Development 121, 429-438 (1995)
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Proneural genes influence gliogenesis in *Drosophila*

Angela Giangrande

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UPR6520-INSERM U.184-ULP, BP163 67404 Illkirch Cedex, CU de Strasbourg, France

SUMMARY

Fly glial cells in the wing peripheral nervous system of *Drosophila melanogaster* originate from underlying epithelial cells. Two findings indicate that gliogenesis is closely associated with neurogenesis. First, it only occurs in regions that also give rise to sensory organs. Second, in mutants that induce the development of ectopic sensory organs glial cells develop at new positions. These findings prompted a genetic analysis to establish whether glial and sensory organ differentiation depend on the same genes. Loss of function mutations of the *achaete-scute* complex lead to a significant reduction of sensory bristles and glial cells. Genes within the complex affect gliogenesis with different strength and display some functional redundancy. Thus, neurogenesis and gliogenesis share the same genetic pathway. Despite these similarities, however, the mechanism of action of the *achaete-scute* complex seems to be different in the two processes. Neural precursors express products of the complex, therefore the role of these genes on neurogenesis is direct. However, markers specific to glial cells do not colocalize with products of the *achaete-scute* complex, showing that the complex affects gliogenesis indirectly. These observations lead to the hypothesis that gliogenesis is induced by the presence of sensory organ cells, either the precursor or its progeny.

Key words: proneural genes, gliogenesis, PNS, *Drosophila*, *achaete-scute* complex

INTRODUCTION

Little is known about the genes inducing the first steps of gliogenesis. In the *Drosophila* wing, glial cells differentiate from epithelial cells, from regions that also give rise to sensory neurons (Giangrande, 1994). Moreover, mutations that induce ectopic sensory organs, also induce gliogenesis at these ectopic positions, indicating a strong association between gliogenesis and neurogenesis (Giangrande, 1994). Since several genes involved in sensory organ differentiation are known (for reviews, see Ghysen and Dambly-Chaudière, 1993; Jan and Jan, 1994), it was important to ask whether they also act on gliogenesis.

The so called proneural genes are necessary for the definition of the proneural cluster, a group of epithelial cells from which one is singled out to become the sensory organ precursor cell (SOP) (for reviews, see Ghysen et al., 1993; Jan and Jan, 1994). To this class of genes belong those of the *achaete-scute* complex (*ASC)*: *achaete* (*ac*), *scute* (*sc*) and *asense* (*ase*) (Garcia-Bellido, 1978; for a review, see Campuzano and Modolell, 1992). *ac* and *sc* are initially expressed in the proneural cluster and then in the SOP cell (Cubas et al., 1991; Skeath and Carroll, 1991, 1992). Their expression fades after the SOP cell has been singled out, when *ase* starts being expressed (Brand et al., 1993; Dominguez and Campuzano, 1993; Jarman et al., 1993). These genes code for bHLH type transcription factors that form heterodimers and activate gene expression (Murre et al., 1989a,b; Cabrera and Alonso, 1991). *ASC* loss of function mutations lead to lack of sensory organs, because the proneural clusters and the SOP cells fail to form (Ghysen and O'Kane, 1989; Romani et al., 1989). Accordingly, *ASC* ectopic expression due to a heat shock promoter or to gain of function mutations such as *Hairy-wing*, (*Hw*), induce ectopic sensory organs, due to the formation of additional SOP cells (Campuzano et al., 1986; Balcells et al., 1988; Rodriguez et al., 1990; Blair et al., 1992). Other genes are needed for sensory organ differentiation. *hairy* (*h*) and *extramacrochaete* (*emc*) repress sensory organ formation at ectopic positions through inhibitory interactions with the *ASC* (Moscoso del Prado and Garcia-Bellido, 1984a,b; Skeath and Carroll, 1991; Van Doren et al., 1991, 1992; Cubas and Modolell, 1992).

Using different glial markers in *ASC* mutant backgrounds I show here that proneural genes as well as *h* are required for proper gliogenesis in the wing PNS. I also show that the products of the *ASC* are not expressed in the early steps of gliogenesis. These results strongly suggest that the mechanisms of action of *ASC* are different in gliogenesis and neurogenesis.

MATERIALS AND METHODS

Stocks

The wild-type stocks used were Oregon R and Sevelen. The mutant stocks were the following: h^1, ase (sc^2 w s), Hw^{49c} (In(1) Hw^{49c} w s/FM7), ac sc (In(1) ac^3 $sc^{10\text{-}1}$ f^{36a}/FM7), sc (In(1) sc^{8L}-sc^{4R} v f/In (1) dl49), ac (In(1)y^{3PL}-sc^{8R}), Hw^1 (y $Hw^1m^2g^2/y^2Y67g$). The sc and ac stocks were gifts from J. Modolell, the Hw^1 stock was a gift from C. Dambly-Chaudière. As glial markers, the following enhancer trap

lines were used: AE2 and rA87 (gifts from V. Auld and C. Goodman; Auld and Goodman, 1992; Giangrande et al., 1993); 2206 (gift from M. Schubiger; Giangrande et al., 1993; Schubiger et al., 1994). The lines A409.1F3/TM3Sb and B72.1M3 (gifts from W. Gehring), which carry a P element on the third chromosome, were identified as being glial specific in a screen in adult flies (Giangrande, unpublished results). All lines label subsets of glial cells, the only exception being rA87, which most likely transiently labels all or nearly all glial nuclei (Giangrande, unpublished results). Mutant heterozygous females were crossed with males of the enhancer trap line. In the F_1 generation, males of the appropriate genotype were selected on the basis of the y+ phenotype. For ase and ac, homozygous females were used in the cross and therefore all males were of the appropriate genotype. For Hw^1, a stock homozygous mutant and heterozygous for the glial insert was obtained, in order to analyse homozygous females, which display a stronger phenotype than males. Glial markers were always analysed in heterozygotes, to avoid possible recessive phenotypes due to the insert. Control flies were obtained by crossing the enhancer trap line with a wild-type stock. The line ase-β-gal (Jarman et al., 1993) was a gift from A. Jarman.

Immunohistochemistry

X-gal labelling in adults was performed as described by Giangrande et al. (1993). Antibody labelling was as described by Giangrande (1994). In double labelling experiments with anti-β-gal and a mouse monoclonal antibody as primaries, polyclonal rabbit-anti-β-gal from Cappel was used at 1:4000, detected with Cy3 conjugated goat-anti-rabbit 1:600 (Jackson). The monoclonal antibody 22C10 (a gift from S. Benzer) was used at 1:100 and detected with FITC-conjugated goat-anti-mouse 1:400 (Jackson). Rabbit-anti-asense (a gift from A. Jarman) was used at 1:8000; mouse-anti-achaete supernatant was prepared from the H990ΣSF1 line (a gift from J. Skeath and S. Carroll) and used at 1:50. These two antibodies were revealed with Cy3-conjugated goat-anti-rabbit 1:300 and FITC goat-anti-mouse 1:300 (both from Jackson).

Confocal images were obtained using a Zeiss axiophot and a Leica DMRE.

RESULTS

ASC loss of function mutations affect gliogenesis

Glial cell organization in *ac sc* mutants

Adult wings carry two major nerves, L1 and L3, travelling along the L1 and the L3 veins respectively (Murray et al., 1984; Hartenstein and Posakony, 1989). These purely sensory nerves contain axons from the chemo- and mechanosensory bristle neurons located on the anterior margin and from the neurons of the large campaniform sensilla located on the L3 vein and on the margin (Figs 1A, 2A). Wing sensory organs differentiate from precursor cells that divide two or more times to give rise to the neuron(s) and to the sensory organ accessory cells (Hartenstein and Posakony, 1989). Glial cells present along the two nerves (Figs 1A, 2B) differentiate from wing epithelial cells, in the same regions in which sensory organs develop, and then migrate for short distances along the underlying nerves (Giangrande et al., 1993; Giangrande, 1994).

Three genes of the *ASC* are necessary for wing sensory organ differentiation: *scute* (*sc*), *achaete* (*ac*) and *asense* (*ase*), in order of importance relative to their mutant phenotype. The $sc^{10\text{-}1}$ mutation lacks functional scute and achaete products (Campuzano et al., 1985; Villares and Cabrera, 1987). In such wings, all chemosensory bristles and many mechanosensory bristles of the anterior margin as well as all the large campaniform sensilla are absent (Fig. 1B). I have crossed the 2206 glial-specific enhancer trap line (Giangrande et al., 1993; Schubiger et al., 1994) with $sc^{10\text{-}1}$ flies and looked at the glial labelling in adult mutant wings. Since 2206, as well as most glial specific lines, mark subsets of glia, two other lines have

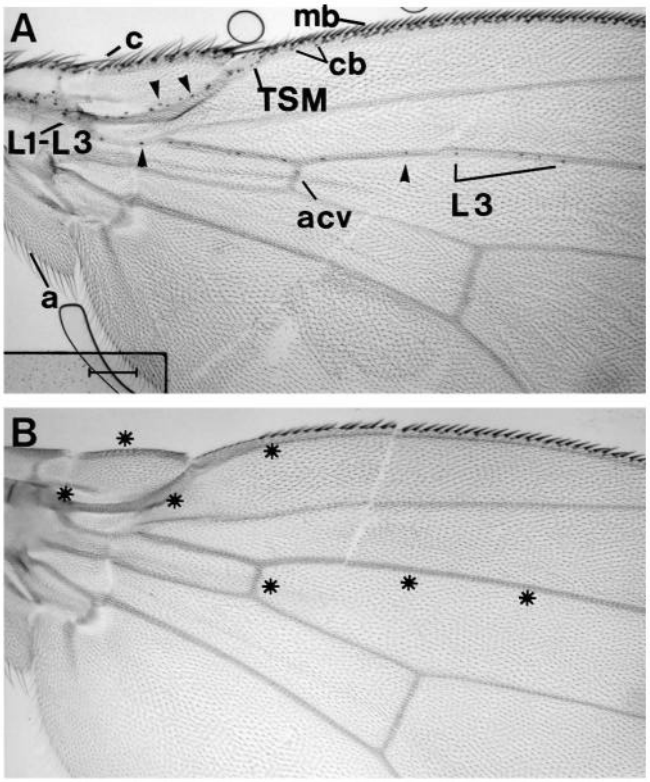
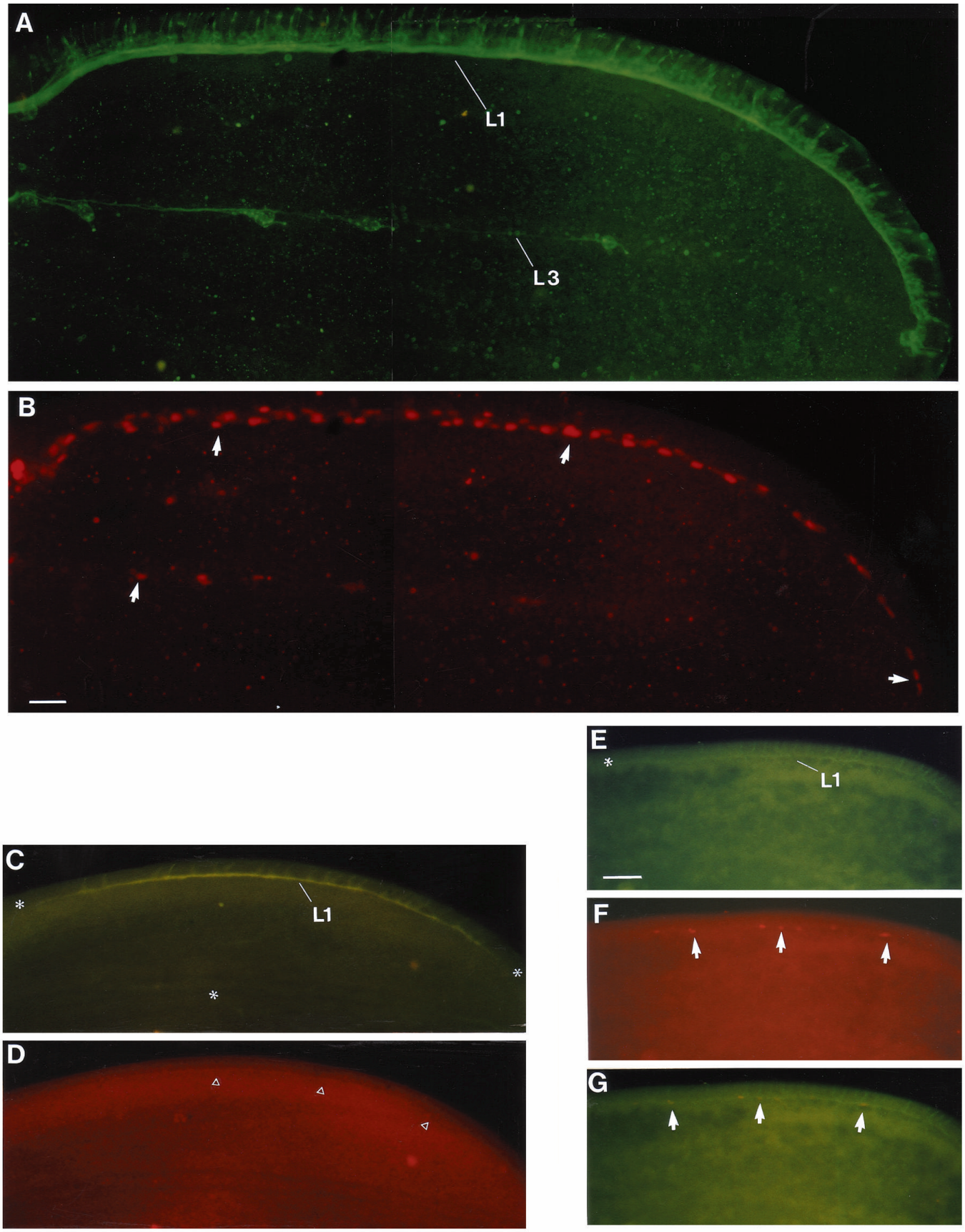


Fig. 1. Glial labelling in wild-type and $sc^{10\text{-}1}$ adults. The *2206* enhancer trap line was used as a glial marker. (A) *2206/+* and (B) $sc^{10\text{-}1}$*/Y; 2206/+* wings, labelled with X-gal. (A) Wild-type array of glial nuclei (arrowheads) and sensory organs. cb and mb indicate the chemo- and mechanosensory bristles on the anterior margin, respectively; acv, the anterior cross vein sensillum; L3, the sensilla present on L3; c and a, the costa and the alula; TSM, the position of the twin sensillum on the margin. L1-L3 shows the junction between veins L1 and L3. (B) Mutant wing shows dramatic defects at sensory organs and glial cells. Chemosensory bristles on the anterior margin and on the costa are absent (see asterisks). Other types of bristles are also partially missing and all sensilla on acv and L3 are absent. No glial nuclei can be detected. To facilitate X-gal penetration, wings were cut at several positions. Bar 100 μm.

Fig. 2. Glial labelling in wild-type and $sc^{10\text{-}1}$ wings at 25-38 hours AP. *2206* and *rA87* enhancer trap lines were used as glial markers. Anti-HRP detects neurons and nerves (A,C,E); anti-β-gal detects glial nuclei (B,D,F). G is a double exposure of E and F. (A,B) *rA87/+* wing: arrows indicate glial nuclei along the L1(L1) and L3 (L3) nerves. Labelled spots outside of L1 and L3 are artefacts as defined by their morphology and their focal plane. (C,D) $sc^{10\text{-}1}$*/Y; 2206/+* wing: asterisks indicate the absence of axons on the distal and proximal parts of L1 nerve and the absence of L3 axons. The L1 nerve, which has lost its polarity and goes proximally and distally, does not carry glial nuclei (open arrowheads). (E-G) $sc^{10\text{-}1}$*/Y; rA87/+* wing, one of the rare cases in which some glial nuclei are present (see also Table 1). (A,B) Bar 50 μm; (C-G) bar 25 μm.


A
L1
L3
B
C
L1
D
E
L1
F
G

also been used in similar crosses: *B72.1M3* and *A409.1F3* (data not shown). In no case can labelling be detected on the L3 and L1 nerves.

Glial cell development in *ac sc* mutants

A possible interpretation for the above results is that, in $sc^{10\text{-}1}$ wings, glial cells differentiate and then degenerate before adulthood. For this reason, glial labelling has been analysed at different stages during pupal development. For the pupal analysis, another glial marker has also been used, *rA87* (Giangrande et al., 1993). In *rA87*, the β-galactosidase (β-gal) labels most if not all glial cells during the early stages of wing development, however labelling progressively decreases and is absent in the adult. In wild-type wings at 24-38 hours after pupariation (AP), the glial cells and the sensory organs have differentiated and the axons have formed the L1 and L3 nerves (Fig. 2A,B). At similar stages, $sc^{10\text{-}1}/Y$ wings show some anti-HRP-positive cells on the anterior margin, which, by their position and the morphology, correspond to the mechanoreceptor neurons (Fig. 2C,E). These neurons differentiate much later than normal ones (data not shown) and send out axons that form a very thin, truncated nerve, which in most cases, does not display glial labelling (Fig. 2). In some cases, 5 out of 25 wings, a few glial labelled nuclei have been observed (Fig. 2 and Table 1).

Glial and neuronal organization has also been analysed earlier in development to determine whether glial labelling ever appears in the mutant background. The earliest stage at which glial labelling can be observed on the anterior margin is at 9-10 hours AP, when the mechanosensory neurons have not yet differentiated whereas some chemosensory neurons are already detectable (Giangrande et. al, 1993). 10 hour AP $sc^{10\text{-}1}$ wings do not show neuronal labelling (data not shown), in agreement with the observation that chemosensory bristles are completely absent in adult wings (see Table 1 and Fig. 1B), nor do they show glial labelling (data not shown). These results indicate that glial labelling is absent throughout pupal development.

The relative roles of *sc* and *ac*

Each member of the *ASC* has a specific role, even though one gene can, at least partially, replace the activity of the others (Rodriguez et al., 1990; Brand et al., 1993; Dominguez and Campuzano, 1993; Hinz et al., 1994). In the wing, *sc* plays a more important role for sensory organ differentiation than *ac*. The *sc* product is necessary and sufficient for the differentiation of most campaniform sensilla (Leyns et al., 1989). On the margin, the effects of *sc* are stronger than those observed in *ac*, especially with respect to the chemosensory bristles (Table 1 and data not shown).

To assess the relative roles of the *ASC* products on gliogenesis, I have analysed the glial pattern in pupae mutant for only one of the genes. 50% and 25% of the glial labelling is absent in *sc* and *ac* mutants, respectively (see Table 1). As expected from the adult phenotype, the number of chemo- and mechanosensory neurons is smaller in *sc* than in wild-type wings (Table 1 and data not shown) and all neurons from the large campaniform sensilla on L3 and L1 are absent. Neuronal defects are less drastic in *ac* wings. The number of chemosensory organs is less reduced compared to *sc* and all campaniform sensilla neurons are present (see Table 1 and data not shown). Overall, in both *ac* and *sc*, the strength of the effects on glial cells parallels that of the effects on neurons.

ac and *sc* loss of function mutations behave as recessive mutations: the number of neurons and glial nuclei is not or only slightly affected in heterozygotes (data not shown).

The role of *ase*

Compared to *sc* and *ac*, *ase* loss of function mutations have weaker effects on the campaniform sensilla and on the mechanoreceptors, but remove as many chemosensory bristles on the anterior margin as *sc* (Dominguez and Campuzano, 1993). The effects of *ase* on sensory organ differentiation also differ qualitatively. In *sc* and *ac*, the lack of sensory organs is due to the absence of their precursors (Romani et al., 1989; Boulianne et al., 1991; Cubas et al., 1991). On the other side, *ase* mutant third larval instar discs do carry chemosensory SOP cells even though their number is slightly decreased compared to wild type (Dominguez and Campuzano, 1993). Later on, some SOP cells do not divide or their progeny are abnormally distributed. In the adult, defects in the sensory organ lineage (duplicated or triplicated shafts) have also been observed (Dominguez and Campuzano, 1993). Therefore, in *ase*, the dramatic reduction of chemosensory bristles along the anterior margin is due in part to a defective singling out of the SOP cell and in part to defects in later steps, either in the SOP divisions or in the differentiation of the SOP progeny.

As for gliogenesis, while in *ac* and *sc* the effects on anterior margin glial cells are comparable to those observed on the early appearing sensory organs (chemosensory bristles), in *ase* there seems to be no such correlation. The number of glial nuclei in *ase* pupal wings is only reduced by about 15% (Table 1 and data not shown). In addition, while $sc^{10\text{-}1}$ wings do not show glial labelling at early stages of development, *ase* wings do. 13 hour AP wings show labelling with neuronal markers (Fig. 3). The labelling is weaker and neurogenesis seems less advanced than in the wild type, but there are no gross rearrangements in the sensory organ organization. Although counting is difficult because the labelling is weak, several glial

Table 1. Effects of *ASC* mutations on the number of labelled glial nuclei

Genotypes (*rA87/+*)	L1	TSM	L3	Chemosensory bristles
+/Y	67 (32)	13 (23)	9 (22)	21 (31)
ac sc/Y	4* (25)	0 (25)	0 (25)	0 (25)
sc/Y	31 (12)	ND	0 (25)	10 (9)
ac/Y	50 (29)	8 (26)	6 (23)	18 (32)
ase/Y	58 (31)	12 (30)	9 (23)	10 (30)
Hw^1/Y	66 (20)	14 (20)	11 (13)	20 (11)

Crosses between wild-type or mutant stocks and the *rA87* line. Stocks in the first column are described in the Materials and Methods. The next three columns indicate the regions where nuclei were scored in pupal wings (24-37 hours AP). L1: glial nuclei from the anterior margin distal tip to the L1-L3 junction; TSM: glial nuclei between the TSM and the L1-L3 junction; L3: glial nuclei between the distalmost neuron of the L3 nerve and the L1-L3 junction. For each region, the average number of labelled nuclei is indicated in the left of the column. In the rightmost column the average number of chemosensory neurons in the dorsal triple row is shown. *The average number of glial nuclei in *ac sc* is calculated from the five wings that display labelling. In all columns, the number of wings analysed is given in parentheses. ND, not determined. Most *sc* wings also present axonal defects such as truncated L1 and L3 nerves and neuromas on L1, similar to those observed in other mutations (Palka et al., 1990; Giangrande et al., 1993, Giangrande, 1994). Axonal defects on L1 have been detected only in one *ac* wing.

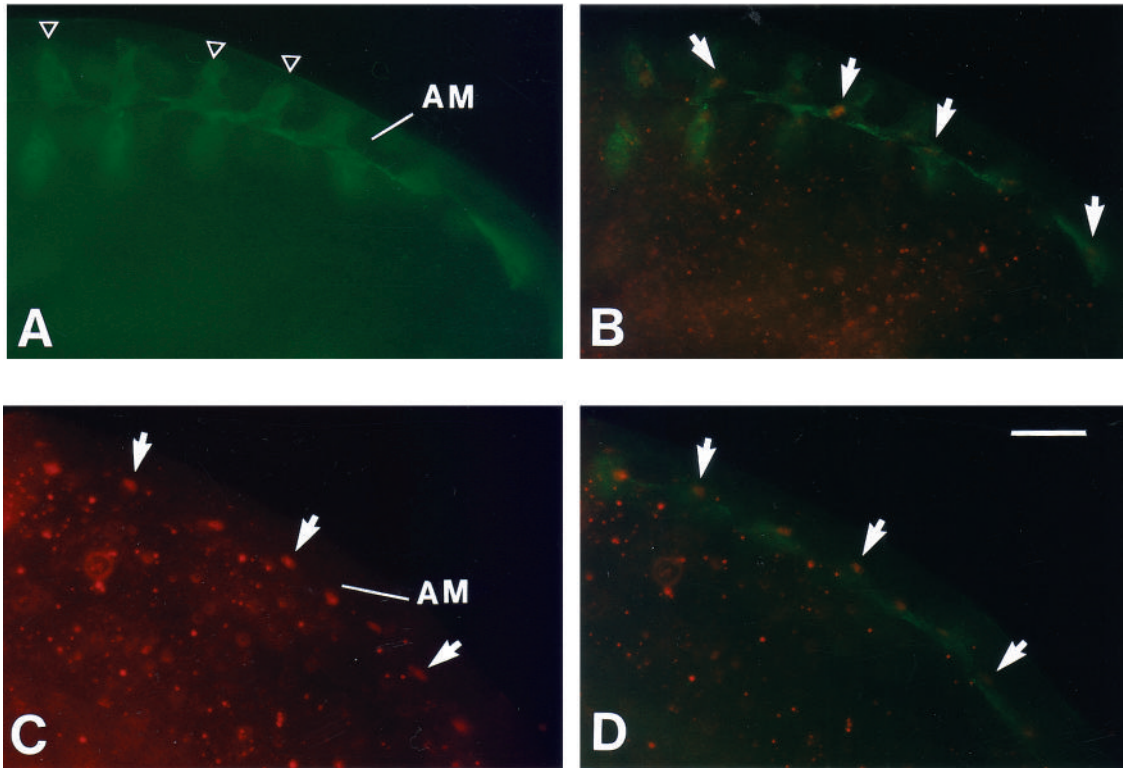


Fig. 3. Neuronal and glial labelling in early *ase* wings. *ase/Y; rA87/+* wings at 13 hours AP, distal region. (A) Wing labelled with mAb22C10, to label neurons and (B) wing showing double labelling with 22C10 and anti-β-gal to see the distribution of glial nuclei along the growing nerve. (C,D) A different wing, labelled with anti-β-gal (C) or (D) showing the double labelling. AM indicates the anterior margin. Neuronal somata of the chemosensory neurons are shown by triangles, glial nuclei by arrows. Bar 25 μm.

nuclei have been observed associated with the chemosensory cells (Fig. 3) and a few nuclei have also been observed at earlier stages (data not shown).

ASC gain of function and *h* mutations induce ectopic glial cells

Hw mutations induce *sc* and *ac* expression at positions at which ectopic sensory organs will later differentiate (Campuzano et al., 1986; Balcells et al., 1988; Blair et al., 1992). Ectopic bristles and campaniform sensilla are present on the entire wing blade except on the anterior margin (Palka et al., 1983). In the wing disc, *ac* ectopic expression appears later than in the normal set of proneural clusters. This is also reflected in the delayed development of the ectopic sensilla (Blair et al.,

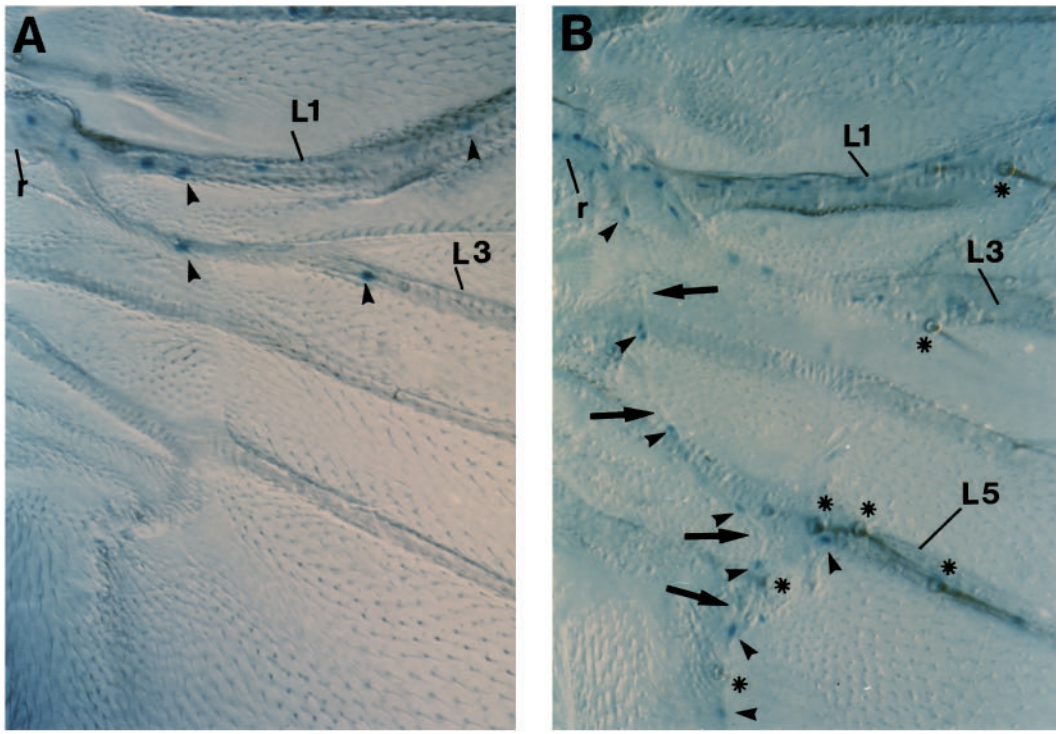


Fig. 4. Glial labelling in wild-type and *Hw* adults. Nomarsky view of wild-type (A) and mutant (B) wings, heterozygous for the *B72.1M3* insertion, labelled with X-gal. (A) Glial nuclei (arrowheads) line the radius (r), L1 (L1) and L3 (L3) veins. (B) Hw^1/Hw^1 wing carrying ectopic sensory organs (asterisks) on the wing blade (see bristles on the L5 (L5) vein). Glial nuclei (arrowheads) line the ectopic L5 nerve (arrows).

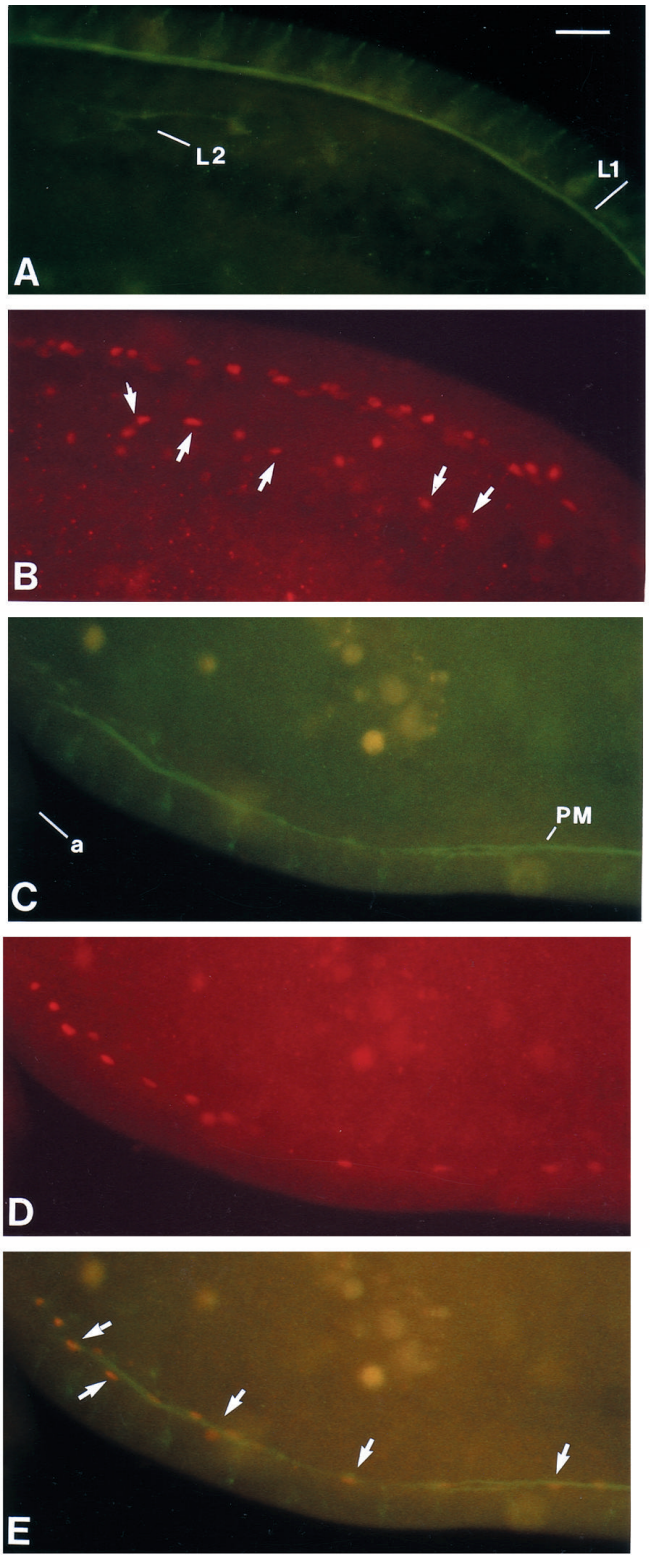


Fig. 5. Glial labelling in *Hw* pupae. *Hw*^{49c}*/Y; rA87/+* wings at around 25 hours AP labelled with (A,C) anti-HRP, (B,D) anti-β-gal. (E) Double exposure of C and D. (A,B) The distal part of the wing carrying the L1 nerve (L1) and an ectopic nerve on the L2 vein (L2). Ectopic glial nuclei are indicated by arrows. (C,D,E) The posterior and proximal part of a mutant wing. a and PM indicate the alula and the posterior margin, respectively. Neurons and axons are present along the PM, forming a nerve wrapped by glial nuclei (arrows). Bar 25 μm.

1992). The ectopic axons join the L1 or L3 nerves or extend in the intervein epithelium without reaching the base of the wing (Palka et al., 1983; Blair et al., 1992). The two mutations, *Hw*^{1} and *Hw*^{49c} (Campuzano et al., 1986; Balcells et al., 1988), display qualitatively similar phenotypes, even though *Hw*^{49c} is stronger than *Hw*^{1} and ectopic *ac* expression appears earlier and at more numerous positions than in *Hw*^{1} (Blair et al., 1992).

Hw flies have been analysed with the same glial-specific markers used to analyse loss of function mutations: a normal pattern of glial labelling is present along the anterior margin nerve (Table 1), in addition, labelled nuclei are present where the ectopic nerves have developed, even though not all ectopic

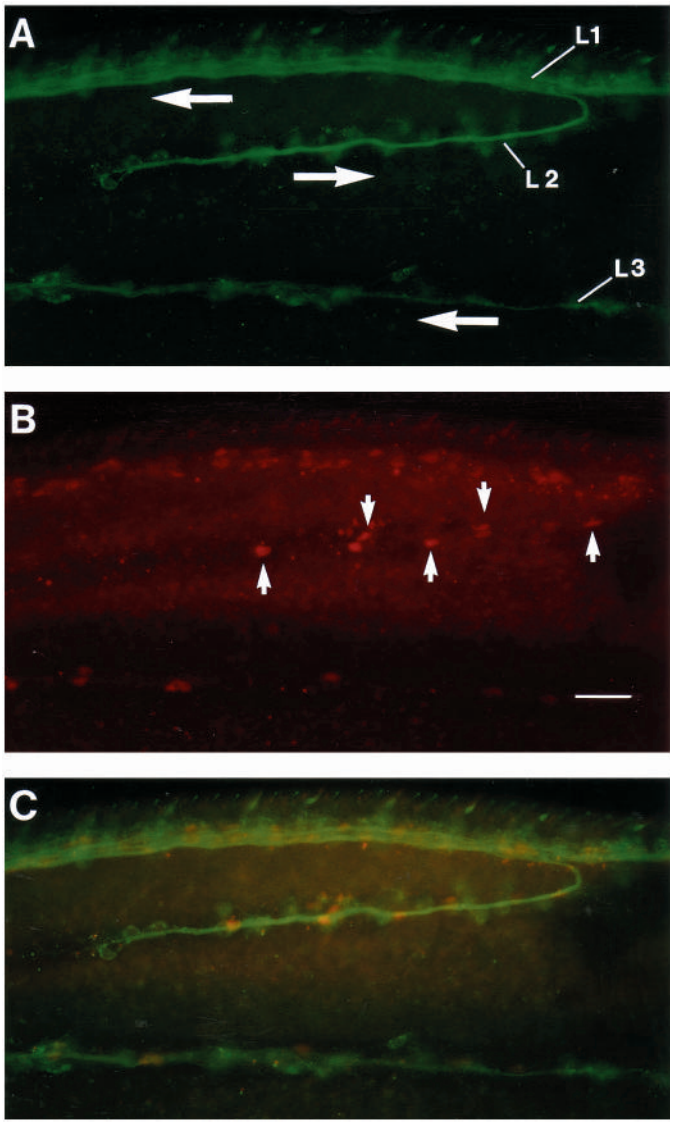


Fig. 6. Ectopic glial labelling in *h* wings. 39 hours AP *h*^{1}*/h*^{1} wing labelled with (A) anti-HRP and (B) RK2, a glial-specific antibody (Campbell et al., 1994; Giangrande, 1994; Xiong et al., 1994). C is a double exposure of A and B. L1, L2, L3 indicate the nerves present along the longitudinal veins L1, L2, L3. (A). The L2 nerve navigates distally, meets the L1 nerve and changes direction. The directions taken by the different nerves are indicated by large arrows. (B,C) Glial nuclei (small arrows) are present along the ectopic nerve. Bar 25 μm.

nerves carry glial nuclei (Fig. 4). I have found no ectopic glial cells using markers for late pupal stages, thus, like the sensory organs, ectopic glial cells develop later than normal glial cells (data not shown). Both mutants display ectopic glial cells (Figs 4, 5), although slightly more have been found in Hw^{49c} (data not shown).

The pair rule segmentation gene *h* (Nüsslein-Volhard and Wieschaus, 1980; Ingham et al., 1985), which codes for an altered bHLH protein (Rushlow et al., 1989; Skeath and Carroll, 1991; Van Doren et al., 1992; Wainwright and Ish-Horowitz, 1992), also affects sensory organ development in the wing (Palka et al., 1983; Skeath and Carroll, 1991; Blair et al., 1992). *h* represses *ASC* expression at ectopic positions and ensures that only the normal set of SOP cells differentiates. In *h* wings, *ac* is initially expressed as in the wild type, but is later activated at positions at which ectopic sensory organs arise

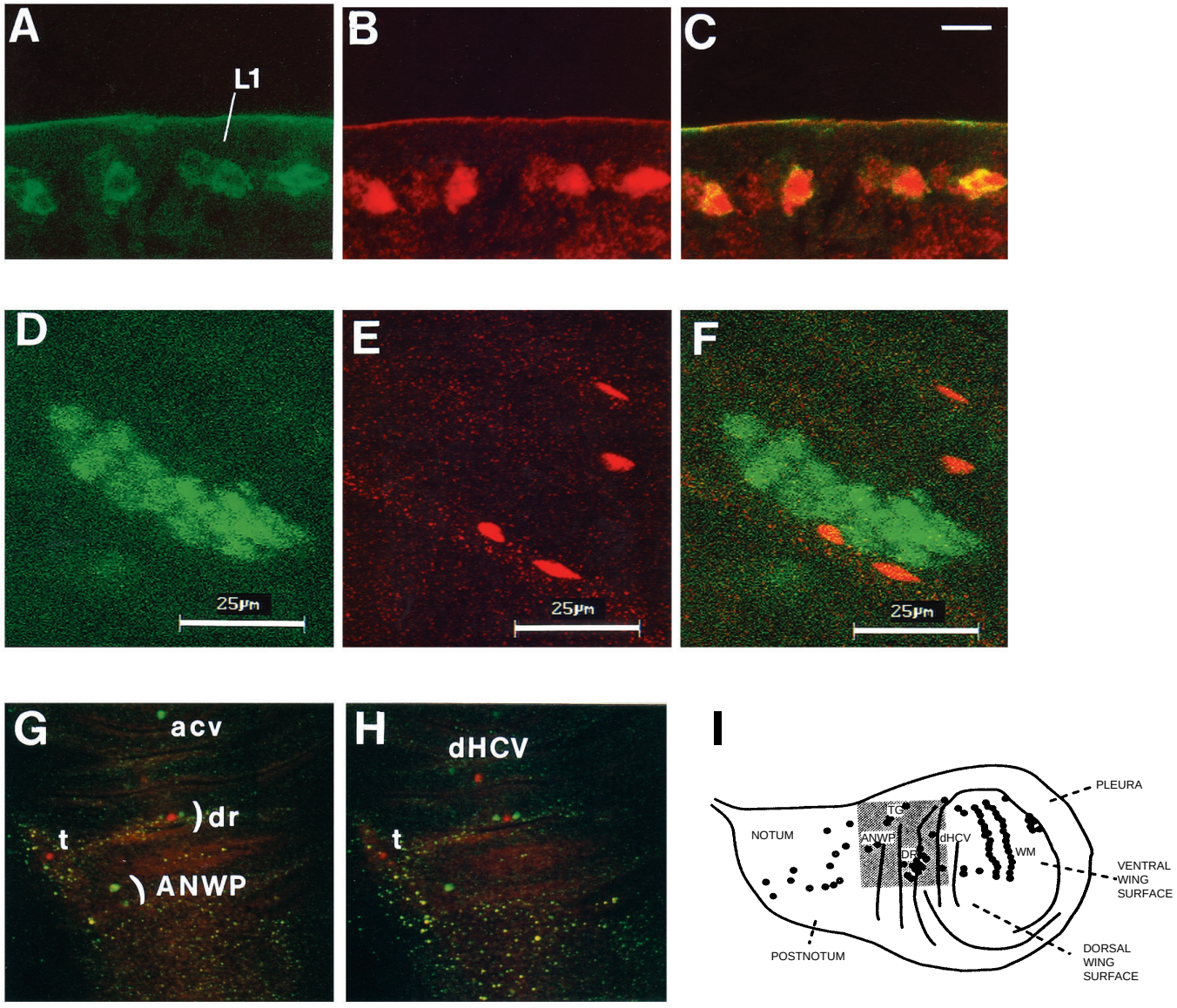


Fig. 7. *ASC* and glial labellings in pupal and larval wings. (A,B,C) 10 hour AP wing from the *ase-lacZ* line labelled with (A) mAb22C10 or (B) with anti-β-gal to detect *ase* expression. C was obtained by superimposing A and B. L1 indicates the anterior margin. The 22C10 antigen is membrane associated whereas the β-gal is localized in the nucleus. All the β-gal is contained within 22C10-positive cells, therefore, *ase* is not expressed in glial nuclei. (A,B,C) Bar, 10 µm. (D,E,F) *rA87/+* wing disc from wandering larva labelled with anti-achaete (D) or anti-β-gal (E) to detect glial nuclei. F is obtained by superimposing D and E. None of the glial nuclei colocalizes with achaete-positive nuclei. The images have been obtained by analysing and superimposing optical sections taken in the region of the dorsal radius (see G,H,I) through 15 µm with a 0.5 µm step. (G,H) Optical sections through a *rA87/+* disc labelled with anti-asense (green) and anti-β-gal (red). The step between each image is of 0.5 µm. (G) acv, dr and ANWP indicate, respectively, the precursors of the sensillum on the anterior cross vein, the sensilla on the dorsal radius and the sensilla campaniformia on the anterior notal wing process. A glial nucleus is present in the tegula (t) region, the SOP cell close to this nucleus is not in the same focus plane. (H) dHCV indicates the precursor of the dorsal large campaniform sensillum on the humeral cross vein. (I) Schematic drawing of the fate map of a third instar wing disc. Modified from Campuzano and Modolell (1992). Filled and open dots indicate the positions of the SOP cells. WM, TG, indicate the wing margin and the tegula region, respectively. DR, dHCV as in (G) and (H). The stippled area indicates the wing region shown in G and H.

(Skeath and Carroll, 1991; Blair et al., 1992). *h* wings carry the normal set of sensory organs and, as in *Hw* wings, ectopic sensory organs develop later (Palka et al., 1983; Blair et al., 1992). The glial pattern has been analysed in *h* pupae. Ectopic neurons are mostly found along the longitudinal veins, sometime sending out axons with reversed polarity (Palka et al., 1983 and Fig. 6). Ectopic glial cells are present along the ectopic nerves, even on those with reversed polarity (Fig. 6).

ASC pattern of expression and glial labelling

The earliest stage at which glial labelling can be observed in the wing disc is during the wandering third larval instar. Initially, one nucleus is present in the presumptive radial region. Later, the number of labelled nuclei in the region increases (Fig. 7E,F). At this stage, a large cluster of achaete-positive cells is located in the presumptive position of the dorsal radius, where many campaniform sensilla will develop (Cubas et al., 1991 and Fig. 7D,E,F,I). The labelled glial nuclei are located near to this cluster but do not express *ac*. Wandering larval discs have also been labelled with anti-asense (Brand et al., 1993) and, similarly, no colocalization of the asense product and the glial marker are observed (Fig. 7G,H).

In the anterior margin, glial cells are first labelled by 10 hours AP. However, at this stage little or no *ac* or *ase* expression is observed in the wing. Therefore I have used a transformant line carrying the *ase* promoter fused to the *lacZ* gene (Jarman et al., 1993). Since the β-gal product is more stable than asense protein, one can use this line to trace the history of the cells that have expressed *ase*. Indeed, in 10 hour AP wings, anti-β-gal and the neuronal specific antibody 22C10 display perfect colocalization (Fig. 7A-C). In contrast, β-gal is not found in glial cells. This shows that *ase* is only expressed in the neuronal lineage.

DISCUSSION

In the present study I show that gliogenesis in the fly PNS is affected by genes that are also required for sensory organ differentiation. Three genes of the *ASC* play a role in wing gliogenesis, *ac*, *sc* and *ase*, the same ones required for wing neurogenesis. The relative requirement of the three products in gliogenesis is different, as is the case for sensory organs. The similarity between neurogenesis and gliogenesis is not limited to the effects of the *ASC* mutations, but extends to genes such as *h*, normally required to repress *ASC* expression at ectopic positions. While the genes of the *ASC* are expressed in the cells that give rise to sensory organs, *ASC* products and glial markers do not colocalize, even at early stages of glial development.

Loss of function mutants of the proneural genes affect neurogenesis and gliogenesis

Previous results have shown that glial cells of the wing only form in regions that give rise to sensory organs. In *engrailed* and *fused* mutants, which have sensory organs at ectopic positions in the wing epithelium, glial cells accompany the ectopic nerves (Giangrande, 1994). In addition, the neurogenic genes, which interact with the proneural genes and are necessary for sensory organ differentiation, also affect gliogenesis in the embryo (Hartenstein et al., 1992) and in the adult (Giangrande, unpublished results). These data suggest a link between neurogenesis and gliogenesis. Therefore it is important to establish whether both processes are controlled by the proneural genes. The fact that the $sc^{10\text{-}1}$ deficiency, a loss of function of *ac* and *sc*, removes most sensory organs and glial cells indicates that neurogenesis and gliogenesis do indeed share a common genetic pathway. The possibility that glial cells are present but do not express a given antigen has been eliminated by using different types of markers. Since in $sc^{10\text{-}1}$ glial labelling is absent throughout development, it is very unlikely that glial cells differentiate and subsequently degenerate. Rather, it strongly suggests that they never form.

If the effects of either *ac* or *sc* mutations alone are analysed, it becomes evident that $sc^{10\text{-}1}$, which lacks both products, displays more than an additive effect on glial cells (see Table 1). This means that, in the single mutants, the remaining gene can in part substitute functionally for the missing one, as has already been observed for the sensory organs (Rodriguez et al., 1990; Brand et al., 1993; Dominguez and Campuzano, 1993; Hinz et al., 1994).

ASC expression at ectopic positions induces gliogenesis

In *Hw, ASC* misexpression induces the formation of ectopic nerves that are lined by glial nuclei. Since glial cells migrate along nerves and in many instances ectopic bundles are not connected with the normal ones, it is unlikely that glial cells originating at normal positions migrate to the ectopic nerves. Moreover, the number of glial cells on the normal nerves is not reduced, as one might expect if glial cells had migrated out of the normal positions.

The expression of the *ASC* only at the correct positions depends on *h* (Rushlow et al., 1989; Skeath and Carroll, 1991; Van Doren et al., 1992; Wainwright and Ish-Horowitz, 1992). The present study shows that glial cell development, like neuronal development, is repressed at ectopic positions due to the activity of the *h* gene. Thus, mutations acting in *cis* or in *trans* to misactivate the *ASC* always result in ectopic gliogenesis.

Does sensory organ differentiation induce gliogenesis?

ASC mutations have a direct effect on neurogenesis. By analogy, the simplest hypothesis is that glial cells arise from a cell that expresses the *ASC*, either the SOP cell, or one of the other achaete-positive cells in the proneural cluster. The present study however shows that none of the genes in the *ASC* are expressed in the glial lineage, unless they are present at such low levels that they are not detectable with the antibodies used. It is also formally possible that these genes are expressed in the glial lineage and switched off before rA87 labelling becomes evident. However, *rA87* is a very early marker, which labels glial cells at the same time as or slightly before RK2, a glial-specific antibody that recognises a homeobox-containing protein (Campbell et al., 1994; Xiong et al., 1994; Giangrande, unpublished results). Studies on the embryonic longitudinal glial cells have shown that RK2 recognises glial precursors (Campbell et al., 1994; Xiong et al., 1994) which suggests that the initial *rA87* labelling is found in glial precursors. These observations make it unlikely that the genes of the *ASC* are expressed during gliogenesis.

In light of these results, I propose that the mechanisms of action of the *ASC* are different in neurogenesis and gliogenesis. The effects of *ASC* mutations on glial cells seem to be indirect, due to the defects in sensory organ differentiation, which means that glial cells require normal sensory organ development to differentiate. Several findings support the hypothesis of an inductive process in glial differentiation. First, the effects of *Hw* on glial cells parallel perfectly those previously observed for sensory organ: both cell types develop at ectopic positions, later than the normal populations. The effects of Hw^{49c} on glial and neuronal cells are stronger and earlier than in Hw^{1}. Although *Hw* and *h* both result in ectopic sensory organs, regional differences exist between them. These same differences are observed for the ectopic glial cells. Second, the effects of *ac* and *sc* loss of function on glial cells also parallel those found for sensory organs. In $sc^{10\text{-}1}$ wings, most sensory organs do not differentiate and the few that are still there differentiate abnormally, since they appear much later than in wild type and send axons that never get to the central nervous system. These major defects in precursor singling out and in sensory organ differentiation result in the absence of glial cells in most mutant wings. Finally, previous data have demonstrated that *ac*, *sc* and *ase* mutations display different strengths with respect to their neuronal phenotype. The present results show that this is also true for gliogenesis and that, for each mutation, the glial phenotype parallels perfectly the neuronal phenotype. For example, *sc* has a stronger effect than *ac* on chemosensory organ and on glial cell development. In the case of *ase*, in which chemosensory neurons and glial cells are differently affected, it is likely that the initial steps of sensory organ differentiation, rather normal in this mutant, are sufficient to promote gliogenesis. Alternatively, since *sc* and *ac*, which affect both mechano- and chemosensory organs, affect glial cells more than *ase*, which affects preferentially chemosensory organs, it is possible that mechano- but not chemosensory organs are mainly required for glial cell differentiation. Further studies will be necessary to establish at what point downstream of *ASC* glial cell induction happens and whether this process depends on post-mitotic cells such as neurons or on premitotic cells, either the SOP or its daughter cells. They will also be important to determine whether glial induction depends on short range cell-cell interactions and whether glial precursors are always induced at reproducible positions.

Thanks to V. Auld and C. Goodman, C. Dambly-Chaudière, W. Gehring, A. Jarman, J. Modolell, M. Schubiger for sending me fly stocks. Thanks also to S. Benzer, J. Skeath and S. Carroll, A. Tomlinson for providing me with antibodies or hybridoma cells. I am grateful to J. Palka, in whose laboratory this work was started, and to P. Heitzler, P. Sassone-Corsi, M. Schubiger and P. Simpson who gave me helpful advice. Thanks to P. Lawrence, J. Modolell, M. Schubiger and P. Simpson for many thoughtful comments on the manuscript. I thank C. Carteret and C. Ackerman for excellent technical assistance and Color System, B. Boulay and S. Metz for help with the figures. J. L. Vonesch helped me with the confocal images. This work was supported by CNRS and INSERM.

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(Accepted 3 November 1994)