Int. J. Dev. Biol. 42: 163-170 (1998)

Original Article

Expression and function of *tap* in the gustatory and olfactory organs of *Drosophila*

VALÉRIE LEDENT[1], FLORENCE GAILLARD[3] PHILIPPE GAUTIER[2], ALAIN GHYSEN[3] and CHRISTINE DAMBLY-CHAUDIÈRE[3]

[1#]*Laboratoire de Génétique du Développement and* [2#]*Laboratoire de Neurobiologie, Université Libre de Bruxelles, Rhode-St-Genèse, Belgium and* [3]*Laboratoire de Neurogénetique Université de Montpellier II, Montpellier, France*

ABSTRACT We have recently described the identification of a gene, tap, which encodes a bHLH protein expressed in one neuron of each larval chemosensory organ. Here we show that *tap* is expressed at a late stage in the development of one type of adult chemosensory organ, the gustatory bristles of the leg, wing and proboscis. We also show that *tap* is expressed very early in the development of a second type of chemosensory receptors, the olfactory organs of the antenna. The results of behavioral experiments suggest that the ectopic expression of *tap* affects the response to sugar and salt.

KEY WORDS: *bHLH, neurogenin, NeuroD, biparous*

Introduction

The development of the peripheral nervous system of *Drosophila* starts with the acquisition of proneural competence by ectodermal cells. This competence is related to the expression of "proneural" genes, all of which encode proteins with a bHLH motif (Jan and Jan, 1993). Among the proneural genes, those of the *achaete-scute* complex are responsible for the development of all larval and adult external sense organs, except for the photoreceptors, and for the olfactory receptors on the adult antennae (Reddy *et al.*, 1997). The other known proneural gene, *atonal*, is involved in the development of the chordotonal organs, of the photoreceptors, and of one subset of olfactory organs, the coeloconic sensilla (Jarman *et al.*, 1993,1994, Gupta and Rodrigues, 1997).

We have recently identified a bHLH gene that is expressed at a much later step in the development of the larval chemosensory organs (Gautier *et al.*, 1997). Because the chemosensory identity of the organs depends on the gene *poxn* (Dambly-Chaudière *et al.*, 1992), this new bHLH gene was called *target-of-poxn (tap). tap* is probably identical to the bHLH gene *biparous (bps)*, which is expressed in specific subsets of neurons and glial cells in the central nervous system (Bush *et al.*, 1996). The probable-identity between *tap* and *bps* was not noticed earlier because the sequence of *bps* was introduced in the databases only in October 1997.

The bHLH motif of *tap* is somewhat different from that of the other known proneural genes of the fly, and resembles most closely that of a newly discovered vertebrate proneural gene, *neurogenin* (Ma *et al.*, 1996). This new subfamily of bHLH motifs also includes that of the vertebrate neuronal differentiation gene, *NeuroD* (Lee *et al.*, 1995). Since *tap* is so far the only representative of this new subfamily in flies, we pursued its characterization. Here we show that *tap* is expressed at a late step in the development of the adult chemosensory organs, much as it is in the larval organs. Unexpectedly, however, we found that it is also expressed at a very early step in the formation of the adult olfactory organs, raising the possibility that the same gene might act as a proneural for some chemosensory organs, and as a neuronal differentiation gene for others. We also present evidence that *tap* might play a role in specifying the properties of the chemosensory neurons where it is expressed, both in the larva and in the adult.

Results

Sequence of tap

The published sequence of *tap* (Gautier *et al.*, 1997) was based on our own sequencing of the cDNA. We have subsequently obtained a sequence of the coding region extracted from the genomic clone by Eurogentec with 99.8% accuracy. The two sequences are identical except in four positions, two of which do not change the amino-acid sequence while the other two lead to conservative changes (Leu 250 to Met, and Asn 393 to His). On the other hand, the sequence of *bps* differs from that of *tap* by 6

Abbreviations used in this paper: APF, after puparium formation; CNS, central nervous system; PNS, peripheral nervous system.

***Address for reprints:** Laboratoire de Neurogénétique, cc103, Université de Montpellier II, place E. Bataillon, 34095 Montpellier Cedex5, France. FAX: 33.467.143928. e-mail: aghysen@univ-montp2.fr

These laboratories have since been closed.

0214-6282/98/$10.00

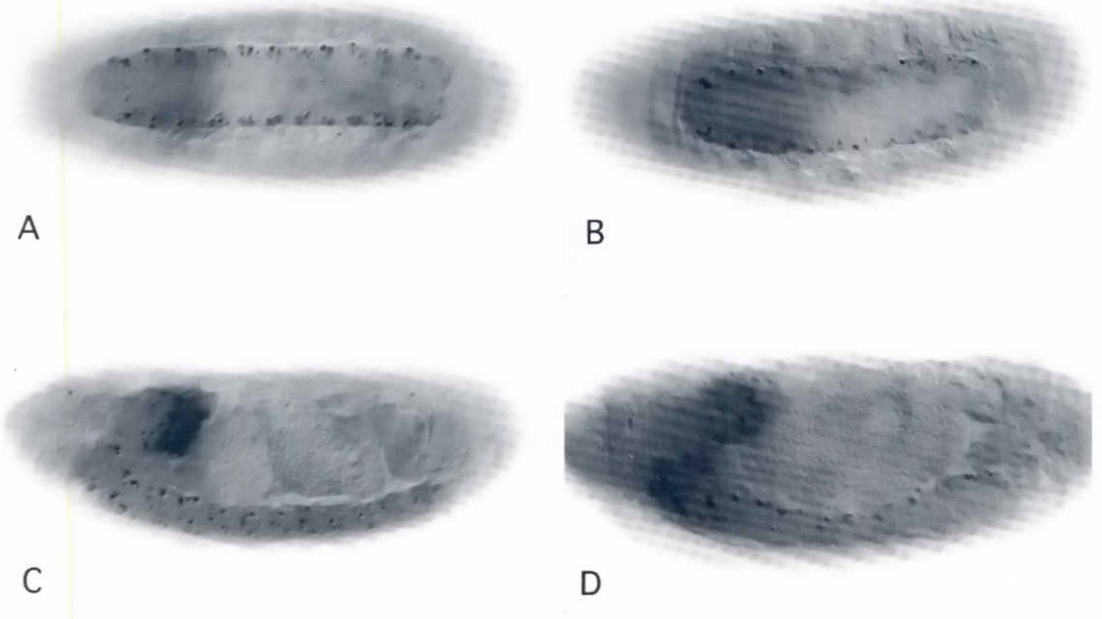

Fig. 1. Anti-Tap immunolabeling of the embryonic CNS in the wild type (left) and in the *tap*^{74} mutant (right). **(A,B)** *Ventral views.* **(C,D)** *Parasagittal views.*

substitutions, 6 losses and one extra base. Thus, while the nucleotide sequences are 99% identical, the amino-acid sequences are only 88% identical (48 substitutions). The major differences are a completely different stretch of 18 aminoacids at positions 56 to 73, another one from 360 to 377, and the C terminal end where a frame shift has changed the last 3 amino-acids and added another 9 in *bps*. The second of these variations (360-377) overlaps the putative *numb*-homology motif (349 to 367) identified by Bush *et al.* The corresponding stretch of *tap* is only 5% homologous to the *numb* motif, and 21% to the similar motif present in *prospero* (Vässin *et al.*, 1991), suggesting that *tap* does not contain a *numb*-homology motif, in line with the observation that the protein is strictly nuclear. The sequence of *bps* agrees with our cDNA sequence at all four positions which differ from the genomic sequence, suggesting either that there is a natural polymorphism at these sites, or that the same variant cDNA was identified in the two labs.

A P insertion near tap

We examined the patterns of *lacZ* expression of an enhancer-trap line where the insert maps near the cytogenetic position of *tap*, at 74B. In this line, P1542, *lacZ* shows a pattern of expression similar to that of *tap*, with one or two cells being labeled at the level of each chemosensory organ. The chromosome bearing the P1542 insertion is homozygous lethal. Labeling with anti-Tap reveals a reduced level of expression of *tap* in one fourth of the embryos, both in the PNS and in the CNS. This reduction did not result in any obvious morphological defect, and in particular we did not detect any abnormality in the presence or morphology of its chemosensory neurons (as seen in embryos where all sensory neurons were labeled by the 22C10 antibody).

We attempted to generate *tap*$^{-}$ mutants by imprecise excision of the P element. Among 169 *ry*$^{-}$ derivatives we found 17 that are homozygous viable, suggesting that the lethality of P1542 is linked to the insertion near *tap*. One of the homozygous lethal *ry*$^{-}$ derivatives, *tap*^{74}, shows a strong reduction in the number of anti-Tap labeled cells (Fig. 1). This effect concerns primarily the expression in the CNS, however, and the expression in the PNS is not detectably lower than in P1542.

We examined whether the reduction of *tap* expression in *tap*^{74} has any effect on the neurons where it is expressed. The pattern of expression of *tap* in the PNS during embryogenesis has been documented previously (Fig. 2). The gene is expressed in one of the neurons of each chemosensory organ. To enhance the mutant phenotype, we examined embryos hemizygous for *tap*^{74} using *Df(3)83K19*, a large deficiency that deletes the *tap* gene. Again no obvious defect could be detected after 22C10 labeling. To test for changes in neuronal identity in the hemizygous embryos, we used the anti-BarH1 antibody (Higashima *et al.*, 1992), which labels one of the two chemosensory neurons innervating the abdominal papilla p6. We observed several types of abnormalities (Fig. 3), including a reduction in neuron cell number (Fig. 3C), or the presence of large cells that might be delayed precursors (Fig. 3D), but no unambiguous evidence for a transformation of one neuronal cell type into another.

Pattern of tap expression in adult chemosensory organs

We extended the analysis of the pattern of *tap* expression to the developing adult sense organs by examining the imaginal discs of the wing, leg and proboscis. Because of the secretion of the pupal cuticle, immunolabeling of the imaginal discs is not possible after 8 h APF. At about 15 h APF the pupal cuticle can be peeled off from the differentiating tissue, making immunolabelings possible again (see material and methods). There is therefore a refractory period extending approximately from 8 to 14 h APF.

In the pupal wing, we observed labeling at the level of the developing chemosensory organs of the anterior margin from 16 to 22 h APF, at the time when the expression of poxn is subsiding. Either single cells or pairs of cells are labeled; in the latter case, one cell is usually more labeled than the other (Fig. 4B). The examination of large numbers of discs convinced us that *tap* is expressed first in a single cell, then in a pair, and finally maintained in only one. The sub epidermal location of these cells suggests that they are neurons, as in the embryo. Our interpretation of this result is that *tap* is be expressed not only in one of the neurons but in its precursor as well, and transiently in both daughter cells. This dynamics would generate the 1-2-1 sequence that we observed.

We did not observe *tap* expression in the pupal legs. This is not surprising, because the development of chemosensory organs proceeds a few hours earlier in the leg than in the wing, as judged e.g., by the onset of expression of *poxn* in the chemosensory precursors: *poxn* is expressed in many precursors in late third instar leg discs, but its expression in the anterior margin of the wing starts only 2 h after puparium formation (Dambly-Chaudière *et al.*, 1992). Likewise at 18 h APF the leg chemosensory neurons are already fully differentiated, while some neurons are still in the process of growing out an axon at 22 h APF in the case of the wing margin organs. We expect therefore the expression of *tap* in leg chemosensory organs to occur around 10-14 h APF, a period when imaginal discs are refractory to immunolabeling. In addition, we found that *tap* is expressed in a few cells of the everting leg disc (Fig. 4C), at positions which suggest that they may correspond to the neurons innervating the Keilin organs (Tix *et al.*, 1989). This is somewhat surprising, since these cells do not express *tap* during embryogenesis. We have not attempted to define the onset of this expression.

In the case of the proboscis, the precursors of the chemosensory organs appear in three waves, at respectively 0, 6 and 16 h APF (Ray *et al.*, 1993). The first wave generates 22C10-labeled neurons by 20 h APF. We examined the pattern of expression of *poxn* from 16 to 24 h APF. At 16 h we observe many clusters of *poxn*-expressing cells (Fig. 5A). Those clusters near the midline comprise several cells, and correspond to the first wave of precursors. Most other cluster comprise 2-3 cells, and correspond to the precursors of the second wave. This pattern is identical to that observed in the enhancer-trap line A37, which labels all cells of the sensory lineages (not shown), suggesting that all organs of the proboscis express *poxn*. We observed the presence of Tap protein from 18 h APF on, in single cells or pairs of cells corresponding to the organs of the first wave (Fig. 5B).

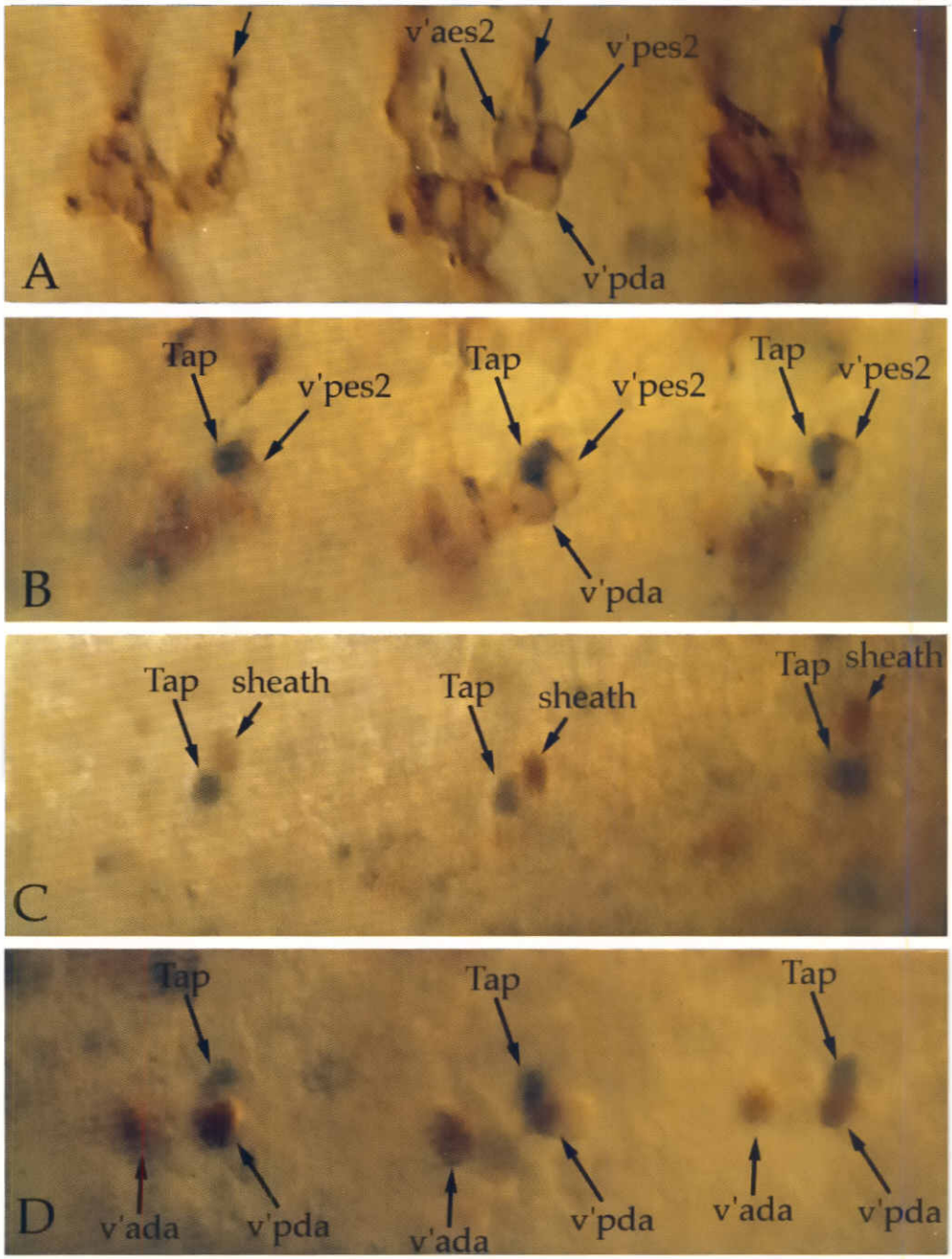

Fig. 2. Identification of the cell that expresses *tap* in the peripheral nervous system. (A) *Labeling of the sensory neurons with the 22C10 antibody, illustrating the appearance of the group of three neurons located posteriorly in the v' cluster. This group contains dorsally the two neurons v'aes2 and v'pes2, which innervate the ventral poly-innervated papilla p6, and ventrally the neuron v'pda, a multidendritic (md) neuron. The arrows point to the two dendrites innervating p6.* **(B)** *Double labeling with 22C10 and anti-Tap (in grey), showing that the cell that expresses tap is the anterior dorsal cell of the cluster, v'aes2, or a cell closely apposed to it.* **(C)** *Double labeling with anti-Tap (grey) and with an antibody that labels the sheath cell (anti-Pros, in brown) showing that the cell that expresses tap is distinct form the sheath cell.* **(D)** *Double labeling with anti-Tap (grey) and with an antibody that labels the md (multidendritic) neurons (anti-βGal in the enhancer-trap line E7-2nd-36, brown), showing that tap is not expressed by the md neuron v'pda. This figure has been printed in black and white in Gautier et al., 1997.*

Thus in the proboscis as in the wing, *tap* is expressed in a pattern that largely overlaps the pattern of *poxn* expression, at stages that are consistent with the idea that *tap* is expressed at the time chemosensory neurons are just about to, or are beginning to differentiate.

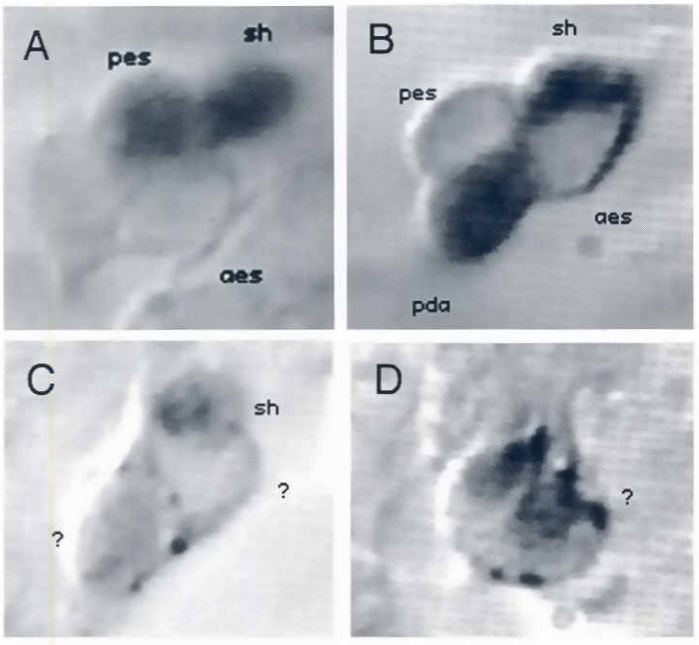

Fig. 3. Anti-BarH1 immunolabeling of the group of three neurons located posteriorly in the v' abdominal cluster. *Two of the neurons, v'aes and v'pes, innervate the ventral chemosensory organ p6; the third neuron, v'pda, is a multidendritic neuron not associated with any sensory structure. In the wild type* **(A)** *the antibody labels the v'pes neuron and the associated sheath cell. In tap^{74}/Df(3)83K19 embryos, various defects are observed: the labeled cell maybe in the position of v'pda* **(B)**, *one of the four cells may be missing* **(C)** *or a very large cell, possibly a precursor cell, may be present* **(D)**.

Effect of the ectopic expression of tap on gustatory organs

Touching the tarsus of starved flies with a sugar solution elicits an extension of the proboscis. If however salt is added to the solution, the response can be blocked (Dethier, 1963). If *tap* plays a role in the specification of one of the gustatory neurons, one might observe a modification of these behavioral responses following ectopic expression. We compared the responses of flies that harbored a hsp-*tap* construct to the responses of their *w* parents (Table 1).

The stimulation of a single tarsal bristle with sugar is usually insufficient to elicit a response, and therefore we routinely stimulated all tarsal bristles simultaneously. Since they do not develop all at the same time, heat-shocks at defined times would presumably affect only a subset of them, and therefore we examined the response to constant exposure to 25°C or 29°C.

The analysis of flies raised at 25°C, a temperature at which the hsp70 promoter is weakly active, reveals an impairment of the response to sugar in the range 10^{-1}-10^{-2} M. If the pupae are kept at 29°C, the highest temperature compatible with survival, even the response to a 1M solution is reduced. In parallel, the inhibitory effect of salt is much reinforced (Table 1).

The results show that the sensitivity of the *hsp-tap* flies to sugar is substantially impaired, while their sensitivity to salt is increased. The reduction of the sensitivity to sugar may be due to a defect in sugar-sensitive neurons, in line with the idea that *tap* plays a role in the specification of gustatory neurons. Alternatively, however, it may be that the decreased response to sugar reflects a general defect in the central nervous system due to the ectopic expression of *tap*. Likewise the seemingly increased sensitivity to salt may reflect a direct effect of *tap* ectopic expression on the specificity of the chemosensory neurons. It must be noted, however, that the response to salt is but an inhibition of the response to sugar, so that the two effects may actually reflect the same cause, rather than being independent evidence in favor of the transformation of one cell type into another.

Pattern of tap expression in adult olfactory organs

The adult comprises two types of chemosensory organs, which seem to develop in very different ways. The chemosensory organs examined so far develop like all other external sense organs, from a single precursor cell that undergoes a defined pattern of divisions to generate a fixed lineage. The formation of the corresponding precursors depends on the expression of the genes of the *achaete-scute* complex, and their identity depends on the expression of

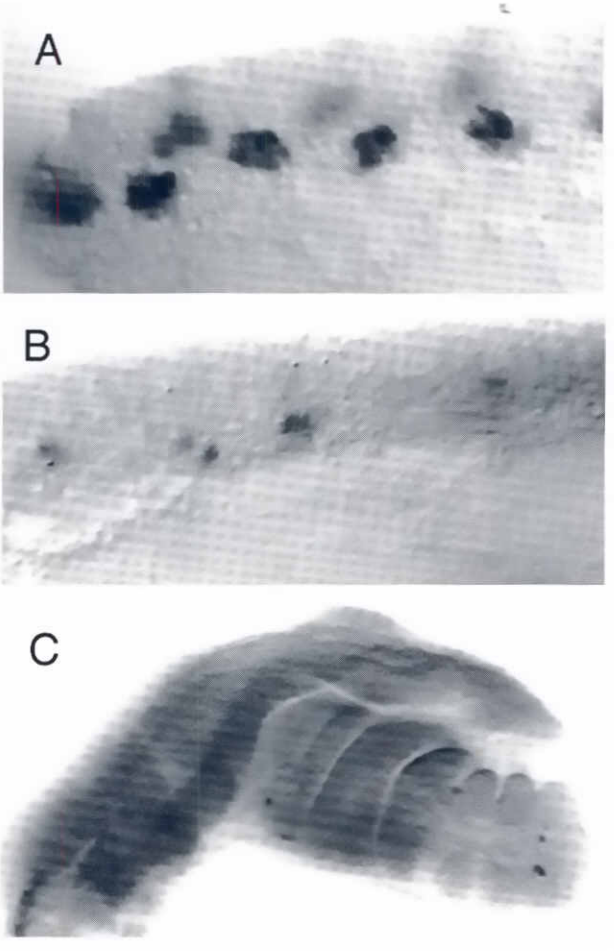

Fig. 4. Patterns of expression during metamorphosis. (A) *Expression of poxn at 16 h APF in the anterior margin of the wing. poxn is expressed in clusters of five or more cells, indicating that the lineage is nearly complete.* **(B)** *Expression of tap at 18 h APF. tap is expressed at the same positions as poxn, but in only one or two cells at each site.* **(C)** *Expression of tap in a leg disc 4 h APF. The position and number of the labeled cells, at the tip of the everting leg, suggests that Tap protein is present in neurons that innervate the Keilin organs.*

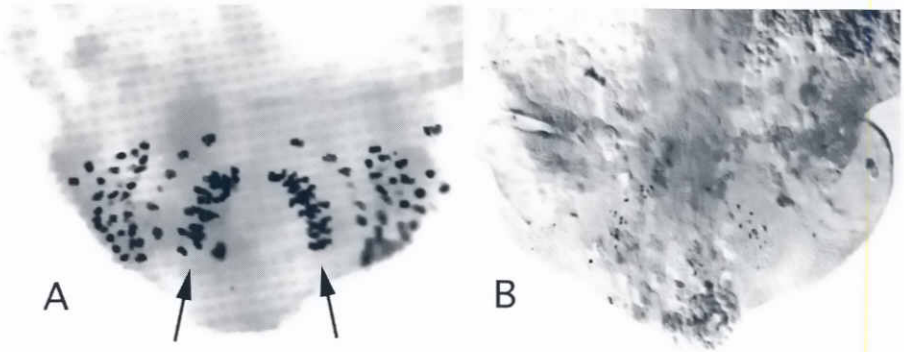

Fig. 5. Patterns of expression in the proboscis. **(A)** *Expression of poxn in the proboscis at 16 h APF. The first wave of precursors, closest to the midline (arrows), has already undergone two or more divisions; some precursors of the second wave have divided once, others have not yet divided.* **(B)** *Expression of tap at 20 h APF. Labeled cells are observed in a pattern that corresponds to the first wave of precursors.*

poxn. These chemosensory organs are gustatory in nature, and respond primarily to sugar and salt.

The second type of adult chemosensory organs are olfactory. There are three main types of organs (Fig. 6D-F): coeloconic, basiconic and trichoid sensilla, (Venkatesh and Singh, 1984). Olfactory organs are found exclusively on the third antennal segment (Fig. 6A-C) and on the palp. They resemble the gustatory organs in that they are poly-innervated, but differ from them in many aspects. Their development depends both on lineage (like the other external sense organs) and on recruitment (like the photoreceptors, and some chordotonal organs): one precursor recruits two or three neighboring cells to form a "presensillum cluster", which then divides to produce the complement of cells corresponding to one organ (Reddy *et al.*, 1997). Contrary to the gustatory organs, *poxn* is not expressed in the olfactory organs, neither in the precursor nor at any other step. Furthermore, none of the olfactory organs depend on the *achaete-scute* genes, contrary to all other external sense organs, including the gustatory ones. The coeloconic sensilla depend on *atonal* (Reddy *et al.*, 1997), the other two types depend on as yet unidentified proneural genes, which are likely are likely to code for bHLH factors, however, as all three types of olfactory organs are affected by the ectopic expression of the HLH antagonist, *emc* (Gupta and Rodrigues, 1997).

Tap-positive cells were observed in everting antennal discs, at three sites near the base of the third antennal segment. The number of labeled cells at each site increases progressively during the first 6 h APF, from 1-3 to more than 10 (Fig. 7). This pattern parallels the appearance of precursor cells in this segment as seen in the A101 enhancer-trap linetrap line (Reddy *et al.*, 1997), suggesting that *tap* is expressed in one subset of olfactory precursors. The position of the labeled cells is consistent with the idea that they may correspond to three large groups of basiconic sensilla found near the junction between the third and the second antennal segment. However this identification is only tentative, as we have no independent marker.

Effect of the early ectopic expression of tap

Given the early expression of *tap* in the antennal discs, corresponding to the period when the precursors are formed, and the high homology of its bHLH sequence to that of genes that induce neural development when ectopically induced, we examined whether the ectopic expression of *tap* might result in the formation of additional sense organs. We heat-shocked mature (late third instar) larvae for 1 h at 39°C, a treatment that resulted in a survival rate that varied between 2 and 30% in different experiments. This treatment caused no detectable abnormality in the pattern of bristles of the resulting adult flies.

If *tap* would induce exclusively olfactory organs, one might imagine that their external structures will not develop normally, or even not at all, in other regions of the fly. Thus we looked for the presence of additional neurons, indicative of supernumerary organs of any type, by immunolabeling with 22C10. We examined at 24 h APF the notum of *hsp-tap* flies that had been heat-shocked as mature larvae, and found no evidence for the presence of supernumerary neurons.

TABLE 1

BEHAVIOURAL RESPONSE OF WILD-TYPE AND *HSP-TAP* FLIES TO SUGAR AND SALT

Raised at 25°C	Su 10 mM	Su 100 mM	Su 1M	response
w	40	14	1	-
	6	32	45	+
	13	*70*	*98*	*% positive*
hs-tap	46	32	1	-
	2	16	47	+
	4	*33*	*98*	*% positive*
Raised at 29°C	Su 10 mM	Su 100 mM	Su 1M	response
w	63	22	5	-
	6	48	64	+
	9		*9*	*93*
% positive				
hs-tap	69	55	31	-
	3	17	41	+
	4	*24*	*57*	*% positive*
Inhibitory effect of NaCl	Su 10 mM	id + NaCl 1M		
Ore-R	23	19		
		17	*% inhibition*	
hs-tap	84	14		
		83	*% inhibition*	

Discussion

The pattern of expression of tap is unique among the bHLH genes identified so far, both in flies and in vertebrates. In the PNS, *tap* is specifically expressed in the lineages that produce chemosensory organs. This expression follows two very different patterns in the two types of chemosensory organs, gustatory and olfactory.

The expression in the gustatory lineages had already been documented in the case of the larval organs, where *tap* has been shown to be expressed in one of the neurons that innervate each organ. Here we show that a very similar pattern is observed in the development of the gustatory organs of the wing margin and of the proboscis, and we assume that only the presence of a refractory period prevented us from observing the same pattern in the legs.

In the developing adult organs, we observe that the labeling follows a 1-2-1 sequence. We interpret this result as reflecting the expression of *tap* in a neuronal precursor and in one of the daughter neurons, with transient labeling of the sib neuron due to the distribution of the Tap protein accumulated in the precursor. This seems different from the case of the larval organs. However, even though we never observed pairs of cells expressing *tap* by *in situ* hybridization on embryos, we occasionally observed pairs of Tap-reactive cells after immunolabeling. Thus we believe that *tap* is expressed in one gustatory neuron and in its mother cell during the development of both larval and adult organs. The reason why pairs of Tap-positive cells are more readily seen during metamorphosis than during embryogenesis may be the faster pace at which the larval organs develop.

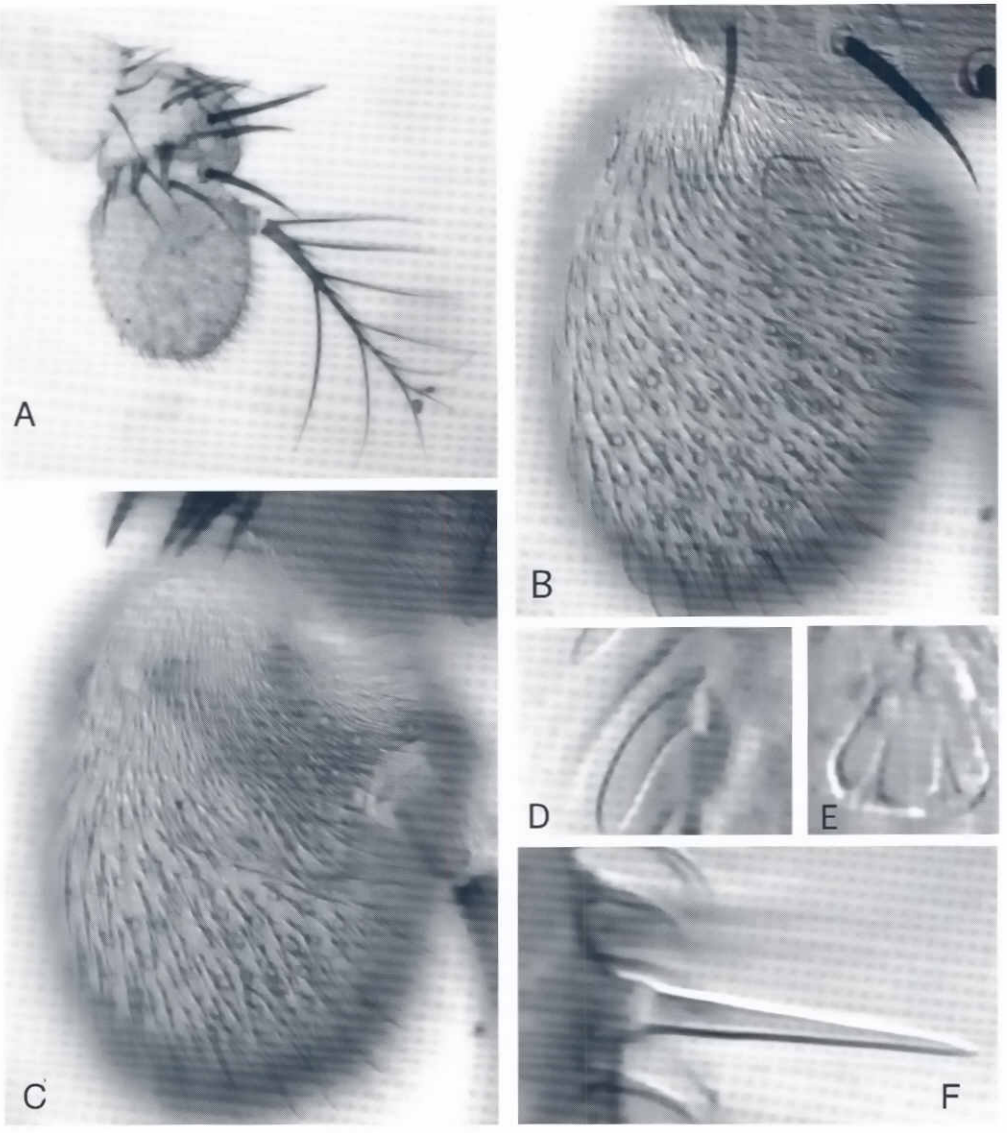

Fig. 6. The adult antenna (A), the anterior (B) and posterior (C) faces of the third antennal segment, and the three types of olfactory receptors: basiconic (D), coeloconic (E) and trichoid (F) sensilla.

A completely different pattern of expression is observed for the olfactory organs, where *tap* is expressed very early, at the time the precursors are forming. We conclude that *tap* is expressed at two different stages of the lineage in gustatory and olfactory organs. As noted previously, *tap* is the only known fly gene to be highly homologous to a subfamily of bHLH factors newly identified in vertebrates. This subfamily includes the genes *NeuroD* and *neurogenin*. Much like *tap* in the gustatory lineages, *NeuroD* is expressed late in neural development, at the time neurons are about to differentiate. And much like *tap* in the olfactory organs, *neurogenin* is expressed at a very early step in neural development, possibly as a proneural gene. As long as we have no clear idea about the function of *tap*, however, any comparison with its vertebrate counterparts, and any speculation about its very diverse times of action in the two types of chemosensory organs is premature.

So far we have not been able to identify the developmental function of *tap*. The available deletion of the *tap* gene removes more than fifty other genes, and therefore does not lend itself easily to an analysis of the loss-of-function phenotype. The P1542 line is but a weak hypomorph, from which we have not yet been able to isolate stronger alleles. In hemizygous condition, P1542 showed some defects at the level of the larval chemosensory neurons, but nothing near a distinct loss or transformation phenotype. This lack of obvious phenotype is not surprising given the weakness of the mutation but prevents us it prevents us from deriving any conclusion about the role of *tap*.

Our gain-of-function analysis suggests that *tap* might play a role in the specification of one of the adult gustatory neurons,

possibly the sugar-sensitive one. The evidence is, however, at best partial. The problem is that the bristles of *Drosophila* are too small electrophysiological analysis, and behavioral assays of gustatory function are subject to indirect alterations resulting from modifications or impairments of the central nervous system.

We have not been able to detect any proneural function of *tap* in gain-of-function experiments. This is surprising, because essentially all bHLH genes of the *achaete-scute* and the *neurogenin-NeuroD* families do show proneural effects in gain-of-function experiments in the fly and in *Xenopus*.

One possible explanation for the lack of gain-of-function effect is that the *hsp-tap* construct used for the gain-of-function analyses may not be fully active. We have verified that the activation of this construct during embryogenesis results in an ectopic expression of *tap* as detected by immunolabeling (not shown). However, there are two amino-acid changes between the putative protein sequences deduced from the cDNA and from the genomic clones, the genomic sequence being most likely the correct one. Although both differences are outside of the bHLH motif, and rather conservative, we cannot exclude that they result in a reduced activity of the protein.

An alternative explanation stems from the observation that the only organs where *tap* is expressed early are concentrated on the third antennal segment. It is conceivable that *tap* will reveal its proneural or precursor-specific function only in the context of this segment, either because of a requirement for antennal co-factors, or because some factor or condition required for the peculiar development of the olfactory receptors is not met in other regions of the body. Here again, a loss-of-function mutation may be indispensable to solve the riddle of the function of *tap*.

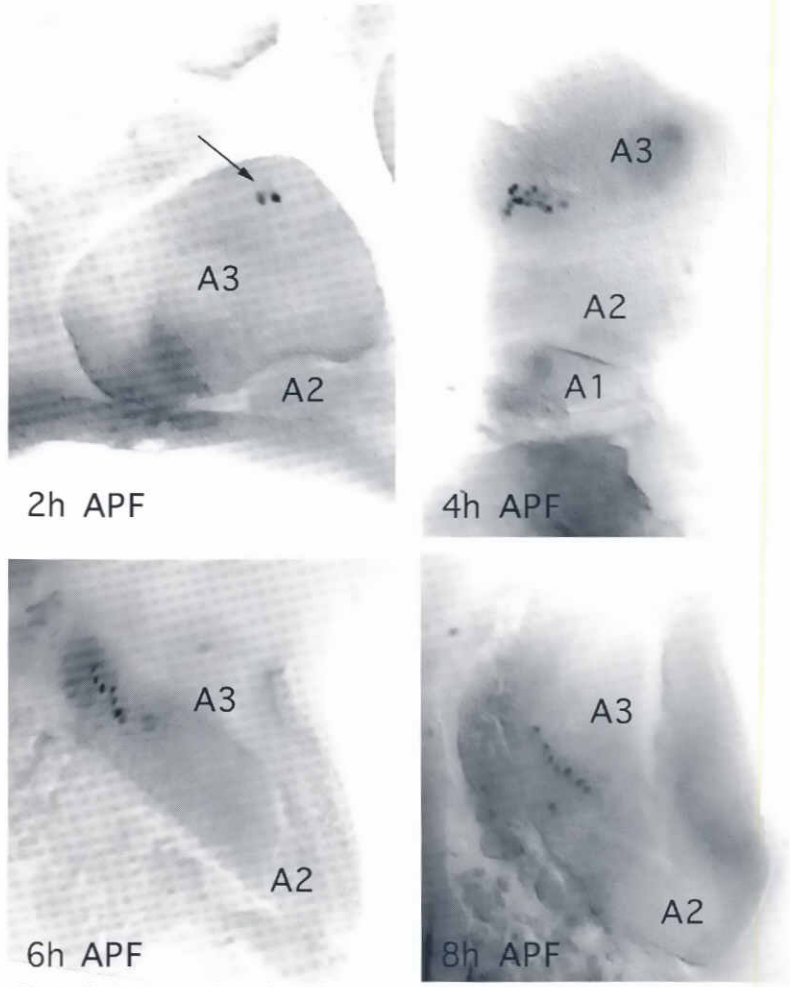

Fig. 7. Early expression of tap in one of the three antennal clusters. *The number of labeled cells increases rapidly during the first 8 h APF.*

Materials and Methods

Drosophila stocks

Stocks were maintained on standard medium at 18°C. Wild-type flies were the Oregon R strain. The ectopic expression of *poxn* was studied with the hsp70-*poxn* strain (Dambly-Chaudière *et al.*, 1992).

Obtention of a hsp-tap transformant line

We recovered the cDNA as an EcoR1 fragment and cloned it in the heat-shock vector pKB256. We tested the orientation of the insert by restriction mapping. The *hsp-tap* construct was then injected together with the pUschD2-3 helper plasmid and a line where the construct had inserted on the X chromosome was recovered. We tested the activity of the construct by exposing this line to a heat-shock of 30 min at 37°C. Thirty minutes after the heat-shock, we observed the generalized presence of Tap protein in most or all nuclei.

Immunohistochemistry

The anti-Poxn labeling was performed according to Dambly-Chaudière *et al.* (1992). All other immunohistochemical detections used PBS instead of phosphate buffer. The antibody 22C10 (Zipurski *et al.*, 1984) was used at a 1:200 dilution, and anti-Tap was used at a 3:1 dilution (150 ml anti-Tap for 50 ml 4x buffer).

Preparation of pupal tissues

After opening the pupal case at both ends, the pupa is left overnight in 3.7% formaldehyde in PBS. The pupal case is then entirely removed, and the pupa is fixed another 30 min in the same fixative. The pupal tissues are dissected in PBS with microscissors and the pupal cuticle is removed with sharpened forceps. All subsequent steps are done in glass dissection dishes.

Behavioral tests

Etherized flies are stuck in myristic acid (MP 50°C) melt locally by approaching a warm needle (in practice, the free end of a thin copper wire coiled around the tip of a welding iron). The flies are held on their back by sticking the wings and meso- and metathoracic legs; the prothoracic legs are left free. The flies are left in a moist chamber at 18°C overnight. The next morning, the flies are offered water in a thin capillary until they don't drink anymore. They are then tested by touching the tarsus of either foreleg with a piece of filter paper soaked in the solution to be tested, and scoring whether they extend their proboscis. The solutions were, in succession, water, sucrose 10 mM, sucrose 100 mM, and finally sucrose 1 M. Flies that respond to water were not tested further; they were offered more water to drink, and subsequently re-tested. Less than 10% of the flies keep on responding to water even when they cannot drink anymore; those were not analyzed further.

Acknowledgments

This work was supported by grants from the Human Frontier Science Organization and from the European Communities to AG and CDC.

References

BUSH, A., HIROMI, Y. and COLE, M. (1996). *biparous*: a novel bHLH gene expressed in neuronal and glial precursors in *Drosophila*. *Dev. Biol. 180:* 759-772.

DAMBLY-CHAUDIÈRE, C., JAMET, E., BURRI, M., BOPP, D., BASLER, K., HAFEN, E., DUMONT, N., SPIELMANN, P., GHYSEN, A. and NOLL, M. (1992). The paired box gene *pox-neuro*: a determinant of poly-innervated sense organs in *Drosophila*. *Cell 69:* 159-172.

DETHIER, V.G. (1963). *The Physiology of Insects Senses*. Methuen, London.

GAUTIER, P., LEDENT, V., MASSAER, M., DAMBLY-CHAUDIERE, C. and GHYSEN, A. (1997). *tap*, a *Drosophila* bHLH gene expressed in chemosensory organs. *Gene 191:* 15-21.

GUPTA, B.P. and RODRIGUES, V. (1997). Atonal is a proneural gene for a subset of olfactory sense organs in *Drosophila*. *Genes to Cells 2:* 225-233.

HIGASHIMA, S., MICHIUE, T., EMORI, Y. and SAIGO, K. (1992). Subtype determination of *Drosophila* embryonic external sensory organs by redundant homeobox genes *BarH1* and *BarH2*. *Genes Dev. 6:* 1005-1018.

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

JARMAN, A.P., GRAU, Y., JAN, L.Y. and JAN, Y.N. (1993). *atonal* is a proneural gene for chordotonal organs in the *Drosophila* peripheral nervous system. *Cell 73:* 1307-1321.

JARMAN, A.P., GRELL, E.H., ACKERMAN, L., JAN, L.Y. and JAN, Y.N. (1994). *atonal* is the proneural gene for *Drosophila* photoreceptors. *Nature 369:* 398-400.

LEE, J.E., HOLLENBERG, S.M., SNIDER, L., TURNER, D.L., LIPNICK, N. And WEINTRAUB, H. (1995). Conversation of *Xenopus* ectoderm into neurons by *NeuroD*, a basic helix-loop-helix protein. *Science 268:* 836-844.

MA, Q., KINTNER, C. and ANDERSON, D.J. (1996). Identification of *neurogenin*, a vertebrate neuronal determination gene. *Cell 87:* 47-52.

REDDY G. V., GUPTA B., RAY K and RODRIGUES V. (1997). Development of the *Drosophila* olfactory sense organs utilises cell-cell interactions as well as lineage. *Development 124:* 703-712.

TIX S., BATE M. and TECHNAU G.M. (1989). Pre-existing pathways in the leg imaginal discs in *Drosophila*. *Development 107:* 855-862.

VÄSSIN, H., GRELL, E., WOLFF, E., BIER, E., JAN, L.Y. and JAN, Y.N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. *Cell 67:* 941-953.

VENKATESH S. and SINGH R.N. (1984). Sensilla on the third antennal segment of *Drosophila melanogaster*. *Int J. Insect Morphol. Embryol. 13:* 51-63.

ZIPURSKI, S.L., VENKATESH, T.R., TEPLOW, D.B. and BENZER, S. (1984). Neuronal development in the *Drosophila* retina: monoclonal antibodies as molecular probes. *Cell 36:* 15-26.

Received: December 1997
Accepted for publication: January 1998