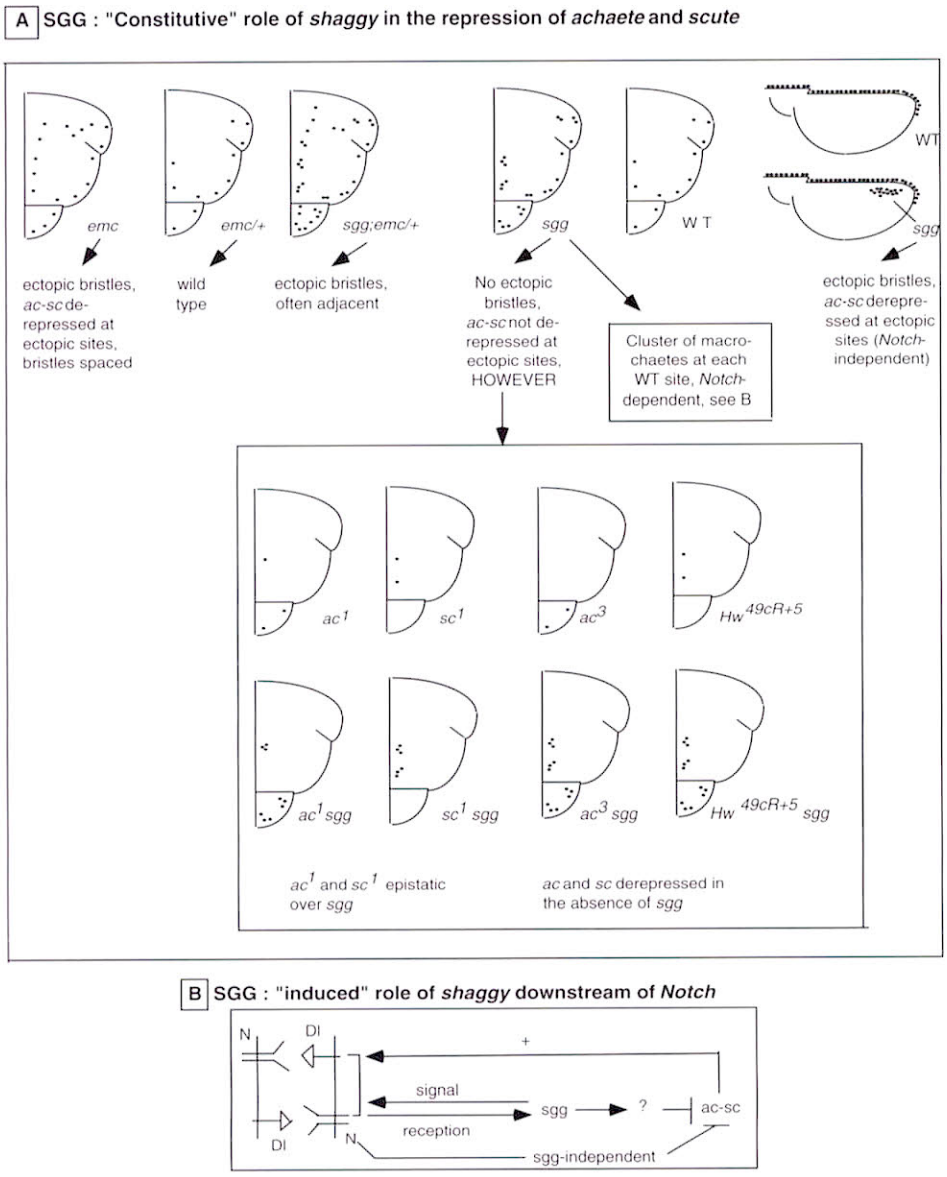


**Fig. 2.** Schematic view of the dual role played by sgg in the repression of *ac/sc*. (A) "Constitutive" and B, "induced" role of *sgg* in the repression of *ac/sc*. On the wing, absence of *sgg* causes the appearance of ectopic bristles due to a derepression of *ac* and *sc*. On the thorax, however, a loss of *sgg* does not lead to ectopic bristles. Nevertheless *sgg* does repress *ac* and *sc* in a similar fashion on the thorax as shown in the inset: $ac^1$ removes the posterior dorsocentral bristle and $sc^1$ the scutellar bristles; both mutants are associated with lesions in cis-regulatory regions responsible for *ac* expression at the dorsocentral site and *sc* expression at the scutellar site, respectively. Both mutants are epistatic over *sgg* and so no bristles form at these sites in $ac^1$ *sgg* and $sc^1$ *sgg* double mutant clones. $ac^3$ $(ac^-)$ and $Hw^{49cR+5}$ $(sc^-)$ have similar phenotypes at these sites (only the dorsocentral and scutellar bristles are represented in these drawings). *shaggy*, however, is epistatic over these two mutants and bristles form in the respective double mutants. This means that *ac* has been switched on at a *sc* site and *sc* has been switched on at an *ac* site in the absence of *sgg*. Therefore *sgg* must normally repress *ac* and *sc* on the notum as well as on the wing. The absence of ectopic bristles in *sgg* mutant thoraces is probably due to the effects of other repression mechanisms, such as that mediated by *emc*. When the amount of *emc* is reduced then absence of *sgg* does result in the appearance of ectopic macrochaetes on the thorax. *shaggy* and *emc* thus act synergistically. The cluster of macrochaetes found at each site (and the higher density of microchaetes) in *sgg* mutants, is, unlike the ectopic bristles of the wing, a *N*-dependent phenomenon. B, Cells in the proneural field have neural potential through the expression of *ac* and *sc*. They also each express both *N*, a putative cellular receptor, and *Dl*, thought to be a ligand for *N*. The activities of *N* and *Dl* within a cell are postulated to be linked via a feedback loop. Binding of the *N* and *Dl* molecules within the same cell membrane is one possible molecular mechanism for this. Quantitative differences between the activities of *N* and *Dl* between cells, amplified by the feedback, result in one cell with a greater amount of the signal protein Dl. Binding of *Dl* to the *N* molecules of neighbouring, inhibited, cells is thought to initiate intracellular signalling events that ultimately results in a cessation of *ac/sc* expression. *shaggy* is required after *N* during signal transduction within the cells. This effect may correspond to an "induced" function of *sgg*. *shaggy* can, however, also modulate the capacity of the cell itself to signal via *Dl*, but only in the presence of *N*. Thus *sgg* is downstream of *N* for transduction of the inhibitory signal, but upstream of *N* for the cells own ability to signal to its neighbours. Other elements in the signalling cascade are as yet unknown. The expression of *Dl* is dependent on the genes of the AS-C. Cell with a higher level of *ac/sc* will generate a greater signal and thus gain an early advantage in the competition for the neural fate. Thus greater accumulation of *ac/sc* by one cell could provide the initial bias for selecting one cell from the group of competent cells.

flies make fewer bristles, the cells instead adopt the epidermal fate (Heitzler and Simpson, 1993). These alleles are associated with single amino acid changes in a cluster of EGF-like repeats in the extracellular domain of the protein (Kelley et al., 1987; Hartley et al., 1987). These altered proteins appear to have an enhanced affinity for the ligand, they are suppressed when the amount of *Dl* is reduced and double mutant *Ax Dl* cells differentiate as neural cells showing that, in order to take up the epidermal fate, *Ax* cells require the ligand (Heitzler and Simpson,

1993). In culture, however, *Ax*-expressing cells continue to adhere to *Dl*-expressing cells but with reduced efficiency (Lieber et al., 1992).

As well as taking up the epidermal fate, and unlike $N^-$ cells, *Ax* cells fail to inhibit their neighbours, so, in these cells with hyperactive *N* molecules the *Dl* molecules are not available to inhibit adjacent cells. This has led to the suggestion that the *N* and *Dl* proteins of the same cell may bind together and that this could be the molecular basis of the feedback between the two (Fig. 2B, Heitzler and Simpson, 1993). When expressed in the same cell after transfection, *N* and *Dl* proteins co-localise suggesting that they can interact within the cell membrane (Fehon et al., 1990). Binding of a cell's *Dl* molecules to its own *N* molecules would reduce the availability of the *Dl* protein to interact with the *N* molecules of neighbouring cells. The Ax molecules could be altered in such a way as to only poorly bind the *Dl* protein of neighbouring cells (Lieber et al., 1992), but strongly bind to that of the same cell. The possibility of autocrine signalling in this manner remains to be tested. For a more complete review see Simpson et al. (1992).

## *shaggy* ACTS DOWNSTREAM OF *Notch* DURING TRANSDUCTION OF THE SIGNAL

The protein kinase nature of sgg is consistent with a role in the intracellular transduction of the inhibitory signal through a signalling cascade. Cells mutant for *sgg*, like *N*, autonomously adopt the neural fate (Heitzler and Simpson, 1991). This suggests that *sgg* is required for reception of the inhibitory signal and that perhaps it is part of a signalling cascade downstream of *N*. The situation is complicated, however, by the fact that cells mutant for *sgg* are also impaired in their ability to inhibit their neighbours, that is to send the inhibitory signal via *Dl*. Thus, in mosaics, mutant bristles can be found adjacent to wild-type ones showing that the mutant cell can neither recieve nor send the signal. Indeed this phenotype is similar to that of clones doubly mutant for *N* and *Dl* (Heitzler and Simpson, 1993). However, *sgg* is not required upstream of *Dl* since double mutant *N sgg* cells continue to behave like *N* $sgg^+$ cells and always signal inhibition to their neighbours. On the other hand, double mutant *sgg Ax* cells take up the neural fate, like *sgg*, and so these hyperactive *N* molecules are unable to transmit the inhibitory signal in the absence of *sgg*. Taken together, these results strongly suggest that *sgg* acts after *N* in the inhibitory pathway (Ruel et al., 1993b; Fig. 2B).

Since $N^+$ $sgg^-$ cells display impaired signalling, but $N^-$ $sgg^-$ cells signal constitutively, it follows that *sgg* modulates the signal but only in the presence of *N* molecules. Therefore, *sgg* is downstream of *N* for transduction of the received signal, but upstream of *N* for the capacity of the cell itself to send the signal (Fig. 2B). Hence it is possible that sgg modifies N which in turn will affect the ability of the cell to signal via *Dl*. Again, such an effect could result from a coupling of the *N* and *Dl* proteins such that inhibited cells become locked into an inhibited state, mediated by an autocrine signal.

## *shaggy* IS REQUIRED FOR A GENERAL REPRESSION OF *achaete* AND *scute* THAT IS EFFECTIVE ON BOTH THE WING AND THE THORAX: REPRESSION MAY BE MEDIATED BY A TRANSCRIPTION FACTOR(S)

Absence of *sgg* leads to a derepression of *ac/sc* and the development of macrochaetes outside of the proneural areas in the wing (Fig. 1F). Like the wild-type bristles found on the margin, these ectopic bristles are adjacent to one another. *Notch* is not required for the epidermal versus neural decision of cells on the wing: clones of *N* null mutants make epidermis over the entire wing blade (with the exception of course of the wing margin) and the study of double mutant *sgg* $N^{ts1}$ clones show that *sgg* is epistatic over *N* in this area (unpublished observations). (Note that *N* is required in the precursors of all bristles at a later step for the differentiation of the four cells of the bristle organ, Hartenstein and Posakony, 1990). This suggests that on the wing (excluding the margin) *sgg* represses *ac/sc* via a mechanism that is *N*-independent and thus different from the mechanism of lateral inhibition described above.

On the thorax, clones mutant for *sgg* do not display ectopic macrochaetes. Nevertheless *sgg* also represses *ac* and *sc* on the notum as shown by the following series of experiments. In *sgg* mutant clones a group of macrochaetes develop at each wild-type site, whereas in *ac/sc* mutants no bristles develop (Santamaria and Garcia-Bellido, 1978). Triply mutant *ac sc sgg* clones are devoid of bristles showing that the *sgg* mutant phenotype requires the activity of *ac* and *sc* (Simpson and Carteret, 1989).

The two mutants $ac^1$ and $sc^1$ are caused by lesions in regulatory sequences that result in a loss of expression of *ac* or *sc*, respectively, at specific sites in the epithelium resulting in the absence of a small subset of bristles on the notum (Modolell et al., 1983; Campuzano et al., 1985; Ruiz-Gomez and Modolell, 1987; Romani et al., 1989). These two mutants are epistatic over *sgg* and, in each case the double mutant clones are devoid of bristles at the $ac^1$ and $sc^1$ mutant sites (Simpson and Carteret, 1989; Fig. 2A). In the wild type, *ac* comes on at the *ac*-dependent sites and it then activates *sc*, and vice versa. Therefore, in this experiment, if *ac* is not expressed at the appropriate site then *sc* will not be activated regardless of the presence or absence of *sgg*. Similarly *sc* will be unable to activate *ac*.

The mutant $Hw^{49cR+5}$ produces a truncated non-functional *sc* protein due to a deletion in the coding sequence (Balcells et al., 1988). The $ac^3$ mutation is associated with a chromosomal inversion that results in a complete loss of all *ac* expression rather than a site-specific loss as in $ac^1$ (Campuzano et al., 1985; Skeath and Carroll, 1991). In contrast to the previous results, *sgg* is epistatic over these two mutants and in $ac^3$ *sgg* and $Hw^{49cR+5}$ *sgg* clones a cluster of bristles arises at each site (unpublished observations; Fig. 2A). In this case then, *sc* has been switched on at *ac* sites in the absence of *ac* itself and *ac* has been switched on at *sc* sites in the absence of *sc* itself. Thus, here, a loss of *sgg* has apparently resulted in a *de novo* expression of *ac* and *sc*. Therefore *sgg* must normally repress their expression.

As the regulatory mutants $ac^1$ and $sc^1$ are epistatic over

*sgg* this suggests that the repression of *ac* and *sc* by *sgg* requires intact enhancer sequences and that *sgg* may act via an intermediate transcription factor(s).

These results suggest that the mechanism by which *sgg* represses *ac* and *sc* in the wing, functions also on the thorax, at least at the proneural sites. Therefore *sgg* represses *ac* and *sc* at many, if not all, sites in the epithelium, but at certain special proneural sites, *ac* and *sc* are expressed in an as yet unknown manner that overcomes this repression.

Mutant *sgg* embryos derived from mutant germ lines are composed exclusively of abnormal cells with neural characteristics (Bourouis et al., 1989). It is possible that this too reflects a requirement for *sgg* in the repression of the genes of the AS-C.

## *extramacrochaetae* PLAYS A ROLE IN THE SPATIAL EXPRESSION OF *achaete* AND *scute* AND ACTS SYNERGISTICALLY WITH *shaggy*

Clones mutant for *sgg* on the notum cause additional bristles to form but only at proneural sites, a consequence of the fact that several cells of the proneural field, instead of one, adopt the neural fate. In contrast clones mutant for *emc* cause ectopic macrochaetes to form at new sites (Moscoso del Prado, 1984a,b; Garcia-Alonso and Garcia-Bellido, 1988; Fig. 1B; 2A). The role of *emc* outside the proneural sites to limit *ac/sc* expression is well documented (Cubas et al., 1991; Skeath and Carroll, 1991; Van Doren et al. 1992). High levels of emc in these regions prevent accumulation of ac and sc (Cubas and Modolell, 1992). We have found that many of the ectopic macrochaetes in *emc* mutants arise later than those at wild-type positions (Heitzler et al., unpublished data). The following observations show that the ectopic macrochaetes do not arise from cells within the proneural fields. In clones mutant for $Dl^{9P39}$, a tuft of about six macrochaetes develops at each site usually occupied by a single one (Heitzler and Simpson, 1991). This is the result of all cells of the proneural field becoming bristle precursors; consequently they stop dividing. Therefore if the later arising ectopic bristles in the case of *emc* were to derive from these same cells after division, they should be absent in double mutant *emc* $Dl^{9P39}$ clones. In fact, in such clones, bristles are still found at ectopic sites in addition to the wild-type ones (unpublished observations).

Hence the additional bristles in *sgg* mutant clones are derived from cells of the proneural field, while those of *emc* are derived from cells outside the proneural fields. Also, we have found that in *emc* mutants, bristles are normally spaced, suggesting that *emc* is not required for lateral inhibition (unpublished observations). Nevertheless a synergism is found between *emc* and *sgg*: *sgg/+; emc/+* double heterozygotes display one additional macrochaete on the thorax (Simpson and Carteret, 1989). Furthermore, whereas *sgg* clones in wild-type flies do not cause ectopic macrochaetes on the thorax, if the amount of emc is reduced (for example in $emc^{1}$/+ flies that by themselves do not have extra macrochaetes) then ectopic macrochaetes do appear in *sgg* mutant clones (Simpson and Carteret, 1989). *shaggy* and *emc* thus act synergistically in spite of the fact that the two genes appear to affect different cell populations. However, it seems likely that the synergism reflects the other, "constitutive", function of *sgg* in the general repression of *ac* and *sc* on the thorax. Both genes therefore act to repress *ac* and *sc* and the question arises as to whether they act together or within independent pathways (see below).

## SIMILARITY, REDUNDANCY AND FUNCTIONAL HOMOLOGY OF sgg TO MAMMALIAN PROTEIN KINASES

Study of the mutant phenotypes has thus led us to propose two roles for *sgg*: a "constitutive" role concerning the repression of *ac/sc* and an "induced" role during lateral inhibition (Fig. 2). Here we discuss molecular and biochemical studies whose ultimate aim is an understanding of the molecular basis of these observations.

A family of protein kinases are encoded at the *shaggy* locus. Two transcription units give rise to ten transcripts and five different proteins (called SGG10, SGG39, SGGY, SGG46, and SGGX) with a common kinase catalytic domain that predicts serine/threonine specificity, and overlapping patterns of expression during development (Ruel et al., 1993a). Mutational analysis of *sgg* defines a single complementation group, lethality of which is associated with the loss of two of the major proteins (SGG10 and SGG39). Phenotypes of flies expressing individual *sgg* proteins revealed that although there is some redundancy between the different forms they do not all carry out identical functions in vivo (Ruel et al., 1993a). Of the three proteins expressed in the epithelium of the wing and thorax, one, SGG10, carries out functions required for the normal segregation of neural precursors.

The *sgg* protein kinases show extended similarities to the rat GSK-3 enzymes (Woodgett, 1991; Fig. 3). The highest level of amino acid conservation is found in a region encompassing the kinase catalytic domain, but also extends to either side (Fig. 3). Homology is lower in a region immediately adjacent to the catalytic domain and is insignificant over the remaining 130 C-terminal amino acids. The rat has two forms of GSK-3, GSK-3α and GSK-3β (Woodgett, 1990), more similar to each other than to the *sgg* kinases. GSK-3β appears slightly more similar to sgg than GSK-3α. Of the *sgg* proteins, SGG10 is the closest to GSK-3β. The *sgg* proteins differ between themselves at the C and N termini which have no counterpart in the rat homologues. There seem to be subtle differences between the different *sgg* enzymes and the two GSK-3 enzymes. The study of transgenic flies showed that GSK-3β, but not GSK-3α can substitute to some extent for *sgg* (Ruel et al., 1993b). Regions outside the kinase catalytic domain may therefore confer differences between the proteins. Although they can phosphorylate the same substrates in vitro, kinetic parameters of phosphorylation by GSK-3α, GSK-3β and SGG10 differ (Plyte et al., 1992). Additionally subcellular localisation or tissue distribution may distinguish them. Cellular compartmentalisation of SGG/GSK-3 is still under investigation.

SGG10 and SGG39 display redundant activities, they only differ by a C-terminal extension present in SGG39. SGG46 is not redundant with other *sgg* proteins. In a

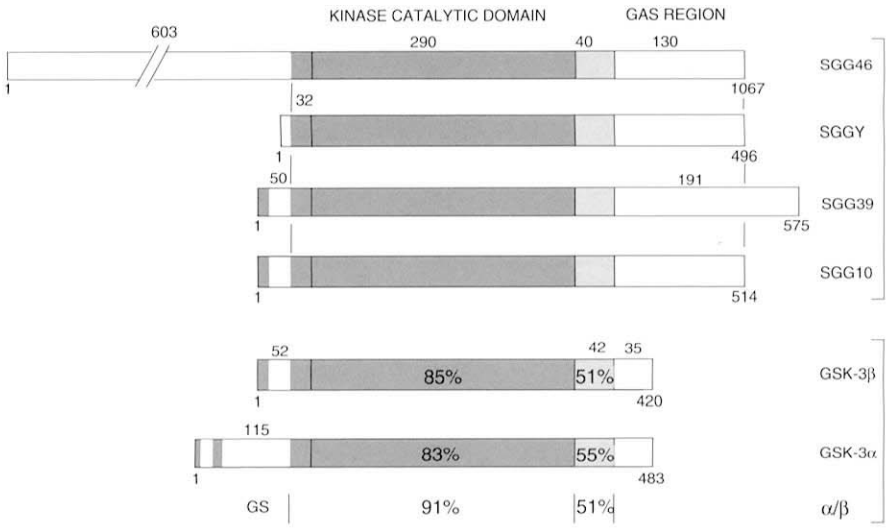


**Fig. 3.** The similarities displayed between the *sgg* proteins and the rat GSK-3 enzymes are drawn. The known sequences of four *sgg* proteins (SGG46, SGGY, SGG39, SGG10) are aligned with respect to an identical central core sequence (delimited by the vertical bars) that includes the kinase catalytic domain. The *sgg* polypeptides differ, however, at the N-terminal or C-terminal regions, which are encoded by alternative exons of the gene. The sequences of the rat GSK-3β and GSK-3α protein kinases are similarly aligned; in this case they are derived from two separate genes. Numbers above the sequences refer to the size, in amino acids, of the domains (depicted as boxes) whereas numbers below indicate the sizes of the polypeptides. The *sgg* proteins have a Gly, Ala, Ser rich C-terminal domain not present in the GSK-3 enzymes, although GSK-3α has a Gly, Ser rich region at its C terminus. Intense and lightly shaded grays areas indicate the extent of sequence similarities detected by computer assisted alignments. The percent of identical residues between the *Drosophila* and the two rat sequences (scored over the respective gray regions), are indicated and compared to the score of identities found between the two rat sequences (indicated below). Note that the region of similarity between *sgg* and the GSK-3 starts at the region common to all of the *sgg* proteins, and that homologies between the two rat sequences does not extend beyond this region. Sequences were taken from Ruel et al, 1993b; Woodgett, 1990; Seigfreid et al, 1992.

mammalian cell transfection assay, GSK-3α, GSK-3β, SGG10 and SGG39, but not SGG46, can modulate the activity of the proto-oncogene c-JUN presumably as a result of direct phosphorylation (deGroot et al., 1992; Nikolakaki et al., 1993), indicating that sgg and GSK-3 can act on the same substrates. The in vivo targets of *sgg* will not, however, necessarily be the same. The phosphorylation sites of c-JUN, conserved in all members of the jun family in mammals, are not conserved in *Drosophila* JUN. GSK-3 is implicated in the insulin response pathway where glycogen synthase is targeted by at least five protein kinases including GSK-3 which has inhibitory effects on the enzyme. Other substrates of GSK-3 are involved in the regulation of cell metabolism or growth control and include transcription factors and components of signal transduction pathways (Woodgett, 1991; Plyte et al., 1992).

In many cases GSK-3 appears to have an inhibitory effect maintaining its targets in a phosphorylated but inactive state in resting cells (Woodgett, 1991). Hormonal stimulation leads to dephosphorylation of the inhibitory GSK-3 sites either by induction of a phosphatase or through inhibition of GSK-3 activity (Plyte et al., 1992). Indeed GSK-3 activity was found contingent to phosphorylation of a tyrosine residue in resting cells (Hughes et al., 1993). These studies suggest that function of these enzymes is itself subject to modulation.

## CELL TRANSFECTION STUDIES FURTHER DEFINE THE MOLECULAR BASIS OF *shaggy* FUNCTION

We have used a cell transfection assay (the transactivation properties of *ac* and *sc* together with *daughterless (da)* on a reporter construct in *Drosophila* S2 cells; Van Doren et al., 1992) in an attempt to identify possible downstream targets of the kinase. The effect of transfection of *sgg* protein kinases was measured in the presence or absence of factors thought to be regulators of *ac/sc*, such as *emc, h*, the *E(spl)* m-proteins and other new putative transcriptional regulators isolated in our laboratory (unpublished observations). Amongst others, *shaggy* was found to modulate the effects of *emc* and *E(spl)* (unpublished observations). Our results provide evidence that *sgg* and *emc* probably do act together in the same pathway and that this is reflected in the synergism observed in the genetic studies.

Constructs expressing one of the basic-HLH proteins of the *E(spl)* complex have also been shown to have negative effects in the same cell transfection assay (unpublished results in collaboration with E. Knust). This is specifically enhanced by co-transfection with *sgg*. The *E(spl)* genes are thought to be negative transcriptional regulators of the genes of the AS-C (Klämbt et al., 1989; Schrons et al., 1992; Knust et al., 1992; Campos-Ortega, 1993), and clones simultaneously mutant for m3, m5, m7 and m8 on the thorax display a *N*-like phenotype (unpublished observations). *shaggy* and *E(spl)* may perhaps function together during lateral inhibition.

Experiments are now in progress to test whether these results could be due to phosphorylation of emc and E(spl) by sgg. Clearly other explanations are possible and furthermore any demonstration of phosphorylation in vitro will have to be followed by more stringent molecular and genetics analysis of target phophorylation sites in vivo.

## CONCLUSIONS

We have presented arguments for a dual role of *sgg* in the regulation of *ac/sc*. *shaggy* is required to repress *ac/sc*

expression on the wing blade, it functions in a similar fashion on the thorax and perhaps even in the embryo. This repression mechanism may be mediated by a transcription factor(s) and is *N*-independent. By analogy to the biological effects of GSK-3, it seems likely that this "constitutive" function is the result of the activity of a phosphorylated form of the *sgg* protein kinase. It is known that some of the targets of GSK-3 are transcription factors, so *sgg* could maintain potential transcriptional activators of *ac/sc* in an inactive state or potential repressors in an active one. At special proneural sites *ac* and *sc* are expressed in spite of the presence of *sgg* by an unknown mechanism that overcomes or antagonizes *sgg* activity. *shaggy* is subsequently required downstream of *N* during lateral inhibition and we postulate that this may be an "induced" function following binding of N to Dl. Molecular studies of GSK-3, as well as sgg, provide evidence for modulation of activity of this enzyme by tyrosine phosphorylation (Hughes et al., 1993). Therefore, shaggy could be regulated by this means at different steps. It is possible that some of the same target proteins may be involved in both aspects of *sgg* function: if, for example, its activity were to be antagonized at the proneural sites, but then "re-activated" after *N* signalling.

Both *emc* and *sgg* repress *ac/sc* on the thorax. High levels of emc, usually occurring at non-proneural sites, prevent accumulation of ac and sc by binding to these proteins and preventing auto and cross-regulation. This phenomenon does not absolutely require *sgg*: derepression of *ac/sc* is not observed in *sgg* mutant clones, repression by *emc* suffices. *shaggy*, on the other hand, may repress *ac/sc* through a mechanism that requires a transcription factor(s) since intact *cis*-regulatory sequences of *ac* and *sc* are necessary for repression. The observed synergism between *emc* and *sgg* could simply reflect the fact that, in the double heterozygotes, both repression mechanisms are less effective and this can cause sufficient derepression of *ac/sc* for an extra bristle to appear. Our cell transfection assay has revealed, however, that the activity of *emc* is modulated by *sgg* and so it is possible that the two genes act together in the same pathway.

It is not known, of course, how a signal is generated after binding of N to Dl, nor how sgg acts in the signalling cascade. Our results are in favour of a role of both *sgg* and *E(spl)* in the same pathway in this process. The *E(spl)* proteins may be transcriptional repressors of *ac* and *sc*. It is also probable that there is more than one pathway leading to the downregulation of *ac/sc* during lateral inhibition since clones mutant for *sgg* display partial penetrance (Fig. 2B).

The physiological significance of the signalling pathway implicating the protein kinases of the SGG/GSK-3 family will undoubtedly be the subject of further investigation. Of major importance will be the demonstration of in vivo modulation of kinase activity following extracellular signalling. shaggy and GSK-3 contain a conserved site for potential phospho-tyrosine regulation and phosphorylation of this site correlates with catalytic activity. Tyrosine (and also threonine) phosphorylation at the equivalent position of the MAP protein kinase family is responsible for their induction during stimulation by various mitogens (Pelech and Sanghera, 1992). This results from activity of an upstream kinase (the MAP kinase kinase) that exhibits a dual specificity for serine/threonine and tyrosine residues and which is itself regulated by serine/threonine phosphorylation. Both the MAP kinase family and the MAP kinase kinase are highly conserved in several organisms including *Drosophila* (Biggs and Zipursky, 1992; Tsuda et al., 1993). Unravelling the entire regulatory cascade could be difficult since it has been documented in at least one case that activation of a GSK-3 target was obtained by the stimulated dephosphorylation of the GSK-3 phospho-sites by a phosphatase (Dent et al., 1990) and furthermore that GSK-3 can itself be inactivated by phosphorylation by PKC and thus is potentially antagonized by the phosphatidyl inositol stimulated pathways (Goode et al., 1992). Genetic dissection together with in vitro studies are important tools that will help to further unravel this complexity.

The technical help of Cathie Carteret, Claudine Ackerman and Véronique Pantesco has been greatly appreciated throughout the years. We thank our colleagues at the LGME for fruitful discussions. Our work is supported by the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Centre Hospitalier Universitaire Régional, the Association pour la Recherche sur le Cancer and the Fondation pour la Recherche Médicale. L. R. is supported by a grant from the Ministère de la Recherche et de la Technologie and P. H. by a grant from the Association pour la Recherche sur le Cancer.

## REFERENCES

**Balcells, L., Modolell, J. and Ruiz-Gomez, M.** (1988). A unitary basis for different *Hairy-wing* mutations of *Drosophila melanogaster*. *EMBO J.* **7**, 3899-3906.

**Benezra, R., Davis, R. Lockshon, D., Turner, D. L. and Weintraub, H.** (1990). The protein Id: A negative regulator of helix-loop-helix DNA binding proteins. *Cell* **61**, 49-59.

**Biggs, W. H. and Zipursky, S. L.** (1992). Primary structure, expression, and signal-dependent tyrosine phosphorylation of a *Drosophila* homolog of extracellular signal-related kinase. *Proc. Nat. Acad. Sci. USA* **89**, 6295-6299.

**Blair, S.** (1992). *shaggy (zeste-white 3)* and the formation of supernumerary bristle precursors in the developing wing blade of *Drosophila*. *Dev. Biol.* **152**, 263-278.

**Blair, S., Giangrande, A., Skeath, J. and Palka, J.** (1992). The development of normal and ectopic sensilla in the wings of *hairy* and *Hairy wing* mutants of *Drosophila*. *Mech. Dev.* **38**, 3-16.

**Bourouis, M., Heitzler, P., El Messal, M. and Simpson, P.** (1989). Mutant *Drosophila* embryos in which all cells adopt a neural fate. *Nature* **341**, 442-444.

**Bourouis, M., Moore, P., Ruel, L., Grau, Y. Heitzler, P. and Simpson, P.** (1990). An early embryonic product of the gene *shaggy* encodes a serine/threonine protein kinase related to the CDC28/cdc2$^+$ subfamily. *EMBO J.* **9**, 2877-2884.

**Cabrera, C.** (1990). Lateral inhibition and cell fate during neurogenesis in Drosophila: the interactions between *scute, Notch* and *Delta*. *Development* **109**, 733-742.

**Campos-Ortega, J.** (1993). In 'Development of Drosophila melanogaster (eds. Bate, M. and Martinez-Arias, A.). Cold Spring Harbor Laboratory Press, New York.

**Campos-Ortega, J. A. and Knust, E.** (1990). Genetics of early neurogenesis in *Drosophila melanogaster*. *Ann. Rev. Genet.* **24**, 387-407.

**Campuzano, S., Carramolino, L., Cabrera, C., Ruiz-Gomez, M., Villares, R., Boronat, A. and Modolell, J.** (1985). Molecular genetics of the *achaete-scute* gene complex of *D. melanogaster*. *Cell* **44**, 327-338.

**Coffman, C., Harris, W. and Kintner, C.** (1990). *Xotch*, the Xenopus homolog of Drosophila *Notch*. *Science* **249**, 1438-1441.

**Cubas, P., de Celis, J.-F., Campuzano, S. and Modolell, J.** (1991). Proneural clusters of *achaete/scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. *Genes Dev.* **5**, 996-1008.

**Cubas, P. and Modolell, J.** (1992). The *extramacrochaetae* gene provides information for sensory organ patterning. *EMBO J.* **11**, 3385-3393.

**de Groot, R., Auwerx, J., Bourouis, M. and Sassone-Corsi, P.** (1992). Negative regulation of Jun/AP-1: conserved function of glycogen synthase kinase 3 and the *Drosophila* kinase *shaggy*. *Oncogene*, **7**, 841-847.

**Dent, P., Lavoinne, A., Nakielny, S., Caudwell, F. B., Watt, P. and Cohen, P.** (1990). The molecular mechanism by which insulin stimulates glycogen synthesis in mammalian skeletal muscle. *Nature* **348**, 302-308.

**Doe, C. Q. and Goodman, C. S.** (1985). Early events in insect neurogenesis. II. Role of cell interactions and cell lineage in the determination of neuronal precursor cells. *Dev. Biol.* **111**, 206-219.

**Duncan, M., Dilicco-Bloom, E. M., Tian, X., Benezra, R. and Chada, K.** (1992). The gene for the helix-loop-helix protein, Id, is specifically expressed in neural precursors. *Dev. Biol.* **154**, 1-10.

**Ellis, H. M., Spann, D. R. and Posakony, J. W.** (1990). *extramacrochaetae*, a negative regulator of sensory organ development in *Drosophila*, defines a new class of helix-loop-helix proteins. *Cell* **61**, 27-38.

**Ellisen, L. W., Bird, J., West, D. C., Lee Soreng, A., Reynolds, T. C., Smith, S. D. and Sklar, J.** (1991). TAN-1, the human homolog of the Drosophila *Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649-661.

**Fehon, R. G., Johansen, K., Rebay, I. and Artavanis-Tsakonas, S.** (1991) Complex cellular and subcellular regulation of *Notch* expression during embryonic and imaginal development of Drosophila: implications for *Notch* function. *J. Cell Biol.* **113**, 657-669.

**Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A. T. and Artavanis-Tsakonas, S.** (1990). Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in *Drosophila*. *Cell* **61**, 523-534.

**Foster, G. G.** (1975). Negative complementation at the Notch locus of Drosophila melanogaster. *Genetics* **81**, 99-120.

**Garcia-Alonso, L. A. and Garcia-Bellido, A.** (1988). *Extramacrochaetae*, a trans-acting gene of the *achaete-scute* complex of *Drosophila* involved in cell communication. *Roux's Arch. Dev. Biol.* **197**, 328-338.

**Garcia-Bellido, A.** (1979). Genetic analysis of the *achaete-scute* system of *Drosophila melanogaster*. *Genetics* **91**, 491-520.

**Garcia-Bellido, A. and Santamaria, P.** (1978). Developmental analysis of the *achaete-scute* system of *Drosophila melanogaster*. *Genetics* **88**, 469-486.

**Garrell, J. and Modolell, J.** (1990). The *Drosophila extramacrochaetae* locus, an antagonist of proneural genes that, like these genes, encodes a helix-loop-helix protein. *Cell* **61**, 39-48.

**Ghysen, A. and Dambly-Chaudiere, C.** (1988). From DNA to form: the *achaete-scute* complex. *Genes Dev.* **2**, 495-501.

**Goode, N., Hughes, K., Woodgett, J. R. and Parker, P. J.** (1992). Differential regulation of glycogen synthase kinase-3β by protein kinase C isotypes. *J. Biol. Chem.* **267**, 16878-16882.

**Hartenstein, V. and Posakony, J. W.** (1990). A dual function of the *Notch* gene in *Drosophila* sensillum development. *Dev. Biol.* **142**, 13-30.

**Hartley, D., Xu, T. and Artavanis-Tsakonas, S.** (1987). The embryonic expression of the *Notch* locus of Drosophila melanogaster and the implications of point mutations in the extracellular EGF-like domain of the predicted protein. *EMBO J.* **6**, 3407-3417.

**Heitzler, P. and Simpson, P.** (1991). The choice of cell fate in the epidermis of *Drosophila*. *Cell* **64**, 1083-1092.

**Heitzler, P. and Simpson, P.** (1993). Altered epidermal growth factor-like sequences provide further evidence for a role of *Notch* as a receptor in cell fate decisions. *Development* **117**, 1113–1122.

**Hughes, K., Nikolakaki, E., Plyte, S. E., Totty, N. F. and Woodgett, J. R.** (1993). Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *EMBO J.* **12**, 803-808.

**Hughes, K., Pulverer, B. J., Theocharous, P. and Woodgett, J. R.** (1991). Baculovirus-mediated expression and characterisation of rat glycogen synthase kinase-3β, the mammalian homologue of the *Drosophila melanogaster zeste-white 3-sgg* homoeotic gene product. *Eur. J. Biochem.* **203**, 305-311.

**Kelley, M. R., Kidd, S., Deutsch, W. A. and Young, M. W.** (1987). Mutations altering the structure of epidermal growth factor-like coding sequences at the Drosophila *Notch* locus. *Cell* **51**, 539-548.

**Kidd, S., Kelley, M. R., and Young, M. W.** (1986). Sequence of the *Notch* locus of *Drosophila melanogaster*: relationship of the encoded protein to mammalian clotting and growth factors. *Mol. Cell. Biol.* **6**, 3094-3108.

**Klämbt, C., Knust, E., Tietze, K. and Campos-Ortega, J. A.** (1989). Closely related transcripts encoded by the neurogenic gene complex *Enhancer of split* of *Drosophila melanogaster*. *EMBO J.* **8**, 203-210.

**Knust, E., Schrons, H., Grawe, F. and Campos-Ortega, J. A.** (1992). Seven genes of the *Enhancer of split complex* of *Drosophila melanogaster* encode helix-loop-helix proteins. *Genetics* **132**, 505-518.

**Kooh, P. J., Fehon, R. and Muskavitch, M. A.** (1993). Implications of dynamic patterns of Delta and Notch expression for cellular interactions during *Drosophila* development. *Development* **117**, 493-507.

**Kopczynski, C. C., Alton, A. K., Fechtel, K., Kooh, P. J. and Muskavitch, M. A. T.** (1988). *Delta*, a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. *Genes Dev.* **2**, 1723-1735.

**Lehmann, R., Jimenez, F., Dietrich, U. and Campos-Ortega, J. A.** (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **192**, 62-74.

**Lieber T., Wesley, C. S., Alcamo, E., Hassel, B., Krane, J. F., Campos-Ortega, J. A. and Young, M. W.** (1992). Single amino acid substitutions in EGF-like elements of *Notch* and *Delta* modify *Drosophila* development and affect cell adhesion in vitro. *Neuron* **9**, 847-859.

**Martinez, C. and Modolell, J.** (1991). Cross-regulatory interactions between the proneural *achaete* and *scute* genes. *Science*, **251**, 1485-1487.

**Modolell, J., Bender, W. and Meselson, M.** (1983). *Drosophila melanogaster* mutants suppressible by the supressor of *Hairy-wing* are insertions of a 7.3-kilobase mobile element. *Proc. Nat. Acad. Sci. USA* **80**, 1678-1682.

**Moscoso del Prado, J. and Garcia-Bellido, A.** (1984a). Genetic regulation of the *achaete-scute* complex of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**, 242-245.

**Moscoso del Prado, J. and Garcia-Bellido, A.** (1984b). Cell interactions in the generation of chaetae pattern in *Drosophila*. *Roux's Arch. Dev. Biol.* **193**, 246-251.

**Murre, C., McCaw, P. S. and Baltimore, D.** (1989a). A new DNA binding and dimerisation motif in immunoglobulin enhancer binding, *daughterless*, *MyoD*, and *myc* proteins. *Cell* **56**, 777-783.

**Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. and Baltimore, D.** (1989b). Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* **58**, 537-544.

**Nikolakaki, E., Coffer, P. J., Hemelsoet, R., Woodgett, J. R. and Defize, L. H. K.** (1993). Glycogen synthase kinase-3 phosphorylates JUN family members in vitro and negatively regulates their transactivating potential in intact cells *Oncogene* **8**, 833-840.

**Pelech, S. L. and Sanghera, J. S.** (1992). Mitogen-activated protein kinases: versatile transducers for cell signaling. *TIBS* **17**, 233-238.

**Perrimon, N. and Smouse, D.** (1989). Multiple functions of a *Drosophila* homoetic gene, *zeste-white 3*, during segmentation and neurogenesis. *Dev. Biol.* **135**, 287-305.

**Plyte, S. E., Hughes, K., Nikolakaki, E., Pulverer, B. J. and Woodgett, J. R.** (1992). Glycogen synthase kinase-3: functions in oncogenesis and development. *Biochem. Biophys. Acta* **1114**, 147-162.

**Portin, P.** (1975). Allelic negative complementation at the *Abruptex* locus of Drosophila melanogaster. *Genetics* **81**, 121-133.

**Portin, P.** (1981). The antimorphic mode of action of lethal *Abruptex* alleles of the *Notch* locus in Drosophila melanogaster. *Hereditas* **95**, 247-251.

**Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, L., Cherbas, P. and Artavanis-Tsakonas, S.** (1991). Specific EGF repeats of *Notch* mediate interactions with *Delta* and *Serrate*: Implications for Notch as a multifunctional receptor. *Cell* **67**, 687-699.

**Romani, S., Campuzano, S., Macagno, E. R. and Modolell, J.** (1989). Expression of *achaete* and *scute* genes in *Drosophila* imaginal discs and their function in sensory organ development. *Genes Dev* **3**, 997-1007.

**Rodriguez, I., Hernandez, R., Modolell, J. and Ruiz-Gomez, M.** (1990). Competence to develop sensory organs is temporally and spatially regulated in *Drosophila* epidermal primordia. *EMBO J.* **9**, 3583-3592.

**Ruel, L., Pantesco, V., Lutz, Y., Simpson, P. and Bourouis, M.** (1993a). Functional significance of a family of protein kinases encoded at the *shaggy* locus in *Drosophila*. *EMBO J.* **12**, 1657-1669.

**Ruel, L., Bourouis, M., Heitzler, P., Pantesco, V. and Simpson, P.**

(1993b). The *Drosophila shaggy* kinase and rat glycogen synthase kinase-3 have conserved activities and act downstream of *Notch*. *Nature* **362**, 557-560.

**Ruiz-Gomez, M. and Ghysen, A.** (1993). The expression and role of a proneural gene, *achaete*, in the development of the larval nervous system of Drosophila. *EMBO J.* **12**, 803-808

**Ruiz-Gomez M. and Modolell, J.** (1987). Deletion analysis of the *achaete-scute* locus of *Drosophila melanogaster*. *Genes Dev* **1**, 1238-1246.

**Santamaria, P. and Garcia-Bellido, A.** (1978). Developmental analysis of the *achaete-scute* system of *Drosophila melanogaster*. *Genetics* **88**, 469-486.

**Schrons, H., Knust, E. and Campos-Ortega, J. A.** (1992). The *Enhancer of split* complex and adjacent genes in the 96F region of *Drosophila melanogaster* are required for segregation of neural and epidermal progenitor cells. *Genetics* **132**, 481-503.

**Siegfried, E., Chou, T-B. and Perrimon, N.** (1992). *wingless* signalling acts through *zeste-white 3*, the *Drosophila* homolog of glycogen synthase kinase-3, to regulate *engrailed* and establish cell fate. *Cell* **71**, 1167-1179.

**Siegfried, E., Perkins, L. A., Capaci, T. M. and Perrimon, N.** (1990). Putative protein kinase product of the *Drosophila* segment polarity gene *zeste-white 3*. *Nature*, **345**, 825-829.

**Simpson, P.** (1990). Lateral inhibition and the development of the sensory bristles of the adult peripheral nervous system of *Drosophila*. *Development* **109**, 509-519.

**Simpson, P., Bourouis, M., Heitzler, P., Ruel, L., Haenlin, M. and Ramain, P.** (1992). *Delta, Notch* and *shaggy*, elements of a lateral signalling pathway in *Drosophila*. In *The Cell Surface*, pp. 391-400. Cold Spring Harbor Symp. Quant. Biol.

**Simpson, P. and Carteret, C.** (1989). A study of *shaggy* reveals spatial domains of expression of *achaete-scute* alleles on the thorax of *Drosophila*. *Development* **106**, 57-66

**Simpson, P. and Carteret, C.** (1990). Proneural clusters: equivalence groups in the epithelium of *Drosophila*. *Development* **110**, 927-932.

**Simpson, P., El Messal, M., Moscoso del Prado, J. and Ripoll, P.** (1988). Stripes of positional homologies across the wing blade of *Drosophila melanogaster*. *Development* **103**, 391-401.

**Skeath, J. B. and Caroll, S. B.** (1991). Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* **5**, 984-995.

**Skeath, J. B. and Carroll, S. B.** (1992). Regulation of proneural gene expression and cell fate during neuroblast segregation in the *Drosophila* embryo. *Development* **114**, 939-946.

**Stern, C.** (1954). Two or three bristles. *Am. Scientist* **42**, 213-247.

**Tsuda, L., Inoue, Y. H., Yoo, M., Mizuno, M., Hata, M., Lim, Y-M., Adachi-Yamada, T., Ryo, H., Masamune, Y. and Nishida, Y.** (1993). A protein kinase similar to MAP kinase activator acts downstream of the raf kinase in *Drosophila*. *Cell* **72**, 407-414.

**Van Doren, M., Ellis, H. M. and Posakony, J. W.** (1991). The *Drosophila extramacrochaetae* protein antagonizes sequence-specific DNA binding by *daughterless/achaete-scute* protein complexes. *Development* **113**, 245-255.

**Van Doren, M., Powell, P. A., Pasternak, D., Singson, A. and Posakony, J.** (1992). Spatial regulation of proneural gene activity: auto- and cross-activation of *achaete* is antagonized by *extramacrochaetae*. *Genes and Dev.* **6**, 2592-2605.

**Vässin, H., Bremer, K. A., Knust, E. and Campos-Ortega, J. A.** (1987). The neurogenic gene *Delta* of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO J.* **6**, 3433-3440.

**Villares, R. and Cabrera, C. V.** (1987). The *achaete-scute* gene complex of *D. melanogaster*: conserved domains in a subset of genes required for neurogenesis and their homology to *myc*. *Cell* **50**, 415-424.

**Weinmaster, G., Roberts, V. J. and Lemke, G.** (1991). A homolog of *Drosophila Notch* expressed during mammalian development. *Development* **113**, 199-205.

**Welshons, W. J.** (1971). Genetic basis for two types of recessive lethality at the *Notch* locus of *Drosophila*. *Genetics* **68**, 259-268.

**Wharton, K. A., Johansen, K. M., Xu, T. and Artavanis-Tsakonas, S.** (1985). Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43**, 567-581.

**Wigglesworth, V. B.** (1940). Local and general factors in the development of "pattern" in *Rhodnius prolixus* (Hemiptera) *J. Exp. Biol.* **17**, 180-200.

**Woodgett, J. R.** (1990). Molecular cloning and expression of glycogen kinase-3/factor A. *EMBO J.* **9**, 2431-2438.

**Woodgett, J. R.** (1991). A common denominator linking glycogen metabolism, nuclear oncogenes and development. *TIBS* **16**, 177-181.