Cell, Vol. 69, 159–172, April 3, 1992, Copyright © 1992 by Cell Press

The Paired Box Gene *pox neuro*: A Determinant of Poly-Innervated Sense Organs in Drosophila

Christine Dambly-Chaudière,* Elisabeth Jamet,†§ Maya Burri,† Daniel Bopp,†‖ Konrad Basler,‡# Ernst Hafen,‡ Nathalie Dumont,* Patrick Spielmann,† Alain Ghysen,* and Markus Noll†

*Laboratoire de Génétique
Université Libre de Bruxelles
1640 Rhode-Saint-Genèse
Belgium
†Institut für Molekularbiologie II and
‡Zoologisches Institut
Universität Zürich
CH-8057 Zürich
Switzerland

Summary

This study describes the structure and function of *pox neuro* (*poxn*), a gene previously isolated by virtue of a conserved domain, the paired box, which it shares with the segmentation genes *paired* and *gooseberry*. Its expression pattern has been analyzed, particularly during development of the PNS. We propose that *poxn* is a "neuroblast identity" gene acting in both the PNS and the CNS on the basis of the following evidence. Its expression is restricted to four neuronal precursors in each hemisegment: two neuronal stem cells (neuroblasts) in the CNS, and two sensory mother cells (SMCs) in the PNS. The SMCs that express *poxn* produce the poly-innervated external sense organs of the larva. In *poxn*⁻ embryos, poly-innervated sense organs are transformed into mono-innervated. Conversely, ectopic expression of *poxn* in embryos transformed with a heat-inducible *poxn* gene can switch mono-innervated to poly-innervated sense organs. Expression of *poxn* in the wing disc is restricted to the SMCs of the poly-innervated sense organs, suggesting that *poxn* also determines the lineage of poly-innervated adult sense organs.

Introduction

The mechanism by which position is translated into a specific cell fate is poorly understood. Yet cell fate specification is a crucial aspect of development, particularly of neurogenesis. An attractive system to study this process is the formation of the peripheral nervous system (PNS) in Drosophila, which comprises few different types of sense organs arranged in highly invariant patterns (Hertweck, 1931; Kankel et al., 1980; Campos-Ortega and Hartenstein, 1985; Dambly-Chaudière and Ghysen, 1986; Ghysen et al., 1986; Bodmer and Jan, 1987).

Most of the external sense organs are innervated by a single neuron (mono-innervated external sense organs, or m-es organs). The m-es organs function as mechanoreceptors and are by far the most abundant external sense organs of the segments in the larva as well as in the adult (Figure 1). The cuticular structure associated with an m-es organ is either a bristlelike trichoid sensillum (also called sensory hair in the larva) or a domelike campaniform sensillum (also called sensory papilla in the larva).

Some external sense organs are innervated by several, usually 2–4, neurons (poly-innervated external sense organs, or p-es organs). The p-es organs are generally thought to act as chemoreceptors (Figure 1). Various types of external sensory structures are associated with p-es organs. In the adult, the external structure is often an open-tipped, recurved bristle. Such bristles are found along the anterior margin of the wing, where they are arranged in two parallel rows, and on the legs, where they form a unique pattern. In the larva, two p-es organs are present in each hemisegment. The most prominent of these are the peglike "kölbchen," first described by Hertweck (1931). Two kölbchen are present in each thoracic hemisegment, one ventral (vk) and one lateral (lk, in T2 and T3) or dorsal (dk, in T1). In the larval abdomen the p-es organs are much less conspicuous: a lateral papilla, p6, and a minute dorsal hair, h3, both of which are innervated by two neurons (Dambly-Chaudière and Ghysen, 1986, 1987). It has been demonstrated that p6 and h3 are the abdominal homologs of the thoracic ventral and lateral kölbchen, respectively (Dambly-Chaudière and Ghysen, 1987). The function of the larval p-es organs is not known.

In addition to the external sense organs, the PNS contains internal sense organs, in particular the chordotonal (ch) organs, which function as stretch receptors, and multiple dendritic (md) neurons, which probably act as touch receptors (Bodmer and Jan, 1987). All internal sense organs are mono-innervated.

Each sense organ is a clone of cells originating from a progenitor cell called a "sensory mother cell" (SMC; reviewed by Bate, 1978; Hartenstein, 1988; Bodmer et al., 1989). SMCs forming m-es organs divide twice to generate four daughter cells. Of these four cells, one differentiates into the sensory neuron, a second forms a sheath surrounding the dendrite, and the remaining two become support cells, which differentiate the structural part of the sense organ, for example, the shaft and the socket of a bristle (schematically illustrated in Figure 1). In the case of p-es organs, additional mitoses generate the additional neurons. Contrary to the diversity of the sense organs, the SMCs are morphologically indistinguishable and can be identified only by their location. It remains to be demonstrated whether the SMCs themselves or their progeny are committed to form a specific sense organ.

It has been discovered recently that the developmental choice between external sense organs and chordotonal

§ Present address: Institut de Biologie Moléculaire des Plantes, CNRS, 67084 Strasbourg, France.
‖ Present address: Department of Molecular Biology, Princeton University, Princeton, New Jersey 08540.
Present address: Howard Hughes Medical Institute, Center for Neurobiology and Behavior, Columbia University, New York, New York 10032.

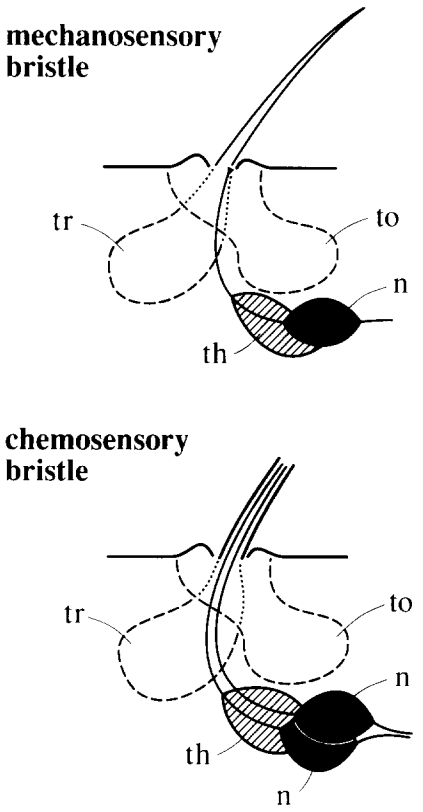

Figure 1. Structure of Adult External Sense Organs in Drosophila

A mono-innervated mechanosensory bristle (a) and a doubly innervated chemosensory bristle (b) are illustrated schematically. Each type of bristle contains two support cells (called tormogen, to, and trichogen, tr) that differentiate the cuticular structures, a socket and a shaft, respectively; one cell (called thecogen, th) forming a sheath around the dendrite; and one or several neurons (n). In the case of the homologous larval poly-innervated sense organs (thoracic kölbchen and abdominal papilla and hair), it is not known whether the dendrites of the neurons are exposed to the outside as shown here for the adult chemosensory bristle.

organs depends on the state of a single gene, *cut* (Bodmer et al., 1987). The *cut* protein is expressed specifically in the cells of the external sense organs (Blochlinger et al., 1988). In its absence, all external sense organs are transformed into chordotonal organs (Bodmer et al., 1987). The *cut* protein can be detected in the SMCs, implying that the decision to form an external sense organ or a chordotonal organ may already have been made in these cells (Blochlinger et al., 1990). The fact that the *cut* protein contains a homeodomain suggests that it imposes the lineage for external sense organs by regulating the expression of a specific gene network.

Another gene that is specifically expressed in a subset of sense organs has been described by Bopp et al. (1989). This gene, *pox neuro* (*poxn*), contains a paired domain and has been isolated on the basis of the gene network concept, which proposes that genes of the same network share a relatively small number of structural and cis-regulatory domains that are specific for the particular network (Frigerio et al., 1986; Bopp et al., 1986). The *poxn* gene encodes a nuclear protein that is expressed in a few clusters of cells in the central nervous system (CNS) and in the periphery. The peripheral cells were proposed to belong to the PNS because they are absent in *daughterless* (*da*) embryos (Bopp et al., 1989), where the PNS is completely abolished (Caudy et al., 1988). Here, we further characterize *poxn* and demonstrate that this gene is responsible for the specification of the poly-innervated external sense organs.

Results

Molecular Characterization of the *pox neuro* Gene

The *poxn* gene, which is uncovered by the large deficiency *Df(2R)WMG* (Bopp et al., 1989), was mapped more precisely within a chromosomal walk to a 30 kb region at band 52D1, separating the proximal *Df(2R)XTE-18* from the distal *Df(2R)KL-32* (Figure 2a). The distal breakpoint of *Df(2R)XTE-18* maps to a 1.4 kb EcoRI fragment, 17 kb proximal to the 3′ end of the *poxn* transcript, while the proximal breakpoint of *Df(2R)KL-32* is within a 3.6 kb EcoRI fragment, 1.6 kb distal to the 5′ end of a nearly full-length *poxn*-cDNA (Figures 2a and 2b). The transcriptional organization of *poxn* depicted in Figure 2b was derived from a comparison of the genomic sequence with that of four *poxn*-cDNAs (cPn1, cPn2, cPn5, and P4c6).

The DNA sequence of *poxn* is shown in Figure 3. The best fit of a start site (6/7 match) to the Drosophila consensus initiation point for RNAs transcribed by RNA polymerase II (ATCAG/TTPy; Hultmark et al., 1986) is found 90 bp upstream of the 5′ end of cPn1 and would produce an mRNA length consistent with the size of a single 2.5 kb mRNA species detected by Northern analysis (data not shown). Two polyadenylation signals (AATAAA) are found 14 bp and 35 bp from the polyadenylation site determined in P4c6. The mRNA is generated from five exons, of which the first and nearly the entire second exon consist of untranslated leader sequences.

The first open reading frame is closed after a potential heptapeptide. It is followed by a short noncoding M- or opa-repeat of 7 CAG/A(T)s and a repeat of 7 pentanucleotides of the sequence ATTGA/G(T). The longest open reading frame starts with the second ATG at the end of the second exon and encodes a protein of 425 amino acids. A paired domain, preceded by only 4 amino acids, is located at the amino-terminal end of the *pox neuro* protein and encoded on three exons (Bopp et al., 1989) interrupted by a 69 bp and a 2.1 kb intron flanking a mini-exon of 80 bp. While the large intron is found at the same position as introns of two isolated human paired domain genes, HuP1 and HuP2 (Burri et al., 1989), no other paired domain gene with an intron at the site of the small intron (after amino acid 47 of the paired domain) has been reported so far.

Following the paired domain, located at the amino-terminal third of the *poxn* protein, the protein contains stretches of poly-ala, which are interrupted by a proline-rich region (15 pro of 45 amino acids), as well as a region of charged amino acids (14/25) followed by a highly acidic region (18 Glu or Asp of 45 amino acids containing no basic residues) at the carboxy-terminal end of the protein. As the *poxn* protein is probably a transcription factor, the

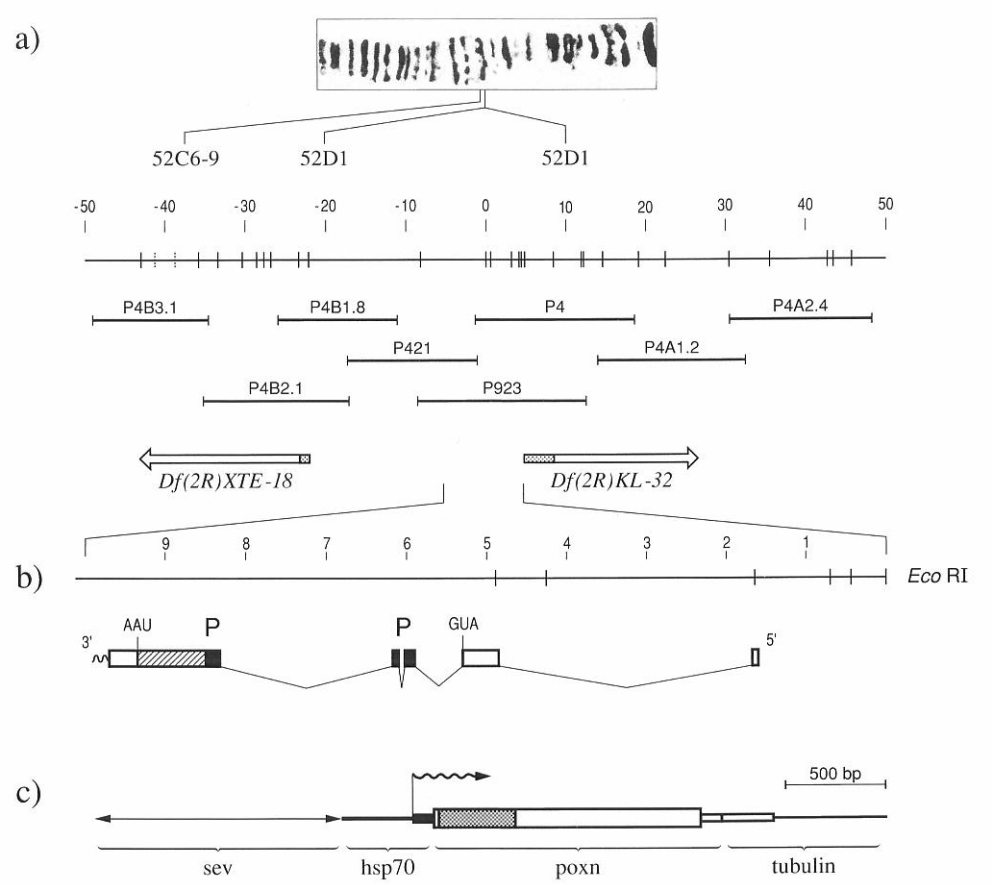

Figure 2. Chromosomal Region at 52C-D Comprising the *pox neuro* Gene, Structural Organization of *poxn* Transcription, and hsp-*poxn* Construct Used for P Element–Mediated Transformation

(a) Chromosomal walk defining *pox neuro* to a region between the proximal breakpoint of *Df(2R)KL-32* and the distal breakpoint of *Df(2R)XTE-18*. *Df(2R)KL-32* possibly includes some cis-regulatory elements of *pox neuro* as discussed in the text. Starting from the end fragments of a cloned region (consisting of the recombinant phages P4, P923, and P421) described previously (Bopp et al., 1989), chromosomal walks were initiated to include these two deficiency breakpoints indicated below the recombinant phages (P4B3.1, P4B2.1, P4B1.8, P4A1.2, and P4A2.4), which were isolated from a genomic *Df(2R)SB1/CyO* library (Baumgartner et al., 1987) constructed in EMBL4 (Frischauf et al., 1983). The breakpoints have been mapped by in situ hybridization to salivary gland chromosomes and by whole genome Southern analysis to the 1.4 kb and 3.6 kb EcoRI fragments marked by the dotted ends of the arrows indicating the direction of the two deficiencies. Above the cloned recombinant phages, a genomic EcoRI map (scale in kb) and the corresponding region on a salivary gland chromosome are shown. The order of neighboring EcoRI fragments bordered by dotted sites around −40 kb has not been determined.

(b) Map of transcriptional unit of *pox neuro*. A blowup of the region around the origin of the EcoRI map is shown above the intron–exon structure of the *pox neuro* transcript, which has been derived from the DNA sequence shown in Figure 3. The beginning and end of the open reading frame, consisting of the paired domain P (closed bars) and the adjacent hatched region in the last exon, are marked by the corresponding initiation and termination codons. Leader (5′) and trailer (3′) sequences are indicated as open bars.

(c) Map of hsp70-*poxn* construct used for generation of transgenic flies. The construct used for P element–mediated transformation is shown. It consists of the 1.2 kb *sevenless* enhancer fragment (sev), the *hsp70* heat shock promoter (hsp70), a trimmed *poxn*-cDNA (poxn), and a 0.8 kb genomic tubulin fragment (tubulin) serving as transcriptional terminator. For a detailed description of the P element vector containing the construct, see Experimental Procedures.

carboxy-terminal acidic domain might act as a gene activator (Ma and Ptashne, 1987) in combination with the paired domain (Bopp et al., 1989). Recently, some support for our suggestion that the paired domain might be a DNA binding domain (Bopp et al., 1989; Burri et al., 1989) has been obtained by in vitro DNA binding studies (Treisman et al., 1991).

Expression of *pox neuro* in the Developing Embryonic Peripheral and Central Nervous System

As illustrated in Figure 4a, the neurons of larval sense organs are arranged in two major patterns, a thoracic (identical in T2 and T3, yet slightly different in T1) and an abdominal pattern (identical in A1 to A7; Ghysen et al., 1986; Dambly-Chaudière and Ghysen, 1987). Bopp et al. (1989) found that *poxn* is expressed, in a segmentally repeated pattern, in some cells of the central and peripheral nervous system of the embryo. A closer examination of the staining pattern with anti-*poxn* antiserum shows that the *poxn* protein is present in four clusters of cells per hemisegment, two each in the PNS and CNS. As is evident from a comparison of Figures 4a and 4b, the positions of the peripheral stained cells coincide with those of the p-es organs in the thoracic as well as in the abdominal seg-

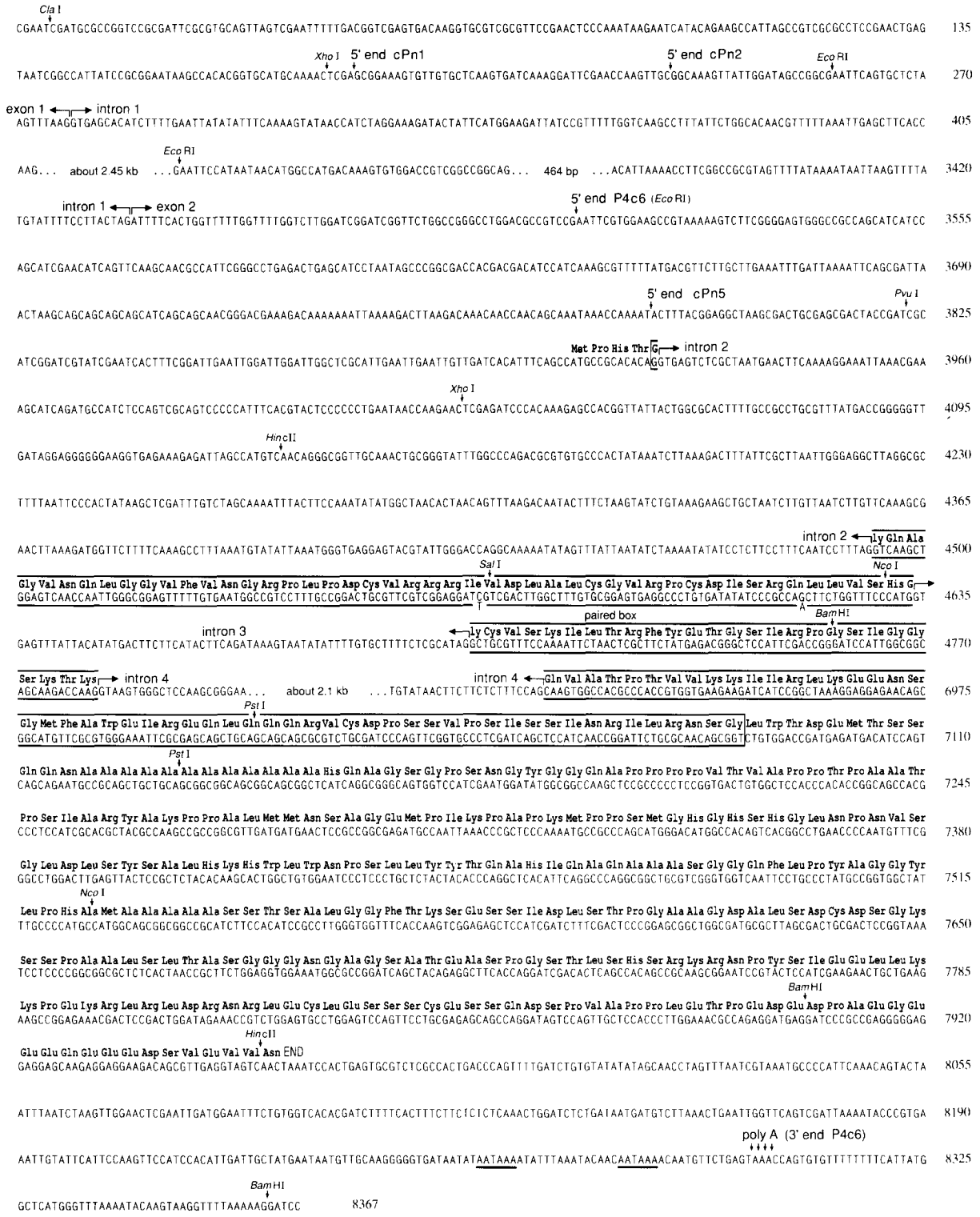

Figure 3. DNA Sequence of the *pox neuro* Gene and Corresponding Amino Acid Sequence of the Putative *pox neuro* Protein

The DNA sequence between the ClaI site upstream of the 5′ end of a nearly full-length cDNA, cPn1, and the BamHI site downstream of the poly(A) addition site found in another cDNA, P4c6, is shown. Three cDNAs (cPn1, cPn2, and cPn5) were sequenced only at their 5′ ends, while P4c6 was sequenced along its entire length on both strands. In addition, the transcribed *poxn* sequence was determined on both strands of the genomic DNA, with the exception of about 2.45 kb of the first and 2.1 kb of the last intron, which have not been sequenced. Hence, the numbering of the nucleotides, indicated in the right margin, is only approximate after this gap of nonsequenced DNA in the first intron. Above the DNA sequence, the amino acid sequence corresponding to the longest open reading frame in the cDNA sequence is shown. The sequences corresponding to the paired domain (Bopp et al., 1989) are boxed. Two deviations of the sequenced P4c6 cDNA from the genomic DNA sequence, which do not result in an alteration of the amino acid sequence, have been found and are indicated below the corresponding positions. A deviation, found in P4c6 by comparison with the genomic and an additional cDNA sequence, cPn5, and consisting of a missing G at the splice junction of the second and third exon, has been

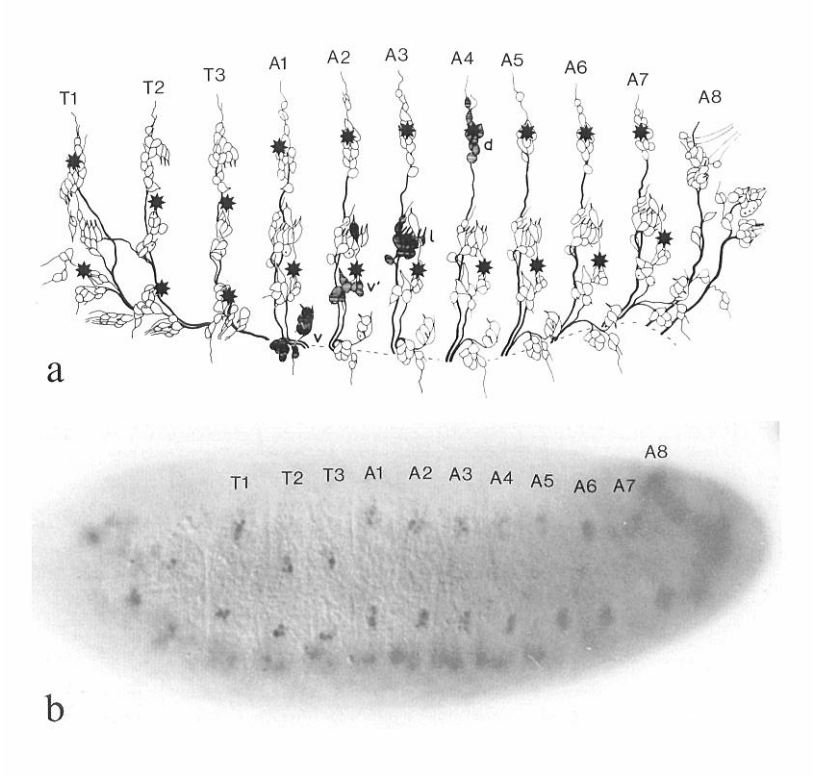

Fig. 4. Correlation of Patterns of *pox neuro* Expression in the Developing PNS and of Larval Poly-Innervated External Sense Organs

(a) Camera lucida drawing of the arrangement of peripheral neurons in an embryo after head involution and dorsal closure (from Ghysen et al., 1986). This is the earliest time at which the differentiated neurons can be detected. The positions of the poly-innervated sense organs (thoracic kölbchen and their abdominal homologs) have been marked with asterisks.

(b) A slightly younger embryo (just before head involution, stage 13; Campos-Ortega and Hartenstein, 1985) immunolabeled with purified anti-*poxn* antiserum as described (Bopp et al., 1989). This is the latest time at which *poxn* protein is still detectable. T1–T3, first to third thoracic segments; A1–A8, first to eighth abdominal segments.

ments. In particular, the pattern of the dorsal-most clusters exhibits the characteristic displacement of the two lateral kölbchen in T2 and T3. The *poxn* gene is also expressed in the gnathal segments and in the terminal abdominal segments A8–A10, which contain p-es organs as well (Campos-Ortega and Hartenstein, 1985; Dambly-Chaudière and Ghysen, 1986; Ghysen et al., 1986).

The expression of *poxn* starts prior to the overt segregation of p-es organs. It is first detected at the extended germband stage (Figure 5a; early stage 11; Campos-Ortega and Hartenstein, 1985) in the PNS, shortly after the first *poxn* transcripts appear (Bopp et al., 1989). At about 5.5 hr of development, staining is observed in a few cells of the gnathal segments as well as in a single cell of each thoracic and abdominal hemisegment (Figure 5a, arrows). At the time when this single cell starts dividing, a second, more ventral cell appears that stains with anti-*poxn* antibodies (Figure 5b, arrowheads), and the first labeled neuroblasts appear in the CNS (arrows). At this stage, *poxn* protein is found predominantly in nuclei, but occasionally cells are observed whose cytoplasm is stained as well (Figure 5f, arrowhead), presumably because these are mitotic cells in which the breakdown of the nuclear envelope has allowed diffusion of *poxn* protein into the surrounding cytoplasm. In slightly older embryos, the ventral cells in the PNS also begin dividing to form pairs of *poxn*-positive ventral cells (Figure 5c, arrows). One neuroblast, or a neuroblast and what appears to be its first ganglion mother cell (GMC), located at the anterior boundary of each hemisegment, are now labeled in the CNS (Figure 5c, arrowheads).

Soon thereafter, during germband retraction (stage 12), the dorsal pair of *poxn*-positive cells in T2 and T3 migrate ventrally to assume their final lateral positions (Figure 5d, arrows). During the fast period of germband shortening (late stage 12), the pattern comprises two clusters of up to 5–7 *poxn*-expressing cells in the PNS (Figure 5e, arrowheads in A2) as well as two neuroblasts and apparently clonally related cells (Figure 5e, arrows) in each hemisegment. Since mitotic figures are regularly observed during the proliferation of the peripheral clusters (Figure 5f), we assume that each cluster originates by divisions from the cell that originally expressed *poxn*. Similarly, we believe that the small labeled nuclei in the CNS (Figure 5g, arrows) belong to GMCs produced by the *poxn*-expressing neuroblasts (Figure 5g, arrowhead). Upon completion of germband retraction, the staining in the PNS fades and has completely disappeared by the time the sensory neurons differentiate.

We have shown that the first *poxn*-expressing cells are SMCs by double-labeling experiments in A37 embryos. A37 is an enhancer trap line that expresses *lacZ* in all SMCs and their progeny (Ghysen and O'Kane, 1989). Double labeling of A37 embryos with anti-*poxn* and anti-β-

corrected by reintroducing a G at this site (cf. Experimental Procedures). The positions of the 5′ end of four cDNAs (cPn1, cPn2, cPn5, and P4c6) and the 3′ end (polyadenylation site) of one cDNA (P4c6), and of the cleavage sites of some restriction enzymes, are marked by vertical arrows. Two canonical polyadenylation signals are underlined.

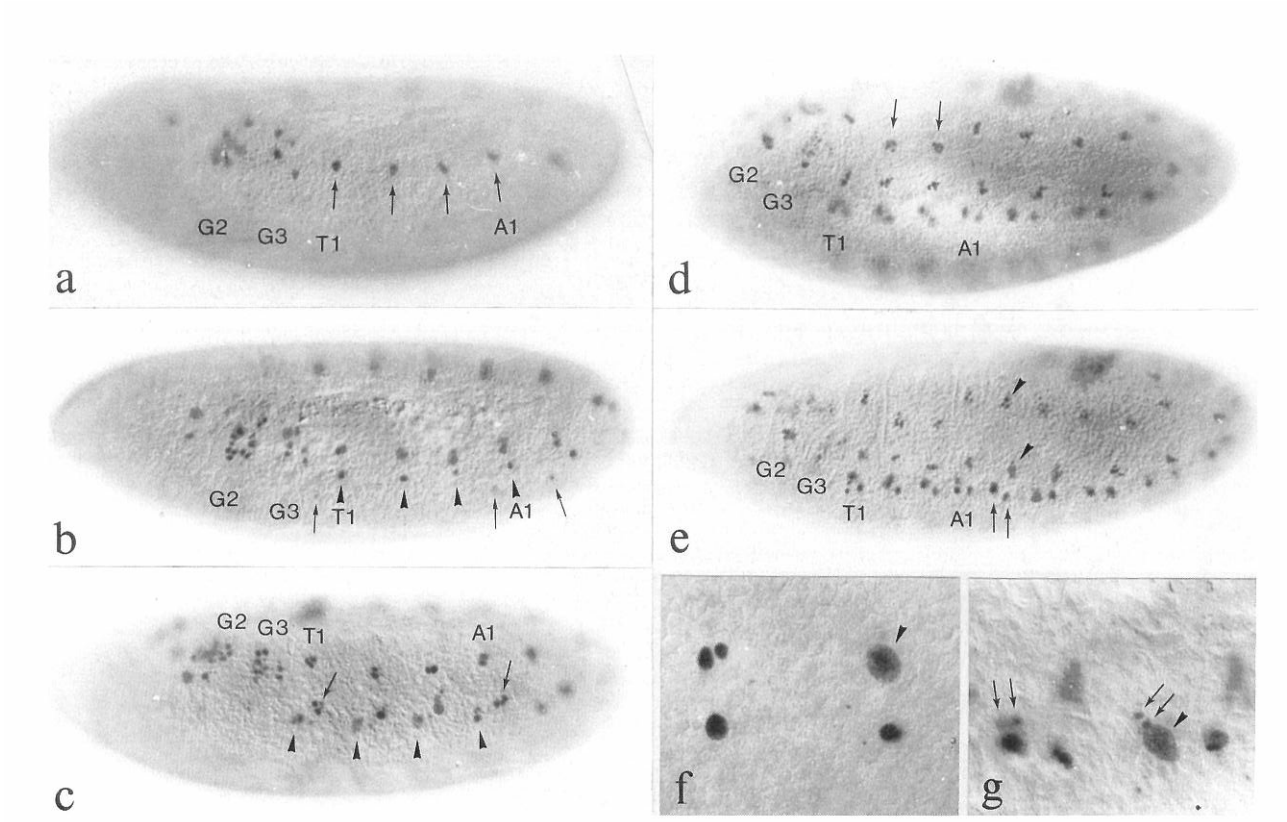

Figure 5. Expression of *pox neuro* during Embryogenesis

Embryos were immunolabeled with purified anti-*poxn* antiserum as described (Bopp et al., 1989).

(a) An embryo at about 5.5 hr of development (at 25°C), showing the early pattern of *poxn* expression. A single cell is labeled in each trunk segment (arrows). T2 and T3, second (maxillary) and third (labial) gnathal segments.

(b) An embryo at about 5.75 hr of development, showing the appearance of a second, more ventral cell in each trunk hemisegment (arrowheads). The first labeled neuroblasts appear (arrows).

(c) A slightly older embryo where the ventral PNS cells in T1 and A1 have also divided (arrows). All stained dorsal cells have already divided at least once. One neuroblast expresses *poxn* in each hemisegment (arrowheads).

(d) At the time of germband shortening, the labeled dorsal and ventral cells of the PNS have generated clusters of several cells. The dorsal cells of T2 and T3 have begun their characteristic ventral migration (arrows), and the two *poxn*-expressing neuroblasts can now be detected in each hemisegment (slightly out of focus).

(e) The final pattern of *poxn* expression is reached upon completion of germband shortening, shortly before neuronal differentiation begins. Each hemisegment contains two peripheral clusters (arrowheads) and two neuroblasts (arrows). In addition to the displacement of the dorsal cluster in T2 and T3, there is also a slight shift of the thoracic and abdominal ventral clusters relative to each other, which is later maintained in the corresponding sense organs of the cuticle (cf. Figure 4).

(f) Two segments (T1 and T2) of an embryo at the stage illustrated in (b), showing a *poxn*-expressing dorsal cell in T2 entering mitosis (arrowhead) and a labeled dorsal pair of cells in T1 after mitosis. The ventral cells stained for *poxn* protein have not yet divided.

(g) *poxn*-expressing neuroblasts and ganglion mother cells in two adjacent segments. The anterior neuroblast in one segment (arrowhead) is entering mitosis. Each anterior neuroblast has produced two ganglion mother cells (arrows).

galactosidase antibodies reveals that *poxn* is expressed in a subset of *lacZ*-expressing cells (A. Goriely, C. D.-C., and A. G., unpublished data).

Expression of *pox neuro* in Imaginal Discs

Similar to the larval PNS, the adult PNS contains m-es and p-es organs, each of which is clonally derived from an SMC. These SMCs can also be visualized by several enhancer trap lines, including A101 (Bellen et al., 1989), a line that, in imaginal discs, is more sensitive than A37. Such lines reveal an invariant pattern of SMCs already at the earliest stage of detection, preceding the onset of metamorphosis (Huang et al., 1991; Cubas et al., 1991). In particular, the SMCs of the two rows of chemosensory bristles on the anterior wing margin are present in wing discs of late third instar larvae. All other SMCs present at this stage will generate m-es organs.

Figure 6a shows an everted wing disc of the enhancer trap line A101 at puparium formation, illustrating the double row of SMCs along the wing margin as well as many other SMCs of notum bristles and campaniform sensilla on the wing blade. As is apparent from Figure 6b, *poxn* is expressed exclusively in the precursors of the poly-innervated wing margin bristles. At the same time, *poxn* is also expressed in a small number of cells in the leg discs (data not shown).

Absence of Poly-Innervated Sense Organs in *poxn*⁻ Embryos

The striking correspondence between the patterns of *poxn*

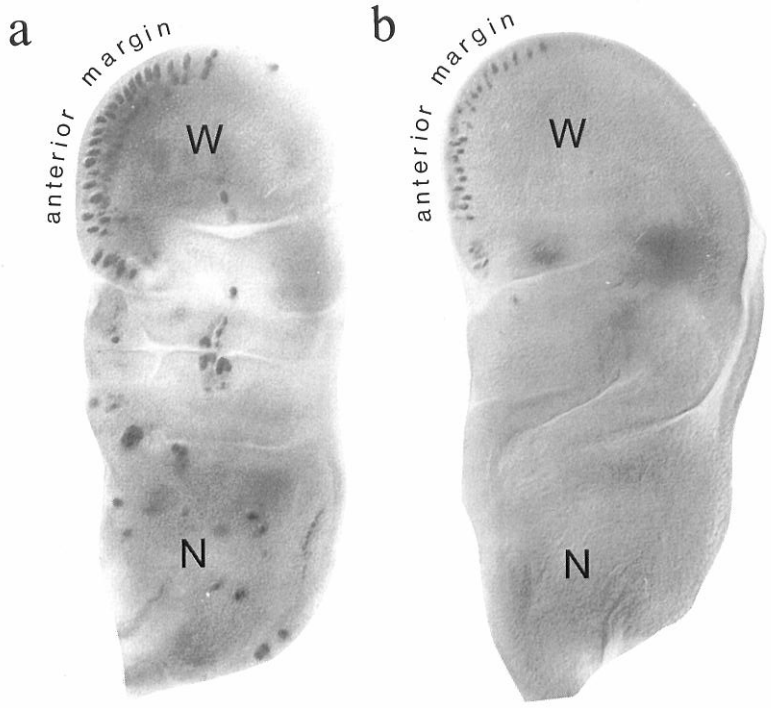

Figure 6. Correlation of Patterns of Sensory Mother Cells and of *pox neuro* Expression in the Wing Disc

(a) Pattern of SMCs in a wing disc at the time of puparium formation, as observed in the enhancer-trap line A101 stained with a monoclonal anti-β-galactosidase antibody (Huang et al., 1991). The double row of SMCs along the anterior wing margin will form two rows of polyinnervated, chemosensory bristles. All other SMCs correspond to m-es organs, either mechanosensory bristles on the notum or campaniform sensilla on the wing blade (Hartenstein and Posakony, 1989). Some of them have already undergone their first division.

(b) Pattern of *poxn* expression in a wing disc of exactly the same age, as revealed by staining with purified anti-*poxn* antibodies. N, prospective notum; W, prospective wing.

expression and those of developing p-es organs suggests that *poxn* expression is involved in the determination of p-es organs. In order to test whether *poxn* is required for the development of these organs, we examined the external sensory structures of embryos deficient for *poxn*. In homozygous *Df(2R)WMG* embryos, the p-es organs are almost always missing: the thoracic kölbchen are always absent (cf. Figures 7a and 7b), the abdominal sensillum p6 never appears, and the abdominal hair h3 is detected 4-fold less frequently than in wild-type embryos (Table 1). The missing p-es organs are often substituted by another type of sense organ, generally a large hair. This is particularly clear for the abdominal sensillum p6 (Figure 7d), where in nearly half the cases a large hair develops instead of a papilla (Figure 7e, arrowhead, and Table 1). All other sensory structures appear to be unaffected with regard to their position and morphology.

Df(2R)WMG is a large deficiency (52C4-E3) uncovering several genes, including *slit* (Rothberg et al., 1988), αGPO (Davis and MacIntyre, 1988), and *poxn*. To locate the gene

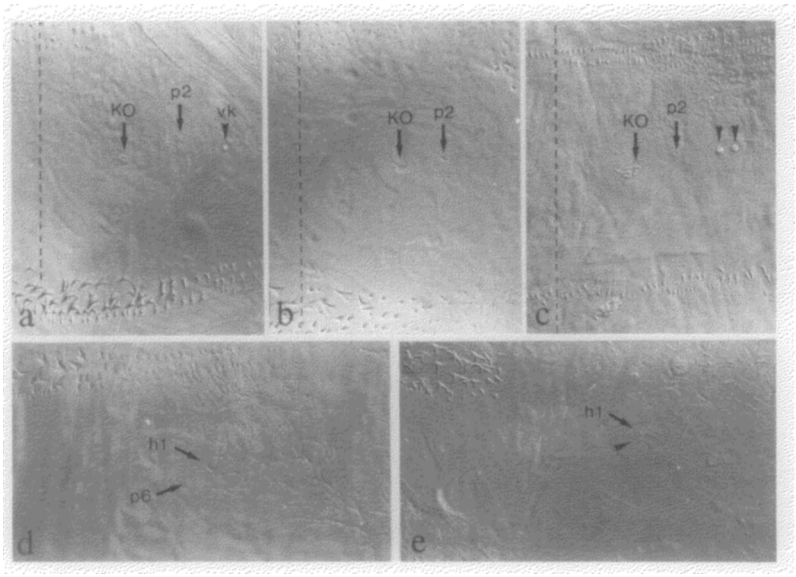

Figure 7. Dependence of p-es Organs on *pox neuro*

(a) Ventral thoracic hemisegment of a wild-type embryo showing Keilin's organ (KO), the papilla (p2), and the ventral kölbchen (vk). Keilin's organ comprises three small hairs and is innervated by five neurons, the kölbchen is innervated by three neurons, and the papilla is innervated by one neuron. The ventral midline is indicated by a broken line.

(b) The same region as in (a) is shown of an embryo deficient for *poxn* (homozygous *Df(2R)WMG*). The ventral kölbchen is missing; the other sense organs are not affected.

(c) The same region is shown of an embryo after ectopic expression of *poxn*. Two ventral kölbchen are present (arrowheads).

(d) The lateral region of an abdominal hemisegment of a wild-type embryo, showing the doubly innervated papilla p6 and the nearby monoinnervated hair h1 (arrows).

(e) The same region as in (d) is shown of an embryo deficient for *poxn* (homozygous *Df(2R)WMG*), demonstrating the substitution of p6 by a large hair (arrowhead).

Table 1. Frequency of the Presence of p-es Organs in Wild-Type Embryos and Embryos Deficient for *pox neuro* or Flanking Chromosomal Regions

	Thoracic Segments		Abdominal Segments		
Genotype	lk	vk	p6	h3	N
Wild-type	1.00	1.00	0.88	0.33	15
Df(2R)WMG	0.00	0.00*	0.00 (0.45)	0.08 (0.5)	18
Df(2R)XTE-18	1.00	1.00	0.7	0.3	10
Df(2R)KL-32	0.95	0.8	0.55	0.3 (0.2)	12

Numbers correspond to frequency at which the appropriate sense organ was detected at the correct site on the cuticle in a total of N homozygous embryos scored. Embryos homozygous for the deficiencies are lethal and, hence, can be easily distinguished from their heterozygous or wild-type sibs. In wild-type embryos, both lateral (lk) and ventral kölbchen (vk) are always visible and the p6 papilla is usually visible, but the small hair h3 is often undetectable. Numbers in parentheses indicate the frequency of substitution by a large bristle. In the case of the frequency marked by an asterisk, we have observed at a low frequency (0.2) a sensillum or a structure resembling a small hair at the position where a kölbchen should have developed. In *Df(2R)KL-32* embryos, the lateral kölbchen in T2 and T3 are found at an unusual dorsal position; the analysis of *poxn* expression patterns at earlier times reveals that this effect is due to the failure of the corresponding cell clusters to migrate ventrally (see text).

required for the formation of larval p-es organs more precisely, we analyzed the cuticle of embryos deleted for either the region to the left (*Df(2R)XTE-18*) or to the right (*Df(2R)KL-32*) of *poxn* (cf. Figures 2a and 2b), uncovering the entire proximal or distal part of *Df(2R)WMG* (Davis and MacIntyre, 1988). These embryos exhibit a complete set of normal external sense organs, in particular of p-es organs (Table 1). Since only about 17 kb remain between the distal breakpoint of *Df(2R)XTE-18* and the 3′ end of the *poxn* transcript (Figure 2b), and in view of the close correspondence between the patterns of *poxn* expression and p-es organs, these results strongly suggest that the inability to form p-es organs of embryos homozygous for *Df(2R)WMG* is caused by the absence of *poxn* itself rather than by the deletion of another gene.

Interestingly, in homozygous *Df(2R)KL-32* embryos the lateral kölbchen of T2 and T3 fail to migrate ventrally as observed in the wild type (Figures 5c and 5d). A possible explanation is that *poxn* is not fully activated in these embryos and, hence, fails to activate genes responsible for the ventral migration. Hence, *Df(2R)KL-32* might remove some cis-regulatory elements required for wild-type expression of *poxn* in the lineage of the lateral kölbchen in T2 and T3.

Ectopic Expression of *poxn* Produces Supernumerary Kölbchen

Since expression of *poxn* appears to be necessary for p-es organ formation, we investigated whether ectopic expression of *poxn* during embryogenesis may also be sufficient for p-es organ development. To this end, stably transformed flies carrying one copy of *poxn*-cDNA under the control of the hsp70 promoter were produced by P element–mediated gene transfer (Figure 2c). In several transgenic lines, the hsp70-*poxn* chromosome was homozygous lethal and could be maintained only over a balancer chromosome.

Embryos of balanced hsp70-*poxn* stocks were heat shocked for 15 min at 37°C after 6 hr of development at 25°C and then allowed to complete embryogenesis at 25°C. As Figures 8b and 8c demonstrate, these embryos formed supernumerary kölbchen in all thoracic segments (cf. also Figures 7a and 7c). Control embryos of the same stock that were not heat shocked (Figure 8a), or heat shocked embryos without the hsp70-*poxn* construct, did not show this phenotype.

The number of additional kölbchen per thoracic hemisegment depends on the time at which the heat shock is applied. Figure 9a shows the effect of a 15 min heat shock at 37°C applied between 3 and 8 hr of development at 25°C. Within this time interval, the strongest effect was observed in embryos heat shocked after 4.5 hr. At this stage, the heat shock results in the formation of an average of about 2.8 instead of the normal 2 kölbchen per thoracic hemisegment. This average has been computed on the basis of all heat shocked embryos, of which half carry one copy and a quarter each carry no or two copies of the hsp70-*poxn* construct. As illustrated in Figure 9b, some embryos exhibit more than twice the normal number of kölbchen in the thorax. In fact, Figure 9b might suggest that the average number of ectopic kölbchen observed in the heat shocked larvae forms a bimodal distribution, probably corresponding to larvae containing one and two doses of the hsp70-*poxn* construct. Our observation that a 15 min heat shock suffices in most instances to generate ectopic kölbchen could have several explanations. It might reflect that the *poxn* product is required only during a short time interval or the *poxn* mRNA or protein is rather stable. Alternatively, upon the initial activation by the heat shock–induced *poxn* protein, the endogenous *poxn* gene might be autoregulated, as has been observed for the *cut* gene (Blochlinger et al., 1991).

The supernumerary kölbchen exhibit a normal morphology. When the heat shock is applied at the beginning of the phenocritical period, only a few additional kölbchen appear, which tend to be located close to the normal kölbchen. During the period of maximal effect, ectopic kölbchen are also formed at more distant positions and even in regions normally devoid of external sense organs. In addition to the ectopic kölbchen in the thorax, supernumerary papillae as well as abnormal distributions and morphologies of the papillae (for example, elongated papillae with two or three contiguous domes instead of one) were observed in the thorax and abdomen, while hairs frequently became undetectable, or their shafts had been considerably reduced.

We have not examined whether, upon ectopic expression of *poxn*, additional h3 or p6 p-es organs are formed similarly in the abdominal segments, because these abdominal homologs of the kölbchen are much less conspicuous than their thoracic counterparts and their appearance at unexpected positions would be much more difficult to detect.

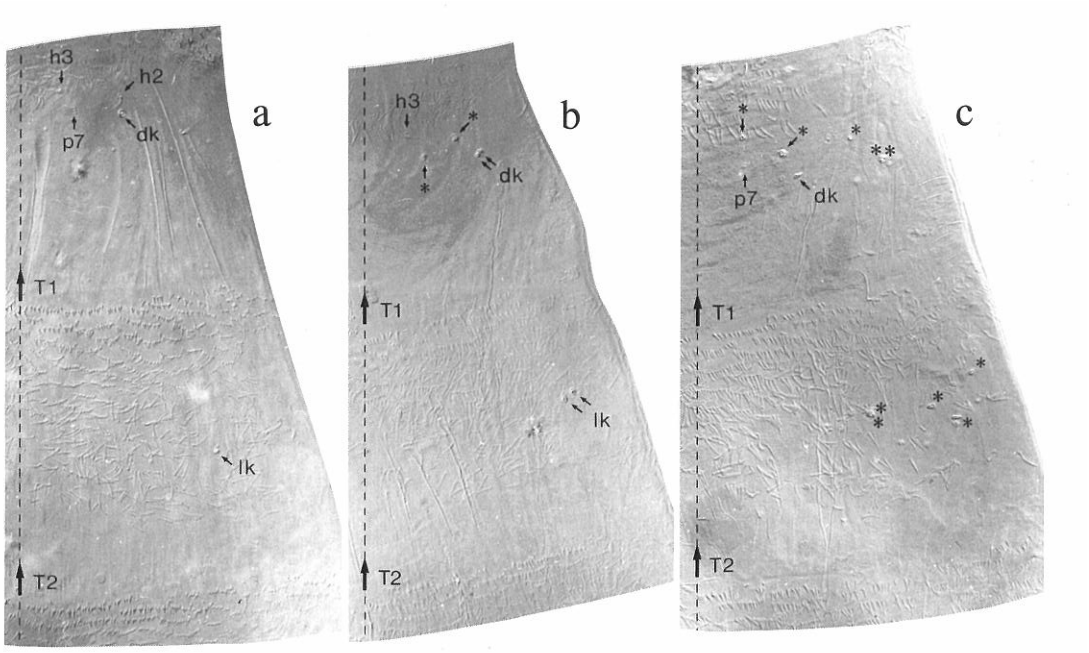

Figure 8. Induction of Supernumerary Kölbchen in the Thoracic Segments by Ectopic Expression of *pox neuro*

(a) Dorsal region of T1 and T2 of a non–heat shocked embryo carrying a *poxn* gene under the control of the *hsp70* promoter (Figure 2c). The embryo shows the normal pattern of sensory structures, including the two dorsal mono-innervated hairs h2 and h3, the dorsal-most mono-innervated papilla p7, and the poly-innervated kölbchen (dk in T1, lk in T2).

(b) The same region as in (a) of a heat shocked embryo which probably contained one copy of the hsp70-*poxn* construct. In T1, the papilla p7 and the hair h2 have been transformed into supernumerary kölbchen. In addition, the dorsal kölbchen has been duplicated both in T1 (dk) and T2 (lk). Other m-es organs have not been transformed, e.g., several papillae near the dorsal kölbchen and the hair h3 in T1, and the hairs h2 and h3 in T2.

(c) An extreme response to heat shock in an embryo which probably contained two copies of the hsp70-*poxn* construct. Several ectopic kölbchen are present in both T1 and T2. Supernumerary kölbchen are marked by asterisks; the dorsal midline is indicated by a broken line.

Discussion

Correlation of *poxn* Expression with Formation of p-es Organs

We have shown that *poxn* is expressed in those cells of the PNS that will form the thoracic kölbchen and their abdominal homologs, p6 and h3. The most obvious feature of this subset of sense organs is their innervation by two or three neurons, as opposed to virtually all other sense organs of the same segments, which are innervated by a single neuron. Although *poxn* is not expressed in Keilin's organs (KO), which are also multiply innervated, they probably constitute no exception to the correlation between *poxn* expression and the development of p-es organs. Several observations suggest that the fine structure of KOs is rather complex, a conclusion consistent with the view that these organs are the remnants of the legs of lower diptera and of insect larvae in general (Keilin, 1911, 1915). In the late embryo, the dendrites of the five KO neurons are arranged as if two of the three hairs were doubly innervated (Ghysen et al., 1986). However, at later times the appearance of the neurons shows that they innervate five distinct sense organs, in Drosophila (Tix et al., 1989) as well as in the blowfly Phormia (Lakes and Pollack, 1990). The abdominal homologs of the KO hairs are mono-innervated sensilla (Dambly-Chaudière and Ghysen, 1987), raising further doubts whether any of these hairs are poly-innervated sense organs.

In the imaginal wing disc, *poxn* is expressed exclusively in the cells that will form p-es organs. In the leg discs, the expression of *poxn* has not been studied in detail, but the overall pattern is suggestive for a specific expression in cells that will form the poly-innervated chemosensory bristles. With the possible exception of the larval Keilin's organs, it appears therefore that the expression of *poxn* is specific for p-es organs both in the larval and in the adult PNS.

poxn Is a Determinant of p-es Organs

Deletion of *poxn* by the deficiency *Df(2R)WMG* results in a complete removal of the conspicuous thoracic kölbchen and in a nearly complete loss of their abdominal homologs, p6 and h3. This effect is almost certainly due to the lack of *poxn* itself, since the loss is observed only when a region of about 30 kb, including at least 8 kb of *poxn* transcript, is deleted. The observation that the absence of a p-es organ is frequently accompanied by the appearance of an additional hair suggests that its SMC formed, yet produced another type of sense organ, and hence that *poxn* is required to specify the particular fate of forming a p-es organ.

The transformation of p-es organs into m-es organs is not always observed. This might be explained, at least in

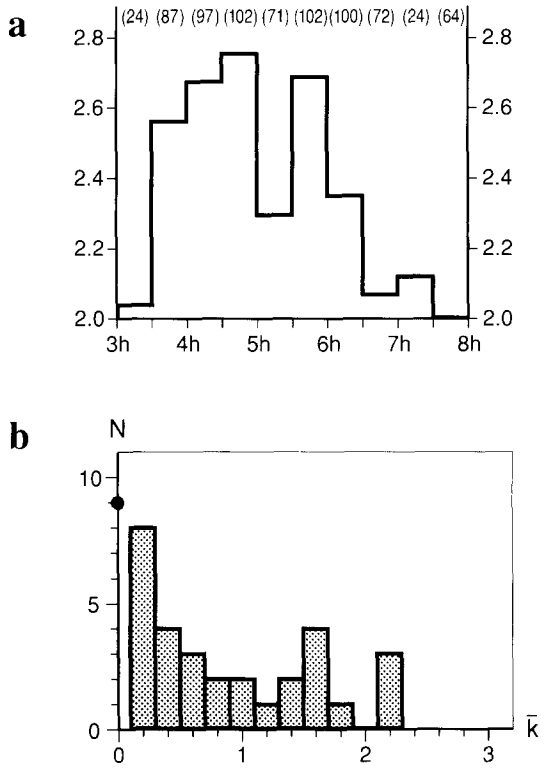

Figure 9. Effect of Ectopic *pox neuro* Expression on Number of Kölbchen

(a) The average number of kölbchen among the analyzed larval hemisegments is plotted on the ordinate as a function of the developmental time (after egg laying at 25°C) at which hsp70-*poxn* had been activated ubiquitously by a 15 min heat shock. Embryos from heterozygous transgenic hsp70-*poxn* parents were subjected to a 15 min heat shock at 37°C during the first or second half of the half-hour time intervals indicated on the abscissa, allowed to complete embryogenesis at 25°C, and examined for the presence of kölbchen. Numbers in parenthesis represent the total number of hemisegments examined in each time interval. In calculating the average number of kölbchen per hemisegment, all embryos were taken into account of which half contains one copy and one quarter each contains two or no copies of the hsp70-*poxn* construct. The data are expressed as kölbchen per hemisegment rather than per embryo, because often not all segments of an embryo can be scored properly, due to folds in the cuticle preparation.

(b) The number of larvae, N, exhibiting an average number of ectopic kölbchen per hemisegment, $\bar{k}$, is shown for the combined time intervals between 4 and 5 hr of development at 25°C when the 15 min heat shock was applied. The number of larvae with no ectopic kölbchen (9) is indicated on the ordinate by a closed circle.

part, by the extent of the rather large deficiency *Df(2R) WMG*, which prevents the embryo from completing dorsal closure and renders the observation of dorsal cuticular structures much less reliable than in normal embryos. In addition, not all m-es organs are as conspicuous as the large hair that often substitutes the doubly innervated p6 and, hence, might escape detection. Finally, it is possible that some p-es organs are transformed into internal sense organs, in which case they could not be detected in cuticle preparations.

To test whether *poxn* is not only necessary but, at least in some regions, also sufficient for p-es organ development, we examined the effect of its ectopic expression. The results show that if a thermoinducible *poxn* gene is activated ubiquitously between 3 and 7.5 hr of development at 25°C, additional kölbchen are produced in the thoracic segments. The sensitivity to ectopic *poxn* expression is highest between 4 and 6 hr of development, the period during which most, if not all, SMCs are singled out. Interestingly, there seems to be a trough in the effect of ectopic *poxn* expression at 5–5.5 hr of development, i.e., at the time when the SMCs that normally express *poxn* arise (Figure 9a). The simplest explanation for this effect is that during this time interval relatively few SMCs arise that are determined for m-es versus p-es organ formation and can be transformed into p-es SMCs by ectopic expression of *poxn*. On the basis of the classical argument that a gene is decisive for the choice of a particular developmental fate if its loss-of-function phenotype results in the loss of the particular fate, while its gain-of-function phenotype results in an excess of that fate (Lewis, 1978), we conclude that *poxn* is crucial in defining the fate of the thoracic kölbchen and their abdominal homologs.

When *poxn* is activated ectopically, supernumerary p-es organs form only at relatively few locations. Clearly, not all m-es organs are transformed into p-es organs. This observation could be explained by two different, yet not mutually exclusive, mechanisms. First, additional factors distinguishing between different m-es organs might exist, and only those SMCs of m-es organs that possess the same combination(s) of factors as SMCs of p-es organs are transformed. Second, the level of ectopic *poxn* protein produced during the 15 min heat shock might not be sufficient to transform all m-es SMCs, because these arise over a much longer time interval (Hartenstein, 1988; Bodmer et al., 1989; Ghysen and O'Kane, 1989) and/or because some of them require higher levels of *poxn* protein to generate p-es organs. Since, upon ectopic expression of *poxn*, p-es organs were also found at locations of the cuticle where no m-es organs form in wild-type embryos, it is possible that they arise from md neurons, many of which also express *cut* (Blochlinger et al., 1990), or ch organs. In addition, it is possible that cell migration is affected by the ectopic expression of *poxn*, as has been observed when ectopic activation of *cut* transformed ch organs into es organs (Blochlinger et al., 1991).

In addition to ectopic kölbchen, altered distributions and morphologies of papillae and hairs were observed upon ectopic expression of *poxn*. These observations might be interpreted to result from incomplete transformations of m-es organs to p-es organs by the ectopic *poxn* protein. In other words, in some regions at a certain developmental time, the level of ectopic *poxn* protein produced during the 15 min heat shock could be sufficient to interfere with normal m-es organ development but fail to transform m-es organs to p-es organs.

The conclusion that *poxn* determines the formation of p-es organs implies that it also specifies the pattern of cell divisions characteristic for p-es organs. The lineage of p-es organs derived from single SMCs differs from that giving rise to m-es organs by additional divisions that generate multiple neurons. Our results suggest that the difference between the two types of sense organs is already specified

at the level of the SMC. Thus, the choice between the two lineages is made at an early stage of development during the end phase of germband elongation, at the time when *poxn* expression is first observed in single cells. Whether this early choice evokes an immediate effect on the pattern of cell divisions or whether it is transmitted through the first division(s) and produces its effect later is not known, because the pattern of divisions in the two types of sense organs (Bodmer et al., 1989) has not been characterized in sufficient detail.

The expression of *poxn* in the PNS declines sharply upon completion of germband retraction. At this time, each cluster consists of 5–7 cells, which corresponds to the final number of cells expected to form the various p-es organs. No *poxn* protein is detectable in the PNS by the time the neurons begin to differentiate, which indicates that *poxn* is not directly involved in the differentiation process of p-es organs per se. This contrasts sharply with the expression of *cut*, which is maintained in es organs throughout development (Blochlinger et al., 1991).

Is *pox neuro* a Neuroblast Identity Gene?

Although we have concentrated in this study on the expression of *poxn* in the PNS, it is important to note that *poxn* is also expressed in the CNS, in a segmentally repeated subset of neuroblasts and probably its progeny (Figure 5). We suggest that *poxn* participates in the determination of the cell lineage of a subset of neuronal precursor cells both in the PNS and in the CNS.

It has been proposed that in the CNS, neuronal identity is determined by the expression of "neuroblast identity" genes in overlapping subsets of neuroblasts. By the expression of a specific combination of such genes, a neuroblast regulates the fate of its progeny and produces a unique cell lineage (Doe, 1991; Doe et al., 1991). We suggest that in the PNS, neuronal identity is determined similarly by combinations of genes expressed in subsets of SMCs, and that *poxn* is one such gene.

A previous example of a gene controlling the identity of SMCs is the homeobox gene *cut* (Bodmer et al., 1987; Blochlinger et al., 1988, 1990, 1991). While the es organ cell identities depend on *cut* gene activity and, in its absence, are transformed into those of chordotonal organs (Bodmer et al., 1987), we have shown here that specification of p-es, but not of m-es, organ cell identities requires the additional expression of *poxn*. Thus, the formation of p-es organs is specified by the combination of *cut* and *poxn* activities, m-es organs are generated when only *cut* is expressed, and chordotonal organs form in the absence of both *cut* and *poxn* expression.

Both *cut* (Blochlinger et al., 1988, 1990) and *poxn* are expressed in subsets of neuroblasts and SMCs. Since *cut* (Blochlinger et al., 1990) and *poxn* are both activated in p-es SMCs, it is possible that the lineages of es organs are specified, in general, at the level of SMCs, much as, in the CNS, the lineages appear to be specified at the level of individual neuroblasts (Doe, 1991).

It is not known whether different neuroblast identity genes act on each other's expression, nor whether their combinatorial specification of cell lineages is hierarchical. The relative simplicity of the PNS might render these questions more easily amenable to analysis.

Role of *poxn* in Light of the Gene Network Concept

It has been proposed that genes that implement an integrated function of the genetic program represented by a functional gene network share a relatively small number of different classes of homologous domains (Frigerio et al., 1986; Bopp et al., 1986). Since *poxn* shares a paired domain with the segmentation genes *prd* and *gsb* (Bopp et al., 1989), this hypothesis predicts that *poxn* belongs to the same network of regulatory genes as *prd* and *gsb* that specify position along the anteroposterior axis during embryogenesis. We have demonstrated here that *poxn* indeed is a member of this network by specifying, in combination with products of other genes belonging to this network (as, for example, the homeobox gene *cut*), the positions of single cells, the SMCs generating p-es organs. As shown previously, the specific expression of *poxn* depends on the activity of other members of the same network, such as on that of *prd* (Bopp et al., 1989).

Eight paired domain genes have been isolated in the mouse, *Pax 1–Pax 8*. We have previously noted a close correspondence of tissue specificity between the most closely related paired domain genes of mouse and Drosophila. Thus, the *undulated* gene (*Pax 1*) of the mouse (Deutsch et al., 1988) and the Drosophila gene *pox meso* (Bopp et al., 1989) are expressed in segmentally repeated patterns of the developing mesoderm. On the other hand, the paired domain of *poxn* is most closely related to that of *Pax 2* and *Pax 8* (Dressler et al., 1990; Plachov et al., 1990), which are both expressed in the developing nervous system. Particularly, *Pax 2* is expressed in two developing external sense organs, the ear and the eye (Nornes et al., 1990). Thus, our findings are consistent with another prediction of the gene network hypothesis, namely that the same sets of conserved domains define analogous gene networks in different organisms (Frigerio et al., 1986; Bopp et al., 1986; Burri et al., 1989).

Experimental Procedures

General Procedures

Standard procedures (Maniatis et al., 1982) such as the construction (Frischauf et al., 1983) and screening of a genomic library, nick-translation (Rigby et al., 1977), whole genome Southern analysis (Southern, 1975), Northern blot analysis, chromosomal walking (Bender et al., 1983), in situ hybridization to salivary gland chromosomes (Langer-Safer et al., 1982), or the isolation of poly(A)$^+$ RNA were carried out essentially as described (Frei et al., 1985; Kilchherr et al., 1986).

Isolation of cDNA Clones

An amplified cDNA library, constructed in λ gt10 of poly(A)$^+$ RNA from 3–12-hr-old embryos (Poole et al., 1985), was kindly provided by T. Kornberg. Another oligo(dT)-primed-cDNA library was constructed in λ ZAPII of poly(A)$^+$ RNA from 8–12-hr-old embryos with the use of a ZAP-cDNA synthesis kit from Stratagene. Three *pox neuro*-cDNAs (cPn1, cPn2, and cPn5) were isolated from the λ ZAPII library and one (P4c6) from the λ gt10 library according to standard procedures (Maniatis et al., 1982).

DNA Sequencing

All DNA sequences were analyzed on both strands. The P4c6-cDNA

was sequenced along its entire length, while only the 5′ ends of the cDNAs cPn1, cPn2, and cPn5 were sequenced. The DNAs were sequenced by the dideoxynucleotide method of Sanger et al. (1977), using single-stranded DNA in M13 (Bopp et al., 1989) or Bluescript vectors (Stratagene).

Preparation of Antibodies against *pox neuro* Antigen and Immunocytochemical Staining of Embryos and Imaginal Discs

A rabbit antiserum was raised against a T7-*pox neuro* fusion protein, purified, and used for immunocytochemical staining of embryos as described (Bopp et al., 1989). Immunostaining of imaginal discs was performed as described by Huang et al. (1991). As primary antibody, either anti-*poxn* antiserum (diluted 1:250) or, in the case of β-galactosidase detection in imaginal discs of the enhancer trap line A101, monoclonal anti-β-galactosidase (Promega; diluted 1:800) was used.

Construction of hsp70-*poxn* Plasmid and Germline Transformation

For the assembly of the hsp70-*poxn* construct, P4c6-cDNA was used. As mentioned in the legend to Figure 3, this cDNA is missing a nucleotide in the fifth codon (numbering starts with the first ATG of the long open reading frame) at the splice junction of the second and third exons. To reintroduce the missing base pair and to provide a convenient restriction site upstream of the first codon, the 0.5 kb EcoRI–SalI fragment at the 5′ end of P4c6 was subcloned into pBluescriptSK$^+$. Using the M13 universal primer and the oligonucleotide 5′-ATGGTACCATTTCAGCCATGCCGCACACAGGTCAAGCTGGAG-3′ (the Asp-718 site, the initiation codon, and the reintroduced G are underlined), a 0.18 kb fragment was amplified by the polymerase chain reaction. The product was digested with Asp-718 and SalI and cloned in front of the 1.3 kb SalI–ScaI P4c6-cDNA fragment (terminating at nucleotides 4570 and 8051 in Figure 3) that had been inserted into pBluescript cleaved by SalI and EcoRV. To obtain the final hsp70-poxn construct in a P element vector (Figure 2c), the corrected and trimmed cDNA of *poxn* was inserted as an Asp-718–EcoRI fragment into pKB255 (K. B. and E. H., unpublished data), a derivative of the P element transformation vector pW8 (Klemenz et al., 1987) which contains the 1.2 kb *sevenless* enhancer fragment (Basler et al., 1989), the *hsp70* heat shock promoter (an XbaI–KpnI fragment consisting of the first 95 bp of the *hsp70* leader and 250 bp upstream sequences; Schneuwly et al., 1987), and a 0.8 kb genomic tubulin fragment serving as transcriptional terminator (kindly provided by G. Struhl; see Lawrence et al., 1987).

DNA of the hsp70-*poxn*–P element construct was injected, together with the helper plasmid pUChsπΔ2–3 (donated by D. Rio), into w^{1118} embryos as described (Rubin and Spradling, 1982). Five independent transformants were obtained, all of which exhibit a dominant rough eye phenotype. This is probably due to the expression of *poxn* in the *sevenless*-expressing subpopulation of ommatidial precursor cells (K. B. and E. H., unpublished data).

Induction of hsp70-*poxn* Expression

Staging of the embryos according to the current scheme (Campos-Ortega and Hartenstein, 1985; Wieschaus and Nüsslein-Volhard, 1986) is of little use in our case, since the entire process, from the appearance of the first SMCs to the production of most or all of their progeny, occurs only during two stages (11 and 12). Hence, we staged each embryo individually as follows. Egg laying was allowed for 1 hr, and the plate with the eggs was left at 25°C for two additional hours. The embryos were then dechorionated in bleach and observed under a dissecting microscope. The staging was based on the completion of the ventral and cephalic furrow, which give rise to a cross-shaped fold when the embryos are viewed ventrally. This stage is easily recognized, lasts only for 1 or 2 min, and occurs at about 3 hr of development at 25°C. The collection of embryos was examined every 5 min. All the embryos which had reached the "crossfold" stage during the preceding 5 min interval were taken to be exactly 3 hr old, transferred to a basket, and incubated in a 25°C water bath (floating poly-basket plates were prepared by removing the bottoms of 24-well culture plates with a razor blade and replacing them with a stainless steel screen, sealed with the plastic wells by heat). When the embryos had reached the appropriate age, the baskets were transferred to a 37°C water bath for 15 min, returned to the 25°C bath, transferred to a petri dish containing fly food, and incubated for 20 hr at 25°C before observation. Embryos that ectopically express *poxn* under the conditions described die as larvae, probably because of severe disorders in their PNS as well as their CNS.

Observation of External Sensory Structures

Late embryos were prepared as described by Dambly-Chaudière and Ghysen (1986) and observed with a Nikon microscope equipped with Nomarski optics.

Fly Strains and Culturing

The following fly stocks were used: *Df(2R)WMG*, *Df(2R)XTE-18*, *Df(2R)KL-32* (Davis and MacIntyre, 1988), P[*lac*, *ry*$^+$]A101 (Bellen et al., 1989), P[*lac*, *ry*$^+$]A37 (Ghysen and O'Kane, 1989), and w^{1118}. Flies were maintained at 18–25°C on a cornmeal, sucrose, dried yeast, and agar medium supplemented with Nipagin and seeded with a suspension of live yeast.

Acknowledgments

We thank R. MacIntyre, M. B. Davis, and W. Gelbart for mutant stocks, H. Bellen for the A101 enhancer trap line, T. Kornberg for a cDNA library, W. Studier for the pAR expression vectors, and H. Noll for comments on the manuscript. We are also grateful to Fritz Ochsenbein for the artwork. This work has been supported by a grant of the Human Frontier Science Program Organization (to C. D.-C. and A. G.), by the Swiss National Science Foundation grants 3.348–0.86, 31–26652.89 (to M. N.), and 31–27709.89 (to E. H.), by both the CNRS and an EMBO long-term fellowship (to E. J.), by a fellowship of the Geigy-Jubiläums-Stiftung (to D. B.), and by the Kantons Zürich and Basel. A. G. is chercheur qualifié of the Fonds National de la Recherche Scientifique (Belgium).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 USC Section 1734 solely to indicate this fact.

Received September 10, 1991; revised January 21, 1992.

References

Basler, K., Siegrist, P., and Hafen, E. (1989). The spatial and temporal expression pattern of *sevenless* is exclusively controlled by gene-internal elements. EMBO J. *8*, 2381–2386.

Bate, C. M. (1978). Development of sensory systems in arthropods. In Handbook of Sensory Physiology, Vol. IX, M. Jacobson, ed. (New York: Springer-Verlag), pp. 1–53.

Baumgartner, S., Bopp, D., Burri, M., and Noll, M. (1987). Structure of two genes at the *gooseberry* locus related to the *paired* gene and their spatial expression during *Drosophila* embryogenesis. Genes Dev. *1*, 1247–1267.

Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Pearson, R. K., and Gehring, W. J. (1989). P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. Genes Dev. *3*, 1288–1300.

Bender, W., Spierer, P., and Hogness, D. S. (1983). Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the bithorax complex in *Drosophila melanogaster*. J. Mol. Biol. *168*, 17–33.

Blochlinger, K., Bodmer, R., Jack, J., Jan, L. Y., and Jan, Y. N. (1988). Primary structure and expression of a product from *cut*, a locus involved in specifying sensory organ identity in *Drosophila*. Nature *333*, 629–635.

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

Blochlinger, K., Jan, L. Y., and Jan, Y. N. (1991). Transformation of sensory organ identity by ectopic expression of *cut* in *Drosophila*. Genes Dev. *5*, 1124–1135.

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

Bodmer, R., Barbel, S., Shepherd, S., Jack, J. W., Jan, L. Y., and Jan, Y. N. (1987). Transformation of sensory organs by mutations of the *cut* locus of D. melanogaster. Cell *51*, 293–307.

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

Bopp, D., Burri, M., Baumgartner, S., Frigerio, G., and Noll, M. (1986). Conservation of a large protein domain in the segmentation gene *paired* and in functionally related genes of Drosophila. Cell *47*, 1033–1040.

Bopp, D., Jamet, E., Baumgartner, S., Burri, M., and Noll, M. (1989). Isolation of two tissue-specific *Drosophila* paired box genes, *pox meso* and *pox neuro*. EMBO J. *8*, 3447–3457.

Burri, M., Tromvoukis, Y., Bopp, D., Frigerio, G., and Noll, M. (1989). Conservation of the paired domain in metazoans and its structure in three isolated human genes. EMBO J. *8*, 1183–1190.

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

Caudy, M., Grell, E. H., Dambly-Chaudière, C., Ghysen, A., Jan, L. Y., and Jan, Y. N. (1988). The maternal sex determination gene *daughterless* has zygotic activity necessary for the formation of peripheral neurons in *Drosophila*. Genes Dev. *2*, 843–852.

Cubas, P., de Celis, J.-F., Campuzano, S., and Modolell, J. (1991). Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. Genes Dev. *5*, 996–1008.

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

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

Davis, M. B., and MacIntyre, R. J. (1988). A genetic analysis of the α-glycerophosphate oxidase locus in *Drosophila melanogaster*. Genetics *120*, 755–766.

Deutsch, U., Dressler, G. R., and Gruss, P. (1988). *Pax 1*, a member of a paired box homologous murine gene family, is expressed in segmented structures during development. Cell *53*, 617–625.

Doe, C. Q. (1991). The generation of neuronal diversity in the *Drosophila* central nervous system. In Determinants of Neuronal Identity, M. Shankland and E. Macagno, eds. (New York: Academic Press), in press.

Doe, C. Q., Chu-LaGraff, Q., Wright, D. M., and Scott, M. P. (1991). The *prospero* gene specifies cell fates in the Drosophila central nervous system. Cell *65*, 451–464.

Dressler, G. R., Deutsch, U., Chowdhury, K., Nornes, H. O., and Gruss, P. (1990). *Pax 2*, a new murine paired-box-containing gene and its expression in the developing excretory system. Development *109*, 787–795.

Frei, E., Baumgartner, S., Edström, J.-E., and Noll, M. (1985). Cloning of the *extra sex combs* gene of *Drosophila* and its identification by P-element-mediated gene transfer. EMBO J. *4*, 979–987.

Frigerio, G., Burri, M., Bopp, D., Baumgartner, S., and Noll, M. (1986). Structure of the segmentation gene *paired* and the Drosophila PRD gene set as part of a gene network. Cell *47*, 735–746.

Frischauf, A.-M., Lehrach, H., Poustka, A., and Murray, N. (1983). Lambda replacement vector carrying polylinker sequences. J. Mol. Biol. *170*, 827–842.

Ghysen, A., and Dambly-Chaudière, C. (1988). From DNA to form: the *achaete-scute* complex. Genes Dev. *2*, 495–501.

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

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

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

Hartenstein, V. (1988). Development of *Drosophila* larval sensory organs: spatiotemporal pattern of sensory neurones, peripheral axonal pathways and sensilla differentiation. Development *102*, 869–886.

Hartenstein, V., and Posakony, J. W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development *107*, 389–405.

Hertweck, H. (1931). Anatomie und Variabilität des Nervensystems und der Sinnesorgane von *Drosophila melanogaster* (Meigen). Z. wiss. Zool. *139*, 559–663.

Huang, F., Dambly-Chaudière, C., and Ghysen, A. (1991). The emergence of sense organs in the wing disc of *Drosophila*. Development *111*, 1087–1095.

Hultmark, D., Klemenz, R., and Gehring, W. J. (1986). Translational and transcriptional control elements in the untranslated leader of the heat-shock gene *hsp22*. Cell *44*, 429–438.

Jan, Y. N., and Jan, L. Y. (1990). Genes required for specifying cell fates in *Drosophila* embryonic sensory nervous system. Trends. Neurosci. *13*, 493–498.

Kankel, D. R., Ferrús, A., Garen, S. H., Harte, P. J., and Lewis, P. E. (1980). The structure and development of the nervous system. In The Genetics and Biology of *Drosophila*, Vol. 2d, M. Ashburner and T. R. F. Wright, eds. (New York: Academic Press), pp. 295–368.

Keilin, D. (1911). Sur certains organes sensitifs constants chez les larves de Diptères et leur signification probable. C. R. Acad. Sci. Paris *153*, 977–979.

Keilin, D. (1915). Recherche sur les larves des Diptères cyclorhaphes. Bull. Scient. France et Belgique *49*, 15–198.

Kilchherr, F., Baumgartner, S., Bopp, D., Frei, E., and Noll, M. (1986). Isolation of the *paired* gene of *Drosophila* and its spatial expression during early embryogenesis. Nature *321*, 493–499.

Klemenz, R., Weber, U., and Gehring, W. J. (1987). The *white* gene as a marker in a new P-element vector for gene transfer in *Drosophila*. Nucl. Acids Res. *15*, 3947–3959.

Lakes, R., and Pollack, G. S. (1990). The development of the sensory organs of the legs in the blowfly, *Phormia regina*. Cell Tissue Res. *259*, 93–104.

Langer-Safer, P. R., Levine, M., and Ward, D. C. (1982). Immunological method for mapping genes on *Drosophila* polytene chromosomes. Proc. Natl. Acad. Sci. USA *79*, 4381–4385.

Lawrence, P. A., Johnston, P., Macdonald, P., and Struhl, G. (1987). Borders of parasegments in *Drosophila* embryos are delimited by the *fushi tarazu* and *even-skipped* genes. Nature *328*, 440–442.

Lewis, E. (1978). A gene complex controlling segmentation in *Drosophila*. Nature *276*, 565–570.

Ma, J., and Ptashne, M. (1987). A new class of yeast transcriptional activators. Cell *51*, 113–119.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Nornes, H. O., Dressler, G. R., Knapik, E. W., Deutsch, U., and Gruss, P. (1990). Spatially and temporally restricted expression of *Pax 2* during murine neurogenesis. Development *109*, 797–809.

Plachov, D., Chowdhury, K., Walther, C., Simon, D., Guenet, J.-L., and Gruss, P. (1990). *Pax 8*, a murine paired box gene expressed in the developing excretory system and thyroid gland. Development *110*, 643–651.

Poole, S. J., Kauvar, L. M., Drees, B., and Kornberg, T. (1985). The *engrailed* locus of Drosophila: structural analysis of an embryonic transcript. Cell *40*, 37–43.

Rigby, P. W. J., Dieckman, M., Rhodes, C., and Berg, P. (1977). Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. *113*, 237–251.

Rothberg, J. M., Hartley, D. A., Walther, Z., and Artavanis-Tsakonas, S. (1988). *slit*: an EGF-homologous locus of D. melanogaster involved in the development of the embryonic central nervous system. Cell *55*, 1047–1059.

Rubin, G. M., and Spradling, A. C. (1982). Genetic transformation of *Drosophila* with transposable element vectors. Science *218*, 348–353.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing

with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Schneuwly, S., Klemenz, R., and Gehring, W. J. (1987). Redesigning the body plan of *Drosophila* by ectopic expression of the homoeotic gene *Antennapedia*. Nature *325*, 816–818.

Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. *98*, 503–517.

Tix, S., Bate, M., and Technau, G. (1989). Pre-existing neuronal pathways in the leg imaginal discs of *Drosophila*. Development *107*, 855–862.

Treisman, J., Harris, E., and Desplan, C. (1991). The paired box encodes a second DNA-binding domain in the *paired* homeo domain protein. Genes Dev. *5*, 594–604.

Wieschaus, E., and Nüsslein-Volhard, C. (1986). Looking at embryos. In *Drosophila*, a Practical Approach, D. B. Roberts, ed. (Oxford, England: IRL Press), pp. 199–227.

GenBank Accession Number

The accession number for the sequence reported in this article is M86927.