Neurogenic and antineurogenic effects from modifications at the Notch locus

J. PALKA, M. SCHUBIGER and H. SCHWANINGER
Department of Zoology, University of Washington, Seattle, WA 98195, USA

Summary
The best studied mutations at the Notch locus produce a neurogenic phenotype, with a massive overgrowth of the nervous system at the expense of epidermis. We report here that, in the development of the adult peripheral nervous system, the Abruptex alleles of Notch have the opposite phenotype, namely an underproduction of sensory organs or sensilla. This arises primarily not from an arrest of the lineages that produce sensilla, from the degeneration of sensillar cells, or from the transformation into neurons of cells that normally secrete the cuticular components of a sensillum (as can happen in Notch alleles). Rather, our evidence argues strongly that the sensillar mother cells never form. This implies that the Notch protein plays a role in the process that first generates a difference between sensillar mother cells and ordinary epidermal cells. The number of sensilla formed on the wing of flies carrying multiple doses of Notch+ is virtually the same as that of wild type, i.e. the Abruptex phenotype is not reproduced to any significant extent. This suggests that the single amino acid substitutions that occur in Abruptex mutants confer on the protein some functionally distinctive feature, possibly more powerful intermolecular binding or altered stability.

Key words: Drosophila, adult peripheral nervous system, Notch locus.

Introduction
In recent years, a set of genes whose mutations have a common phenotype – production of nervous tissue at the expense of epidermis – have been identified in Drosophila and collectively called the neurogenic genes (Campos-Ortega, 1988). It is believed that the products of these genes are involved in cell-cell communication, and that the switching of cells from the epidermal to the neural pathway of differentiation in the mutants reflects the breakdown of this communication process.

Historically, the first of the neurogenic genes to be recognized as such was Notch (Poulson, 1937, 1940). The adult, dominant phenotype is a slight notching of the wing, but homozygous Notch embryos die because epidermal cells in the neurogenic region are replaced by nerve cells and no cuticle is produced there. There is now strong evidence that the basic process affected by Notch has to do with cellular interactions (Artavanis-Tsakonas, 1988; Campos-Ortega, 1988). The Notch product is a membrane-associated protein with substantial similarity to mammalian epidermal growth factor (EGF), and is therefore a molecule that could well be involved in signalling (Wharton et al. 1985; Kidd et al. 1986). Notch expression is seen throughout the embryo, both within and outside the neurogenic areas (Hartley et al. 1987; Kidd et al. 1989; Johansen et al. 1989). This is consistent with the extremely complex phenotype that Notch mutants can assume (Shellenbarger and Mohler, 1978), and with detailed observations in the retina which indicate that this phenotype is not limited to an epidermal-to-neural transformation (Cagan and Ready, 1989).

We have examined the neural phenotype of a series of Abruptex mutations, Notch alleles that are recognized because of their effects on wing venation (Nasar-enko, 1930; Foster, 1975; Portin, 1975), and in which the molecular lesions consist of single amino acid substitutions in the EGF-like repeat regions of the Notch protein (Hartley et al. 1987; Kelley et al. 1987). We find that there is a consistent loss of specific sensory structures found on the wing’s cuticular surface, and a concomitant loss of the neurons that innervate them. We present evidence that this represents an antineurogenic phenotype in which sensillar mother cells do not form, not the replacement of cuticle-secreting epidermal cells by neurons as might have been expected from the neurogenic phenotype of Notch mutations (Hartenstein and Campos-Ortega, 1986; Hoppe and Green-span, 1986).

Materials and methods
Stocks
Viable Abruptex alleles have been classified into two groups
on the basis of their interaction with Notch mutations: as transheterozygotes, some enhance the Notch phenotype in the adult wing, and others suppress it (Foster, 1975; Portin, 1975). In addition, some alleles are lethal in the homozygous condition. We have examined two enhancers (Ax51d and Ax28a), one suppressor (Ax309) and one lethal (Ax55b). The stocks were obtained from M.W. Young (Rockefeller University) and W.D. Welshons (Iowa State University). They were used directly, without any attempt to generate a uniform genetic background. Qualitatively similar effects were seen in all the stocks. The data presented here were obtained primarily from the two enhancer stocks.

For developmental studies, Ax51d was crossed into the enhancer trap line A37 whose bacterial β-galactosidase insertion marks all 4 cells of a developing sensillum (Ghysen and O’Kane, 1989). The male progeny of this cross were hemizygous for Ax51d and heterozygous for A37; a single copy of the β-gal insertion was sufficient to give clear staining of the nuclei. Double mutant stocks were also produced with hairy1 (h1) in order to test the effect of Ax on a novel population of sensilla. Both of these constructions either placed the autosomes of the originally homozygous Ax stocks in a heterozygous condition or replaced them, and in neither case was any effect on the mutant sensillar phenotype observed. Thus, the effects of genetic background on this phenotype must be rather small and/or associated selectively with the X chromosome.

The following crosses were made to obtain individuals with different doses of Notch+: From the cross w/w;+/- X y w N264-40 rb/Y; Dp(1,2)S16b/+, male progeny carrying the duplication that covers the Notch and w locus were selected on the basis of their red eyes. They were then backcrossed to w/w females. The resulting female offspring carrying 2 doses of Notch+ were recognized by their white eyes, those with 3 doses of Notch+ by their red eyes, since they carried the w+ gene (and N+) in the duplication. Similarly, males with 1 copy of Notch+ had white eyes and those with 2 copies had red eyes. y w N264-40 rb/+ females were considered to carry 1 dose of Notch+ since this Notch allele is a recessive embryonic lethal.

All data on adult wings were obtained from animals reared at 25°C, because the strength of the Ax phenotype is influenced by temperature (Portin and Siren, 1976).

Immunocytochemistry

Developing animals of desired ages were obtained by selecting white prepupae and maintaining them at 25°C; ages are given as h after pupariation (AP). Neurons were labelled with anti-HRP antiserum (Cappell Labs) or monoclonal antibody 2C10 (kindly provided by S. Benzer), followed by an FITC-conjugated secondary antibody (Murray et al., 1984), or by a biotin-avidin system (Vectastain ABC kit, Vector Labs, used according to the manufacturer’s instructions). The bacterial β-galactosidase of the A37 strain was revealed using an appropriate monoclonal antibody (Promega) followed by the Vectastain ABC (Elite) procedure (Vector Labs).

Scoring

Sensilla in the TSM location and distally on L3 cannot reliably be identified as individuals when one or several of them are missing. Therefore, these sensilla and their neurons were always scored as a group: for example, in 10 specimens 30 L3 sensilla would be expected, and if only 20 were found the probability of occurrence would be given as 67% (Figs 1, 3, 4 and 6; Table 2). There was only one exception to this procedure. In Fig. 3, plotting the probability of finding adult sensilla against the probability of finding the corresponding clusters of marked cells in the pupa, we were unable to distinguish reliably between 1 and 2 clusters in the TSM location because the clusters are so close together. Therefore, we only scored for the presence or absence of any marked nuclei. Correspondingly, in the adult wings, we scored for the presence of at least 1 TSM sensillum, and did not distinguish between 1 and 2.

Results

The sensory complement of the wild type wing

The cuticular sense organs of the wild type Drosophila wing blade are of two types: three rows of mechanosensory bristles along the anterior edge, and strain-detecting campaniform sensilla found in specific locations along several wing veins as shown in Fig. 1. The cuticular component of each campaniform sensillum consists of a central dome surrounded by a circular socket. Its single sensory neuron has a dendrite that attaches to the center of the inner face of the dome and an axon that projects into the CNS (Palka et al. 1986).

<table>
<thead>
<tr>
<th>GSR</th>
<th>TSM</th>
<th>L3-v</th>
<th>ACV</th>
<th>L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+/+ (Sev)</td>
<td>100</td>
<td>95</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A37</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>28a/Y (S)</td>
<td>100</td>
<td>85</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>E2/Y (E)</td>
<td>100</td>
<td>85</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td>71d/Y (E)</td>
<td>100</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>59b/+ (L)</td>
<td>100</td>
<td>0</td>
<td>45</td>
<td>6</td>
</tr>
</tbody>
</table>

![Fig. 1. The location of campaniform sensilla on the wing blade and the probability of their occurrence in wild type (+/+; Sev: Sevellen strain); the enhancer-trap strain A37; Ax51d males (28a/Y), a suppressor of N (S); Ax28 (E2/Y) and Ax51d (71d/Y) males, both enhancers (E) of N; and heterozygous Ax55b females (59b/+ carrying a lethal (L) Ax allele. GSR, Giant Sensillum of the Radius; TSM, the two Twin Sensilla of the Margin; L3-v, Ventral Sensillum of Vein L3; ACV, Anterior Cross Vein Sensillum; L3, the three Sensilla of Vein L3. Early-developing sensilla are shown with closed circles, late-developing ones with open circles (see text). The sensory nerves are shown in black; the thick marginal nerve carries the axons of all the marginal bristle neurons, the thin nerve in vein L3 the axons of its campaniform neurons and E-cells (see text). n=20-40.](image)
The campaniform sensory neurons of the wing blade arise within two time windows during the first 24 h of metamorphosis (Murray et al. 1984; Fig. 1). The early neurons (GSR, one of the two TSM, ACV and one of the three L3 sensilla) are recognized by their immunoreactivity to anti-HRP and 22C10 by zero h AP, while the late neurons (L3-v, the two other L3 sensilla and the second TSM) become immunoreactive between 4 and 10 h AP. The two groups also differ in their physiology and axonal projections (Palka et al. 1986; Dickinson and Palka, 1987).

Developing with the early TSM, ACV and the early L3 are so-called ‘extra’ or E-cells, neurons of unknown function whose multiple dendrites are not associated with cuticular specializations but rather remain confined within the hemolymph of the wing veins; they resemble the multiple dendrite (md) neurons described in Drosophila embryos (Bodmer and Jan, 1987). Because they have no visible cuticular component, we have not analyzed these cells closely in the present study. However, they are affected by Ax mutations in much the same way as are the nearby campaniform sensilla.

The adult Ax phenotype
The classic Ax phenotype (Nasarenko, 1930) is a region-specific effect on vein formation, and our observations agree with the original description. The tip of vein L5 was absent in all cases. The tips of both veins L4 and L5 were missing in the majority of wings, and the distal half of vein L2 was often interrupted. Vein L3 and proximal parts of veins L2 and L4 were affected only rarely, and vein L1 was spared in all cases. The venation phenotypes of the different alleles were very similar, except that Ax^{50} was very weak and of low penetrance. When Ax was combined with hairy, the venation phenotype became more variable and often stronger (more veins showed longer gaps).

There was little or no effect of any Ax allele on the sensory bristles of the anterior margin, but the population of campaniform sensilla was significantly altered. Fig. 1 shows the frequencies with which specific sensilla were encountered in the wings of adult wild-type flies of two strains and in the several Ax mutant stocks. The probability of occurrence was both sensillum-specific and strain-specific: at the extremes, the GSR was completely spared in all strains, while the L3-v and both TSMs were completely lacking in specific Ax mutant stocks. Members of both the early- and the late-developing groups of sensilla were affected by Ax. It is striking that the sensillar phenotypes could differ so widely among strains even though the venation phenotypes were largely indistinguishable. Among the homozygous viable stocks, Ax^{7ld} had the strongest sensillar phenotype and was used in many of the analyses described below.

A similar antisensillar effect was observed when Ax was combined with hairy, a mutation that causes the development of numerous supernumerary bristles and campaniform sensilla in ectopic locations during a third wave of differentiation between 17 and 26 h AP (Palka et al. 1983). As an index of this effect, Table 1 compares the number of sensory structures along L2 (which normally carries no sensilla at all) in h and in Ax; h double mutants, in individuals selected for minimal disruption of this vein. Both campaniform sensilla and bristles of this novel sensillar population were reduced in number, and again Ax^{7ld} proved to be more potent than Ax^{E2}.

Sensillar development tracked by means of the A37 reporter gene
The A37 stock carrying a bacterial /3-galactosidase insertion has been described as marking all the nuclei of a developing sensillum, from the single cell (mother cell) stage to the full complement of 4 cells per sensillum (Ghysen and O'Kane, 1989). Thus, it can be used to evaluate the several possible alterations in cell lineage that might be responsible for a sensillum’s failure to appear in the cuticle of the adult wing. We will argue that the principal effect of Ax is to reduce the probability that the mother cell of a sensillum will form (Figs 2-4).

Table 1. Ax-h interaction evaluated by scoring the average number of bristles and campaniform sensilla found on vein L2, a vein that normally carries no sensilla of either type

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax^{7ld};h</td>
<td>46</td>
<td>33</td>
</tr>
<tr>
<td>Ax^{E2};h</td>
<td>22</td>
<td>48%</td>
</tr>
<tr>
<td>Ax^{7ld};h</td>
<td>7</td>
<td>16%</td>
</tr>
</tbody>
</table>

The animals were homozygous for h and homozygous (females) or hemizygous (males) for Ax. n=6.
Fig. 2. Preparations of pupal wings from A37 (A and C) and Ax<sup>71d</sup>; A37 animals (B and D), stained with an antibody to β-galactosidase. (A and B) 2–3 h wings; (C and D) 25 h wings. Sensilla identified as in Fig. 1. In A37 animals at 25 h AP (C), there are three groups of stained nuclei belonging to the L3 sensilla; in Ax<sup>71d</sup> (D) there are only two. The failure of cells to stain in mutant pupal wings (arrowheads) anticipates the failure of the corresponding sensilla to appear in the cuticle of adult wings (Fig. 3). In B, the nuclei along the margin are not conspicuous because they are out of focus. Scale bar in A and B: 50 μm; in C and D: 100 μm.

Fig. 3. Regression analysis of the probability of finding an adult sensillum on the probability of finding the corresponding A37-positive pupal cell cluster at 25 h AP (closed circles) in Ax<sup>71d</sup> males. The slope of the line is well within the 95% confidence of the theoretical slope of 1.000, and the occurrence of clusters predicts the occurrence of adult sensilla virtually perfectly (R<sup>2</sup>=0.963). Open circles: probability of cell clusters in 2–3 h pupal wings from Ax<sup>71d</sup> males. n=30–33.
was at the other extreme, being present in only a single wing in this sample of Ax-A37 animals; significantly, the single cluster that was found contained the normal 4 nuclei. As shown earlier in Fig. 3, the probability of occurrence of an L3 cluster in mutant animals was close to 0.5 (approximately half the cases fell in the zero column). However, the mean number of nuclei per cluster when a cluster was present was indistinguishable from the control, and the distributions for mutant and N+ wings were again overlapping (Mann-Whitney U-test; \( P=0.4-0.5 \) for both GSR and L3). On the hypothesis of degeneration or arrested lineages, an increased incidence of clusters containing just 1 or 2 nuclei might be expected; this was not found.

In sum, the observation that marked clusters are either present with the full number of nuclei or are absent altogether strongly suggests that Ax affects the probability that a mother cell will be selected from the general epithelium.

**The pupal neuronal phenotype**

A cell cluster might be present and contain the proper number of cells, but differentiation within it might be abnormal. In particular, the formation of multiple neurons at the expense of other cell types within the sensillar lineage, such as can occur in Notch flies (M. Schubiger, unpublished observations; V. Hartenstein, personal communication), might be expected. To examine this possibility, we stained pupal wings for neurons using both anti-HRP and 22C10 antibodies (Fig. 5). We found no instances of multiple neurons in single locations in 6 or 36 h wings, where individual neurons can be identified with minimal ambiguity, and therefore reject this interpretation of the antisensillar phenotype.

In addition, we observed an extremely strong corre-

---

**Fig. 4.** Distribution of number of A37-positive nuclei per sensillar cluster in N+ and in Ax-A37 25 h AP wings, exemplified by the GSR, ACV and L3 sensilla. GSR is unaffected by this mutation, and the distributions of N+ (A37; solid bars) and mutant (hatched bars) nuclear counts overlap completely. The ACV virtually never appears in Ax-A37; in this sample, it is represented only in one case outside the zero column. The L3 sensilla appear with a combined probability of about 0.5 both in the adult and in the pupa (cf. Fig. 3), but the distribution of nuclear number in the clusters that do occur overlaps with N+ (A37). The Mann-Whitney U test yielded \( P=0.4-0.5 \) for all comparisons of mean number of nuclei (excluding zeros) in N+ and mutant clusters. \( n=32-33 \).

**Fig. 5.** Abnormalities in nerve formation. In wild type flies, the pattern of peripheral nerves in 36 h AP pupal wings anticipates that illustrated for adults in Fig. 1. In Ax-A37 flies, the marginal nerve (A) often shows neuroma-like swellings (arrow), and may also be truncated (arrowhead), or the marginal nerve may be normal while the L3 nerve (B) leaves vein L3 (arrowhead), wanders into the posterior regions of the wing which are normally completely nerve-free and often branches (arrows). Scale bars: 50 \( \mu \)m.
lation between the probability of finding a specific sensillum in the adult and finding a single neuron at the corresponding location in the pupa (Fig. 6). Finding pupal neurons with lower probability than adult sensilla (data points above the 45° line) would suggest a failure of the neurons to differentiate, or possibly their selective degeneration. Finding neurons with a higher probability than sensilla (data points below the 45° line) would imply a deficiency of trichogen and tormogen secretory receptors at the anterior edge of the wing, and a thin nerve along vein L3 formed by the axons of its cuticle-secreting cells.

Abnormalities in nerve formation

Fig. 1 shows the simple pattern of peripheral nerves seen in the wing blade: a thick nerve along the margin formed by the axons of the mecano- and chemosensory receptors at the anterior edge of the wing, and a thin nerve along vein L3 formed by the axons of its campaniform sensilla. As illustrated in Fig. 5, in Ax71d the marginal nerve frequently showed anomalies such as truncation or the formation of a neuroma, and the L3 nerve was often misrouted, particularly in the region between the ACV and the GSR. Neither of these classes of abnormalities has been analyzed in detail, but they do raise the possibility that the Notch protein may have some involvement in axonal pathfinding.

Effects of gene dosage

Loss of function Notch mutations are neurogenic, while Ax mutations in the same protein are antineurogenic. Is the Ax phenotype a specific property of the altered Notch protein, or does it simply reflect an increase in Notch function such as would also result from more wild type protein being made?

We examined this question by constructing stocks with variable doses of Notch+, 1–3 doses for females and, assuming dosage compensation, 2 or 4 doses for males. Table 2 shows the outcome of the experiment: females with a single dose of N+ (N+/N−) showed slightly elevated numbers of sensilla, whereas both females and males with multiple doses showed a loss, but this was limited to the TSM. For comparison, Table 2 also shows the effect of Ax71d in single and double dose, i.e. Ax71d/N+ and Ax71d/Ax71d. The antineurogenic effect arising from even a single copy of the mutant gene far exceeded that of an increased dose of the wild type gene, was pronounced in all sensilla except the GSR, and was substantially enhanced (in fact, approximately doubled) when both copies of N+ were replaced by Ax71d.

Discussion

Ax suppresses the formation of specific sensilla

In the first formal description of Abruptex, Nasarenko (1930) described a reduction in the probability of occurrence of specific macrochaetes on the head and thorax, in addition to the short-vein phenotype. We have found a loss of specific campaniform sensilla on the wing blade, an effect not recognized in previous phenotypic descriptions. In the adult wing blade, Ax mutations remove both early- and late-developing campaniform sensilla; as in the case of the macrochaetes, some sensilla are more susceptible to removal than others. The expression of this phenotype varies in the different Ax stocks. The triple row bristles along the leading edge of the wing were not significantly affected by Ax in any genotype we examined.

This suppressive effect of Ax on normal macrochaetes and campaniform sensilla is paralleled by the suppression of supernumerary sensory structures, both bristles and campaniform sensilla, on the wings of double mutants with hairy (Ax;h). The effect is clear even when the supernumeraries are located on veins

---

**Table 2. Effect of dosage of Notch+ and of Ax71d on the formation of sensilla in the wing**

<table>
<thead>
<tr>
<th>Number of copies</th>
<th>Notch+ males</th>
<th>Notch+ females</th>
<th>Ax71d males</th>
<th>Ax71d females</th>
<th>ACV</th>
<th>L3-v</th>
<th>L3-v</th>
<th>L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>106</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

t=22–46.

*Note that, because of dosage compensation, 1 copy in a male corresponds to 2 copies in a female.
that are not markedly affected by the venation phenotype of Ax, or when they are between veins. Since the supernumeraries develop at a later time as well as in different places than do the normal sensilla (Palka et al. 1983), this result demonstrates that the antisensillar effect of Ax in the wing is not limited to a single class of sensory structures, to a single vein-defined region, or to the normal time windows of sensillar differentiation.

Evidence that sensillar mother cells do not form

The failure of the cuticular components of a sensillum to form could be due to any of several processes, including:

(i) A cuticular-to-neural transformation within the lineage.
(ii) Failure of successfully formed sensillar cells to differentiate.
(iii) Degeneration within the lineage.
(iv) Arrest of the lineage at one or two cells.
(v) Failure of the mother cell to form.

Our data allow us to argue strongly for the dominant importance of the last alternative.

Process (i), a Notch-like transformation of cuticle-secreting cells to neurons, does not occur because no multi-neuronal clusters are found, and because the probability of finding neurons in pupal wings does not exceed the probability of finding the corresponding sensilla in the adult (Fig. 6). Processes (ii), (iii) and (iv), resulting in a loss of trichogen and tormogen cells or in their inability to secrete cuticle, would predict that the probability of finding an A37-positive cell cluster in the pupa should be greater than the probability of finding the corresponding sensillum in the adult, and this is not the case (Fig. 3). Processes (iii) and (iv) would predict a low final number of cells per cluster, but Fig. 4 shows that if cells are detectable at all in a specific location, approximately the normal number of cells in the cluster is reached by 25h. Essentially no clusters containing just 1 or 2 nuclei were found.

On a statistical basis, a cluster is either present, contains a neuron and is associated with a dome and socket in the adult, or it is absent altogether (contains no A37-positive nuclei, or anti-HRP-positive neuron, and forms no corresponding adult cuticular structures). Given this all-or-none relationship, we conclude that Ax\textsuperscript{21d} causes a failure of sensillum formation primarily by preventing the formation of sensillar mother cells (as recognized by A37 staining) rather than by interfering with subsequent processes. We have seen no reason to suspect that any of the other alleles acts differently.

'Bipolar' phenotypes

Even though Notch mutants can have varied and complex phenotypes, it is clear that the null phenotype includes an extremely powerful neuralizing effect in the embryonic ventral neurogenic region where the CNS forms (Lehmann et al. 1983), as well as causing supernumerary neurons to form in the embryonic PNS (Hartenstein and Campos-Ortega, 1986; Hoppe and Greenspan, 1986). Extra photoreceptors are found in Notch\textsuperscript{+} clones in adult eyes (Dietrich and Campos-Ortega, 1984) and in Notch\textsuperscript{b} mutants exposed to the appropriate temperature shift (Cagan and Ready, 1989). We have found at least a slight tendency to form extra campaniform sensilla on the wings of Notch heterozygotes (Table 2). Thus, it is clear that molecular lesions in the Notch gene frequently produce a powerful neurogenic phenotype, even though other transformations in cell fate can occur as well (Cagan and Ready, 1989).

The Ax phenotype stands in marked contrast to this, even though it is caused by lesions in the same gene. Nasarenko (1930) described a loss of specific macrochaetes on the thorax, in the present study we have documented a loss of specific campaniform sensilla and their neurons in the wing, and we have also seen a loss of neurons in the embryonic PNS (M. Schubiger, unpublished). Clearly, changes in different parts of the Notch gene can cause opposing effects.

A phenotypically similar situation has been described for Enhancer of split (E(spl)), another of the neurogenic genes (Knust et al. 1987a). Loss-of-function mutations of E(spl) cause a neurogenic phenotype in both the CNS and the PNS of the embryo. The allele E(spl)\textsuperscript{D} is interpreted as a gain-of-function mutation, in part because its phenotype is mimicked by three doses of E(spl)\textsuperscript{+}. It causes the opposite phenotype, namely a reduction of the CNS and PNS. Thus, lesions in both Notch and E(spl) can cause both neurogenic and antineurogenic effects. A detailed interpretation of this parallel is complicated by the internal complexity of the E(spl) region, from which as many as 6 (Preiss et al. 1988) or 11 (Knust et al. 1987b) transcripts have been reported. Nevertheless, the observation of 'bipolar' phenotypes among the mutations of two genes whose products are both thought to participate in the same cell communication process may prove to be useful in understanding the role of that process in the control of differentiation.

Beyond neurogenesis

The prevailing interpretation of the role of the neurogenic genes, including Notch, has been that they play a critical role in processes that generate alternative decisions regarding cell fate, conspicuously but not exclusively the decision between neural and epidermal fate (Campos-Ortega, 1988; Artavanis-Tsakonas, 1988; Cagan and Ready, 1989). However, a strong argument has been presented that the Notch protein is also required for the maintenance of such decisions over time (Hoppe and Greenspan, submitted). Our data on Ax are certainly consistent with the decision-making interpretation, but also raise the possibility of a role of the protein in later processes such as axon extension and/or pathfinding.

The formation of peripheral nerves along their specified pathways is a robust process (e.g. Blair et al. 1987). Without further study it is not clear whether such drastic alterations in the nerve pattern as are illustrated in Fig. 5 reflect a change in the interaction between the axons and their epithelial substrate, or whether the internal architecture of the wing is altered and the
effects on axons are secondary. Should the former prove to be the case, however, then the Notch protein would be shown to have an important developmental role at a time far beyond the initial decision concerning neural or non-neural fate. It would also suggest a possible functional explanation for the observation that the protein is heavily expressed in developing axon bundles within the CNS as well as in the very regions of the pupal wing in which the axons of Ax flies lose their way (Kidd et al. 1989; Johansen et al. 1989).

Abruptex and Notch

Foster (1975) and Portin (1975) studied the interactions of Notch and Ax alleles in transheterozygotes, and produced a 3-fold classification on the basis of adult wing morphology and of lethality: some Ax alleles enhance the Notch phenotype (e.g. Ax$^{E2}$ and Ax$^{71d}$), some suppress it (e.g. Ax$^{286}$), and some are lethal (e.g. Ax$^{29b}$). However, we find that all these stocks show an antisensillar phenotype, no matter which of the three groups they fall into; in fact Ax$^{71d}$, a Notch enhancer, has a particularly strong antisensillar effect. Thus, the diverse interactions of Ax alleles with Notch mutations do not predict their consistent effect on the generation of sensilla. The reason for this contrast is not clear.

Molecular cloning and DNA sequencing (Kelley et al. 1987; Hartley et al. 1987) has shown that these Ax alleles differ from each other only in precisely which single amino acid of the extracellular domain of the Notch protein is altered. Furthermore they all, enhancers, suppressors and lethals alike, fall within a 226 amino acid region containing only 6 of the 36 EGF-like repeats. Kelley et al. have suggested that all Ax proteins are more active than the wild type Notch protein on some functional criterion, possibly having to do with intermolecular binding. This view is consistent with our finding that the phenotype of all Ax alleles tested is the opposite of the loss-of-function Notch phenotype. Our gene dosage analysis shows that the phenotype resulting from up to 4 doses of N$^{+}$ is similar to the wild type (unlike the case in E(spl)). It does not approach the powerful antisensillar phenotype of Ax mutants (Table 2), but the slight deviations that do occur are in the antineurogenic direction. In contrast, increasing the dosage of Ax$^{71d}$ from 1 to 2 markedly increases the already strong antineurogenic effect. Taken together, these observations are consistent with the interpretation that their single amino acid substitutions confer on the Ax proteins some property, perhaps significantly higher binding activity (Kelley et al. 1987) or altered stability (R.G. Fehon, pers. comm.), that is not easily mimicked by simply making more of the wild type protein.

We thank Michael Young for extremely helpful discussions, and Peter Kareiva and Scott Freeman for advice on statistics. We extend our thanks to Richard Fehon, Ralph Greenspan, Michael Young and members of our laboratory for comments on the manuscript. Supported by grants BNS88-12865 (NSF) and NS07778 (NIH Jacob Javits Award) to JP; HS was supported by REU supplement BNS87-43527 (NSF).

References


neurons in relation to their time of development. J. Neurosci. 6, 1822–1830.


(Accepted 6 February 1990)