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The *big brain* gene of *Drosophila* functions to control the number of neuronal precursors in the peripheral nervous system

YI RAO[1], ROLF BODMER[2], LILY Y. JAN and Y. N. JAN

Howard Hughes Medical Institute and the Departments of Physiology and Biochemistry, University of California, San Francisco, CA 94143-0724, USA

Present addresses: [1]Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138 USA; [2]Department of Biology, University of Michigan, Ann Arbor, Michigan 48109-1048, USA

Summary

***big brain* (*bib*) is one of the six known zygotic neurogenic genes involved in the decision of an ectodermal cell to take on the neurogenic or the epidermogenic cell fate. Previous studies suggest that *bib* functions in a pathway separate from the one involving *Notch* and other known neurogenic genes. For a better understanding of the *bib* function, it is essential first to characterize the mutant phenotype in detail. Our mutant analyses show that loss of *bib* function approximately doubles the number of neuronal precursors and their progeny cells in the embryonic peripheral nervous system. Mosaic studies reveal a hypertrophy of sensory bristles in *bib* mutant patches in adult flies. Our observations are compatible with a function of *bib* in specifying neuronal precursors of both the embryonic and adult sensory nervous system. This is in contrast to the function of *Notch*, which continues to be required at multiple stages of neural development subsequent to this initial determination event.**

Key words: neurogenesis, neurogenic gene, *big brain*, *Drosophila*.

Introduction

The determination of neuronal precursors in both the central and the peripheral nervous system of insect embryos is thought to involve cell-cell interactions; as one cell (or a few cells) of an initially equivalent group of competent cells takes on the fate of the neuronal precursor, it prevents the neighboring cells from doing the same, so that they become epidermoblasts (Doe and Goodman, 1985; Hartenstein and Campos-Ortega, 1984; Technau and Campos-Ortega, 1987; for reviews, see Campos-Ortega, 1988; Artavanis-Tsakonas, 1988; Campos-Ortega and Jan, 1991). This "lateral inhibition" process involves the zygotic gene function of at least six genes in *Drosophila;* loss of function of any one of these neurogenic genes results in neural hyperplasia and epidermal hypoplasia (Poulson, 1937; Lehmann et al., 1981, 1983).

With the notable exception of *big brain* (*bib*), most other known neurogenic genes show genetic interactions suggesting that they act in a common pathway (Vaessin et al., 1985; de la Concha et al., 1988; Shepherd et al., 1989; Brand and Campos-Ortega, 1989, Xu et al., 1990). Previous studies also indicate that *bib* mutations cause a less severe neural hyperplasia than other neurogenic mutations (Lehmann et al., 1983; Hartenstein and Campos-Ortega, 1986), that there is no detectable maternal contribution of *bib* function (Jimenez and Campos-Ortega, 1982), and no adult phenotype in mosaic flies with patches of *bib* mutant tissue (Dietrich and Campos-Ortega, 1984), in contrast to the findings concerning other neurogenic genes. Taken together, these observations suggest that *bib* functions in a pathway separate from the one involving other known neurogenic genes.

The majority of the known neurogenic genes have been characterized at the molecular level. Thus *Notch* (*N*) and *Delta* (*Dl*) encode transmembrane proteins with EGF-like repeats (Wharton et al., 1985; Kidd et al., 1986; Vaessin et al., 1987; Kopczynski et al., 1988), which seem to be able to mediate cell-cell interaction directly (Fehon et al., 1990), whereas potential nuclear regulatory proteins are encoded by *mastermind* (*mam*) (Smoller et al., 1990), *neuralized* (*neu*) (Boulianne et al., 1991), and some of the genes in the *Enhancer of split* (*E*(*spl*)) gene complex (Knust et al., 1987; Klambt et al., 1989) and a protein of sequence similarity to the β-subunit of G-proteins is encoded by another gene in the *E*(*spl*) complex (Preiss et al., 1988; Hartley et al., 1988). The predicted *bib* gene product contains multiple stretches of hydrophobic residues that are potentially membrane spanning and shows sequence similarity to membrane proteins known to be involved in transport of small molecules across the membrane (Rao et al., 1990), consistent with the hypothesis that the *bib* product mediates cell-cell interaction, perhaps in a pathway separate from that involving the *Notch* and *Delta* gene products.

In addition to the proposed function of neurogenic genes in the early process of neuronal precursor formation

(Lehmann et al., 1981, 1983), *Notch* has been found to act at successive stages of cell fate determination in the development of the eye and other sensory organs of the adult fly (Cagan and Ready, 1989; Hartenstein and Posakony, 1990). In addition, *Notch* function may be important for the maintenance of epidermal differentiation in embryos (Hoppe and Greenspan, 1990; Greenspan, 1990), muscle development (Corbin et al., 1991) and oogenesis (Ruohola et al., 1991). These studies underscore the importance of careful examination of the phenotypes of neurogenic mutants at different developmental stages; such mutant analyses provide the basis for the study of the underlying genetic and cellular mechanisms.

To test whether *bib* acts at the stage of neuronal precursor determination and whether *bib* also acts at later stages in the cell fate determination of the progeny cells of the neuronal precursors, we chose to analyze the null mutant phenotype of *bib* in the embryonic peripheral nervous system (PNS) because it is possible to identify every type of sensory neuron and its support cells (Campos-Ortega and Hartenstein, 1985; Ghysen et al., 1986; Dambly-Chaudiere and Ghysen, 1986; Bodmer and Jan, 1987; Bier et al., 1989) and to follow the cell-lineages that give rise to these cells in the PNS (Bate, 1978; Bodmer et al., 1989). Since neurogenic mutants show hypertrophy in both the central nervous system (CNS; Poulson, 1937; Lehmann et al., 1981, 1983) and the PNS (Hartenstein and Campos-Ortega, 1986), detailed analysis of the mutant phenotypes in the embryonic PNS offers an opportunity to compare the mutant effects of different neurogenic genes at single cell resolution. In this report we show that *bib* mutations increased both the sensory neurons and their support cells by a factor of about two, suggesting that *bib* normally functions to control the formation of neuronal precursors in the embryonic PNS, in agreement with the observations of Goriely et al. (1991). Furthermore, loss of *bib* function also increased the number of adult sensory bristles in mosaic flies, suggesting that *bib* is likely to serve similar functions in the formation of the adult PNS.

Materials and methods

Drosophila *strains*

The three *bib* alleles analyzed are $bib^{III9\text{-}5}$, a deletion mutant (Rao et al., 1990), bib^{FX1}, an inversion mutant, and bib^{IDO5}, which has been extensively analyzed in the study of the CNS phenotype (Lehmann et al., 1983). All three alleles showed similar phenotypes with respect to the hypertrophy of the embryonic nervous system as revealed by staining using antibodies against horseradish peroxidase. The enhancer-trap lines used are B7 2nd 2 (at cytological location 33CD), which expresses β-galactosidase specifically in the md sensory neurons that have multiple dendrites, and C8 3rd 38 (cytological location on the 3rd chromosome not determined), which shows expression in the cap cell, one of the three types of support cells in the chordotonal (ch) organ. Immunocytochemical analyses were carried out primarily on the $bib^{III9\text{-}5}$ allele. Because no recombinants carrying both $bib^{III9\text{-}5}$ and the PlacW transposon on the second chromosome in B7 2nd 2 were obtained, the bib^{ID05} allele was used in experiments involving the B7 2nd 2 insertion.

Analysis of the embryonic sensory organs

Immunocytochemistry was carried out using antibodies against horseradish peroxidase (anti-HRP), which recognize all neuronal membranes (Jan and Jan, 1982), antibodies against the Cut protein, which stain all cells of the external sensory (es) organs (Blochlinger et al., 1990), and the monoclonal antibody 21A6, which stains a dot at the dendritic cap of the es organ and the scolopale of the chordotonal (ch) organ (Zipursky et al.,1984). The procedure for immunocytochemical staining was as described previously (Bodmer et al., 1987; Rao et al., 1991).

External sensory structures on the surface of the embryo were examined in cuticle preparations as described by Dambly-Chaudiere and Ghysen (1986).

Labelling of replicating precursor cells

Wild-type or $bib^{III9\text{-}5}$ embryos isolated in 1 hour collections from cultures maintained at 18˚C were aged in a moist chamber at 25˚C to the desired developmental stage, treated with the thymidine analog BrdU (Sigma), and stained immunocytochemically for replicating cells that have incorporated BrdU, as described by Bodmer et al. (1989). Briefly, embryos were dechorionated, permeabilized with octane for 4 minutes, then transferred to a wide tube containing 1 mg/ml BrdU in Grace's insect medium (Gibco/BRL) so as to allow the octane to evaporate. After incubation for 30 minutes at 25˚C the embryos were fixed, devitellinized, treated with 2 N HCl for one hour, then with anti-BrdU antibodies (1:50 dilution, Becton-Dickinson) and processed for immunocytochemical staining.

Generation of bib *mosaic flies*

The FLP/FRT method developed by Golic and Linquist (1989) was used. Multiple recombinant second chromosomes carrying Flip Recombinase Target (FRT) (at 33; Golic and Lindquist, 1989) and bib^{ID05} or bib^{FX1} (at 30F), but not $bib^{III9\text{-}5}$, were isolated and analyzed to ensure that they all show the same phenotype. Mosaic flies were generated by crossing *y w* FLP; p[y^+]FRT/+ males to *y w*/*y w*; *bib* FRT/CyO females, and heat-shocking the progeny at 37˚C to activate FLP which is under the control of a heat-shock promoter. Heat shock at embryonic, the three larval, and pupal stages gave rise to mutant patches of different sizes; heat shock at the early larval stages produced large mutant patches in mosaic flies.

Results

The highly invariant patterns of sensory organs in embryos as well as adult flies allow one to determine whether a mutation alters the total number of these sensory organs (Dietrich and Campos-Ortega, 1984; Hartenstein and Campos-Ortega, 1986; Caudy et al., 1988; Ghysen and Dambly-Chaudiere, 1986) or whether it causes certain cells of the sensory organ to adopt an alternative fate (e.g. Bodmer and Jan, 1987; Uemura et al., 1989; Hartenstein and Posakony, 1990). In this study, we first characterized the different types of sensory neurons and their support cells in *bib* mutant embryos, using antibodies that are specific to various cell types, or enhancer-trap lines that allow β-galactosidase expression in specific cell types, and then examined the external sensory structures on the cuticle of these mutant embryos. The ventral clusters of sensory neurons and their support cells were missing, probably due to the overgrowth of the CNS in the *bib* embryos. The lateral and dorsal clusters showed an increase of sensory neurons and

their support cells, as well as the external sensory structures, raising the possibility that the number of neuronal precursors was increased. This possibility was tested by measuring the number of replicating precursor cells that had incorporated BrdU. Finally, mosaic flies were generated using the FLP/FRT method to determine whether loss of *bib* function also leads to an increase of adult sensory organs.

All three types of embryonic sensory neurons were increased by up to twofold in bib *mutants*

The three major types of sensory neurons in the *Drosophila* embryo are recognizable using specific markers. The external sensory (es) neurons that innervate mechanosensory or chemosensory structures on the cuticle express *cut* and therefore are stained immunocytochemically using antibodies against the Cut protein (Blochlinger et al., 1988; 1990). The chordotonal (ch) neuron that innervates an elongated internal sensory structure is recognizable by its characteristic shape using Nomarski optics; its dendrite is aligned in the same direction as the tube-shaped internal sensory structure it innervates and ends within a specialized structure, the scolopale (Ghysen et al., 1986; Bodmer and Jan, 1987; Hartenstein and Campos-Ortega, 1986). Whereas each of the es and ch neurons has a single dendrite, the md neurons exhibit multiple dendrites (Bodmer and Jan, 1987). In an enhancer-trap line characterized previously (Bier et al., 1989), B7 2nd 2, β-galactosidase is expressed specifically in the md neurons.

Compared to the wild-type embryo, the *bib* mutant embryos showed an increase in each of the three types of sensory neurons. The supernumerary neurons were found in roughly the same location as their normal counterparts and the increase in neuronal numbers in individual cases was up to, but not beyond, twofold; the averaged number of ch, es and md neurons was increased to 1.7, 1.5 and 1.5, that of the respective neurons in wild-type embryos (Table 1, Fig. 1).

The support cells in the sensory organs were increased to a similar extent in bib *embryos*

The es or ch sensory organ contains one (or more) neuron(s) and several (typically three) support cells that form the sensory structures and the cap around the dendritic tip of the neurons. The neuron(s) and support cells of a sensory organ are derived from a common precursor cell via a fixed pattern of cell division (Bate, 1978; Hartenstein, 1988; Bodmer et al., 1989). Transformation of a subset of cells within a sensory organ to cells with the identity of their sister cells has been observed in certain mutants, including a mutant of the neurogenic gene, *Notch* (Uemura et al., 1989; Hartenstein and Posakony, 1990).

To determine whether *bib* acts primarily at the level of determination of neuronal precursors or whether it also acts at later stages in the cell fate determination of their progeny cells, we examined the effect of *bib* mutations on the number of support cells of the sensory organs; in the former case but not in the latter case the number of support cells should be increased to the same extent as the number of sensory neurons. We used antibodies against the Cut protein, which is expressed in every neuron and support cell of the es organ, the monoclonal antibody 21A6, which stains a dot formed by the inner support cell at the tip of the es dendrite and the scolopale formed by the sheath cell (a support cell) at the tip of the ch dendrite, and an enhancer-trap line with ß-galactosidase expression in the cap cell (also a support cell) of the ch organ, and found that these support cells in *bib* embryos were increased to the same extent as the sensory neurons, by up to twofold (Fig. 1, d-n). The averaged number of a support cell of ch neuron or es neuron is increased to 1.7 and 1.6 that of the respective support cells in the wild-type embryo (Table 1).

Table 1. *Hyperplasia of three types of sensory neurons and their support cells in* bib *mutant embryos*

ch neurons in the dorsal cluster of a thoracic segment		es neurons in the dorsal-most group of an abnominal segment		md neurons in the dorsal cluster of an abdominal segment	
wild type	*bib*	wild type	*bib*	wild type	*bib*
3.0±0.02 (3-4) *n*=37	5.0±0.13 (3-6) *n*=60	2.0±0.01 (1-2) *n*=56	3.0±0.09 (2-4) *n*=65	7.8±0.05 (7-8) *n*=55	11.9±1.9 (10-16) *n*=60

cap cells in the dorsal cluster of a thoracic segment		inner support cells in the dorsal-most group of an abdominal segment	
wild type	*bib*	wild type	*bib*
3.0±0 (3) *n*=30	5.6±0.16 (3-6) *n*=32	2.0±0 (2) *n*=22	3.15±0.14 (2-4) *n*=20

Numbers given are mean ± standard error of the mean.

Numbers in parenthesis indicate the minimum and maximum numbers of cells per cluster.

n=total number of cluster of sensory neurons scored.

ch neurons counted correspond to those shown in Fig. 1 j, es neurons correspond to those in the dorsal-most group of the dorsal cluster shown in Fig. 1c, i.e. the two simple es organs of which one is shown in Fig. 1 d,e, and md neurons correspond to those in Fig. 1 h,i.

The ratio of the number of neurons observed in *bib* mutants over that observed in wild-type embryos is 1.7 for chordotonal neurons, 1.5 for es neurons and 1.5 for md neurons. The ratio of the number of support cells is 1.7 for the cap cell, support cell of the ch neuron, and 1.6 for the inner support cell of the es neuron. These differences between the *bib* mutant and wild-type embryos are statistically significant (Student's t test $P<0.01$).

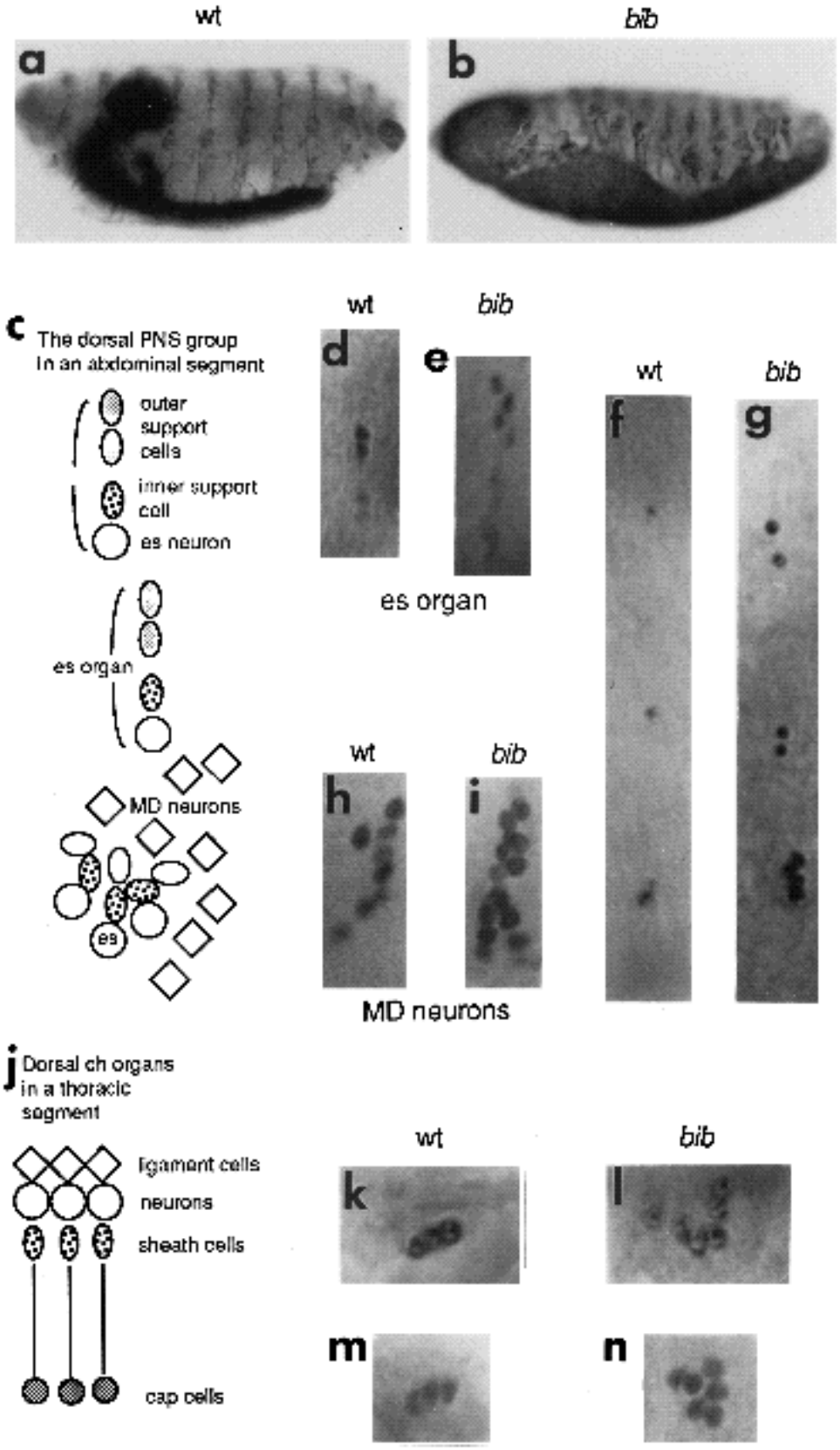

Fig. 1. The embryonic phenotype of *bib*. (a,b) The CNS and the PNS of embryos homozygous for the null allele $bib^{III9\text{-}5}$ (b) is hypertrophic as compared with those in the wild-type (wt:a). The nervous system was labelled with anti-HRP antibodies that stain the surface of all neurons (Jan and Jan, 1982). (c) A diagram of all the md neurons and cells of the es organs in the dorsal cluster of an abdominal segment. Cells shown in d-i are all from this region (d, f, h) are from wt embryos, and (e, g, i) are from mutant embryos. (d, e) Every cell in an es organ is duplicated in $bib^{III9\text{-}5}$ (shown here is the dorsal-most es organ), as shown by an antibody to the Cut protein which is expressed in every cell of the es organs (Blochlinger et al., 1990). (f, g) The dot-like dendritic cap structures made by the inner support cells of the es organs are also duplicated as shown by staining with the monoclonal antibody 21A6 (Zipursky et.al.,1984). (h, i) The md neurons are duplicated as revealed by a PlacW transformant line that expresses β-galactosidase specifically in the md cells (Bier et al., 1989). (j) A diagram of cells of the ch organs in the dorsal cluster of a thoracic segment. Cells shown in k-n are from this region; k, m are from wt embryos while l and n are from mutants. (k, l) The number of the sheath cell, a support cell of the ch organ, is increased. The structures shown here are scolopales made by the sheath cells, stained with the monoclonal antibody 21A6. (m, n) The number of the cap cell, another support cell in the ch organ, is also increased in *bib* mutants, as revealed by a PlacW transformant line expressing β-galactosidase specifically in the cap cells (Bier et al., 1989); the null allele, $bib^{III9\text{-}5}$, is shown in b, e, g and l, while another allele with similar phenotype, bib^{ID05} is shown in i and n.

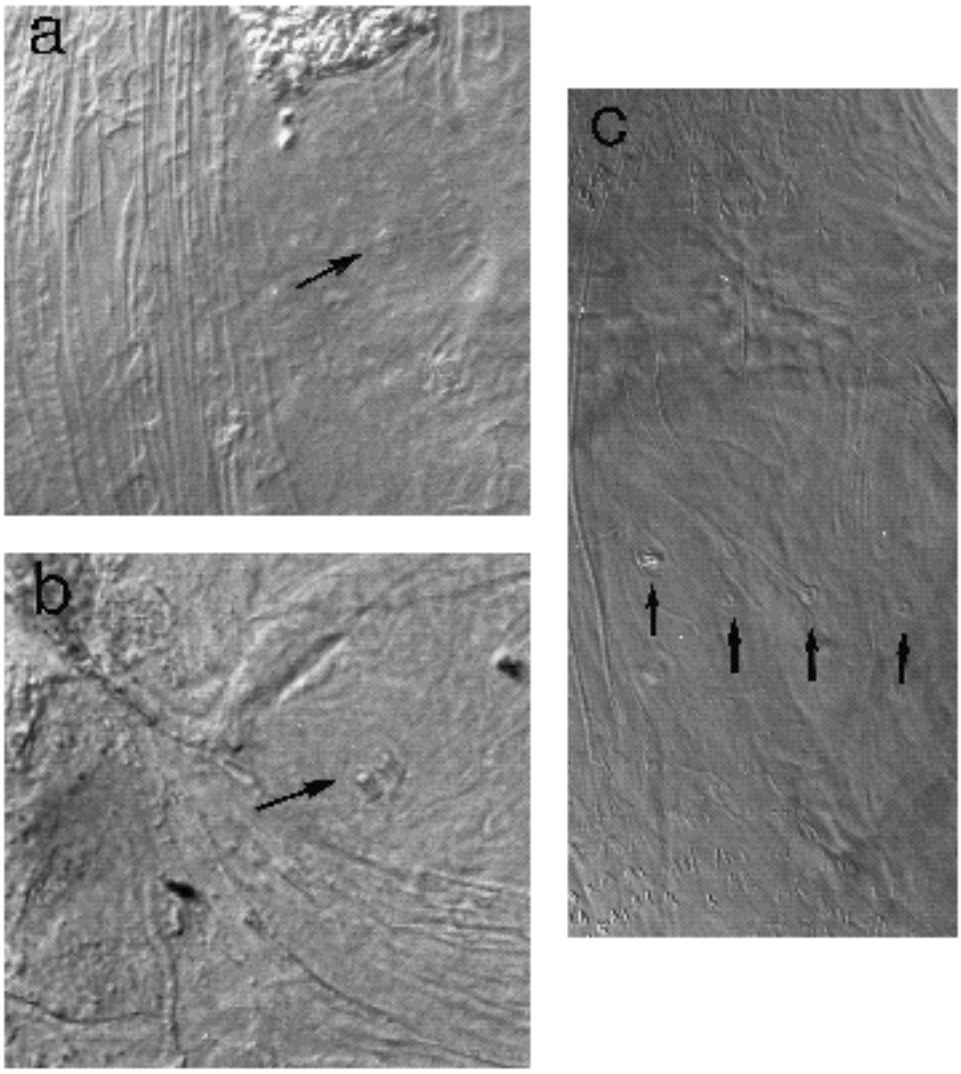

Fig. 2. Formation of duplicated sensory structures in *bib* mutant embryos. Cuticle preparations of *bib* mutant (a, b) and wild-type (c) embryos show the cuticular sensory structures (indicated by arrows) that are part of the external sensory organs. c shows the sensory structures in the lateral cluster of a thoracic segment: from the left are the lateral peg (a chemosensory sensillum basiconicum), a campaniform sensillum, a mechanosensory hair, and another campaniform sensillum (Dambly-Chaudiere and Ghysen, 1986). The reduced cuticle of *bib* embryos contains only the dorsal region and has a tendency to wrinkle, making the identification of the position of sensory structures ambiguous. Nevertheless, duplicated sensory structures can be found in *bib* mutant embryos, such as duplicated campaniform sensilla (a) and duplicated sensillum basiconia (b).

Duplication of external sensory structures in bib *mutant embryos*

Another indication for the increase of support cells was obtained by examining the sensory structures on the cuticle, which are formed by the outer support cells of the es organs. In contrast to strong alleles of other neurogenic genes, which often contain poorly differentiated neurons (Hartenstein and Campos-Ortega, 1986), the *bib* mutant embryos not only contained neurons and support cells that expressed the appropriate markers but also had duplicated sensory structures on the cuticle (Fig. 2), indicating that the supernumerary support cells in *bib* mutants were located at appropriate positions and fully differentiated.

Loss of bib *function caused an increase of replicating PNS precursors*

The approximate duplication of sensory neurons, their support cells, and the external sensory structures formed by the support cells suggests that the primary effect of *bib* mutations is an increase of the precursor cells that give rise to both the neurons and the support cells. This could be best tested by examining the pattern of replicating cells in the embryos between 6.5 and 10 hours after fertilization (at 25˚C, stage 11-13, as defined in Campos-Ortega and Hartenstein, 1985); most or all of the replicating cells in the dorsal ectoderm at these stages are precursors of sensory organs, since the last mitotic wave of epidermal cells in this region is complete by 6.5 hours (Bodmer et al., 1989). Replication and cell division of these sensory precursors take place in a dorsal to ventral gradient temporally in the wild-type embryo, and require about one hour or longer to complete one cell cycle, as has been found for most cells in the embryo after cycle 14 (cellular blastoderm; Foe, 1989). Thus an incubation period of 30 minutes in BrdU would be adequate for the labelling of precursors in S-phase, before they complete mitosis (Bodmer et al., 1989).

Incorporation of BrdU at 6.5, 7.5 and 8.5 hours after fertilization revealed an increased number of replicating precursors in *bib* mutants as compared with the wild-type embryo (Table 2, Fig. 3). In both cases the number of replicating cells declined with developmental time, as more and more precursors completed their phase of DNA replication, and at these different stages there was a roughly constant ratio of the number of precursors in *bib* embryos relative

Table 2. *Hyperplasia of replicating PNS precursor cells in* bib *mutant embryos*

Age (hours)	Embryo	The dorsal cluster in an abdominal segment* Number±SE (*n*)	The lateral chordotonal cluster in an abdominal segment Number±SE (*n*)
6.5	wild type	8.5±0.24 (34)	4.7±0.08 (36)
	bib	13.0±0.31 (51)	6.5±0.21 (53)
7.5	wild type	5.3±0.14 (38)	2.2±0.1 (36)
	bib	9.3±0.26 (58)	3.7±0.14 (49)

SE indicates standard error of the mean.
n gives the number of segments counted.
*The dorsal cluster of replicating cells corresponds to those marked in Fig 3. The increase of replicating PNS precursor cells observed in *bib* mutants (by a factor of 1.4 to 1.8-fold) is statistically significant ($P < 0.01$).

to that in the wild-type embryo. After 8.5 hours of embryogenesis, there were virtually no replicating cells in wild-type or mutant embryos (Fig. 3 e,f) with the exception of a few such cells in the more ventral region of the wild-type embryo; the ventral clusters of the PNS did not form in *bib* embryos, presumably due to overgrowth of the CNS (see Fig. 3f). Taken together, these results suggest that the precursors of sensory neurons are more numerous in *bib* embryos but their division patterns are similar to those in the wild-type embryo.

Effect of bib *mutations on the adult sensory organs*

Our studies of the embryonic PNS indicate that *bib* mutations affect mainly the number of the precursor cells, without altering the location or the differentiation of these cells and their progeny. To see whether similar mutant pheno-

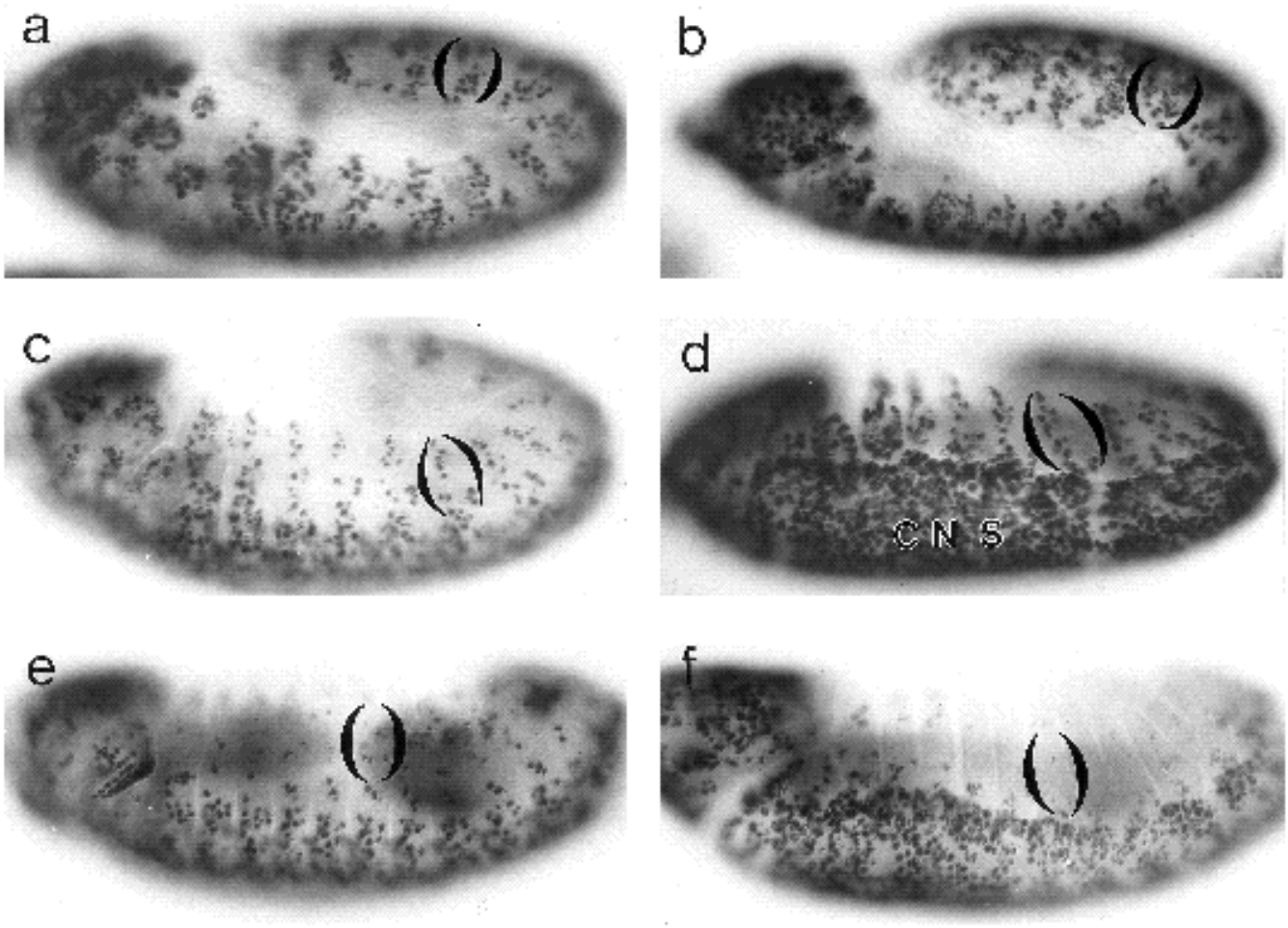

Fig. 3. Pattern of replicating PNS precursors in wild-type and *bib* mutant embryos. BrdU-labelled embryos at 6.5 (a, b), 7.5 (c, d) and 8.5 (e, f) hours of development (middle stage 11, late stage 11 and stage 12, respectively). All embryos had been incubated with BrdU for 30 minutes before fixation and anti-BrdU staining. a, c and e show replication patterns of wild-type embryos, while b, d and f are those of $bib^{III9\text{-}5}$. Anterior is to the left and ventral is down. All embryos are shown in a lateral superficial view. Homozygous mutant embryos are easily recognized by a large excess of stained CNS nuclei, best illustrated in the tilted embryo in d and by the failure of these CNS neuroblasts to segregate from the ectodermal layer (the last wave of ectoderm replication is at 6 hours) (Campos-Ortega and Hartenstein, 1985; Bodmer et al., 1989). The dorsal ectoderm in an abdominal segment is bracketed in each panel. These regions contain the precursors for the lateral and dorsal clusters of sensory organs; the precursors for the ventral cluster are obscured by replication in the CNS.

Table 3. *Extramacrochaete formation in flies carrying both* bib *FRT and FLP and in flies with* bib *FRT but no FLP*

	$\frac{yw\text{FLP}}{yw}, \frac{bib^{FX1}\text{FRT}}{p[y^+]\text{FRT}}$	$\frac{yw}{yw}, \frac{bib^{FX1}\text{FRT}}{p[y^+]\text{FRT}}$	$\frac{yw\text{FLP}}{yw}, \frac{bib^{ID05}\text{FRT}}{p[y^+]\text{FRT}}$	$\frac{yw}{yw}, \frac{bib^{ID05}\text{FRT}}{p[y^+]\text{FRT}}$
No. of flies with y^- extra macrochaete	50 (68.5%)	0	25 (43.1%)	0
No. of flies with y^+ extra macrochaete	8 (10.9%)	8 (17.8%)	9 (15.5%)	5 (8.8%)
No. of flies without extra macrochaete	15 (20.5%)	37 (82.0%)	24 (41.4%)	52 (91.2%)

Flies without FLP serve as control and should not have any clones of mutant tissue; the same heat treatment (37˚C, for 1 hour at second instar larval stage) was applied to both groups of flies. Number in parenthesis are percentage of flies with or without extra macrochaetae. They sometimes add up to more than 100% because some flies have both y^+ and y^- extra bristles. Since *bib* mutant clones could only be recognized by y^- bristles, we also counted the number of bristles in those areas of the mosiac fly that did not contain detectable y^- patches. We found the normal number of bristles (2.13 ± 0.3 dorsal centrals on each side of the notum, n=23, and 2.09 ± 0.29 scutellars on each side of the scutellum, n=43, mean ± standard deviation), supporting the conclusion that there is no reduction in bristle number in *bib* mutant clones.

types could be observed in adult tissue, we used the FLP/FRT method developed by Golic and Linquist (1989), which utilizes the yeast recombinase FLP, driven by a heat-shock promoter, to promote site-specific recombination. The site recognized by FLP, FRT, has been inserted into chromosome location 33 and was brought to the same second chromosome that carries either the bib^{ID05} or the bib^{FX1} allele (at 30F) by genetic recombination. The chromosome carrying the wild-type allele of *bib* was marked with the *yellow* gene (y^+), so that the homozygous *bib* mutant clones in the mosaic fly would be recognized by being mutant for *yellow* (see Materials and methods for details). The external sensory organs in these mosaic flies could be identified as bristles on the body and the wing (Hartenstein and Posakony, 1989), and their genotype was indicated by the bristle color, specified by *yellow*.

The *bib* mutant clones showed an increased number of macrochaete in all of the body parts examined, including the notum (Fig. 4 a,b), the scutellum (Fig. 4 c,d), the sternopleura, the head, and the wings (Fig. 5). An increase in the number of microchaete in these locations was also apparent, but has not been quantitated. The supernumerary bristles were only found in locations where bristles normally form, e.g. on the sides but not the medial region of the scutellum (Fig. 4d), and at the location of the "dorsal-central" macrochaete, on either side of the midline of the notum (Fig. 4 a,d). This was also true for the extra recurved bristles on the anterior wing margin (Fig. 5 a,b) and the supernumerary campaniform sensillae on the second wing vein (two of the three found in the wild-type tissue are shown in Fig. 5c and marked with arrowheads, whereas six are present in a *bib* mutant clone, as shown in Fig. 5d).

The *bib* mutant patches showed either an increased number or the normal number of bristles, but never a reduced number of bristles (Table 3), unlike *Notch* (Hartenstein and Posakony, 1990). This observation was consistent with the hypothesis that *bib* functions primarily to determine the number of sensory precursor cells. The observed increase of bristles in *bib* mutant tissues exceeded the background level. Identical heat shock treatments of control flies carrying no FLP, or no *bib* mutations, occasionally resulted in the formation of one, and rarely two, extra bristles, in contrast to the increase by up to seven bristles in clones carrying either the bib^{ID05} or the bib^{FX1} allele (Table 4, see also Fig. 4b). The omission of FLP in control flies also drastically reduced the fraction of flies with extra bristles (Table 3).

Table 4. *Formation of extra macrochaete in* bib *mosaic patches*

allele	Number of clones with extramacrochaete	Number of extra macrochaete per clone±s.d. (range)
bib^{FX1}FRT		
Recombinant line 1	52*	2.0±0.97 (1-6)
Recombinant line 3	33*	2.8±1.52 (1-4)
bib^{ID05}FRT		
Recombinant line 6	15	1.7±0.87 (1-4)
Recombinant line 7	28	2.1±1.49 (1-7)

Two recombinant lines for each bib allele were examined; besides *bib* mutant clones with extra y^- bristles, a single isolated extra y^+ bristle was found in each of two flies of line 3, four flies of line 6, five flies of line 7, and eight flies of line 1, and two separate extra y^+ bristles were found in each of two flies of recombinant line #1. The frequency of occurrance of extra y^+ bristles is at the background level.

Macrochaetes are scored at different locations, including dorsocentrals, scutellars, postalars, supra alars, sternopleuras, presuturals, notopleurals, orbitals and post verticals. The size of the clones varied from 1/10 to 1/3 of notum, or 1/4 to the entire scutellum.

*In two of the 52 clones of line 1 and two of the 33 clones of line 3, a single extra y^+ bristle was found near the border of the clone of *bib* mutant tissue containing extra y^- bristles.

s.d. indicates standard deviation.

"range" shows the minimum and maximum numbers of extra macrochaete

Discussion

A previous study has shown that bib^{ID05} caused hypertrophy of both CNS and PNS. Moreover, this phenotype of bib^{ID05} is weaker than those of the strong alleles of other neurogenic mutants *N*, *Dl*, *mam*, *neu* and *E*(*spl*) (Hartenstein and Campos-Ortega, 1986). We have extended this observation in several aspects.

(1) In the previous study (Hartenstein and Campos-Ortega, 1986), only a single allele, bib^{ID05}, was examined. It was not known whether bib^{ID05} was a null mutant, hence whether the weaker phenotype observed corresponded to a partial loss of function. In the present study, we used several alleles, including a molecularly characterized null mutant (Rao et al., 1990). This allows us to conclude that total loss of function of *bib* leads to a phenotype which is weaker and qualitatively different from that of other neurogenic mutants.

(2) In the CNS, *bib* is known to act at the level of neu-

ronal precursors to control the number of neuroblasts (Lehmann et al., 1983). In the PNS, *bib* mutations have also been shown to affect the number of sensory organ precursors (Goriely et al., 1991). To determine whether *bib* acts at multiple stages of neural development, we used a number of cell-specific markers, and we have found that loss of *bib* function increases the number of embryonic sensory organ precursors to the same extent as their individual progeny cell types, by approximately twofold, whereas the location and cellular differentiation of these cells are not altered significantly. Furthermore, BrdU labelling experiments indicate that the division patterns of the sensory organ precursors are similar to those in the wild-type embryo. Thus all of the *bib* mutant PNS phenotypes could be accounted for by the increase in neuronal precursor cells.

The numbers of neuronal precursor cells in *bib* mutant embryos are increased by twofold in the strongest cases and are unaltered in the weakest case. We cannot rule out the possibility of reduced expressivity of increases in precursor cell numbers in these *bib* null mutants. It is also possible that partially redundant functions of other genes reduce the expressivity of precursor cell number defects in *bib* mutants.

Comparison of bib *and other neurogenic genes*

Considerable amount of evidence has been gathered in support of the idea that development of the sensory organ is a progressive process (for review see Ghysen and Dambly-Chaudiere, 1989; Jan and Jan, 1990). The process is initiated by the proneural genes such as those in the *achaete-scute* complex which endow a group of ectodermal cells (the proneural clusters) with the competence to become precursors of sensory organs. The "proneural pattern" is then refined into a "sensory organ precursor pattern" when one (or a few) cell(s) within each proneural cluster is singled out to be the sensory organ precursor(s) (Cubas et al., 1991; Skeath and Carroll, 1991). Previous studies support the idea that neurogenic mutations affect the "singling out" process but not the setting up of proneural clusters (Goriely et al., 1991).

It is generally thought that the refinement of "sensory organ precursor pattern" involves lateral inhibition, i.e. the sensory organ precursor, once singled out, can inhibit other cells within the proneural clusters to become sensory organ precursors (see, however, Goriely et al., 1991 for an alternative idea of "mutual inhibition"). Previous studies indicate that in null mutants of neurogenic genes such as *N* and *Dl*, most and possibly all cells in the proneural clusters become sensory organ precursors (Brand and Campos-Ortega, 1989; Cabrera, 1990; Heitzler and Simpson, 1991; Goriely et al., 1991). The most striking difference between *bib* and other neurogenic genes such as *N* and *Dl* is that in the embryonic PNS, *bib* null mutations only cause a roughly twofold increase of the number of sensory organ precursors as compared to the 8 to 12-fold increase in *N* or *Dl* null mutants (Goriely et al., 1991).

What might be the explanation of this approximately twofold increase in the number of sensory organ precursors in *bib* mutant embryos? There are several possible scenarios. It could be that *bib* mediates the interaction between the sensory organ precursor and its sister cell, rather than all cells in the proneural clusters, so that a failure of this cell-cell interaction would increase the number of neuronal precursors by a factor of two. It is also possible that *bib* controls mitotic arrest of the sensory organ precursor so that an extra round of division takes place in *bib* mutants, leading to duplication of these precursors. We find this scenario less attractive because we have seen no evidence of an extra round of divisions in experiments in which the replicating precursor cells are labelled with BrdU. Also, this scenario could not be used to explain the CNS phenotype which is coupled with a loss of the epidermis. Another possibility is that perhaps within a proneural cluster the cells are not strictly equivalent; *bib* is only required in cell-cell interaction between a subset of these cells. In this context, it is interesting to note that Goriely et al. (1991) found that in *N* and *Dl* mutant embryos, not all cells within the proneural cluster become sensory organ precursors simultaneously. Often 2-3 cells become sensory organ precursors first. These clusters of neuronal precursors then increase progressively in size, up to a final size of 8-12 sensory organ precursors. It is conceivable that *bib* is required only in the selection of a cell among the subset of 2-3 cells to become a sensory organ precursor. At present, we cannot distinguish between these possibilities. Nevertheless, our results clearly show that *bib* is not required for the refinement of the entire proneural clusters and thus its function is qualitatively different from that of other neurogenic genes such as *N* and *Dl*.

A second observation that distinguishes *bib* from *Notch* is that all of the *bib* mutant PNS phenotypes could be accounted for by assuming a *bib* function in neuronal precursor formation. In contrast to the effects of the *Notch* mutation, which causes either an increase or a decrease of the bristles in mosaic flies depending on whether the *Notch* function manifested is at the level of the precursors or at the level of the sensory neurons and their support cells (Cagan and Ready, 1989; Hartenstein and Posakony, 1990); *bib* mutations caused an increase of sensory bristles regardless of the time when the *bib* mutant clones were induced. Thus, the failure to observe bristle loss in *bib* mutant clones implies that there is no evidence of transformation of bristle-forming or socket-forming support cells into neurons as in the case of *Notch* mutant clones.

bib *is also required in the adult fly*

Previous studies of X-ray induced mitotic clones did not reveal any *bib* mutant phenotype in adult tissues (Dietrich and Campos-Ortega, 1984). Our result is in contradiction with this conclusion, since our mosaic analysis clearly shows hyperplasia of sensory bristles. In the earlier study, the chromosome that carries the wild-type allele of *bib* also contains *Minute* and a duplication of a portion of the *achaete-scute* complex (Dp(1:2)*sc*^{19}M(2)201). Whether any of the differences in the genetic background accounts for the discrepancy remains to be determined.

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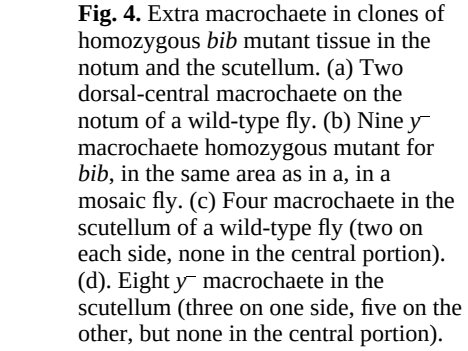

Fig. 4. Extra macrochaete in clones of homozygous *bib* mutant tissue in the notum and the scutellum. (a) Two dorsal-central macrochaete on the notum of a wild-type fly. (b) Nine y^- macrochaete homozygous mutant for *bib*, in the same area as in a, in a mosaic fly. (c) Four macrochaete in the scutellum of a wild-type fly (two on each side, none in the central portion). (d). Eight y^- macrochaete in the scutellum (three on one side, five on the other, but none in the central portion).

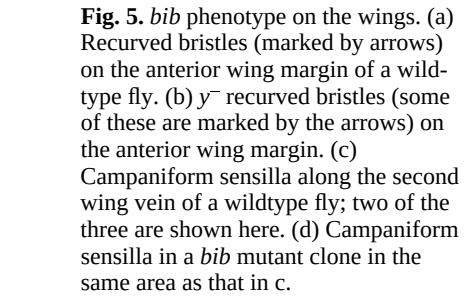

Fig. 5. *bib* phenotype on the wings. (a) Recurved bristles (marked by arrows) on the anterior wing margin of a wild-type fly. (b) y^- recurved bristles (some of these are marked by the arrows) on the anterior wing margin. (c) Campaniform sensilla along the second wing vein of a wildtype fly; two of the three are shown here. (d) Campaniform sensilla in a *bib* mutant clone in the same area as that in c.