Development 124, 2819-2828 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
DEV7578

Genetic basis of the formation and identity of type I and type II neurons in *Drosophila* embryos

Michel Vervoort[1,3,*], David J. Merritt[2], Alain Ghysen[3] and Christine Dambly-Chaudière[1,3]

[1]Laboratoire de Génétique du Développement, Université Libre de Bruxelles, 67 rue des Chevaux, 1640 Rhode-St-Genèse, Belgium
[2]Department of Entomology, University of Queensland, St Lucia, Qld 4072, Australia
[3]Laboratoire de Neurogénétique, Unité 432 INSERM, Université de Montpellier II, Place E. Bataillon, case courrier 103, 34095 Montpellier Cedex 5, France

*Author for correspondence at address 3 (e-mail: mvervoor@dbm.ulb.ac.be)

SUMMARY

The embryonic peripheral nervous system of *Drosophila* contains two main types of sensory neurons: type I neurons, which innervate external sense organs and chordotonal organs, and type II multidendritic neurons. Here, we analyse the origin of the difference between type I and type II in the case of the neurons that depend on the proneural genes of the *achaete-scute* complex (ASC). We show that, in *Notch*$^-$ embryos, the type I neurons are missing while type II neurons are produced in excess, indicating that the type I/type II choice relies on *Notch*-mediated cell communication. In contrast, both type I and type II neurons are absent in *numb*$^-$ embryos and after ubiquitous expression of *tramtrack*, indicating that the activity of *numb* and the absence of *tramtrack* are required to produce both external sense organ and multidendritic neural fates. The analysis of *string*$^-$ embryos reveals that when the precursors are unable to divide they differentiate mostly into type II neurons, indicating that the type II is the default neuronal fate. We also report a new mutant phenotype where the ASC-dependent neurons are converted into type II neurons, providing evidence for the existence of one or more genes required for maintaining the alternative (type I) fate. Our results suggest that the same mechanism of type I/type II specification may operate at a late step of the ASC-dependent lineages, when multidendritic neurons arise as siblings of the external sense organ neurons and, at an early step, when other multidendritic neurons precursors arise as siblings of external sense organ precursors.

Key words: *Drosophila*, sense organ, neuronal identity, cell fate determination

INTRODUCTION

The sensory neurons of arthropods belong to either of two main types, called type I and type II (Zacharuk, 1985). Type I neurons are characterized by their single dendrite whose distal part is a modified cilium and are associated with accessory cells which form the non-neuronal part of the sense organs. In contrast, type II neurons possess several dendrites lacking ciliated structures, and are not associated with accessory cells. This division into type I and type II neurons extends to other phyla, and could represent an ancient feature of sensory systems (Bullock and Horridge, 1965; Lentz, 1968; Blackshaw, 1981).

Type I and II neurons are easily distinguished in the *Drosophila* embryo, where the sensory neurons are arranged in a highly stereotyped pattern (Fig. 1A; Campos-Ortega and Hartenstein, 1985; Ghysen et al, 1986; Bodmer and Jan, 1987; Hartenstein, 1988). Type I neurons innervate two main types of sense organs: the **e**xternal **s**ense organs (es organs) and the **ch**ordotonal organs (ch organs; Fig. 1A; Ghysen et al, 1986; Hartenstein, 1988), and are called es and ch neurons, respectively. Type II neurons have been named **m**ulti**d**endritic neurons (md neurons; Fig.1; Bodmer and Jan, 1987), as most of them have profuse dendritic arborizations which end freely under the epidermis (Bodmer and Jan, 1987). A large body of cellular, genetic and molecular analyses has produced a wealth of information about the development of the ch and es organs and their neurons (Jan and Jan, 1993). In contrast, little is known about the origin and development of the md neurons.

All type I neurons derive from precursor cells (Bate, 1978) through a fixed lineage that also produces the accessory cells of the sense organs (Bate, 1978; Bodmer et al., 1989; Brewster and Bodmer, 1995). By analogy, it has been suggested that md neurons also originate from precursor cells, likely to be born around the same time as es and ch precursors (Bodmer et al., 1989). Recently, the origin of md neurons has been directly assessed using FLP/FRT-induced *lacZ* clones (Struhl and Basler, 1993). The results (Fig. 1B; Brewster and Bodmer, 1995) suggest that some md neurons are produced by precursors that also generate ch or es cells. In that case, the md neuron appears to be the sister cell of the es or ch neuron. Other md neurons appear to have precursors which do not produce es or

ch neurons ('solo' md neurons). Finally, some es and ch lineages do not produce md neurons ('solo' es and 'solo' ch lineages).

The competence to become a precursor cell depends on the expression of one or more 'proneural genes' (Ghysen and Dambly-Chaudière, 1989). Among the known proneural genes, those of the *achaete-scute* complex (ASC) are required for the formation of the es organs (Garcia-Bellido and Santamaria, 1978; Garcia-Bellido, 1979; Garcia-Alonso and Garcia-Bellido, 1986). In the embryo, ASC genes are required not only for the formation of all es organs, but also for most md neurons (Dambly-Chaudière and Ghysen, 1987). Another gene, *atonal* (*ato*), is responsible for the formation of the ch organs and of a small subset of md neurons (Jarman et al., 1993).

In this paper, we investigate the origin of the difference between es and md identities among the ASC-dependent lineages. Our results indicate that this specification uses the same mechanism that assigns the different cell fates within the es and ch lineages (for review, see Posakony, 1994, Jan and Jan, 1995; Vervoort et al., 1997), and suggest that the default state of the ASC-dependent lineages is the md fate. This default state requires the activity of *numb* (Uemura et al., 1989) and the silence of *tramtrack* (*ttk*; Read and Manley, 1992). The alternative fate (es) requires *Notch*-mediated cell communication (Fortini and Artavanis-Tsakonas, 1993), as well as a new factor that we named *X1*.

MATERIALS AND METHODS

Drosophila strains

Oregon R was used as wild-type strain. We used the following mutant stocks: w^a N^{55e11} / *FM7C* and *Df(1)N8* / *In(1)dl49, y Hw* m^2 g^4 for the analysis of *N* phenotypes; $numb^1$ / *CyO* (Uemura et al., 1989) for the analysis of *numb*; ttk^{1e11} / *TM3 Sb Ser* (Xiong and Montell, 1993) and *hs-ttk*^{P69} (Read and Manley, 1992) for *tramtrack*; *st e* stg^{7B69} / *TM3 Sb Ser* (Jurgens et al., 1984) for *string*; pbl^{70} / *TM3 Sb Ser* and pbl^{11D} / *TM3 Sb Ser* (Lehner, 1992) for *pebble*. *X1* was used as a *y X1 f* / *FM7c* stock.

As markers, we also used the following enhancer trap lines: *E7-2nd-36*, in which *lacZ* is expressed exclusively in the md neurons, *A1-2nd-29*, in which *lacZ* is expressed specifically in the outer accessory cells of the es organs (Bier et al., 1989), and *A37*, in which *lacZ* is expressed in all sense organ precursors and in their progeny (Ghysen and O'Kane, 1989). We assessed the effects of various mutations on these lines in the progeny of flies doubly heterozygous for the mutation and for the enhancer trap insertion.

Characterization of the *X1* deficiency

The stock containing the *X1* deficiency was originally sent to us as *Df(1)sd*^{72b}, a deficiency which deletes the 13F-14B region of the X-chromosome. We found, however, by using mutations and rearrangements of this region that our stock was not *sd*^{72b} but harboured another X-linked lethal mutation and renamed it *X1*. We localized the lethality by recombining *X1* with a *y w ct m f* chromosome. The lethality could not be separated from the w^+ allele among 612 males progeny. We confirmed the linkage between w^+, the lethality and the *X1* phenotypes by testing individually 25 *y* w^+ *ct m* / *y w ct m f* females, all of which failed to produce w^+ males and gave one fourth of the embryos with the cuticular (see below) and neuronal X1 phenotype. We used rearrangements of the *w* region (position 3C) to localize *X1* more precisely. The lethality due to X1 is rescued by the duplications *Dp(1;3)w*^{67k27} (3A4 to 3E8-F1) and *Dp(1;2)51b7* (3C1-2 to 3D6-E1), localizing the locus to the 3C1-2 to 3D6-E1 interval. We observed that *X1* does not complement the deletions *Df(1)N*^{71h} (3C4 to 3D5) and *Df(1)N*^{8} (3C2-3 to 3E3-4), but does complement *Df(1)N*^{69h9} (3C6 to 3D1-4) and *Df(1)w*$^{258–42}$ (3A4-6 to 3C5-6). Taken toghether, these results suggest that the X1 mutation maps between 3D1-4 to 3D5 or, if it were a deletion, that it is comprised between 3D1-4 and 3D6-E1.

We characterized the phenotypes of *X1* at the level of the es organs using additional markers to those described in the results section. First, we observed on cuticular preparations (Dambly-Chaudière and Ghysen, 1986), that the external structures of the es organs are absent, while all other cuticular derivatives are present. We confirmed that this absence is related to the absence of the es outer cells by using the *A1-2nd-29* enhancer trap line (Bier et al., 1989) and anti-Cut labelling (Blochlinger et al., 1990). We also observed that the es sheath cell markers anti-BarH1 (Higashima et al., 1992) and anti-Prospero (Vaessin et al., 1991) fail to label any cell in *X1* embryos.

Immunolabelling

Immunolabellings were made as described (Ghysen et al., 1986) using PBS as buffer. Double-labellings were made following Higashima et al. (1992) but using both Nickel and Cobalt ions. All antibodies were used at the published concentrations. We used a rabbit polyclonal anti-β-Gal antibody (Cappel; diluted 1:200) or, in some experiments, a mouse monoclonal anti-β-Gal antibody (named 40-1a; diluted 1:200) developed by J. R. Sanes, and a mouse monoclonal anti-Elav antibody (dilution 1:400) developed by G. Rubin. Both antibodies were purchased from the Hybridoma Bank (University of Iowa). The antibody against Poxn is a mouse hybridoma supernatant (G. Hassanzadeh, K. S. K. De Silva, C. Dambly-Chaudière, L. Brys, A. Ghysen, P. De Batselier, S. Muyldermans and R. Haemers, unpublished data; diluted 1:20) produced in the Algemene Biologie laboratory at the Vrij Universiteit Brussel (Belgium). Secondary antibodies were used according to the manufacturer's indications (Promega).

Heat-shock treatments

5-6 hours embryos containing two copies of the *hsp70*-p69 cDNA construct were placed for 1 hour in a water bath at 37°C and then transferred to 18°C until the end of embryogenesis.

DiI labelling

Single sensory neurons of X1 embryos at stage 17 were stained by in vivo injection with the carbocyanine dye, 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate (DiI; Molecular Probes) according to Merritt and Whitington (1995). Briefly, the tips of microelectrodes were filled with an ethanolic solution of DiI, brought into contact with a sensory neuron cell body and depolarizing current applied. After fixation, preparations were photoconverted in the presence of 0.2% diaminobenzidine to give a permanent dark reaction product and mounted in 100% glycerol.

RESULTS

Loss of es neurons and overproduction of md neurons in *N*⁻ embryos

Fig. 1A summarizes the pattern of sensory neurons in the abdominal segments of the embryo, and the proneural genes on which these neurons depend. 27 neurons (open profiles in Fig. 1A) depend on one or more genes of the *achaete-scute* complex (ASC genes), 11 neurons (stippled profile) depend on *atonal* and 3 neurons (dotted profile) depend on neither ASC nor *ato*. We have focused our analysis on the ASC-dependent sensory neurons because most larval md neurons fall in this category. ASC precursors display three major types of lineages, as illustrated in Fig. 1B: some of them produce both es and md cells (es/md precursors) while others produce only one of the

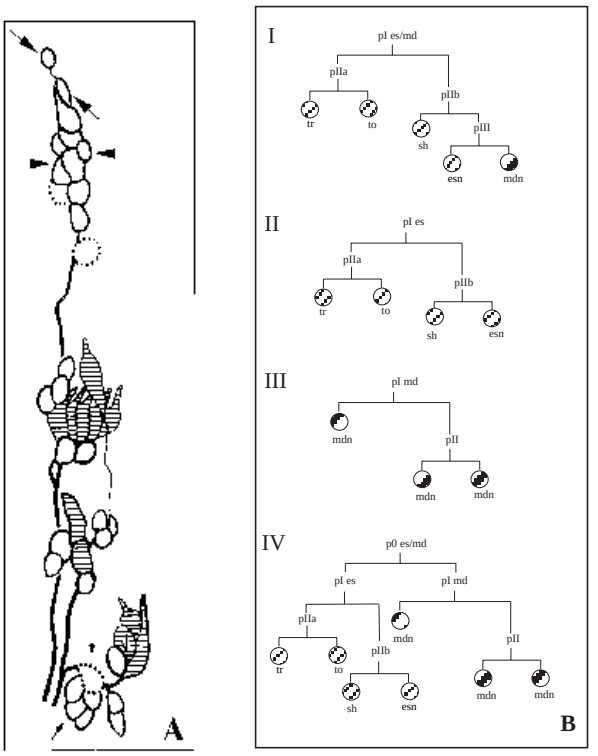

Fig. 1. (A) Drawing of the sensory neurons of an abdominal segment. Neurons with an open profile are ASC-dependent, striped ones are *ato*-dependent, dotted ones depend neither on ASC nor on *ato*. In this and all subsequent figures, dorsal is up and anterior is left. The lower arrows point to a group of ventral neurons which comprises four ASC-dependent md neurons and one ASC- and *ato*-independent neuron. The dorsal cluster comprises ten ASC-dependent neurons, five of which are md and five are es. The upper arrows point at the two dorsalmost es neurons, the arrowheads to two other es neurons of the dorsal cluster. (B) Schematic representation of the types of lineages of ASC-dependent precursors. Abbreviations are: esn, neuron innervating an es organ; mdn, md neuron; p0 es/md, protoprecursor; pI es/md, 'mixed' es/md precursor; pIIa, pIIb and pII are second-order precursors; pIII is a third-order precursor; sh, sheath cell (thecogen); to, tormogen (socket cell); tr, trichogen (shaft cell). I, II and III represent three types of lineages described by Brewster and Bodmer (1995). We propose (see discussion) that precursors following lineages I and II are related to each other, as shown in IV.

two types ('solo' es and 'solo' md precursors; Brewster and Bodmer, 1995).

The assignment of different cell fates in the es lineages requires cell interactions mediated by the product of the gene *Notch* (*N*; Posakony, 1994; Jan and Jan, 1995, Vervoort et al., 1997). In the absence of a functional *N* gene, sensory neurons are overproduced at the expense of the accessory cells (Hartenstein and Campos-Ortega, 1986; Hartenstein and Posakony, 1990b; Fig. 2A,B). We wondered whether *N* could also be involved in the assignment of the es and md fates. We analysed the identity of the neurons in N^- embryos, using specific markers of es or md neurons. The anti-BarH1 antibody labels es neurons (as well as their associated sheath cells) but none of the md neurons (Higashima et al., 1992). The enhancer-trap line *E7-2nd-36* expresses *lacZ* in most md neurons but not in es ones (Bier et al., 1989).

We observed in N^-; *E7-2nd-36* embryos large numbers of cells expressing *lacZ* (Fig. 2C,D) while no *BarH1*-expressing cells are present (Fig. 2E,F). In the dorsal cluster of neurons, which comprises only es and md neurons in the wild type, all neurons are of the md type in N^- conditions. To confirm that the supernumerary md neurons seen in N^- embryos are derived from ASC-expressing precursors, we used the anti-Cut antibody, which labels all ASC precursors and their progeny, but not the ASC-independent precursors (Blochlinger et al., 1990). We observed large numbers of *cut*-expressing cells in N^- embryos (Fig. 2G,H), suggesting that most supernumerary md neurons derive from ASC precursors.

We conclude from these experiments that N^+ is required to specify the es fate, since in N^- conditions only md neurons are produced. This is true not only in the 'mixed' es/md lineages, but also in the lineages that normally generate only es neurons (the 'solo' es lineages). This result suggests that all ASC-dependent precursors have the potentiality to produce md neurons and that the N^+ function is required in all types of ASC-dependent lineages to specify the es alternative.

Effects of *numb* on the formation of md neurons

The progeny of es precursors comprises three types of accessory cells and one or more neurons (Bate, 1978; Bodmer et al., 1989; Brewster and Bodmer, 1995). It is thought that the attribution of different identities to the daughter cells relies both on cellular communications, mediated by *N*, and on the unequal distribution of an intrinsic determinant, *numb* (reviewed in Posakony, 1994; Jan and Jan, 1995; Vervoort et al., 1997). In the former section, we showed that *N* is involved in the specification of es versus md fates among the neurons. We examined whether *numb* could also be involved in this specification.

Of the two daughters of es precursors, the first to divide (pIIa) produces two accessory cells, while the second one (pIIb) generates the neuron(s) and the associated sheath cell. In the absence of *numb*, the two daughters usually adopt the same fate, pIIa (Uemura et al., 1989). In some cases, the pIIb cell forms but its two daughters differentiate as sheath cells (Brewster and Bodmer, 1995). Both defects result in the loss of the es and md neurons, which belong to the es/md lineages (Brewster and Bodmer, 1995; our own observations). We observed that, in addition, most or all of the 'solo' md are also missing (Fig. 3A; see also Brewster and Bodmer, 1995). For example, in the abdominal ventral and dorsal clusters, the only remaining md neurons are those that do not depend on ASC. In order to determine whether the loss of 'solo' md neurons is due to their conversion into es accessory cells, we labelled $numb^-$ embryos with anti-Cut. The es sheath cells and neurons are converted into accessory cells as judged by their increased expression of *cut*. We observed few or no other cell expressing *cut* (Fig. 3B), suggesting that the md cells do not form, or loose their ability to express *cut*, or die. We conclude that *numb* is required to produce all es and md neurons including the 'solo' md ones.

Effects of *tramtrack* on the formation of md neurons

Based on the epistatic relationship between *numb* and *tramtrack* (*ttk*), it has been suggested that the main function of *numb* is to repress the expression of *ttk* (Guo et al., 1995). Embryos mutant for *ttk* have a phenotype opposite to that of $numb^-$ embryos: the

neurons and sheath cells of the es and ch organs are duplicated, at the expense of the support cells (Salzberg et al., 1994; Guo et al., 1995). We examined whether *ttk* could be involved in the formation of md neurons by extending the analysis of the *ttk* phenotypes to the md neurons. We used the ttk^{1E11} mutation (Xiong and Montell, 1993) which behaves as a strong hypomorphic mutation (Guo et al., 1995), as no null allele is available. We observed a doubling of both md and es neurons of the es/md lineages (by using anti-BarH1 and 22C10 labelling; not shown). This amplification is the expected result of the transformation of pIIa into pIIb. In contrast, we did not detect any modification of the 'solo' md neurons. This is most clearly seen in the case of the ventral abdominal md neurons (arrowed in Fig. 1) which are well separated from all other neurons and hence easily distinguishable. These neurons are present in normal number and pattern in ttk^{-} embryos (not shown).

A gain-of-function analysis revealed that the ubiquitous expression of *ttk*, at stages when the precursors of adult sense organs form, results in a loss of all precursors and consequently in the disappearance of the adult sensory structures (Ramaekers et al., 1997). We examined whether, while not required for the formation of embryonic md neurons, *ttk* could interfere with it. The ubiquitous expression of *ttk* at 5-6 hours of development results in an almost complete loss of neurons, including all the md ones (Fig. 3C). At the level of the es organs, we found two phenotypes. Some es organs are completely missing (absence of both 22C10 and anti-Cut labelling; Fig. 3C), probably reflecting the disappearance of the precursors. Other es organs are present and comprise four accessory cells (no 22C10 but strong anti-Cut labelling; Fig. 3C) as in $numb^{-}$ embryos. The extent of these two phenotypes is variable, probably depending on the exact stage at which the embryos were treated. In all cases, we observed a nearly complete loss of both 'solo' md neurons and of the md neurons coming from md/es precursors.

We conclude that the ubiquitous expression of *ttk* prevents the acquisition of both es and md neural fates, and therefore that *ttk* is not involved in the choice between es and md identities.

es and md neurons in the absence of cell divisions

Taken together, the previous results suggest that the primary fate of ASC precursors is to produce md neurons and that N-mediated cell communication is required to produce the alternative (es) fate. In order to assess directly the primary fate of ASC precursors, we examined the identity of the neurons produced in the absence of precursor divisions. In embryos mutant for the *string* gene (stg^{-}, Jurgens et al., 1984), which is required for the entry in M phase (Edgar and O'Farell, 1989), all divisions are blocked after the blastoderm stage, when the maternal supply of *stg* product is exhausted (Edgar and O'Farell, 1989). In such embryos, sense organ precursors do not divide but differentiate into neurons (Hartenstein and Posakony, 1990a; Fig. 4A).

We labelled stg^{-} embryos with es- or md-specific markers (anti-BarH1 and the *E7-2nd-36* enhancer trap line). Fig. 4B and C show that both md and es neurons are present. However, the proportion of the two types is dramatically altered. A wild-type abdominal hemisegment comprises 13 es organs and 21 md neurons. In stg^{-} embryos, we observed on the average 6.7±0.9 md cells (n=31) and only 0.7 es cells (0 cells in 60% of the observed segments, 1 in 15%, 2 in 15% and 3 in 10%, n=49). The positions of these cells vary from one embryo to another. The total number of ASC-dependent precursors, as revealed by anti-Cut labelling, was 7.1±1.0 (n=32; Fig. 4D). We conclude that most ASC-dependent precursors, and sometimes all of them, differentiate into md neurons in the absence of a functional *stg* gene.

stg mutations block not only cell divisions but also the progression through the cell cycle, as Stg protein is required to exit G_2 and enter M phase (Edgar and O'Farell, 1989). Recently, this G_2 arrest has been shown to be important for cell

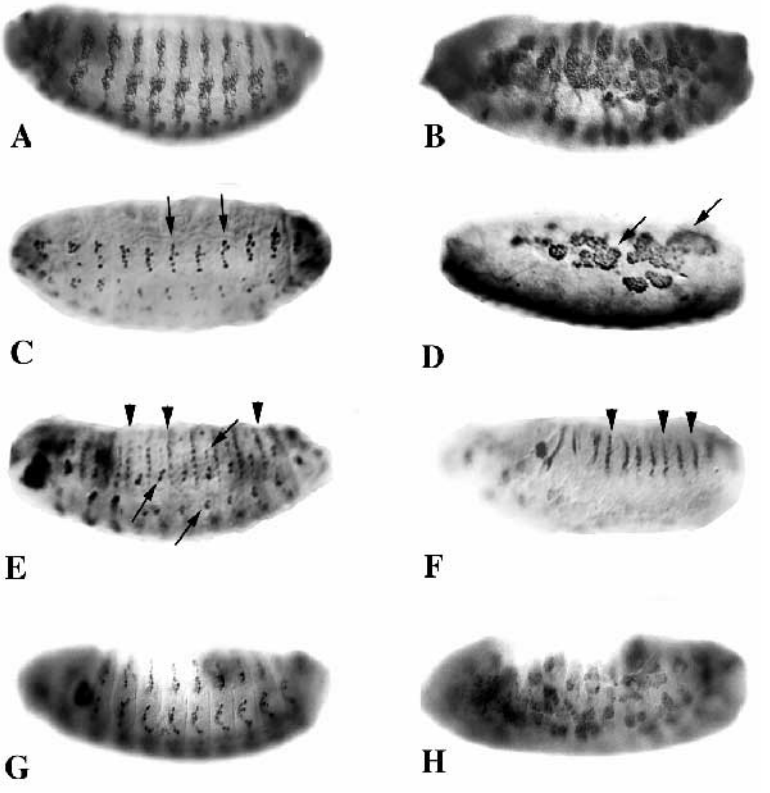

Fig. 2. In N^{-} embryos, ASC-dependent precursors produce md neurons only. (A,C,E,G) Wild-type;(B,D,F,H) N^{-} embryos. (A,B) Sensory neurons labelled with the 22C10 antibody (Fujita et al., 1982; Ghysen et al., 1986). (B) Note the large excess of neurons due to the *N* mutation. (C,D) *lacZ* expression in the *E7-2-36* enhancer trap line. Arrows (C) point to the dorsal clusters of md neurons in two abdominal segments. In N^{-} embryos (D), a large excess of labelled cells is observed, suggesting an overproduction of md neurons. Arrows point to two large clusters of dorsal neurons (compare with C). (E,F) Anti-BarH1 labellings. In the wild type (E), neurons and sheath cells of es organs (arrows) are observed between the stripes of labelled epidermal cells (arrowheads). In N^{-} embryos (F), the epidermal labelling is normal (arrowheads), but no es neuron or sheath cell can be detected. (G,H) Anti-Cut labelling, specific for the ASC-dependent lineage, is observed both in wild type and in N^{-} embryos.

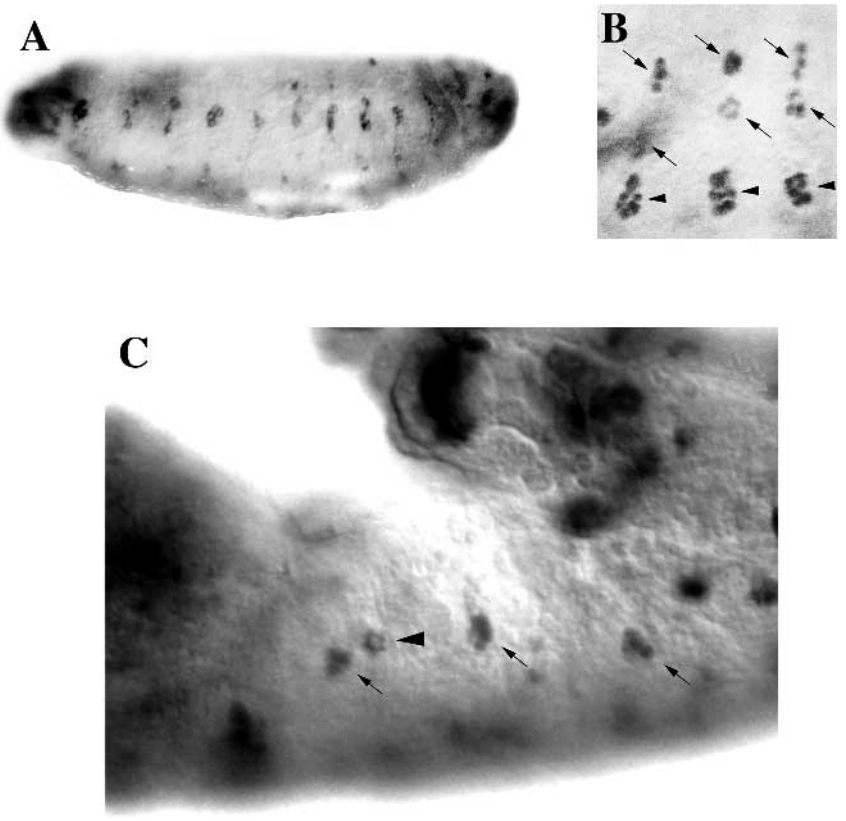

Fig. 3. Effects of *numb* and *ttk* on ASC-dependent sense organs. (A) 22C10-labelled *numb*$^{-}$ embryo. Most neurons are absent (compare with Fig. 2A). (B) Anti-Cut-labelled *numb*$^{-}$ embryo. The panel shows the dorsal clusters of three consecutive abdominal segments. The arrows point to clusters of 3-4 labelled cells, which correspond to the two dorsalmost es organs (see Fig. 1A). The arrowheads point to a cluster of up to eight cells which corresponds to the other two dorsal es organs. No other labelled cells are observed, indicating the absence of the dorsal md neurons. (C) Double labelling with anti-Cut (nuclear staining) and 22C10 (membrane staining) of an embryo containing the *hs-ttk*^{P69} construct and heat-shocked at 5-6 hours of development. This embryo did not complete the retraction of the germ band, presumably as a result of the heat shock. The arrows point to small groups of up to four cells expressing *cut*. These cells are not labelled by 22C10. The arrowhead points to the single neuron observed in this preparation, which does not express *cut*.

specification as it prevents the expression of the neuroblast identity gene, *even-skipped*, in some neuroblasts of the embryonic central nervous system (Cui and Doe, 1995; Weigmann and Lehner, 1995). We examined whether the loss of es neurons observed in *stg* mutants could be related to the blocking of the precursors in G_2 phase, and not to their inability to divide. For that purpose, we analysed *pebble* mutant embryos, in which cytokinesis (and hence cell division) but not cycle progression is prevented (Lehner, 1992; Hime and Saint, 1992). We observed that the precursors differentiate predominantly into md neurons (not shown), exactly as in *stg*$^{-}$ mutants.

These results indicate that all ASC-dependent precursors have a tendency to form md neurons, and that both cell division and cell communication are required to generate es neuronal identity. In that view, the occasional appearance of es neurons in *stg*$^{-}$ embryos could be a direct consequence of cell contacts between adjacent precursors. We tested that possibility by examining *N*$^{-}$; *stg*$^{-}$ double mutants where we would expect to observe a complete loss of es neurons if the hypothesis is correct. We observed 7.4±1.9 cells expressing the neuronal marker 22C10 (Fig. 4E) and 5.8±1.8 *cut*-expressing cells (Fig. 4F) per hemisegments in *N*$^{-}$*; stg*$^{-}$ embryos, but no *BarH1*-expressing cell was ever detected (not shown).

In a previous section, we showed that *numb* is required for the formation of all neurons. We wondered whether this requirement is maintained for the differentiation of precursors as neurons, when cell division is blocked. We analysed *numb*$^{-}$; *stg*$^{-}$ embryos and observed that they lack most of the sensory neurons: only occasionally do 1 or 2 cells express neuronal markers (22C10, Fig. 4G; anti-Elav, not shown). In contrast, the number of *cut*-expressing cells is similar to that in *stg*$^{-}$ embryos (7.3±1.2; n=24; Fig. 4H). Furthermore, the high level of *cut* expression in these cells is typical of the es accessory cells (Blochlinger et al., 1990). We conclude that, in *numb*$^{-}$; *stg*$^{-}$ embryos, the ASC precursors differentiate into accessory cells, and therefore that *numb* is required for the acquisition or expression of the neuronal fate even in the absence of cell division.

X1, a mutation that prevents the acquisition of es fate

During a study of several genomic deletions (M. V., unpublished data), we found a line where the external derivatives of the es organs are absent in one fourth of the embryos. This phenotype is due to a previously unreported X-chromosome mutation (see Methods), which we named *X1*. As expected from the cuticular phenotype, we observed a loss of the markers of the es accessory cells (not shown; see Methods). Nevertheless, neurons are present in numbers slightly larger than normal (Fig. 5A,B). We assessed the identity of the neurons with the anti-BarH1 antibody. No cells are labelled in the hemizygous embryos (Fig. 5C), indicating that the es neuronal identity is lost. On the contrary, many neurons express *lacZ* in *X1*; *E7-2nd-36* mutant embryos (Fig. 5D). In the dorsal abdominal cluster, which comprises only es and md neurons in the wild-type, all neurons express *lacZ* (Fig. 5E). The conclusion that all or most dorsal neurons are of the md type was confirmed by filling dorsal neurons with DiI (Merritt et al., 1993). All the neurons that were filled (n=13) show the branched dendritic processes typical of md neurons (Fig. 5F). In the lateral regions, besides md neurons, several ch neurons were also observed (D. J. M., unpublished data).

Some md neurons arise from lineages that are ASC-independent; these neurons are overproduced in *X1* embryos (D. J. M. and M.V., unpublished). The excess of md neurons in *X1* embryos might therefore be solely due to the overproduction of ASC-independent precursors, and the loss of es cells could be caused by a loss of ASC-dependent precursors. Alternatively, the ASC-dependent precursors might appear normally but form only md neurons. In order to discriminate between these two possibilities, we used the anti-Cut antibody, which labels exclusively cells of the ASC-dependent lineages. We observed the presence of 6-7 md neurons expressing *cut* in the dorsal cluster of *X1* embryos (6.7±1.6 cells, n=29; not shown). These results suggest that ASC-dependent precursors appear normally in *X1* embryos and produce md neurons only.

We confirmed that ASC-dependent precursors are formed in *X1* embryos in two ways. First, we combined *X1* with the A37 enhancer trap line, which allows the detection of all precursors (Ghysen and O'Kane, 1989). At the earliest stage of precursor formation, two pairs of cells are formed within each segment: an anterior pair (A cells), which corresponds to ASC-dependent precursors, and a posterior pair (P cells), which corresponds to *ato*-dependent precursors (Ghysen and O'Kane, 1989). Soon after the A and P cells are formed, additional precursors appear in more ventral and dorsal positions (V and D cells). At this early stage, we cannot distinguish the hemizygous *X1* embryos from their normal siblings. However, we never observed embryos that lack A cells when the first D or V cells start appearing. Furthermore, in some embryos, we observed one A cell and 3-4 P cells before any D and V cells were formed, confirming both the excess of *ato*-dependent precursors (P cells) and the presence of ASC-dependent precursors (A cells) in mutant embryos.

Second, we used the anti-Poxn antibody which labels specifically some of the es precursors (those that will give rise to poly-innervated sense organs; Dambly-Chaudière et al., 1992) and their progeny. We observed one, or rarely a few labelled cells per segment (not shown), suggesting that the precursors appear and may occasionally divide, but always lose *poxn* expression much earlier than normal, consistent with the idea that the es fate cannot be maintained in *X1* embryos.

We conclude from these experiments that, in *X1* embryos, ASC-dependent precursors appear normally but produce only md neurons and no es cells, suggesting that the mutation removes one or more factor(s) required for the acquisition or maintenance of the es fate as an alternative to the md fate within the ASC-dependent lineages. The *X1* factor is required for the production of the es identity even when the precursors are unable to divide, as *stg*$^{-}$; *X1* double mutants lack all es neurons (anti-BarH1 labelling, not shown).

DISCUSSION

Asymmetric cell divisions and cell fates

Sense organ precursors produce a set of different cell types through fixed lineages. For example, in the case of 'solo' es organs, four different cells are generated: two accessory cells,

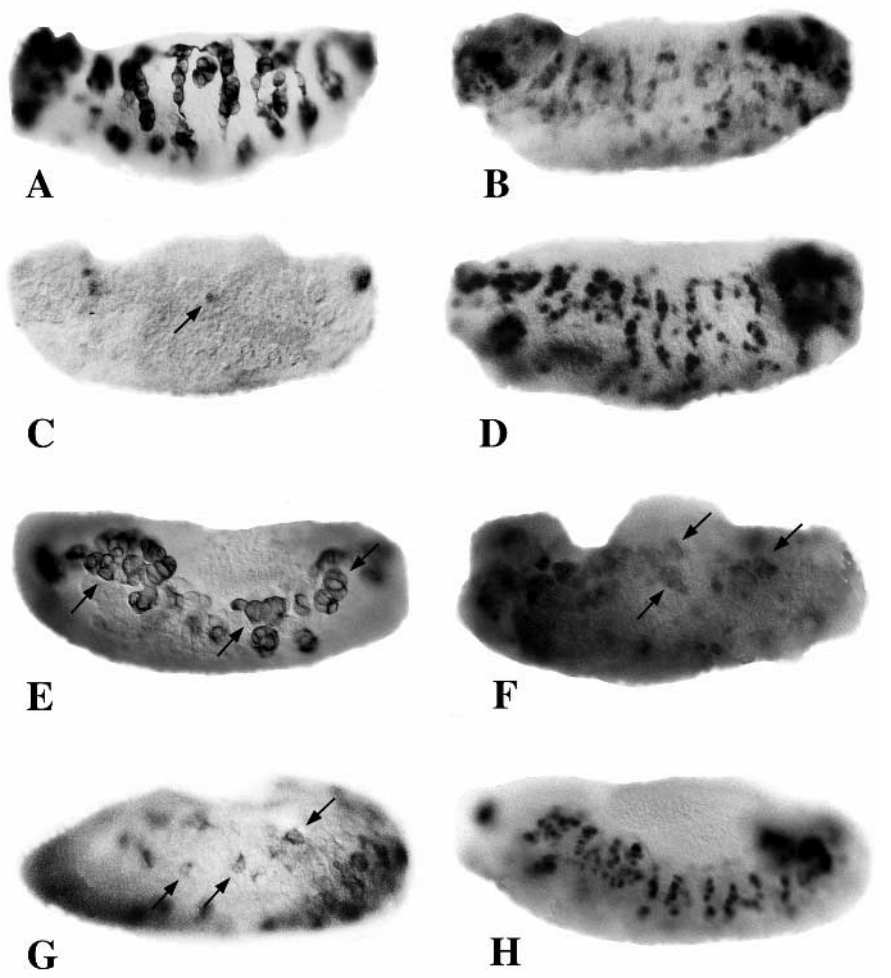

Fig. 4. Differentiation of the precursors in the absence of cell divisions. (A) In *stg*$^{-}$ embryos, precursors differentiate into neurons as shown by 22C10 labelling. (B,C) Both md and es neurons can be observed, but while several md neurons are present (B; anti-β-Gal labelling of *E7-2-36*, *stg*$^{-}$ embryos), few es neurons can be observed (C; anti-BarH1 labelling of *stg*$^{-}$ embryos). The embryo in C is an extreme case: only one es cell (arrow) is present (see text for numbers). (D) Anti-Cut labelling of *stg*$^{-}$ embryos showing the presence of ASC-dependent sensory cells. (E) In *N*$^{-}$*; stg*$^{-}$ double mutants, precursors differentiate into neurons (labelled by 22C10; arrows). (F) Several of these neurons come from ASC^{-}dependent precursors, as they are labelled by anti-Cut labelling (arrows). (G,H) In *numb*$^{-}$; *stg*$^{-}$ embryos, ASC-dependent precursors usually differentiate into es accessory cells: very few neurons are labelled with 22C10 (arrows in G) while many cells express strongly *cut* (H).

a sheath cell and a type I neuron (Bate, 1978; Bodmer et al., 1989, Brewster and Bodmer, 1995; Fig. 1). It is thought that the specification of these different identities is made progressively, through a succession of asymmetric cell divisions (Posakony, 1994; Jan and Jan, 1995; Vervoort et al., 1997). In a first step, the pI precursor produces two equipotent pII cells whose potentiality is subsequently restricted such that one (pIIa) will generate the two accessory cells while the other (pIIb) will generate the inner cells. In a second step, each pII cell divides to produce two equipotent cells which subsequently adopt two different fates: the daughters of pIIa differentiate as tormogen and trichogen cells, while the daughters of pIIb differentiate as sheath cell and neuron.

Several genes are involved in the allocation of the different cell types constituting the sense organ (Posakony, 1994; Jan and Jan, 1995; Vervoort et al., 1997). Mutations of these genes lead to the formation of abnormal sense organs with one or a few cell types overproduced at the expense of the others. For example, in the absence of *N*, two pIIb cells and no pIIa are produced, and the pIIb cells give rise to neurons only, and not to sheath cells (Hartenstein and Posakony, 1990b). The same set of genes functions at the different steps of the lineage, acting as 'symmetry breaker' (Garcia-Bellido, 1996) at each step.

The specification of different fates would then be due to the reiteration of the same developmental operation and the lineage itself can be viewed as a concatenation of asymmetric divisions (Horvitz and Herskovitz, 1992). This concatenation would ensure that at each step one daughter cell will adopt the default state corresponding to this step while its sister will necessarily adopt the alternative fate, and therefore that the entire complement of cells necessary for the proper functioning of the organ will be unerringly generated.

In this paper, we asked whether and how the type II (md) neuronal fate fits in this fate allocation system. We show that when the asymmetry-generating system is inactivated, in N^- embryos, ASC-dependent precursors generate md neurons at the expense of the other cell types. This result suggests that the specification of the md fate is made through the same system used to specify the other cell fates and that the md neuronal fate is the primary or default state in these lineages.

'mixed' and 'solo' lineages

We will consider the case of the 'mixed' md/es lineages first. In this case, one of the daughters of pIIb behaves as a third order precursor, $pIII_n$, which further divides to produce a md neuron and one (or more) es neuron(s) (Brewster and Bodmer, 1995). Since es neurons are absent and only md neurons are produced in N^- embryos, we conclude that N^+ is required to allow one of the daughters of $pIII_n$ to become an es neuron. The easiest interpretation of this effect is that the specification of es and md identities involves the same type of asymmetric division that acts in the previous steps, and that the $pIII_n$ precursor cell gives rise to two equipotent daughter cells which then adopt two different fates, one of them becoming a md neuron, the other one an es neuron.

We have to consider, however, that not all ASC-dependent

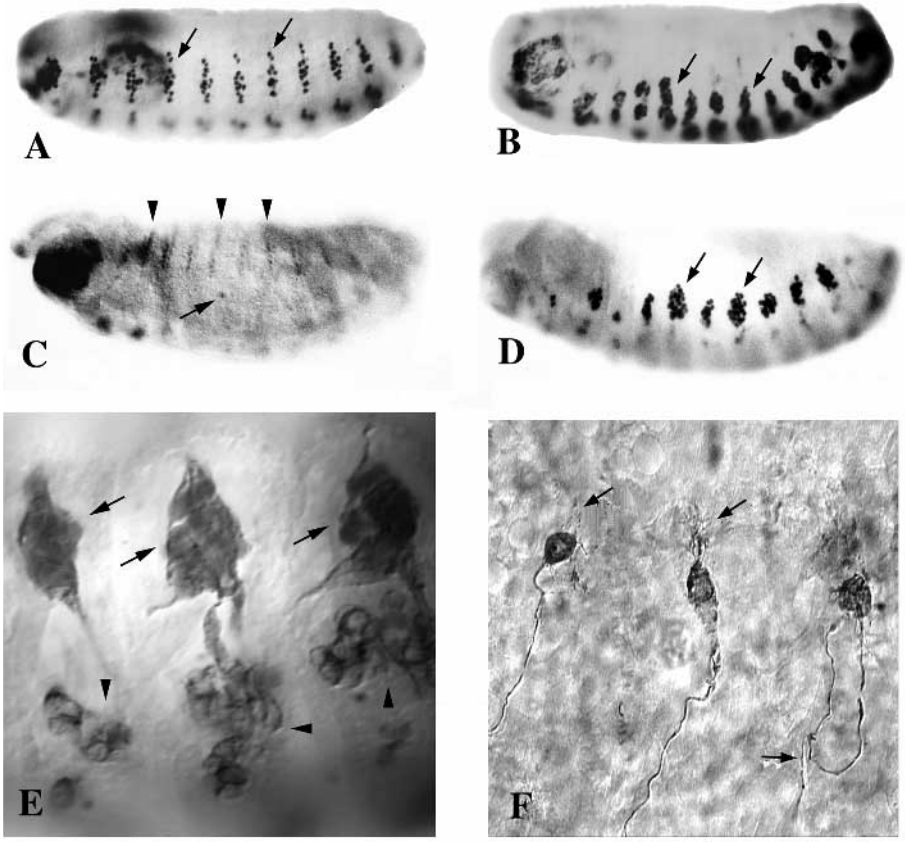

Fig. 5. The *X1* mutation induces the loss of es neurons and the overproduction of md ones. (A,B) Anti-Elav labellings of wild-type and *X1*/Y embryos. Anti-Elav labels all the neurons. A significant increase in the number of cells labelled by anti-Elav is observed in *X1*/Y embryos. Arrows point to the dorsal clusters of sensory neurons of two abdominal segments. (C) *X1*/Y lacks most of the es neurons and sheath cells (anti-BarH1 labelling; compare with Fig. 2E). Arrowheads highlight epidermal staining. The arrow points to a strongly labelled cell that belongs most probably to an es organ, highlighting the incomplete expressivity of the *X1* phenotype (see text). (D) md neurons are overproduced in *X1*/Y; *E7-2-26* as seen by anti-β-Gal labelling (arrows point to two dorsal abdominal clusters; compare with Fig. 2C). (E) The dorsal and lateral clusters of three successive abdominal segments of a *X1*/Y; E7-2-26 embryo double-labelled with 22C10 (membrane labelling) and anti-β-Gal (nuclear staining). The arrowheads point to the dorsal clusters where all neurons express *lacZ* and are probably of the md type. In the lateral clusters (arrows), many cells do not express *lacZ*, and probably belong to the ch type. (F) Randomly selected sensory neurons from three adjacent segments of an X1/Y embryo were stained with DiI. All of them are md neurons, as evidenced by the presence of finely branched dendrites (arrows). Axonal misrouting is frequently seen in these embryos: the lower arrow indicates a bifurcating axon with one branch running ventrally and the other dorsally.

md neurons arise from 'mixed' md/es lineages: some precursors produce only es cells and others only md neurons (Fig. 1; Brewster and Bodmer, 1995). We have observed that es neurons coming from 'solo' es precursors are also lost in N^- mutants.

One possible explanation would be that all ASC-dependent precursors follow a lineage of the 'mixed' type and that, in some of them, the md neuron fails to differentiate ('solo' es lineages) while, in others, the es neuron and support cells fail to differentiate ('solo' md lineages). In this case, however, one would expect that, in N^- embryos, where the two daughters of pIIIn take on the md fate, both should fail to differentiate in the 'solo es' lineages. This does not happen, since we observed an excess of md neurons. For this and other reasons listed below, we favour an alternative explanation.

We propose that these 'solo' es precursors (pI-es) are in fact sisters to the 'solo' md precursors (pI-md), and arise from the division of a protoprecursor cell (p0) which has both es and md potentiality (Fig. 1B). In the absence of *N*, the two daughters of the protoprecursors will adopt the same fate, the md precursor fate. The specification of es and md fates in these lineages would hence be similar to that proposed for the 'mixed' es/md precursors lineages and rely on an asymmetric cell division of the protoprecursor cell.

The existence of protoprecursors is supported by three lines of evidence. The first line is based on the fact that partial deletions of ASC lead to the absence of a subset of es organs, including 'solo' es organs (Dambly-Chaudière and Ghysen, 1987), and of a subset of md neurons, including 'solo' md neurons (M. V., unpublished observations). For example, in $Df(1)sc^H$, the three ventralmost es and four of the five ventral 'solo' md neurons are usually absent. A segment-by-segment analysis reveals that the two phenotypes are correlated, as the four md neurons are never present when the three es neurons are absent and are always present when the three es neurons are formed (M. V., unpublished observations).

Second, the analysis of the embryonic expression of *achaete*, a member of the ASC, reveals the existence of 11 proneural clusters (and precursors) for ASC-dependent sense organs (Ruiz-Gomez and Ghysen, 1993). This is much too low to account for the formation of the 13 es organs and the 7 'solo' md neurons (inferred from Brewster and Bodmer, 1995) if those originated from independent precursors, and suggests that some precursors give rise to both es and 'solo' md lineages.

Third, Brewster and Bodmer (1995) often observed FLP/FRT-induced *lacZ*-positive clones containing both 'solo' es organs cells and 'solo' md neurons, indicating that the corresponding precursors may derive from the same cell.

It may seem surprising that the decision to choose between es and md identity might, in the case of the 'solo' lineages, precede the choice between pIIa and pIIb identities, for it seems a departure from the rule that cells make progressive choices in a specific order. Yet the capability of the precursors to differentiate as neurons in *stg* and *pebble* mutants suggests that the precursor and neuronal fates may be closely related. Furthermore, precursors can differentiate as either md or es neurons, indicating that they can opt between es and md identities. The reason why this choice would operate at the level of the precursors in some lineages, and at the level of the neurons in others, remains however mysterious.

md neurons as default fate

The product of the gene *Notch* is part of a cellular communication system used for the specification of fates among equipotent cells (Fortini and Artavanis-Tsakonas, 1993; Artavanis-Tsakonas et al., 1995). Its implication in the sensory lineages suggests that, after each division, the two daughter cells form a small equivalence group where fates are determined through cellular communications. In such a group, the fate taken by the cells in the absence of signals is called the 'default state' (Greenwald and Rubin, 1992). Our results suggest that, in the lineages produced by ASC-dependent (proto)precursors, the default state is the md neuron and that the es identity is the alternative state.

We confirmed the idea that the md neuron is the 'default state' by looking at the situation where the lineage is reduced to a single cell. This is possible thanks to mutations (*string* and *pebble*) which block the division of the (proto)precursors and let them differentiate in the absence of any influence from equivalent (sister) cells. We found that the (proto)precursors predominantly differentiate into md neurons, confirming the md as the default state.

We have however to explain that es neurons are occasionally produced, though at very low frequency, in the absence of precursor divisions (see Results). Our interpretation is based on the idea that whenever two ASC (proto)precursors are in direct contact the md fate is inhibited and the es identity is acquired in one of them. In wild-type conditions, this happens between the daughters of $pIII_n$ or between the daughters of pO, leading to the determination of an es and a md fate. We propose that this interaction can also happen in stg^- between different (proto)precursors, so that one of them will differentiate as md neuron, while the other as es one. These interactions between (proto)precursors are made more likely by their close proximity, due to the reduced number of ectodermal cells in *stg* embryos. We observed that the number of es neurons is low and variable in stg^- embryos (as monitored by anti-BarH1 labelling), indicating that the interactions between precursors happen randomly and at a rather low frequency. The idea that the occasional presence of es neurons is due to cell communication is supported by the observation that only md neurons form in stg^- embryos where cell communication is prevented (in N^-; stg^- embryos).

In conclusion, the function of N^+ in the sensory lineages would be to force after each division one daughter cell to adopt the 'alternative state': in mixed lineages, pIIa versus pIIb, sheath cell versus $pIII_n$, and es versus md neuron; in protoprecursors lineages, es-pI versus md-pI.

Function of *numb* and *ttk*

Besides the *N*-mediated communication system, other genes are also involved in cell fate specification in the sensory lineages (Posakony, 1994; Jan and Jan, 1995, Vervoort et al., 1997). One of them is *numb* whose product is asymmetrically distributed to one daughter of pI (Rhyu et al., 1994), as well as probably to one daughter of pIIb. Numb determines the fate of the cell in which it is located and promotes pIIb versus pIIa fate, and neuron versus sheath cell fate (Jan and Jan, 1995; Brewster and Bodmer, 1995). Numb appears to repress *tramtrack* (*ttk*), a gene that promotes pIIa versus pIIb and sheath cell versus neuron (Guo et al., 1995; Ramaekers et al., 1997).

We have analysed the requirement for these two genes in the formation and specification of the md neurons. We first looked in the 'mixed' es/md lineages. In *numb*$^-$ embryos, both md and es neurons from 'mixed' lineages are usually missing (Brewster and Bodmer, 1995). Their absence is likely due either to the absence of $pIII_n$ cells, which results from the conversion of pIIb into pIIa (Uemura et al., 1989) or to the conversion of $pIII_n$ into a sheath cell (Brewster and Bodmer, 1995). This absence makes it impossible to determine whether *numb* plays a role in the specification of es versus md neuronal identity. In *ttk*$^-$ embryos, two pIIb cells are produced, both of which generate a sheath cell, an es and a md neuron. This result is consistent with the observation that *ttk* is never expressed in neuronal cells (Guo et al., 1995; Ramaekers et al., 1997) and indicates that *ttk* plays no role in the specification of neuronal types.

In the case of the protoprecursors lineages, the inactivation of *numb* or the ectopic expression of *ttk* results in the absence of the pI-md cells. We do not know whether this absence is due to cell death, or to transformation into another cell type. One possibility would be that pI-md are transformed into pI-es. Each pI-es resulting from this transformation, should produce four es accessory cells (as in mixed lineage), and we should then observe clusters of eight cut-expressing cells in *numb* mutants. We never observed such clusters, however, (Fig. 3B), suggesting that md precursors fail to form neurons, rather than being transformed into es precursors.

These results confirm that the presence of Numb and the absence of Ttk are required to produce both es and md neural fate. As in the case of the 'mixed lineages', our results neither support nor rule out a possible function of *numb* in es/md specification, as no neuronal types can be observed in the absence of *numb*. In *ttk*$^-$, both pI-es and pI-md appear and generate respectively es and md cells. This result confirms that *ttk* is not involved in es/md specification. We can therefore say that, if *numb* has any role in the es/md specification, it must be through a pathway that does not include *ttk*.

In conclusion, we have shown that the presence of *numb* and absence of *ttk* are required for the acquisition of both es and md neuronal fates. In the case of *numb*, this requirement is maintained when the (proto)precursors are unable to divide. This result is consistent with the conclusion that the expression of *ttk*, which probably depends on *N* activation (Guo et al., 1996), prevents a cell from adopting a neuronal identity (Guo et al., 1995; Ramaekers et al., 1997). The role of *numb* would be to prevent *ttk* from exerting this inhibition, probably by blocking the transcription of *ttk* (Guo et al., 1996). Our observations suggest, therefore, that the determination of es and md neuronal identity comprises two aspects: the acquisition of a neuronal fate, which involves *N*, *numb* and *ttk*, and the specification of the type I/type II identity, which involves *N* but not *ttk* and probably not *numb*.

X1 defines a new factor required for the alternative es fate

We found a new factor required to produce the es fate from ASC-dependent precursors. This factor has been defined through the analysis of a X chromosome mutant line, X1. In the mutant embryos, the ASC precursors appear normally but only produce md neurons, and all es neurons as well as accessory cells are missing. This is similar to the effect of *N* inactivation and suggests that the *X1* factor may function in the same pathway as *N*. However, while *N* mutations lead to the emergence of many ASC-dependent precursors (Brand and Campos-Ortega, 1989; Goriely et al., 1991; Ruiz-Gomez and Ghysen, 1993), no such phenotype is observed in *X1* embryos. We do nevertheless observe that *X1* embryos present neurogenic phenotypes at the level of the ASC-independent md neurons, and in the central nervous system (M. V. and D. J. M., unpublished). These phenotypes are also present in *N*$^-$ embryos (Hartenstein and Campos-Ortega, 1986). These similarities suggest that the 'X1 factor' may be one among several effectors of *N*-mediated cell communication, and therefore could be involved only in some of the processes in which *N* acts. Further genetic and molecular analyses will be required to understand the function of the 'X1 factor' during sense organ formation.

We are grateful to the people who sent us fly stocks and antibodies; L. Ackerman, C. Bazinet, K. Blochlinger; W. Chia, S. Fujita, Y.N. Jan, A. Katzen, T. Kojima, C. Lehner, C. Nüsslein-Volhard, C. Poodry, S. Shepard and K. Saigo. We are specially grateful to the *Drosophila* stock centers of Umea and Bloomington from which we received numerous stocks, and to their respective curators K. Ekström and K. Matthews. We also aknowlegde the Hybridoma Bank for the anti-Elav (developed by G. Rubin) and anti-β-gal (developed by J. R. Sanes) antibodies. We thank G. Hassanzadeh and R. Haemers for the anti-Poxn; M. Guo and G. Ramaekers for communicating unpublished observations, and K. and A. Usui for advice on antibody labelling. This work was supported by a contract from the Human Frontier Science Programme Organization (to C. D.-C. and A. G.). M. V. has been a fellow of the IRSIA and a grantee from the FNRS and the ULB.

REFERENCES

Artavanis-Tsakonas, S., Matsuno, K. and Fortini, M. E. (1995). Notch signaling. *Science* **268**, 225-232.

Bate, C. M. (1978). Development of sensory systems in arthropods. In *Handbook of Sensory Physiology'*, vol.9 (ed. M. Jacobson), pp. 1-53. Berlin, New-York: Springer Verlag.

Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel,S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., Jan, L. Y. and Jan, Y. N. (1989). Searching for pattern and mutation in the *Drosophila* genome with a P-*lacZ* vector. *Genes Dev.* **3**, 1273-1287.

Blackshaw, S. E. (1981). Morphology and distribution of touch cell terminals in the skin of the leech. *J. Physiol.* **320**, 219-228.

Blochlinger, K., Bodmer, R., Jan, L. Y. and Jan, Y. N. (1990). Patterns of expression of Cut, a protein required for external sensory organ development in wild-type and *cut* mutant *Drosophila* embryos. *Genes Dev.* **4**, 1322-1331.

Bodmer, R., Carretto, R. and Jan, Y. N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. *Neuron* **3**, 21-32.

Bodmer, R. and Jan, Y. N. (1987). Morphological differentiation of the embryonic peripheral neurons in *Drosophila*. *Roux's Arch. Dev. Biol.* **196**, 69-77.

Brand, M. and Campos-Ortega, J. A. (1989). Two groups of interrelated genes regulate early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **197**, 457-470.

Brewster, R. and Bodmer, R. (1995). Origin and specification of type II sensory neurons in *Drosophila*. *Development* **121**, 2923-2936.

Bullock, T. H. and Horridge, G. A. (1965). *Structure and function in the nervous system of invertebrates*. San Fransisco: W. H. Freeman and Company..

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic Development of Drosophila melanogaster.* Berlin, Heidelberg, New-York, Tokyo: Springer Verlag.

Cui, X. and Doe, C. Q. (1995). The role of cell cycle and cytokinesis in regulating neuroblast sublineage gene expression in the *Drosophila* CNS. *Development* **121**, 3233-3243.

Dambly-Chaudière, C., Jamet, E., Burri, M., Bopp, D., Basler, K., Hafen, E., Dumont, N., Spielmann, P., Ghysen, A. and Noll, M. (1992). The paired box gene *pox-neuro*: a determinant of poly-innervated sense organs in *Drosophila*. *Cell* **69**, 159-172.

Dambly-Chaudière, C. and Ghysen, A. (1987). Independent subpatterns of sense organs require independent genes of the *achaete-scute* complex in *Drosophila* larvae. *Genes Dev.* **1**, 297-306.

Dambly-Chaudière, C. and Ghysen, A. (1986) The pattern of sense organs in the *Drosophila* larva and its relation to the embryonic pattern of sensory neurons. *Roux's Arch. Dev. Biol.* **195**, 222-228

Edgar, B. A. and O'Farrell, P. H. (1989). Genetic control of cell division patterns in the *Drosophila* embryos. *Cell* **57**, 177-187.

Fortini, M. E.and Artavanis-Tsakonas, S. (1993). *Notch*: neurogenesis is only part of the picture. *Cell* **75**, 1245-1247.

Fujita, S. C., Zipursky, S. L., Benzer, S. Ferrus, A. and Shotwell, S.L. (1982). Monoclonal antibodies against the *Drosophila* nervous system. *Proc. Natl. Acad. Sci. USA* **79**, 7929-7933.

Garcia Alonso, L. and Garcia-Bellido, A. (1986). Genetic analysis of *Hairy-wing* mutations. *Roux's Arch. Dev. Biol.* **195**, 259-264.

Garcia-Bellido, A. (1996). Symmetry breaking along organic evolution. *Proc. Natl. Acad. Sci. USA* **93**, 14229-14232.

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.

Ghysen, A. and O'Kane, C. (1989). Neural enhancer-like elements as specific cell markers in *Drosophila*. *Development* **105**, 35-52.

Ghysen, A. and Dambly-Chaudière, C. (1989). Genesis of the *Drosophila* peripheral nervous system. *Trends Genet.* **5**, 251-255.

Ghysen, A., Dambly-Chaudière, C., Aceves, E., Jan, L. Y. and Jan, Y. N. (1986). Sensory neurons and peripheral pathways in *Drosophila* embryos. *Roux's Arch. Dev. Biol.* **195**, 281-289.

Goriely, A., Dumont, N., Dambly-Chaudière, C. and Ghysen, A. (1991). The determination of sense organ in *Drosophila*: effect of the neurogenic mutations in the embryo. *Development* **113**, 1395-1404.

Greenwald, I. and Rubin, G. M. (1992). Making a difference: the role of cell-cell interactions in establishing separate identities for equivalent cells. *Cell* **68**, 271-281.

Guo, M., Jan, L. Y. and Jan, Y. N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron* **17**, 27-41.

Guo, M., Bier, E., Jan, L. Y. and Jan, Y. N. (1995). *tramtrack* acts downstream of *numb* to specify distinct daughter cell fates during asymmetric cell divisions in the *Drosophila* PNS. *Neuron* **14**, 913-25.

Hartenstein, V. (1988). Development of *Drosophila* larval sensory organs: Spatio-temporal pattern of sensory neurons, peripheral axonal pathways and sensilla differentiation. *Development* **102**, 869-886.

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

Hartenstein, V. and Posakony, J. W. (1990b). Sensillum development in the absence of cell division: The sensillum phenotype of the *Drosophila* mutant *string*. *Dev. Biol.* **138**, 147-158.

Hartenstein, V. and Campos-Ortega, J. A. (1986). The peripheral nervous system of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **195**, 210-221.

Higashima, S., Michiue, T., Emori, Y. and Saigo, K. (1992) Subtype determination of *Drosophila* embryonic external sensory organs by redundant homeobox genes *BarH1* and *BarH2*. *Genes Dev.* **6**, 1005-1018

Hime, G. and Saint, R. (1992). Zygotic expression of the *pebble* locus is required for cytokinesis during the postblastoderm mitoses of *Drosophila*. *Development* **114**, 165-171.

Horvitz, H. R. and Herskovitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. *Cell* **68**, 237-255.

Jan, Y. N. and Jan, L. Y. (1995). Maggot's hair and Bug's eye: role of cell interactions and intrinsic factors in cell fate specification. *Neuron* **14**, 1-5.

Jan, Y. N. and Jan, L. Y. (1993). The peripheral nervous system. In *The Development of Drosophila melanogaster*. (ed. M. Bate and A. Martinez-Arias). pp 1207-1244. Cold Spring Harbor Laboratory Press.

Jarman, A. P., Grau, Y., Jan, L. Y. and Jan, Y. N. (1993). *atonal* is a proneural gene that directs chordotonal organ formation in the *Drosophila* perpheral nervous system. *Cell* **73**, 1307-1321.

Jürgens, G., Wieschaus, E., Nüsslein-Volhard, C. and Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. II. Zygotic loci on the third chromosome. *Roux's Arch. Dev. Biol.* **193**, 283-295.

Lehner, C. F. (1992). The *pebble* gene is required for cytokinesis in *Drosophila*. J. *Cell Sci.* **103**, 1021-1030.

Lentz, T. L. (1968). *Primitive Nervous System*. New Haven and London: Yale University Press.

Merritt, D. J. and Whitington, P. M. (1995). Central projections of sensory neurons in the *Drosophila* embryo correlate with sensory modality, soma position and proneural gene function. *J. Neurosci.* **15**, 1755-1767.

Merritt, D. J., Hawken, A. and Whitington, P. M. (1993). The role of the *cut* gene in the specification of central projections of sensory axons in *Drosophila*. *Neuron* **10**, 741-752.

Posakony, J. W. (1994). Nature versus nurture: asymmetric cell divisions in *Drosophila* bristle development. *Cell* **76**, 415-418.

Ramaekers, G., Usui K., Usui A., Ramaekers, A., Ledent, V., Ghysen, A. and Dambly-Chaudière C. (1997). Lineage and fate in *Drosophila*: Role of the gene *tramtrack* in sense organ development. *Development, Genes and Evolution*, in press.

Read, D. and Manley, J. (1992). Alternatively spliced transcripts of the *Drosophila tramtrack* gene encode zinc finger proteins with distinct DNA binding specificities. *EMBO J.* **11**, 1035-1044.

Rhyu, M. S., Jan, L. Y. and Jan, Y. N. (1994). Asymmetric distribution of Numb protein during division of sensory organ precursor cell confers distinct fates to daughter cells. *Cell* **76**, 477-491.

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**, 1121-1130.

Salzberg, A., D'Evelyn, D., Schulze, K., Lee, J.-K., Strumpf, D., Tsai, L. and Bellen, H. J. (1994). Mutations affecting the pattern of the PNS in *Drosophila* reveal novel aspects of neuronal development. *Neuron* **13**, 269-287.

Struhl, G. and Basler, K. (1993). Organization of *wingless* protein in *Drosophila*. *Cell* **72**, 527-540.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L. Y. and Jan, Y. N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. *Cell* **58**, 349-360.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L. Y. and Jan, Y. N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. *Cell* **67**, 941-954.

Vervoort, M, Dambly-Chaudière, C. and Ghysen, A. (1997). Cell fate determination in *Drosophila*. *Curr. Opin. Neurobiol.* **7**, 21-28.

Weigmann, K. and Lehner, C. F. (1995). Cell fate specification by *even-skipped* expression in the *Drosophila* nervous system is coupled to cell cycle progression. *Development* **121**, 3713-3721.

Xiong, W-C. and Montell, C. (1993). *tramtrack* is transcriptional repressor required for cell fate determination in the *Drosophila* eye. *Genes Dev.* **7**, 1085-1096.

Zacharuk, R. Y. (1985). Antennae and sensillae. *In Comprehensive Insect Physiology, Biochemistry and Pharmacology* (ed. L. I. Gilbert and G. A. Kerkut), Vol.**6**, 1-69. Oxford; Pergamon Press.

(Accepted 14 May 1997)