Precocious Expression of the Glide/Gcm Glial-Promoting Factor in Drosophila Induces Neurogenesis

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ABSTRACT

Neurons and glial cells depend on similar developmental pathways and often originate from common precursors; however, the differentiation of one or the other cell type depends on the activation of cell-specific pathways. In Drosophila, the differentiation of glial cells depends on a transcription factor, Glide/Gcm. This glial-promoting factor is both necessary and sufficient to induce the central and peripheral glial fates at the expense of the neuronal fate. In a screen for mutations affecting the adult peripheral nervous system, we have found a dominant mutation inducing supernumerary sensory organs. Surprisingly, this mutation is allelic to glide/gcm and induces precocious glide/gcm expression, which, in turn, activates the proneural genes. As a consequence, sensory organs are induced. Thus, temporal misregulation of the Glide/Gcm glial-promoting factor reveals a novel potential for this cell fate determinant. At the molecular level, this implies unpredicted features of the glide/gcm pathway. These findings also emphasize the requirement for both spatial and temporal glide/gcm regulation to achieve proper cell specification within the nervous system.

In the nervous system, many precursors generate both neurons and glial cells (Lever et al. 1990; Williams et al. 1991; Condron and Zinn 1994; Davis and Temple 1994; Bossing et al. 1996; Schmidt et al. 1997; Bernardoni et al. 1999; Schmid et al. 1999; Ragone et al. 2001). Glial and neuronal fates, however, are mutually exclusive, each depending on specific factors that promote and implement one fate at the expense of the other. Some developmental pathways, however, are common between the two cell types. Indeed, both neurons and glia need the activity of proneural and neurogenic genes (Ghysen and Dambly-Chaudière 1989; Romani et al. 1989; Simpson 1990; Campuzano and Modolell 1992; Giangrande 1995).

The expression of proneural transcription factors triggers the neural competence in groups of cells also called “proneural clusters.” In the peripheral nervous system (PNS), one cell of the cluster adopts a sensory organ precursor (SOP) fate and prevents neighboring cells from becoming SOPs by a process called lateral inhibition, which involves the interplay of proneural and neurogenic genes (Ghysen and Dambly-Chaudière 1989; Hartenstein and Posakony 1990; Jan and Jan 1990; Simpson 1990; Heitzler and Simpson 1991; Campuzano and Modolell 1992; Ghysen et al. 1993; Parks and Muskavitch 1993; Artaianis-Tsakonas et al. 1999). In flies, the best-characterized proneural genes belong to the Achaete-Scute Complex (AS-C). These genes—achaete (ac), scute (sc), lethal of scute (l'sc), and asense (ase)—are required for the differentiation of mechano- and taste sensory organs (Campuzano et al. 1985; Ruiz-Gomez and Modolell 1987; Romani et al. 1989; Rodriguez et al. 1990; Cubas et al. 1991; Gomez-Skarmeta et al. 1995). Another proneural gene, atonal (ato), is required for the differentiation of types of sensory organs: olfactory receptors, photoreceptors, and chordotonal organs (Jarman et al. 1993, 1994; Reddy et al. 1997; Hassan and Bellen 2000). Two classes of mechanosensory organs exist with respect to their neuronal/glial composition: gliogenic sensory organs, which contain one neuron and several peripheral glial cells, and nongliogenic sensory organs, which contain two neurons but no glial cell.

The glial-promoting activity is provided mostly by a transcription factor, Glide/Gcm (Hosoya et al. 1995; Jones et al. 1995; Akiyama et al. 1996; Vincent et al. 1996; Bernardoni et al. 1997, 1998; Schreiber et al. 1997; Miller et al. 1998; Van De Bor et al. 2000; Ragone et al. 2001). Glide/Gcm misexpression induces ectopic gliogenesis, indicating that glide/gcm must be tightly regulated spatially. This gene implements the lateral glial fate in the central nervous system (CNS) as well as the glial fate in the PNS.

Here we present the phenotype of a dominant mutation, Polythryx (Pyx), obtained in a screen to identify mutations affecting sensory organ differentiation, which specifically exhibits supernumerary sensory organs on the notum. To our surprise, we found that Pyx is an allele of glide/gcm. In the wild type, glide/gcm is expressed in the gliogenic sensory organs of the notum during the pupal life (Gho et al. 1999; Reddy and Rodrigues 2000).
In Pyx animals we have found that glide/gcm is more precociously expressed (third larval instar) in that part of the body. Precocious glide/gcm activates the expression of the A5-C, which subsequently triggers neurogenesis. By using the UAS-Gal4 system, we show that the Pyx mutant phenotype can be reproduced in other regions, but only when glide/gcm is induced prior to its normal time of expression. Thus, regulation of glide/gcm expression in space and time are fundamental to instruct specific cell fates. These results also demonstrate that a heterochromatic mutation transforms a glionic factor into a sensory organ-promoting factor.

MATERIALS AND METHODS

Fly stocks: The wild-type strain was Sevelen. The ac se mutant stock was lin(1) ac sc[1995] F1 M7 (CAMPUZANO et al. 1985). glide/gcm w; P(eve-2;glide/gcm, w) is an amorph (Vincent et al. 1996; Miller et al. 1998). Complementation tests were made using Df(2L)13A-C Df(2L)166, l(2)DA2, l(2)DB1, glide/gcm w; P(eve-2;glide/gcm, w) for the 30B region; Df(2L)M11 and Df(2L)w11 for the 24D region; and Df(2)ren30 and Df(2)ren-B for the 48B region (Lindsley and Zimm 1992; Lane and Kalderon 1993). Pyx is an X-ray-induced dominant transposition with the following cyto- logical order: 21A-24D/30C-48A/24E-30B/48B-60F. The 24D break is associated with a pupal lethal fat (β) allele. The Tp(2;2)Pyx chromosome is viable in the presence of a ft duplica- tion [Dp(2;Y)odd11]. Cytological analysis of the X-ray- induced revertants PyxR-1 and PyxR-2 reveals the following new order: 21A-24D/41F-30C/88F-100F; 61A-85E/42A-48A/ 24E-30B/48B-60F and 21A-24D/101F-101A; 102F-101F/30C- 48A/24E-30B/48B-60F, respectively. Dp(2;2)Pyx and Df(2L) Pyx segregants were recovered by simple exchange between Tp(2;2)Pyx and a wild-type chromosome. Since the deficiency covers the M(2)24F dominant mutation, recombination was recovered upon screening for the M(2)24F lethal phenotype. In particular, Df(2L)Pyx was screened for the M(2)24F dominant phenotype using Dp(2;3)dpβ2, which prevents haplo- lethality induced by aneuploidy. Dp(2;2)Pyx was screened as dominant suppressor of M(2)24F using Df(2L)M24-B. Pyx flies were always used in heterozygous condition.

The following lines were used in gain-of-function experi- ments: [w; P(hev-4;glide/gcm, w)]; [w; P(UAS-glide/gcm, w)]; (Bern-

ardoni et al. 1997), [w; P(hev-4;glide/gcm, w)]; P(hev-4;glide/ gcm, w); P(hev-4;glide/gcm, w)] /TM3 (Bernardoni et al. 1997), [yw, hs-FLP122; +; P(UAS<CD2, y<glide/gcm[wH10]); w]) and [w; P(hev-4;glide/ gcm, w)].

Mutagenesis: Pyx revertants were induced in Pyx/CyO males with ethyl methanesulfonate (EMS) according to the protocol of Lewis and Bacher (1968) or by X rays at a dose of 4000 rad (100 kV, 10 mA for 5 min, 1.5-mm aluminum filter, Philips MG102 constant potential X-ray system, beryllium window; Serenbrovsky and Dubin 1930).

Immunolabeling and in situ hybridization: Fixation, dissection, and antibody incubation were performed as in GRANDE et al. (1993). In situ hybridization was performed as in Bernardoni et al. (1997) and Vande Bor et al. (2000). Wings and embryos were mounted in Vectashield medium (Vector, Burlingame, CA). The following primary antibodies were used: the anti-ELAv (1:2000; provided by G. Rubin), rabbit anti-Repo (1:8000; provided by A. Travers), rat anti-RK2 (1:1000; provided by A. Tomlison), rat anti-β-Gal 55976 (1:2000; Cappel), sheep anti-digoxigenin (1:1000; Boehringer Mannheim, Indianapolis), rabbit anti-Ase-1 (1:8000; pro- vided by A. Jarman), mouse anti-Achaete (1:50; provided by S. Carroll), and mouse anti-CD2 (1:2000; Serotec, Oxford, UK). Secondary antibodies coupled with Oregon Green (Molecular Probes, Eugene, OR), Cy3, Cy5, and FITC (Jackson) were used at 1:400. Preparations were analyzed using a confocal microscope (DMRE, Leica).

Ubiquitous and clonal overexpression of glide/gcm: Ubiquitous overexpression was obtained by crossing w; P(hs- glide/gcm, w) flies with the w; P(UAS-glide/gcm, w) line, or by using the w; P(hs-glide/gcm, w); P(hs-glide/gcm, w)/TM3 line. Prepupae or larvae coming out from this cross or from the w; P(hs-glide/gcm, w); P(hs-glide/gcm, w)/TM3 line were collected and challenged with different heat-shock regimens. Pupal wings or wing discs were dissected and labeled as above. To induce glide/gcm clonal overexpression we used the "flp-out cassette" technique. The flp-out cassette is fused to the glide/gcm coding sequences and is cloned downstream to Gal4 yeast transcription factor target sites (UAS sequences). The cassette contains the y+ gene and the CD2 protein coding sequences, flanked by targets for the yeast FLP recombinase. These targets (FRT) have the same orientation. Induction of FLP expression leads to a recombination event that induces the excision of the DNA located between the FRT sites. After recombination, the UAS sequences are fused to the glide/gcm coding sequences. Full-length glide/gcm cDNA was subcloned into pCasper UAST as an XbaI fragment. The flp-out cassette (FRT, y+ FRT or <CD2, y<; kindly provided by K. Basler; Zecca et al. 1995) was then inserted as a Kpn1 fragment between the UAS sequences and the glide/gcm coding sequences. Transgenic lines were generated carrying the UAS<CD2, y<glide/gcm construct. The so-called M411 transgenic line carrying the flp-out cassette on the third chromosome was crossed with w; hs-FLP122 flies. The male progeny w; hs-FLP122; +; P(UAS<CD2, y<glide/gcm[wH10], w) were crossed with females Act5C-gal4. Adults were allowed to lay eggs for one day. Female larvae of the genotype w; hs-FLP122/+; Act5C-gal4/w; P(UAS< y<glide/gcm[wH10], w)/+ were collected and heat-shocked 21 hr later at 38°C for 30 min to induce mitotic recombination early during the second larval instar. These females were then kept at 25°C until 24 hr after puparium formation (APF) to be dissected or until the adult stage. Clones overexpressing glide/gcm were recognized by the absence of anti-CD2 labeling or by the presence of the adult yellow cuticle marker. Adult nota and wings were mounted in Euparal (Fischer). Searches for Glide/Gem binding sites were carried out using MatInspector program against the TRANSFAC database (Heinemeyer et al. 1999).

RESULTS

Pyx induces supernumerary sensory organs: A genetic screen was performed to identify mutations affecting the differentiation of the adult peripheral nervous system. The fly notum exhibits two types of sensory organs: microchaetes, small bristles that are regularly spaced, and macrochaetes, large bristles that are located at stereotyped positions (Figure 1A). X-ray mutagenesis led to the identification of dominant mutations altering the number of bristles. One of these mutations, Pyx, induces the differentiation of supernumerary macrochaetes. Wild-type nota contain 11 macrochaetes per heminota (in this and in the following genotypes 20 heminota were analyzed). In Pyx flies, we found, on average, 19 large bristles (female average, 21; male average, 17; Figures 1 and 2). Both bristle number and position vary.
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**Figure 1.**—*Pyx* affects bristle differentiation on the notum. (A) Schematic representation of an adult notum. Anterior is to the top. Small and large dots represent microchaetes and macrochaetes, respectively. PS, the presutural macrochaete; aNP, the anterior notopleural; pNP, the posterior notopleural; ASA, the anterior supraalar; pSA, the posterior supraalar; aPA, the anterior postalar; pPA, the posterior postalar; aDC, the anterior dorso-central; pDC, the posterior dorso-central; aSC, the anterior scutellar; pSC, the posterior scutellar. (B–F) Adult nota from wild type (WT; B), *Pyx/+* (C), *Pyx/glide/gcmN7-4* (D), *PyxR+1/+* (E), and *PyxR+3/+* flies (F). Bar, 200 μm.

even within the two heminota of the same animal, making it difficult sometimes to distinguish supernumerary bristles from those that are normally found in the adult fly. Throughout the adult body, supernumerary bristles are present only on the scutum and on the scutellum (see Figure 1, B and C). Also, we never found adjacent supernumerary bristles, indicating that the process of lateral inhibition has not been affected by the mutation.

**Pyx affects the glide/gcm locus:** To characterize the mutation causing the bristle phenotype, we performed cytological analyses and found that *Pyx* flies carry a transposition from the left to the right arm of chromosome 2 (Figure 3). The transposed region, which goes from 24D to 30B, is inserted at 48A. *Tlp(2;2)Pyx* is viable and fertile over deletions covering 30B or 48A. The breakpoint at 24D induces late pupal lethality that can be rescued by using a duplication of the 24D region. *Pyx* homozygous flies carrying this duplication are viable and show a bristle phenotype stronger than heterozygous adults (data not shown). We then investigated which breakpoint, 24D, 30B, or 48A, is responsible for the bristle phenotype. *Pyx* is an intrachromosomal transposition (Figure 3). By simple exchange with a wild-type chromosome, we recovered both the deletion and the duplication derivatives (see MATERIALS AND METHODS). Clearly, we found that the bristle phenotype is associated with the *Df(2L)Pyx* derivative and not with even within the two heminota of the same animal, making it difficult sometimes to distinguish supernumerary bristles from those that are normally found in the adult fly. Throughout the adult body, supernumerary bristles are present only on the scutum and on the scutellum (see Figure 1, B and C). Also, we never found adjacent supernumerary bristles, indicating that the process of lateral inhibition has not been affected by the mutation.

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Mutagenesis carried on *Tlp(2;2)Pyx* flies enabled us to recover three revertants of the thoracic phenotype. One revertant was obtained by EMS (1/2000; *PyxR+3*) and two by X ray (2/9000; *PyxR+1* and *PyxR+2*; Figure 1, E and F, and Figure 2). All *Pyx* revertants (including the EMS-induced one) are embryonic lethal in homozygous conditions and over a deficiency covering the 30B region, whereas the parental *Pyx* chromosome complements 30B deletions. Consistently, the two X-ray-induced revertants are associated with a new breakpoint at the...
24D-30B junction of the parental chromosome. Since the reversion of the dominant Pyx phenotype is invariably associated with a recessive lethality located at 30B, we propose that Pyx affects a vital gene from the 30B, a section that has been studied extensively (Lane and Kalderon 1993). Complementation tests using deficiencies and lethal mutations in the 30B region revealed that Pyx revertants specifically affect the glide/gcm locus (see MATERIALS AND METHODS). Moreover, Pyx revertants do complement (2)DB1 and (2)DA2, the two complementation groups that flank glide/gcm. Finally, the analysis of the PyxR+3 revertant revealed that both glial development and glide/gcm expression are drastically reduced (Figure 4, C and F). Pyx embryos, on the other side, do not show defects (Figure 4, A–E).

All these results indicate that Pyx is an allele of glide/gcm and we refer to it as glide/gcmPyx. The facts that glide/gcmPyx is a dominant mutation and that glide/gcmN7-4 clones do not show bristle phenotypes (data not shown) strongly suggest that glide/gcmPyx is not a loss-of-function mutation. Since no deletions at 24D or 30B are dominant, we hypothesized that glide/gcmPyx is not haplo-insufficient but rather corresponds to a gain-of-function mutation. Moreover, glide/gcmPyx/glide/gcmN7-4 flies display the same phenotype as glide/gcmPyx/+ flies, suggesting that the mutation is a neomorph (Figure 1D and Figure 2).

**glide/gcmPyx induces precocious glide/gcm expression** in the notum: The finding that a mutation in a gliogenic gene produces supernumerary sensory organs is most surprising, since we know that Glide/Gcm is necessary and sufficient to implement the glial fate (Hosoya et al. 1995; Jones et al. 1995; Vincent et al. 1996; Bernardoni et al. 1998; Van De Bor et al. 2000). Peripheral glial organs originate from a class of sensory organs (Gho et al. 1999; Reddy and Rodrigues 1999; Jhaveri et al. 2000; Orgogozo et al. 2001; Van De Bor and Giangrande 2001). After singling out, the SOP divides repeatedly to produce the cells that compose the sensory organ (Hartenstein and Posakony 1989; Huang et al. 1991). In the tissues in which gliogenic sensory organs have been studied, glide/gcm is expressed during the pupal life (Gho et al. 1999; Reddy and Rodrigues 1999; Jhaveri et al. 2000; Van De Bor et al. 2000; Van De Bor and Giangrande 2001).

In the wing, the fifth cell of the sensory organ lineage is also called glial precursor (GP). This cell requires glide/gcm to differentiate, divides several times, and produces the glial cells that migrate along peripheral nerves (Giangrande et al. 1993; Giangrande 1994; Van De Bor et al. 2000). Mutations at the glide/gcm locus lead to fate transformations within the sensory organ lineage: lack of Glide/Gcm converts glial cells into neurons, whereas ectopic Glide/Gcm results in the opposite transformation: namely, neurons convert into glial cells (Hosoya et al. 1995; Jones et al. 1995; Van De Bor et al. 2000).
Gain- or loss-of-function mutations, however, do not produce supernumerary sensory organs as those found in glide/gcm<sup>Pyx</sup>. All these results indicate that glide/gcm triggers the fate choice between two cell types but is required neither for inducing the neural competence nor for SOP singling out. They also suggest that, within the neurogenic regions, neuron is the default fate due to the absence of glide/gcm expression.

To clarify the cause of the supernumerary sensory organs, we analyzed the molecular nature of the glide/gcm<sup>Pyx</sup> mutation. Transposition breakpoints bring together genomic fragments belonging to different loci. This may result in misexpression of the gene close to the breakpoint or in the expression of fusion products. In situ hybridization performed on glide/gcm<sup>Pyx</sup> chromosomes allowed us to show that glide/gcm has not moved to the right arm of the second chromosome (data not shown). Southern blot, PCR, and sequence analyses confirmed that, in glide/gcm<sup>Pyx</sup>, the glide/gcm locus has preserved its integrity and that at least 2 kb 3’ to glide/gcm are intact (Figure 3 and data not shown). Thus, glide/gcm<sup>Pyx</sup> is not due to the presence of a fused gene. Moreover, the glide/gcm profile of expression is not altered during pupal life. The remaining explanation was that, in glide/gcm<sup>Pyx</sup> flies, glide/gcm is temporally misexpressed. To test this hypothesis, we used two approaches: (i) we induced early misexpression by using hs-glde/gcm or hs-gal4/+; UAS-glde/gcm/+ transgenic lines and analyzed the bristle phenotype (Figure 5) and (ii) we determined the profile of glide/gcm expression in glide/gcm<sup>Pyx</sup> larvae (Figure 6).

Third instar larvae or white pupae expressing glide/gcm under the heat-shock promoter were submitted to heat pulses varying in length and/or temperature. Adult nota and wings were mounted in order to count the number of sensory organs (Figure 5, A and B). The effects of the different regimens were quantified, as shown in Figure 5C. In general, the use of the UAS-GAL4 system induced stronger defects and more lethality than the hs-glde/gcm transgene, most likely due to the amplification obtained in the sandwich technique.

However, the qualitative results obtained with the two types of transgenes are similar. Third instar larva (L3) and white pupa (WP) heat shocks did induce supernumerary bristles on the notum as well as on the wing (Figure 5C), whereas heat shocks during the pupal life did not (data not shown). The regimen that more closely mimics the glide/gcm<sup>Pyx</sup> phenotype is obtained upon L3 heat shock for 90 min at 37°C (Figure 2 and Figure 5, A and C).

In agreement with these data we found that glide/gcm<sup>Pyx</sup> larvae show consistent glide/gcm-specific labeling in regions that later display supernumerary bristles (Figure 6, A and B). No glide/gcm-specific signal was detected at this stage in wild-type discs.

**Early Glide/Gcm activates proneural genes:** The phenotype induced by precocious glide/gcm expression prompted us to ask, How does a glial-promoting factor induce supernumerary sensory organs? We analyzed the profile of expression of the AS-C, the proneural genes that induce the neural competence in the wing disc. To do so, we used anti-Ac, the profile of which also reflects that of the second major proneural gene in the wing, Sc (Romani et al. 1989; Rodriguez et al. 1990; Cubas et al. 1991) and anti-ase, which is expressed in the SOPs. glide/gcm<sup>Pyx</sup> discs show ectopic expression of these genes in the part of the presumptive notum where glide/gcm had been previously observed (Figure 6, C and D). Interestingly, the number of Ase-positive cells is lower than that of the supernumerary bristles observed in the adult,
sugesting that more SOPs arise at later stages. As in the adult, it is often difficult to distinguish the supernumerary SOPs from the endogenous ones (Figure 6, E and F).

We then asked whether glide/gcmPyx supernumerary bristles depend on the expression of the AS-C. The ac\textsuperscript{3} sc\textsuperscript{101} strain carries an amorph sc mutation and displays a strong reduction of ac and l\textasciitilde{e}c expression. Nota from mutant escapars are completely devoid of bristles (Campuzano et al. 1985). ac\textsuperscript{3} sc\textsuperscript{101}/y; glide/gcmPyx/+ males resemble ac\textsuperscript{3} sc\textsuperscript{101}/y males, since zero to three bristles are present on the nota of adult males (Figure 2). The few remaining bristles are most likely due to the subsisting expression of l\textasciitilde{e}c and ac. The effects can already be seen in ac\textsuperscript{3} sc\textsuperscript{101}/+; glide/gcmPyx/+ females (Figure 2). These results clearly indicate that precocious glide/gcm needs the AS-C to form bristles.

The data obtained with glide/gcmPyx are in agreement with those found upon heat-shock induction of glide/gcm. In 6-hr APF wild-type wings, Ac is detectable only on the anterior margin, where sensory organs are still differentiating (Figure 7A: WT), whereas wings expressing glide/gcm precociously (Figure 7C: hs-gal4; UAS-glide/gcm) show labeling throughout the blade. Thus, precocious Glide/Gcm induces the expression of proneural genes.

To follow sensory organ differentiation we also used the neuronal-specific marker anti-Elav (Robinow and White 1988) and the antibody against the Reverse polarity (Repo) product, which is specifically expressed in glial cells (Campbell et al. 1994; Xiong et al. 1994; Halter et al. 1995). In the wild type, neurogenesis and glioniogenesis take place along L1 (or anterior margin) and L3 veins (Figure 7B). On the contrary, mutant wings display supernumerary neuronal and glial labeling within and outside L1 and L3, including the posterior compartment (Figure 7D). These defects are never observed in wild-type wings submitted to the same heat-shock treatment as mutants and processed in parallel. As for the supernumerary sensory organs seen in the adult, neurons are never adjacent, indicating that the lateral inhibition process has not been affected.

Adult mechano-sensory organs are of two types, gligenic and nongliogenic, glide/gcm expression being restricted to the first type (Gho et al. 1999; Reddy and Rodrigues 1999; Van De Bor et al. 2000). Interestingly, we found that while some supernumerary neurons were accompanied by Repo labeling (Figure 7D; see arrows), some others were not (Figure 7D; see arrowheads), suggesting that precocious Glide/Gcm induces both gligenic and nongliogenic lineages.

One possible explanation for the bristle phenotype is that glide/gcm activates the AS-C during normal development. Such a late requirement for the AS-C may have
been overlooked because of the early effects of AS-C mutations. By using anti-Ase or anti-Ac we analyzed wings at the time at which glide/gcm is normally expressed but we did not detect any labeling, strongly suggesting that glide/gcm does not induce AS-C expression in the GP (data not shown).

**Glide/Gcm acts autonomously:** To determine whether glide/gcm has an autonomous activity, we induced its expression in clones. We constructed a flip-out transgenic line that allowed us to express glide/gcm conditionally: UAS<CD2, y<glide/gcm. Larvae of the following genotype were generated: hs-fLP/+; Act5C-gal4/+; UAS<CD2, y<glide/gcm/+ . Such larvae were submitted to heat shock at second instar to induce FRT cassette excision and thereby glide/gcm expression. Clones expressing glide/gcm were recognized by the lack of yellow expression in the adult and by the lack of CD2 labeling in pupal tissues (Figure 7, E and F).

As in the case of ubiquitous expression, supernumerary glial cells and neurons were observed; however, we found fewer neurons than Repo-positive cells, which often formed large clusters (Figure 7F). The difference between this and the previous phenotypes most likely resides in the transgene used. In the clonal experiment, once an excision event takes place in a cell and switches glide/gcm on, the gene remains activated in that cell and in its progeny throughout development. Precocious Glide/Gcm initially triggers the neural competence and the differentiation of sensory organ precursors, as seen with the hs-glide/gcm transgene. Late activation of glide/gcm, however, is known to induce glial cells at the expense of neurons within the SOP lineage (Van De Bor et al. 2000). Thus, continuous expression affects two different and independent processes and results in massive glial differentiation. Autonomous glide/gcm activity was also confirmed by the observation that all supernumerary sensory organs lie within the clones.

Precocious glide/gcm expression experiments also revealed that the type of sensory organ induced depends on the context. In wild-type flies, the thorax exhibits only bristles whereas the wing carries both bristles and campaniform sensilla. Accordingly, precocious Glide/Gcm induces bristles on the thorax but bristles and campaniform sensilla in the wing (Figure 5 and Figure 7E). It should be noted that continuous expression of glide/gcm using the FRT cassette on the notum also allowed differentiation of microchaetes, which differentiate later than macrochaetes (data not shown). Thus, glide/gcm does not specify the type of sensory organs.

Finally, we asked whether glide/gcm induces only sensory organs depending on the AS-C. In the antennae, the differentiation of olfactory receptors does not depend on the AS-C (Reddy et al. 1997; Jhaveri et al. 2000). Indeed, some of them require the ato proneural gene (Jarman et al. 1993; Gupta and Rodrigues 1997;...
Reedy et al. 1997). Interestingly, the number of olfactory receptors as well as the ato profile of expression is unchanged but supernumerary bristles are induced upon glide/gcm activation (data not shown). Thus, the potential to induce sensory organs seems limited to sensory organs that are normally induced by the proneural genes of the AS-C.

**DISCUSSION**

**Precocious Glide/Gcm induces expression of the AS-C genes:** In vertebrates and in invertebrates the differentiation of the nervous system is under the control of proneural genes that define clusters of neural competent cells (Ghysen and Dambly-Chaudière 1989; Romani et al. 1989; Campuzano and Modolell 1992; Kageyama et al. 1997; Guillemot 1999; Hassan and Bellen 2000). In flies, it has been shown that one cell of the cluster adopts the neural fate and produces the neural precursor, stem cells in the CNS and SOPs in the PNS, whereas the others become epidermis. In the absence of the AS-C products, the neural competence is not acquired and SOPs are not formed. Within the gliogenic sensory organ lineage, glide/gcm is expressed and required in the GP well after the SOP is singled out (Gho et al. 1999; Reedy and Rodrigues 1999; Van De Bor et al. 2000; Van De Bor and Giangrande 2001). Thus, glide/gcm is expressed when proneural genes have already accomplished their function and is not required in sensory organ formation.

The present results show that glide/gcm is able to induce PNS formation. Interestingly, supernumerary sensory organs are induced only when glide/gcm is expressed precociously, due to the activation of the AS-C genes. Indeed, the stage at which glide/gcm induces PNS formation is the same at which ectopic expression of the AS-C does (Rodriguez et al. 1990). Together with the analysis of the epistatic relationship these results suggest that all the effects observed in glide/gcm and in hs-glide/gcm flies are mediated by ectopic AS-C expression. It is possible that Glide/Gcm acts on the AS-C directly, since several Glide/Gcm-binding sites are present in the promoter sequences that induce ac and sc expression (data not shown). For example, we found GBSSs in the regulatory elements that promote AS-C expression in the dorso-central and scutellar clusters (Romani et al. 1989; Cubas et al. 1991; Gomez-Skarmeta et al. 1995). A search for GBSSs in the AS-C promoter from Drosophila virilis has revealed that several sites do exist (data not shown). Some of them are located in the region that corresponds to the dorso-central enhancer, even though the precise position of GBSSs is not

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**Figure 7.—** Precocious glide/gcm activates PNS differentiation in an autonomous manner. Anterior is to the top, distal to the right. (A–D) Wings heat-shocked at WP for 1 hr at 37°C. (A and C) 6-hr APF wings labeled with anti-Achaete antibody: (A) wild type (WT); (C) hs-gal4/+; UAS-glide/gcm/+.. (B, D, and F) 24 hr APF heat-shocked wings. (B and D) Wings simultaneously labeled with anti-Repo (red) and anti-Elav (blue), a neuronal-specific marker: (B) wild type, (D) hs-gal4/+; UAS-glide/gcm/+.. A and P indicate the anterior and the posterior compartments, respectively. The white line in between indicates the compartment boundary. Arrowheads and arrows indicate isolated supernumerary neurons and neurons associated with glia, respectively. The inset shows a high magnification of a wing region containing ectopic neurons (arrowhead) and ectopic neurons associated with glial cells (arrows). (E) Adult wing carrying a clone overexpressing glide/gcm across the L4 vein (L4). The black dashed line indicates clone borders. Note the presence of supernumerary sensory organs: sensilla campaniformia (arrow) and bristles (arrowheads). (F) Wing carrying the flip-out cassette and submitted to heat shock simultaneously labeled with anti-Repo (red), anti-Elav (blue), and the clonal marker anti-CD2 (green). The patches of cells overexpressing glide/gcm are those that lack CD2 expression. Dorsal and ventral clone borders are indicated by white dashed and yellow dotted lines, respectively. L1 and L3 indicate the L1 and the L3 innervated veins, respectively. Flp-out transgenic lines were heat-shocked during the second instar. Note that the patch of cells expressing glide/gcm straddles the anterior/posterior compartment, indicating that it contains several clones. Bar, 52 μm in A and C; 78 μm in B, D, and F; 40 μm in E.

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conserved between D. virilis and D. melanogaster. It will be interesting to determine whether the sites present in the ASC promoter are functional and important during development. This might provide insight into a previously unknown regulation of the ASC genes by Glide or related proteins. It is worth mentioning that precocious glide/gcm induces the ASC, but not other proneural genes such as ato (V. Van De Bor and A. Giangrande, unpublished observations).

It is tempting to speculate that the supernumerary bristles induced by precocious glide/gcm are due to defects in positional information. A number of genes controlling the ASC and sensory organ differentiation are required to define specific territories in a given tissue. This class of genes, to which pannier and the iroquois complex belong (Romain et al. 1993; Gomez-Skarmeta et al. 1996; Heitzler et al. 1996; Grillenzoni et al. 1998; Diez del Corral et al. 1999; Garcia-Garcia et al. 1999), have also been called prepattern genes. Glide/Gcm shares some features with such genes in the sense that it affects the expression of proneural genes and thereby PNS development. Interestingly, however, while pannier mutations also affect notum differentiation, precocious glide/gcm does not seem to alter the general structure of the tissue in which it is expressed. It is unlikely that glide/gcm acts by regulating prepattern genes, since its precocious expression does not modify the expression of pannier (data not shown). Thus, even though glide/gcm and pannier affect the same sensory organs (dorso-central bristles), it is likely that they act independently.

Supernumerary bristles and ectopic ASC expression are found specifically on the thorax of glide/gcmBts flies. The simplest explanation is that the breakpoints at 24D/30B have created a regulatory element that induces glide/gcm expression in the thorax. One possible candidate for this novel regulation is the fat locus, which is located at 24D (Garola et al. 2000) and is affected by the glide/gcmBts mutation (see Materials and Methods). Since the fat gene is expressed in the notum (Bryant et al. 1988), we speculate that, in the mutant, glide/gcm expression is regulated partly by fat regulatory sequences. More importantly, however, the results obtained with hs-glide/gcm flies show that the potential to activate the ASC is a general phenomenon, rather than being allele specific.

**Dominant mutation reveals novel potentials for the Glide/Gcm protein:** Loss- and gain-of-function mutations had previously shown that glide/gcm acts as a glial-promoting factor in the CNS and in the PNS (Hosoya et al. 1995; Jones et al. 1995; Vincent et al. 1996; Bernardoni et al. 1998; Van De Bor et al. 2000). Indeed, glide/gcm expression throughout the neurogenic region of the embryonic CNS represses neurogenesis (Bernardoni et al. 1998, 1999; data not shown). Yet, the present results show that precocious Glide/Gcm during postembryonic development leads to PNS formation due to activation of the ASC. We found no ASC expression in the GP of wild-type animals, indicating that Glide/Gcm does not normally control ASC, unless the ASC is activated very transiently or at levels that are undetectable with anti-Ac. As for the other members of the complex, previous analyses showed that ase-lacZ transgenic flies do not display β-gal expression in the glial lineage (Giangrande 1995), sc expression has been shown to be similar to that of ac, and lsc is specifically expressed in the embryo (Garcia-Bellido and Santamaria 1978; Romani et al. 1989; Cubas et al. 1991; Martin-Bermudo et al. 1991; Hinz et al. 1994; Skeath and Doe 1996).

A likely explanation for the glide/gcmBts phenotype is that Glide/Gcm is indeed able to control the ASC but its expression is normally induced too late to activate the complex. It is likely that the ASC promoter is accessible only during the third larval instar, due to chromatin modification, and that this hampers activation by Glide/Gcm at late pupal stages (see, for review, Farkas et al. 2000). Indeed, we have seen no ASC activation upon late ectopic glide/gcm expression (V. Van De Bor and A. Giangrande, unpublished observations). The glide/gcmBts phenotype may reveal a potential that has been lost during evolution. In the future, it will be interesting to determine whether in more primitive species the role of glide/gcm is to activate proneural genes. The present results also hint at the possibility that during normal development Glide/Gcm activates promoters that are similar to those of the ASC. Such promoters induce the expression of genes that are involved in glial differentiation. Upon glide/gcm precocious expression, however, these targets cannot be expressed because their promoters are not yet “competent.” Differential screens will allow us to identify such glide/gcm targets.

Finally, Glide/Gcm could mimic a similar but unknown protein that normally regulates the proneural genes, or it may inhibit a negative regulator of the ASC. Irrespective of the molecular mechanisms involved, the characterization of the glide/gcmBts mutation has made it possible to discover a novel role of glide/gcm. Indeed, none of the mutations so far analyzed had anticipated a potential to induce sensory organs for the Glide/Gcm protein. The unexpected phenotype revealed by the glide/gcmBts mutation warns us about the results obtained solely by using dominant mutations and those obtained upon screenings for modifiers of dominant phenotypes. Nonetheless, when combined with loss-of-function phenotypes, gain-of-function mutations do allow a better understanding of the mode of action of a given gene.

**Heterochronic mutations and the Glide/Gcm pathway:** The differentiation of multicellular organisms depends on the strict temporal and spatial control of gene expression, which ensures the coordination of developmental events. Breaking the laws results in heterochronicity. Clear examples of this have been observed in worm, in which mutations in the heterochronic path-
ways cause altered temporal patterns of larval development, due to the fact that larval cells divide or differentiate according to programs specific to other stages (Ambros and Horvitz 1984; Ambros 2000; Reinhart and Ruvkun 2001). Thus, worm heterochronic mutations produce a time shift in the developmental program. The heterochronic phenotype observed in glide/gcm<sup>56s</sup> and hs-glide/gem flies also indicates that timing of expression is crucial. The two examples of heterochronic mutations, however, are fundamentally different. In worms, such mutations cause temporal transformations that alter stage-specific gene networks; that is, stage-specific events are either reiterated or omitted. In the case of glide/gem, the gene is normally expressed during both embryonic and postembryonic development to promote a specific cell fate. Thus, temporal misregulation of Glide/Gcm makes this glial-promoting factor become “proneural” (in the sense that it induces sensory organs).

Interestingly, temporal misexpression of proneural genes, which normally induce neural differentiation, does not seem to trigger different developmental programs. Indeed, early expression of the AS-C in the wing disc either induces sensory organs at ectopic positions or has no effects. In no case, however, does the AS-C induce other cell types (Rodriguez et al. 1990). These results suggest that tissues must be competent to respond to the activity of the proneural genes. In molecular terms, the neural competence has been interpreted as a need for cofactors that make the expression of proneural genes apt to trigger PNS differentiation. Thus, some “master” genes are able to trigger a developmental pathway in a “naïf” territory, whereas some others are not. This may reflect the fact that some transcription factors (like Glide/Gcm) are more potent activators/repressors than others (for example, the AS-C) and may overrun the AS-C need for cofactors. The AS-C gain-of-function phenotypes also indicate that proneural gene expression in ectodermal cells can induce only neurogenesis. In molecular terms, this means that the AS-C can interact only with specific cofactors. Factors like Glide/Gcm, instead, may be more promiscuous and may interact with different cofactors. The developmental pathway triggered by the ectopic expression of this type of transcriptional regulator will then be influenced by the kind of cofactor that is available. This may explain why a glial-promoting factor induces PNS formation. Interestingly, glide/gem is also expressed and required in the embryonic CNS and in the hemocytes; however, its overexpression in those domains does not seem to induce proneural gene expression (Bernardoni et al. 1999; data not shown). Thus, the effects observed in glide/gem<sup>56s</sup> reflect differences in the mode of action and in the requirement of glide/gem throughout development.

In the future, it will be interesting to determine the molecular bases of the phenotype observed upon early glide/gem expression. We speculate that one of the strategies used to control nervous system development is to repress illegitimate expression of glide/gem, which is a potent transcription factor. Massive ectopic glide/gem expression is indeed fatal to the organism, indicating that a tight gene regulation must take place during development (Bernardoni et al. 1998; Miller et al. 1999). The analysis of the glide/gem promoter will tell us whether positive as well as negative regulation controls the expression of glide/gem.

Finally, glide/gem is located 27 kb upstream to glide/gem<sub>2</sub>, a transcription factor that shows homology in the DNA-binding domain. glide/gem<sub>2</sub> is expressed in the glial lineage at later stages and at lower levels as compared to glide/gem (Kammerer and Giangrande 2001). Moreover, glide/gem<sub>2</sub> is necessary and sufficient to induce glial differentiation, even though its potential is more limited than that of glide/gem. Finally, the two genes work as a complex, in the sense that they share regulatory sequences and provide all the activity that allows lateral glial differentiation. The expression of glide/gem<sub>2</sub> does not seem to be affected by the glide/gem<sup>56s</sup> mutation (V. Van De Bor and A. Giangrande, unpublished observations), which is in agreement with the finding that the translocation occurs 3′ to glide/gem, far away from the glide/gem<sub>2</sub> gene. Moreover, the ectopic expression of glide/gem<sub>2</sub> does not produce ectopic bristles (V. Van De Bor and A. Giangrande, unpublished observations), indicating that the proneural potential seems specific to one member of the complex. Since the homology is restricted to the DNA-binding domain, it is likely that the different potential relies on different pathways. For all these reasons, it will be very important to determine the partners as well as the targets of Glide/Gcm and Glide/Gcm<sub>2</sub>.

Altogether, the unexpected phenotype observed upon precocious glide/gem expression calls for a better understanding of the regulation and the mode of action of the Glide/Gcm transcription factors. This will allow us to unravel the molecular cascade that triggers glial differentiation.

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