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Lateral inhibition and the development of the sensory bristles of the adult peripheral nervous system of *Drosophila*

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Summary

Cells in the neurectoderm of *Drosophila* face a choice between neural and epidermal fates. On the notum of the adult fly, neural cells differentiate sensory bristles in a precise pattern. Evidence has accumulated that the bristle pattern arises from the spatial distribution of small groups of cells, proneural clusters, from each of which a single bristle will result. One class of genes, which includes the genes of the *achaete–scute* complex, is responsible for the correct positioning of the proneural clusters. The cells of a proneural cluster constitute an equivalence group, each of them having the potential to become a neural cell. Only one cell, however, will adopt the primary, dominant, neural fate. This cell is selected by means of cellular interactions between the members of the group, since if the dominant cell is removed, one of the remaining, epidermal, cells will switch fates and become neural. The dominant cell therefore prevents the other cells of the group from becoming neural by a phenomenon known as lateral inhibition. They, then, adopt the secondary, epidermal, fate. A second class of genes, including the gene *shaggy* and the neurogenic genes mediate this process. There is some evidence that a proneural cluster is composed of a small number of cells, suggesting a contact-based mechanism of communication. The molecular nature of the protein products of the neurogenic genes is consistent with this idea.

Key words: *Drosophila*, lateral inhibition, sensory bristle, peripheral nervous system.

Introduction

The development of the nervous system of insects requires a great deal of precision. The number of precursor cells, or neuroblasts, from which the central nervous system (CNS) is derived is well conserved between different insect species and furthermore the general layout and even specific neurons are found to be similar from one species to the next (Thomas *et al.* 1984). Each neuroblast is unique and produces a defined part of the CNS. The peripheral nervous system (PNS) of *Drosophila* is equally precise. There is an absolutely fixed number of sensory organs in the larval PNS (Dambly-Chaudière and Ghysen, 1986), and many of the sensory bristles of the adult PNS can be identified individually and occupy fixed positions. This bristle pattern is widespread among the Diptera and the relative constancy of bristles has long been used for classification (Imms, 1960; Sturtevant, 1970). Furthermore, the invariant bristle pattern reflects an underlying specificity of neuronal connections in the CNS; individual bristles, when stimulated, can be shown to evoke specific reflexes on behalf of the fly (Vandervorst and Ghysen, 1980) and to display specific axonal connections in the CNS (Ghysen, 1980). Most tissues develop from a contiguous group of cells that together enter into the same initial fate, e.g. the mesoderm that develops from a ventral strip of adjacent cells. The CNS and PNS develop differently: individual neuroblasts or sensory mother cells segregate from over a large area of ectoderm (Hartenstein and Campos-Ortega, 1984; Hartenstein and Posakony, 1989). There is evidence that the specific part of the nervous system that is produced from an initial precursor cell is a function of the position in the animal at which it was born (Ghysen, 1980; Walthall and Murphey, 1984; Taghert *et al.* 1984; Doe and Goodman, 1985; Patel *et al.* 1989; Doe *et al.* 1988*a*; Doe *et al.* 1988*b*). An origin of precursor cells from over a wide area of the animal would therefore provide for a greater diversity of positional identities. Such a requirement for diversity may explain the unique mode of development.

The specificity of positional information that would be necessary to allow individual precursor cells to adopt developmental fates different from those of their immediate epidermal neighbours, would have to be remarkable. The decision to make a neuroblast or a sensory mother cell, however, may initially be taken by a small group of cells that are collectively determined. Such a cluster of cells can be called an equivalence group by analogy to a similar mode of determination in the nematode (Kimble, 1981; Sulston and White, 1980;

Palka, 1986; Cabrera *et al.* 1987; Simpson and Carteret, 1989). Subsequent local cell interactions occurring between the equivalent cells would lead to the singling out of only one cell that would come to predominate. The dominant cell would then inhibit the other members of the group from realising their neural potential, they then adopt the secondary, epidermal fate, by a mechanism known as lateral inhibition. This presumably involves the production of a signal by the dominant cell and the reception of this signal by the remaining members of the group. A failure of this signalling mechanism would lead to all cells of the group adopting the primary, dominant, neural fate. Such a mechanism ensures that only a single cell ultimately differentiates into a neuroblast or sensory mother cell but at the same time provides a safeguard against the loss of the neural precursor, the integrity of which is essential. Laser ablation of developing neuroblasts in the grasshopper leads to the production of a new one from an adjacent cell (Doe and Goodman, 1985).

The sequence of events outlined above leads one to predict the necessity for at least two classes of genes. One class of genes is required for the establishment of the equivalent groups of cells in response to positional cues present in the embryo. Another class of genes is required for the cell interactions that will ensure that only one cell actually adopts the neural fate. In this paper, I shall review the evidence both for this sequence of events and for the existence of genes of both categories with particular reference to the gene *shaggy* and the development of the sensory bristles of the adult PNS of *Drosophila*.

Earlier studies on bristle spacing

In the insect integument, bristles arise through the determination of bristle mother cells that subsequently undergo two differentiative divisions giving rise to the four cells of the sensory organ: tormogen, trichogen, neurilemma cell and sensory cell (Lawrence, 1966*a*; Hartenstein and Posakony, 1989). One can distinguish two classes of bristles: large bristles (often called macrochaetae), of which there are a fixed number in a stereotyped pattern, and smaller bristles (microchaetae), slightly variable in number which are evenly spaced. A number of early investigators studied the mechanisms leading to the spacing of bristles (see review by Lawrence, 1973). I will first consider the simple case of a pattern of dispersed but evenly spaced bristles of a uniform size. Hemimetabolous insects go through a series of moults as the animal increases in size and at each moult new bristles are added to the existing pattern. The first point to establish is that these new bristles arise from a population of homogeneous epidermal cells by the singling out of particularly spaced individuals. The evidence for this is threefold. First, after wound healing, the epidermis has the capacity to regulate and produce new bristles (Wigglesworth, 1940). Second, an artificial increase in the number of cells, caused by a distension of the cuticle (Wigglesworth, 1940) or a reduction in cell size (Santamaria, 1983; Held, 1979), leads to an increase in the number of bristles whereas a decrease in multiplication of epidermal cells due to starvation (Lawrence, 1966*a*) leads to fewer bristles. Third, even after development has finished epidermal cells retain the ability to make bristles (Lawrence, 1966*b*). It therefore follows that most, and probably all, epidermal cells have the potential to make bristles, but that only a fraction of evenly spaced cells actually does so. Wigglesworth (1940) studied the way in which new bristles arise in the bug *Rhodnius*. He discovered that early in the moult cycle bristle mother cells are determined and that this is followed by cell division of the intervening epidermal cells prior to the deposition of cuticle at moulting. Therefore, at each cycle new bristles can be added in the spaces between the preexisting ones that have been provided by the growth of the epidermis in the preceding cycle. In this way, the density of bristles remains approximately constant as the insect grows. This process of adding new bristles in between the old ones means that as growth continues, the earliest formed bristles become more and more dispersed. Lawrence and Hayward (1971) showed that the differentiation of bristles in *Oncopeltus* occurred in a non-random order so that the first to develop were an overdispersed subset of the total. Furthermore, Wigglesworth showed that new bristles arise at those points at which the extant bristles were the most widely separated (see Fig. 1). When the

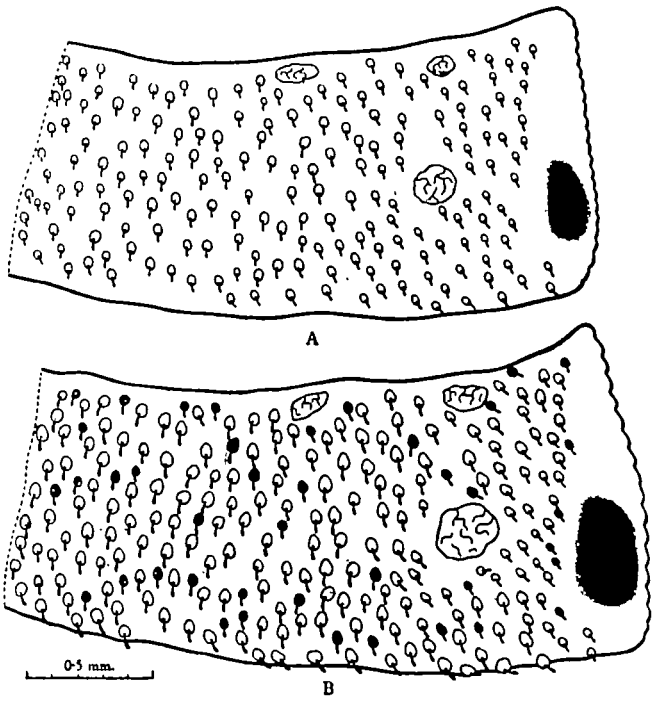

Fig. 1. The distribution of bristles on the right half of the third tergite of a *Rhodnius* nymph at the 4th (A) and 5th (B) instars. New bristles arising after the moult are shaded. It can be seen that they arise where the old bristles were the most widely spaced. Reprinted from Wigglesworth (1940), courtesy of Sir V. B. Wigglesworth and The Company of Biologists Ltd.

number of epidermal cells between bristles exceeds a certain limit, a new bristle will arise. Studies of bristle patterns in animals in which cell size has been varied by triploidy or haploidy, have also shown that the interval between microchaetae in *Drosophila* is measured as a fixed number of cells (Held, 1979; Santamaria, 1983).

Wigglesworth (1940) proposed the existence of a diffusible substance distributed in the epidermis that is necessary for the formation of bristles. As bristles become determined they would absorb the substance thus depleting the area immediately surrounding them. Cells nearby being deprived of the bristle determining substance would therefore remain epidermal. More recent models (Claxton, 1964; Lawrence, 1969; Richelle and Ghysen, 1979) suggest that each competent cell produces a bristle-inducing substance that diffuses away in the epithelium. The concentration of this would be highest in the centre of a group of competent cells. The first cell whose concentration reaches a threshold would initiate differentiation. This cell would then inhibit those nearby by the production of a diffusible inhibitory substance. The size of the inhibitory field would specify distance between bristles and lead to an even spacing.

From their studies on the spacing of heterocysts along a filament in *Anabaena*, where new ones arise midway between the others as the filament grows, Wilcox *et al.* (1973) postulated a similar threshold model based on the production of an inhibitor from each heterocyst. They noticed, however, that often more than one proheterocyst initiated development and that it was not always the first to appear that finally became the heterocyst. They therefore introduced a notion of competition between proheterocysts and suggested that this could occur if, even after a proheterocyst has begun to produce inhibitor, it remains itself susceptible to the effects of the inhibitor and will regress if the latter exceeds a certain critical level. Thus, if two proheterocysts are developing close together, each will cause an increased level of inhibitor in the other and within a critical distance, one will eventually win out. Such a notion of competition can also be applied to bristle spacing.

Some bristle patterns are composed of a fixed number and disposition of bristles. For example, on the notum of *Drosophila* there are 11 macrochaetae placed in an invariant pattern (see Fig. 2A). Even for these accurately placed bristles, however, there is evidence that the cells forming them are not unique in that neighbouring cells can take their place should the extant bristle be removed. Stern (1954) produced flies mosaic for mutant *achaete* (*ac*) territories and wild-type territories. The mutation *ac* removes the posterior dorsocentral bristle and its action is cell autonomous in that no bristle is made if the mutant cells occupy the position at which it normally forms. If, however, the mutant territory only just covered the bristle site, then occasionally a nearby wild-type cell will make the bristle in a slightly displaced location. Two important conclusions can be drawn from this observation. First, the failure to form a bristle at the normal site followed by the development of a bristle close to that site is

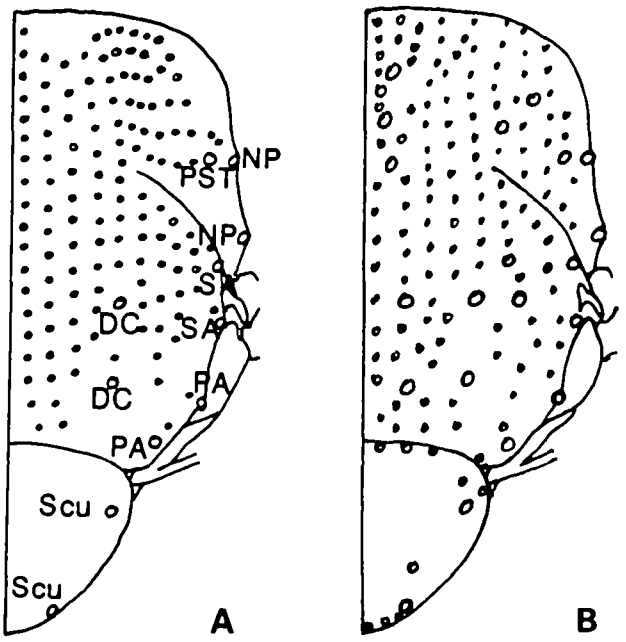

Fig. 2. (A) Standard diagram of the wild-type heminotum of *Drosophila* showing the positions of macrochaetae, large circles, and microchaetae, small circles. The macrochaetae are named as follows: DC, dorsocentral; Scu, scutellar; PA, postalar; Sa, supraalar; NP, notopleural; PST, presutural. (B) Diagram of the heminotum of a Hw^{J} fly. Hw^{J} is a gain-of-function allele of the AS–C, it causes the development of extra macrochaetae and microchaetae in ectopic locations.

evidence in favour of the notion that the normal bristle usually inhibits nearby cells from themselves making a bristle. This is formally the experimental equivalent of the ablation of a neuroblast in the embryo (Doe and Goodman, 1985). Second, the experiment shows that the potential to form that specific, precisely positioned bristle is a property of a group of cells at that location. This suggests that in normal development one cell from that group is singled out to become the bristle mother cell. Stern suggested that wild-type cells are competent to respond to a prepattern of factors which specify the formation of bristles in particular places. Later models involved gradients (Lawrence, 1973) used in a more general theory of positional information (Wolpert, 1969) to provide a system of coordinates to the developing tissue.

Several authors have investigated the role of the genes of the *achaete–scute* complex (AS–C) in bristle formation. Different alleles of these genes cause the loss of specific bristles (see next section). Stern (1956) suggested the *scute* (*sc*) gene either altered the prepattern itself or the cells' response to the prepattern. Ghysen and Richelle (1979) showed that the behaviour of these genes was compatible with their being responsible for the synthesis of the diffusible bristle-promoting substance suggested earlier. Older models also assumed an even distribution of *sc* product, the amount of which varied with the different alleles. The specificity of the phenotype was thought to be due to differential sensi-

tivities of individual bristles (see review by Ghysen and Dambly-Chaudière, 1988).

The remainder of this paper will be spent discussing the recent work on the notum of *Drosophila* and its use as a model system. The adult heminotum is composed of about 10000 cells. There are the 11 named macrochaetae and about 100 evenly spaced microchaetae. By means of double-labelling experiments using monoclonal antibodies on whole mounts of pupal wing discs, Hartenstein and Posakony (1989) counted approximately 14 epidermal cells for each microchaete. This number corresponds well with the number of hairs on the differentiated cuticle where microchaetae are separated by 5 to 6 hairs. It is therefore likely that each hair is the product of a single epidermal cell as has been shown to be the case on the wing blade (Dobzhansky, 1929). Any useful discussion of the mechanism involved in bristle spacing would require an accurate estimation of the number of cells separating bristle precursors at the time of their determination. This may occur a considerable time before the differentiative divisions which take place after pupariation, between 7 and 12 h for the macrochaetae and between 16 and 20 h for the microchaetae (Hartenstein and Posakony, 1989). First, the epidermal cells divide further after the bristles first become morphologically distinguishable (ibid). This means that in the final differentiated notum the bristles are separated by a greater number of intervening cells than they were at the time of their segregation. Second, in a study of marked clones initiated by means of X-ray-induced mitotic recombination, Garcia-Bellido and Merriam (1971) suggested that microchaetae become committed as early as 40 h before pupariation, since after this time no clones including both bristles and epidermal cells could be obtained. [Using the same method of mosaic analysis, the estimation of these authors for the division separating the trichogen and tormogen of microchaetae agrees well with the direct morphological observations of Hartenstein and Posakony (1989)]. This would imply that the bristle precursors remain in a non-dividing state for some hours. A zone of mitotically quiescent cells has been observed in the larval wing disc at the position of the rows of wing marginal bristles (O'Brochta and Bryant, 1985). Taken together these results suggest that, at the time of determination, the spacing of microchaetae precursors is in radial distances only two or three cells. This rather small distance suggests that, the inhibitory signal does not need to diffuse over several cell diameters.

The decision to make a bristle is taken by a small group of equivalent cells. The genes of the *achaete–scute* complex govern the positioning of such proneural clusters

I shall now review evidence that the decision to make a bristle is initially a collective one taken by several adjacent cells. So far the only genes for which clear evidence exists for their involvement in this process are those of the AS–C. This complex has now been shown to produce four transcripts, one of which is important for the development of parts of the CNS (lethal of scute, T3), another is involved in the determination of larval sense organs (asense, T8), while the remaining two (T4 and T5) correspond to *scute* (*sc*) and *achaete* (*ac*), respectively, and appear to be necessary for both central neurons and sensory neurons of the larval and the adult PNS (Alonso and Cabrera, 1988; Carramolino *et al.* 1982; Campuzano *et al.* 1985). *achaete* and *sc* are responsible for the determination of all of the bristles on the notum. It should be noted, however, that not all sensory organs of the larva or the imago and not all central neurons arise through the activity of the AS–C; other genes must be involved too (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; Caudy *et al.* 1988). Ghysen and Modolell (see Ghysen and Dambly-Chaudière, 1990 and Romani *et al.* 1989) have proposed that genes of this class be termed "proneural". I shall therefore use the words "proneural cluster" to describe the group of equivalent cells responsible for the determination of a bristle.

A large number of mutations of *ac* and *sc* have been obtained over decades of genetic analysis (see Garcia-Bellido, 1979 and Ghysen and Dambly-Chaudière, 1988). Deletion of both *ac* and *sc* leads to a loss of all bristles on the notum. This is not due to death of the bristle-producing cells because all of the cells differentiate into epidermis (Fig. 3A). Selective loss of either *ac* or *sc* reveals that ac^+ is required for three macrochaetae and all of the microchaetae, whereas sc^+ is required for nine macrochaetae. Many different, viable mutant *sc* alleles exist and each one removes a specific subset of macrochaetae. In all cases, however, the remaining bristles occupy their correct locations showing that the development of a macrochaete at one site is independent of the presence of bristles at other positions. This suggests regional control of the T4 transcript in different parts of the notum. Many of these *sc* alleles are due to chromosomal rearrangements with break points at some distance from the coding region (Campuzano *et al.* 1985). The breakpoints are thought to affect *cis*-acting site-specific control elements that govern the expression of the *sc* transcript (Ruiz-Gomez and Modolell, 1987; Leyns *et al.* 1989). 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. Romani *et al.* (1989) confirmed this supposition by direct observation: *sc* transcripts were found to be in clusters of cells in the areas of the imaginal disc where each bristle will later form. Furthermore, they found that, in a particular *sc* mutant allele, *sc* transcripts were absent in the appropriate cluster of cells. *scute* transcripts are also found in groups of cells in the embryo from which one will presumably segregate as a neural precursor (Cabrera *et al.* 1987). However, transcripts are not detected in differentiating neurons consistent with the idea that the AS–C is involved in the initial decision and not in the differentiation (ibid). Therefore, unlike earlier models, molecular evidence suggests that *sc* is locally expressed in discrete groups of cells, even though only a single bristle will be produced

Fig. 3. (A) A twin spot resulting from two clones that each develop from one of the two sister cells arising from a cell in which mitotic recombination occurred. The clone at the top is mutant for *sgg* and shows a cluster of closely spaced bristles, they are marked with *forked*. The area to the right of the *sgg* clone is lacking the genes of the AS–C. No bristles develop but the cells secrete epidermis. Flies of the genotype $sgg^{D127}\ f^{36a}/Df(1)sc19,\ y^-,\ AS\text{–}C^-$ were irradiated with 1000R of X rays. (B) A mutant *sgg* clone in which extra macrochaetae have formed. Although found in the vicinity of the extant bristle, the *sgg* macrochaetae are spread out and separated by intervening hairs, and sometimes microchaetae are also found interspersed between them. Flies of the genotype $sgg^{D127}\ w\ f^{36a}/+$ were irradiated. (C) A clone of cells mutant for Df^{6B37}. In this instance, a closely packed cluster of adjacent macrochaetae are formed at a site where usually one develops. Flies of the genotype $st\ Df^{6B37}\ e/M(3)w^{124}$ were irradiated.

from each cluster. Furthermore, the *sc* product, which shows homology to a transcription factor, is unlikely to be a diffusible molecule (Villares and Cabrera, 1989; Cabrera, personal communication).

In contrast to loss-of-function alleles of *ac* and *sc* that cause a loss of bristles, there are other mutants that result in over expression of *ac* and/or *sc*, such as *Hairy wing* (*Hw*), *hairy* (*h*) and *extramacrochaetae* (*emc*) (Garcia-Bellido, 1979; Garcia-Alonso and Garcia-Bellido, 1986, 1988; Campuzano *et al.* 1986; Balcells *et al.* 1988; Moscoso del Prado and Garcia-Bellido, 1984). These mutants cause the appearance of extra bristles and two important aspects of their phenotypes are relevant. First, although the density of bristles increases considerably on the notum, bristles are evenly spaced and never immediately adjacent. They are always separated by intervening hairs. In the case of mutants affecting lateral inhibition, bristles can often be adjacent (see next section). Second, many of the additional bristles are found in the wrong locations. This is particularly obvious for the macrochaetae since the wild-type ones occupy such precise positions. It is also true of the microchaetae, however, and ectopic ones can be found for example on the scutellum that is usually devoid of them. An obvious explanation for these phenotypes would be that the regional control of *ac* and *sc* has been altered leading to the establishment of proneural clusters in the wrong places. Indeed, it has been observed that in Hw^1 and Hw^{49c} mutants within the AS–C, *ac* and *sc* transcripts accumulate in ectopic locations (Balcells *et al.* 1988). The ectopic, supernumerary, bristles in *h* mutants form later than the normal ones during a third wave of differentiation lasting up until 26 h after pupariation (Palka *et al.* 1983; see also Rushlow *et al.* 1989). It seems likely that, after determination of the normal complement of bristles, growth of the epidermal cells creates spaces between bristles large enough to accomodate a supplementary

wave of extra bristles. This would lead to the observed pattern of even spacing but with increased density.

It can be concluded, then, that the *ac* and *sc* genes govern the positions of bristles through a precise spatial distribution of proneural clusters. Further evidence for this is given in the next section. Loss-of-function mutants cause the loss of specific clusters while gain-of-function mutants cause the appearance of new ectopic ones. A failure of establishment of proneural clusters results in the cells differentiating epidermis, which means that the epidermal state is the default state of the epithelium. The pattern of bristles, therefore, relies on the accurate pattern of expression of *ac* and *sc*. The *hairy* and *emc* genes may play a role in this process, which I will not discuss.

Mutation of genes involved in lateral inhibition leads to the differentiation of a cluster of adjacent bristles at the sites where usually only one develops. These clusters correspond to the domains of expression of achaete and scute

A number of genes have been described that are thought to play a role in lateral inhibition in both embryos and adults. The best studied of these are those of the neurogenic class (Lehman *et al.* 1983; Campos-Ortega, 1985; 1989). Mutations in such genes cause neural hyperplasia in embryos that is thought to result from a defect in the cellular interactions leading to a separation of the neural and epidermal cell lineages. Evidence in favour of this comes from a study of the expression of the protein product of the T3 transcript of *lethal of scute*. In wild-type embryos, this protein is restricted to developing neuroblasts whereas the corresponding RNA is present in cells of both the neural and epidermal lineages (C. Cabrera, personal communication). In *Notch* (*N*) and *Delta* (*Dl*) mutant embryos, however, the T3 protein is present in most, if not all, of the RNA-producing cells (ibid). This suggests that the *N* and *Dl* genes normally function to ensure a correct differentiation between adjacent neural and epidermal cells. Rather little is known about the role of these genes in bristle development but they have been reported to cause the differentiation of extra bristles when mutant (Dietrich and Campos-Ortega, 1984; Cagan and Ready, 1989). Another gene, *shaggy* (*sgg*), mutants of which cause hyperplasia of both central and peripheral nervous systems (Simpson *et al.* 1988; Bourouis *et al.* 1989) has been more carefully analysed for its role in bristle development. Mutants that interfere with the cellular interactions normally leading to the selection of one bristle mother cell from each proneural cluster, would be expected to cause the differentiation of more than one bristle per cluster. This means that the neural fate is the default state *within* proneural clusters. This would lead to a phenotype of extra bristles, superficially similar to a *Hw* phenotype, which itself results from the definition of extra clusters. *shaggy* mutants are lethal but their phenotype on the adult epidermis can be studied in mutant clones in otherwise

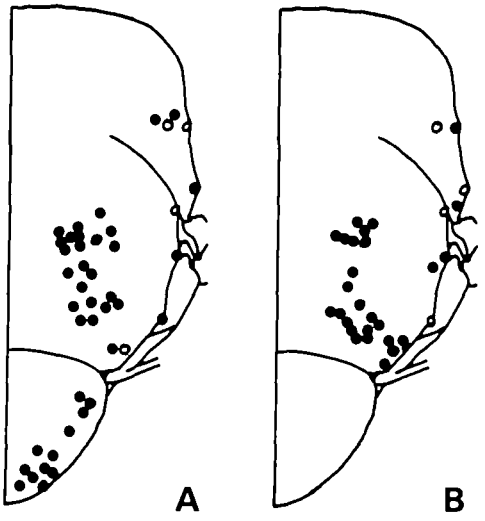

Fig. 4. (A) The distribution of macrochaetae observed in a study of mutant *sgg* clones in otherwise wild-types flies. These can be seen to be clustered around the positions of the extant macrochaetae. (B) The distribution of macrochaetae seen in *sgg* clones in sc^{1} flies. The sc^{1} mutant removes the two scutellar bristles. Here the *sgg* cells fail to differentiate bristles anywhere on the scutellum unlike those in A. Elsewhere the clones show the typical *sgg* phenotype. From Simpson and Carteret (1989).

sgg^{+} flies. Such clones differentiate large numbers of supernumerary bristles, (see Fig. 3A), but the patterns formed are quite unlike those seen in *Hw* mutants. Both extra microchaetae and macrochaetae are formed but they develop at precise spatial locations. Microchaetae develop only on those parts of the thorax that are normally covered with them; they are not formed in ectopic positions. Similarly macrochaetae are only found clustered around the positions of the extant macrochaetae (Simpson and Carteret, 1989; see Fig. 4A). Therefore, bristles do not form at ectopic positions. Furthermore, although many clones retain some spacing between the bristles, in others the bristles are immediately adjacent. A gene dosage study and observations of clones mutant for both *sgg* and the genes of the AS–C established that *sgg* plays no role in the regulation of *ac* and *sc*; on the contrary, the spatial expression of the *ac* and *sc* genes is unaltered in *sgg* mutants. It is therefore possible that the clusters of macrochaetae caused by *sgg* are the result of the differentiation of several bristles from each proneural cluster. This idea is reinforced by the observation that specific *sc* alleles that remove only one or a small subset of macrochaetae suppress the entire *sgg* cluster of macrochaetae at those specific locations in double mutant clones (ibid, see Fig. 4B). This reveals that the expression of *sc* extends over a wider area than just the single cell that normally forms the bristle and is consistent with the observation that *sc* is expressed in small clusters of cells in the discs. Therefore, while sc^{+} is necessary for the cluster of equivalent cells, sgg^{+} is necessary for the segregation of only one bristle mother cell within the cluster.

The results just discussed provide evidence that

bristle determination is initially a property of a group of cells. An important prediction that arises from this notion is that all cells of the group must be functionally equivalent. That is, if, through a failure in lateral inhibition, all or many members of the group adopt the primary, neural fate, then they should all produce the same identical part of the nervous system and display identical neuronal specificity. It should be possible to test this in the case of several clusters of *sgg* macrochaetae on the notum, since the extant bristles in the wild type have been shown to elicit specific reflexes on behalf of the fly, when stimulated (Vandervorst and Ghysen, 1980) and to display bristle specific axonal projections in the CNS (Ghysen, 1980).

How many cells constitute a proneural cluster and what mechanisms can be envisaged for the phenomenon of lateral inhibition?

The question of the precise roles of individual genes in the segregation of neural and epidermal lineages has been mainly investigated for the neurogenic genes with reference to the CNS. The inhibitory effects of neuroblasts upon the surrounding cells (Doe and Goodman, 1985) will probably involve a molecular signal produced by the neuroblast and receptor molecules for the signal in the inhibited cells. Campos-Ortega and his colleagues reasoned that mutant cells defective for the receptor mechanism would always adopt the neural fate, in other words they would be cell autonomous and remain neural even if surrounded by wild-type cells. Mutant cells defective only in the signal, on the other hand, would retain the possibility of being inhibited by signalling wild-type cells and could adopt the epidermal fate in mosaics. By means of a new technique of transplantation of individual cells between embryos, Technau and Campos-Ortega (1987) studied mosaic embryos and their results suggested that, amongst the known neurogenic mutants, only *Enhancer of spl*, *E(spl)*, behaved cell autonomously and the others gave rise to both neural and epidermal lineages. Hoppe and Greenspan (1986), however, presented results suggesting cell autonomy for mutant *N* cells and recent studies by these authors on the cell-by-cell expression of *N* protein along the borders between *N* and N^+ cells in mosaic embryos provides good evidence for such cell autonomy (P. Hoppe and R. Greenspan, personal communication). Much more detailed information can be obtained from the study of mosaic adult cuticles but few such studies have been reported yet for the neurogenic mutants. I will illustrate this potential with reference to *sgg*.

On the notum, clones of cells mutant for *sgg* differentiate both bristles and epidermal hairs. If the hairs were the result of non-autonomy, they should be found round the edges of the clones where the cells are in contact with rescuing wild-type neighbours. This is not the case. Rather, the hairs result from an incomplete penetrance. Not all cells of the cluster are determined to become bristles, some make epidermis and this leads to a small number of hairs between some bristles. On the wing blade, clones produce exclusively bristles and behave entirely autonomously (Simpson *et al.* 1988). Clones of cells mutant for *sgg* small enough to differentiate a single bristle can be made. Such single *sgg* bristles can be found immediately adjacent to a wild-type bristle. This means that the initial mutant bristle mother cell was insensitive to inhibition from the wild-type bristle mother cell and suggests that *sgg* cells are rendered independant of the inhibitory signal. On the other hand, if the *sgg* cells are condemned by their mutant state to adopt the neural fate then one might expect them to become the dominant members of the group and to prevent other, wild-type, cells from becoming bristles. This is clearly not the case either, since wild-type bristles can be adjacent to the mutant one, and therefore it seems that cells can neither send nor receive the inhibitory signal. Further observations on mosaic clusters reveal occasional cases of nonautonomy of wild-type tissue: two wild-type bristles are found associated with *sgg* bristles within the same cluster. Two cases were observed from 47 mosaic clusters. No cases of two wild-type bristles in purely wild-type clusters were observed in these animals (i.e. 470 wild-type clusters). It would seem that in these cases the presence of the mutant cells has prevented communication between two wild-type cells of the same proneural cluster. From such detailed observations of mosaic clusters, we may suppose that *sgg* does not play a specific role in the inhibitory signal but that this gene product is required more generally for this type of cell signalling. Similar analyses with mutants of the neurogenic class will perhaps lead to a greater understanding of their role in lateral inhibition.

If we wish to consider possible molecular mechanisms for lateral inhibition, then it is important to know the number of cells that constitute a proneural cluster. In the case of *sgg*, entirely mutant clusters differentiate on average 3.0 macrochaetae, but we know that in this case not all of the cells make bristles. As a result of the incomplete penetrance, the *sgg* bristles of a cluster become dispersed through subsequent division of intervening epidermal cells (see Fig. 3B and Fig. 5). If, however, a mutant were to have complete penetrance and all the cells of the cluster became bristle mother cells, then there would be no further growth and the resulting bristles should be tightly grouped and adjacent to one another. Such a phenotype is in fact observed in clones mutant for some alleles of *N* and *Dl* (Dietrich and Campos-Ortega, 1984; see Fig. 3C and Fig. 5). Clusters of tightly packed bristles are observed in the place of each macrochaete. Although it has not been shown, as in *sgg*, that these clusters represent the domains of expression of *ac* and/or *sc*, this would seem to be likely. We have looked at clones mutant for Dl^{6B37} and N^{ts1} and find that the clusters are composed on average of 6 to 7 bristles (P. Heitzler and P.S., unpublished observations). If this really represents the entire cluster then the number of cells might be small enough for the mechanism of lateral inhibition to rely on direct cell contact. There would be no need to

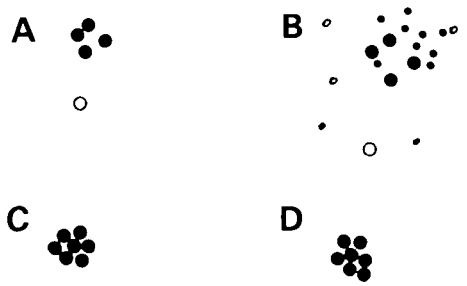

Fig. 5. (A) The deduced situation of the segregation of macrochaetae precursor cells in the epithelium that would give rise to the mosaic *sgg* cuticle seen in (B). A single wild-type precursor develops at the location of the posterior dorsocentral bristle (large open circle). Instead of a single precursor for the anterior dorsocentral bristle, four mutant ones are formed (large solid circles). This would not represent all of the cells of the proneural cluster, however, and these four precursors would be interspersed with epidermal cells (not shown). Division of the intervening epidermal cells leads to the dispersion of the four macrochaetae seen in the clone in B. The subsequent segregation of microchaetae precursors can then arise through the establishment of new proneural clusters between the macrochaetae (small circles represent microchaetae). (C) The deduced situation of the segregation of macrochaetae precursor cells in the epithelium that would give rise to the Df^{6B37} clone seen in D. At the position of the posterior postalar bristle, all cells of the proneural cluster segregate as bristle mother cells. There are therefore no intervening epidermal cells to provide further growth. These precursors thus remain adjacent to one another and produce the cluster of bristles depicted in D.

invoke molecules that diffuse over more than one cell diameter. Similarly, we argued earlier that, at the time of determination, microchaetae may be only two to three cells apart. Cagan and Ready (1989) also argue in favour of a local contact-based mechanism of inhibition from their study of the effects of *Notch* on the retinal bristles.

Some of the genes of the neurogenic class have been cloned and sequenced. The products of these genes may allow the equivalent group of cells to interact through their surfaces leading to the selection of a dominant one. Consistent with this, the *N* product is a transmembrane protein with an extracellular domain that contains 36 cysteine-rich, epidermal growth-factor-like repeats (Wharton *et al.* 1985; Kidd *et al.* 1986). The predominant embryonic and maternal *Dl* transcripts were also found to encode a putative transmembrane protein with an extracellular domain containing nine cysteine-rich repeats homologous to the EGF-like repeats present in the *N* polypeptide (Vassin *et al.* 1987; Kopczynski *et al.* 1989). Many transcripts are made at the *E(spl)* locus and the relation of each one to neurogenesis is not clear. One has been found to show homology to a G-protein subunit and some code for *myc*-like protein domains that could potentially act as transcription factors (Hartley *et al.* 1988; Klambt *et al.* 1989). Thus *E(spl)* too may function in intercellular communication. Transcripts of these genes appear in many tissues and seem to accumulate in all cells in the neurogenic ectoderm (Hartley *et al.* 1987; Hartley *et al.* 1988; Knust *et al.* 1987; Kopczynski and Muskavitch, 1989). The *N* protein is found on the cell surface of both epidermal and neural cells (Kidd *et al.* 1989; Johansen *et al.* 1989).

It is of interest to mention here results pertaining to the *lin-12* gene of the nematode, since these point to an analogous cell communication phenomenon, the details of which are better known. *N* and *Dl* show sequence homology to *lin-12* (Yochem *et al.* 1988). This gene codes for a cell surface receptor and is also involved in mediating decisions governing cell fate. In the vulva of the nematode, the cell next to the anchor cell in the overlying gonad becomes a primary cell as a result of an inducing signal from the anchor cell. The primary cell then prevents the two cells on either side of it from becoming primary cells by means of an inhibitory signal. These two cells then become secondary cells (Sternberg, 1988). *lin-12* encodes a receptor for the inhibitory signal and in loss-of-function mutants the secondary cells adopt the primary or dominant fate (Ferguson and Horwitz, 1985). In gain-of-function mutants, in contrast, primary cells are converted into secondary cells, presumably because of activation of *lin-12* in the primary cell (Greenwald *et al.* 1983). The way in which such a signalling system may work is illustrated by the role of *lin-12* in another decision between two precursor cells in the gonad. One of these becomes an anchor cell (AC) and the other a ventral uterine cell (VU). A signal from AC activates *lin-12* in the other cell which then becomes a VU. This event is stochastic. In mosaic animals, if one cell is $lin\text{-}12^-$ and the other wild type, then the $lin\text{-}12^-$ cell will always become the AC and the wild-type cell will always become the VU. Therefore, a cell without *lin-12* activity will always activate *lin-12* in its neighbour (Seydoux and Greenwald, 1989). This means that in normal development the amount of *lin-12* activity influences the ability of a cell to signal, which means that a small difference in receptor activity could result in a large difference in signal production (see reviews by Kenyon and Kamb, 1989 and Greenwald, 1989).

Conclusions

I have suggested that the need for a wide diversity of positional identities may be the reason why neural precursors segregate individually from over a large area of the animal. Furthermore, since this requires single cells, the neural precursors, to adopt different developmental fates from those of adjacent cells, initially probably a small group of cells is collectively determined and acquires neural potential. This, then, necessitates a second process, lateral inhibition, whereby cell interactions between the group of equipotential cells lead to the singling out of one dominant cell that adopts the neural fate. The dominant cell then prevents the other cells from becoming neural by means

of an inhibitory signal and they then adopt the secondary epidermal fate. Modern studies provide evidence that bristle determination occurs *via* this two-step process. I have also discussed evidence that different genes are required for each of these steps. Genes of the AS–C are involved in establishing the number and position of proneural clusters in response to topological cues. *shaggy* and the genes of the neurogenic class are required for the cell interactions necessary to promote one dominant cell, and to enable that cell to inhibit its neighbours. In the absence of expression of genes of the AS–C, no proneural clusters are formed and all cells differentiate into epidermis which means that the epidermal fate is the default state of these cells. In the absence of expression of genes of the neurogenic class, all cells within proneural clusters adopt the neural fate which means that the neural fate is the default state within proneural clusters. One prediction that can be made concerning such a mode of development is that the initial state of determination of each cell within the equivalent group of cells should be rigorously identical and, if all cells adopt the neural fate due to a failure of lateral inhibition, they should produce identical neurones.

There is some evidence that the number of cells separating bristle precursors at the time they are determined is rather small and that consequently the number of cells that constitute a proneural cluster is small. I therefore suggest that lateral inhibition may occur by means of direct contacts between neighbouring cells. The molecular nature of the protein products of the neurogenic genes is consistent with this idea. Therefore this type of cell signalling may be similar to that involved in the development of ommatidia (Rubin, 1989) and to a suggested mechanism of cell interactions that may generate the pattern within segments (Martinez-Arias *et al.* 1988).

For the moment, the neurogenic mutants offer the greatest opportunity for a further understanding of lateral inhibition. There is much to be gained from a cell-by-cell analysis of mosaic flies. Also unequivocal identification of the receptor and its ligand will no doubt arise from the continued molecular analysis of these genes.

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