Subtype determination of *Drosophila* embryonic external sensory organs by redundant homeo box genes *BarH1* and *BarH2*

Shin-ichi Higashijima, Tatsuo Michiue, Yasufumi Emori, and Kaoru Saigo1

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

*BarH1* and *BarH2* are two closely related homeo box genes that form a small complex at the *Bar* locus on the X chromosome of *Drosophila*. By immunostaining, we showed that *BarH1* and *BarH2* proteins are coexpressed in cells belonging to the central and peripheral nervous systems in embryos. In external sensory (es) organs, their expression was particularly apparent in thecogens (glial cells) and neurons at late development. Although deletion of *BarH2* caused no appreciable morphological change in es organs, the simultaneous deletion of *BarH1* and *BarH2* led to a homeotic change in these organs with consequent conversion from campaniform-like sensilla to trichoid sensilla. In contrast, the overexpression of either *BarH1* or *BarH2* resulted in opposite morphological change. It would thus follow that *BarH1* and *BarH2* are a pair of redundant homeo box genes required for the subtype specification of es organs.

[Key Words: *Drosophila melanogaster*; sensory organ; *Bar* homeo box genes; redundant genes; nervous system]

Received February 6, 1992; revised version accepted March 16, 1992.

The peripheral nervous system (PNS) of the *Drosophila* embryo consists of various sensory organs having precise modes of arrangement (Campos-Ortega and Hartenstein 1985; Ghysen et al. 1986; Bodmer and Jan 1987). In the thoracic and abdominal segments, locations of virtually all PNS neurons have been determined, and the relationship between larval sensory organs and embryonic PNS cells has been clarified (Campos-Ortega and Hartenstein 1985; Dambly-Chaudiere and Ghysen 1986). Sensory organs are of two major groups: external sensory (es) organs, which may function as mechanosensors or chemosensors, and internal chordotonal (ch) organs, which function as possible stretch receptors (McIver 1985; Zacharuk 1985; Hartenstein 1988). Neurons of either es or ch organs have single dendrites, whereas the third class of neurons in the PNS have multiple dendrites (md) and are not associated with definite sensory structures (Ghysen et al. 1986; Bodmer and Jan 1987). es organs have several subtypes, among which the campaniform-like sensillum, with a papilla, and trichoid sensillum, with a long hair surrounded by a socket, are two major groups in thoracic and abdominal segments (Dambly-Chaudiere and Ghysen 1986; Hartenstein 1988). Irrespective of differences in morphology, they are highly homologous to each other in various respects (Ghysen et al. 1986; Bodmer et al. 1989; Blochlinger et al. 1990).

Genetic analysis of PNS formation has led to the identification of several relevant genes. The *cut* gene encodes a homeo domain protein, probably acting as a homeotic selector for es-ch determination (Bodmer et al. 1987; Blochlinger et al. 1988, 1991), whereas the absence of the *numb* gene product with zinc fingers results in conversion of the neuron and thecogen [glial cell] to outer support cells (Uemura et al. 1989). The *prospero* protein, which is essential for the axonal outgrowth and path-finding of the PNS and central nervous systems (CNS), has a putative DNA-binding domain similar in sequence to the DNA-recognition helix 3 of the homeo domain (Doe et al. 1991; Vaessin et al. 1991; Matsuzaki et al. 1992). However, no gene has been found related to the subtype determination of es organs.

*BarH1* and *BarH2* are two closely related homeo box genes that form a small complex at the *Bar* locus on the X chromosome of *Drosophila* (Higashijima et al. 1992). Their overexpression at the late third instar causes reduced eye morphology similar to that noted in the *B* mutation (Kojima et al. 1991; unpubl.), in which *BarH1* is duplicated (Sturtevant 1925). *BarH1* and *BarH2* proteins are not only coexpressed but also functionally required in two photoreceptors, R1 and R6, along with primary pigment cells (Higashijima et al. 1992). In addition to their roles in normal eye development, both genes are known to be extensively expressed during embryogenesis (Kojima et al. 1991; Higashijima et al. 1992).
In this study the expression and functions of these Bar homeo box genes in the embryo were examined. BarH1 and BarH2 were coexpressed mainly in a subset of neurons and thecogens in the PNS and the CNS. In the PNS, their expression was particularly prominent in es organs. The simultaneous deletion of BarH1 and BarH2 resulted in homeotic changes in es organs, that is, the conversion from campaniform-like sensilla to trichoid sensilla, although the deletion of BarH2 caused no appreciable change in es organs. In contrast, their overexpression had the opposite result. Thus, it is suggested that BarH1 and BarH2 are paired, redundant homeo box genes, acting as homeotic selectors for the subtype specification of es organs.

Results

Coexpression of BarH1 and BarH2 proteins in a limited number of embryonic cells

BarH1 and BarH2, sibling homeo box genes in the Bar region, are mainly expressed during embryogenesis and metamorphosis (Kojima et al. 1991; Higashijima et al. 1992). To examine their temporal and spatial expression patterns in embryos, polyclonal antibodies were generated. Antibody S12 raised against BarH1 protein could recognize BarH1 and BarH2 proteins [Fig. 1A, lanes 1,2], whereas the Y2 antibody raised against the BarH2 protein was BarH2-specific [lanes 5,6]. The BarH1-specific antibody S1-1 {lanes 3,4) was generated by absorption of the S12 antibody with extracts of Escherichia coli cells producing the BarH2 protein.

Figure 1, B-G, shows immunostaining patterns of wild-type embryos at 12-14 hr of development. Staining with the three antibodies was virtually the same in all cases. Segmentally repeated, isolated signals, intersegmental stripes of the dorsal region, and intensively stained clusters in the anterior one-third could always be recognized. Note that the BarH2-embryo [see below] can be stained by the S1-1 antibody [Fig. 1I] but cannot be stained by the Y2 antibody at all [Fig. 1J]. Close examination indicated that segmental patterns were classifiable into four types, representing T1-T3, A1-A7, and A8-telson. No significant differences in signal positions and intensity in the staining patterns with the three antibodies could be found in the case of the segmental patterns [E-G for lateral signals in an abdominal segment].

Anterior signal-clusters, located at various focal planes in Figure 1, were found to be near the surface of embryos at earlier stages [Fig. 2]. During late embryogenesis, anterior signals change their locations considerably owing to head involution (Campos-Ortega and Hartenstein 1985). BarH1 and/or BarH2 proteins began to express in anterior segments, labium, maxilla, and procephalic lobe at 5.5-6.5 hr of development [Fig. 2A]. In 6.5- to 7.5-hr embryos, S12-antibody staining reached its maximum level and other stained regions could be seen in mandible, clypeolabrum, hypopharynx, and procephalic lobe [Fig. 2B]. The staining patterns by S1-1 and Y2 antibodies were quite the same [data not shown]. BarH1 and BarH2 would thus appear to be coexpressed in a particular set of
cells even in anterior signal clusters. Because of this strict coexpression, only staining patterns produced by the S12 antibody appear in the following sections. The results in most cases could be confirmed by staining with S1-1 and Y2.

Expression of BarH1/BarH2 proteins in the CNS and PNS

Except for stripes of the dorsal region, BarH1/BarH2-positive signals appeared distributed in parallel with the...
Higashijima et al.

CNS and PNS. To find a possible relation between BarH1/BarH2 expression and nervous systems, 12- to 14-hr embryos were examined by double staining with the S12 antibody and the neuron-specific monoclonal antibody, mAb22C10 (Fujita et al. 1982; Zipursky et al. 1984), which stains both the cytoplasm and membrane so that axons and dendrites can be traced easily (Fig. 3B, F). In thoracic and abdominal repeats, strong and weak BarH1/BarH2-positive signals had a tendency to make pairs (see Fig. 3C, G); but owing to technical difficulties, only stronger signals could be reproducibly detected as black dots upon double staining (Fig. 3B, F).

Figure 3. BarH1/BarH2 expression in the PNS of 12- to 14-hr embryos. Anterior is left; dorsal is up. (A–D) Thoracic segments T2 and T3. (E–H) Abdominal segments A1 and A2. Locations of es neurons were determined based on the results of Dambly-Chaudiere and Ghysen (1986) and Blochlinger et al. (1990). (B, F) Double staining with S12 (black signal) and mAb22C10 (brown signal) antibodies. Explanations are shown schematically in A and E. (Red cells) es neurons; (blue circles) nuclei of the putative es thecogens; (green circles) nuclei of the putative outer support cells that exceptionally express BarH1/BarH2 proteins; (purple circles) nuclei of es neurons strongly stained with the S12 antibody. Location and number of thecogens are unclear in the case of the Keilin’s organ (KO). (Arrows a and b) Nuclei of a putative lesC thecogen and lesB neuron, respectively; (arrow c) one of the nuclei of outer support cells of the les3 organ. (C, D, G, H) S12 staining patterns of es neurons and thecogens. A part of C was replaced by the counterpart of another embryo for clarity. (D, H) Signal intensities in nuclei of es neurons (red circles), along with structural features of corresponding sensillum. (W, V) Very weak; (W) weak; (M) moderate. Blue circles correspond to nuclei of thecogens, in which BarH1 and BarH2 proteins are strongly expressed. “Complex” es organs (les3, v’es3, and KO in the thoracic segment, and des2 and v’es2 in the abdominal segment) were not analyzed. Abdominal desB and thoracic v’esA/v’esB could not be analyzed because of their close proximity.

Figure 4, H and I, respectively, shows patterns of some anterior clusters and those near the posterior end. The most prominent BarH1/BarH2-positive cluster in the anterior region corresponded to the antennomaxillary complex, the largest sensory organ in the embryo and larva, where nearly all es neurons and their support cells apparently expressed BarH1/BarH2 proteins. Although no detailed analysis was conducted, other sensory organs from labium, hypopharynx, and clypeolabrum appeared to be positively stained with the anti-BarH1/BarH2 antibody. All complex es organs situated at the posterior terminal appeared to include BarH1/BarH2-positive, es-related cells (Fig. 4I).

Figure 4H also shows that the brain (supraesophageal ganglion) contained a few BarH1/BarH2-positive clusters, most likely derivatives of BarH1/BarH2-positive clusters located medially in the procephalic lobe (thick arrow in Fig. 2D). The expression of BarH1 and BarH2 proteins was also observed in the ventral ganglion, where BarH1/BarH2-positive signals formed segmentally repeated patterns as in the case of PNS (Fig. 4F). In addition to thoracic and abdominal segments, three gnathal segments, C1–C3, were BarH1/BarH2-positive as evident from Figure 2C. Although a detailed analysis was not carried out, locations of most BarH1/BarH2 protein-expressing cells, which did not express the engrailed gene (Fig. 4G), were restricted to the ventral surface of the ganglion.

Cells corresponding to dorsal, intersegmental stripes [Figs. 1 and 2] have yet to be shown on any embryonic fate map. However, the coincidence of their locations with those of dorsal cuticle hairs in the anterior and/or posterior regions of each segment in larva would suggest that cells forming these 11 stripes may correspond to the primordia of two of the three types of dorsal hairs (Campos-Ortega and Hartenstein 1985).

In summary, except for intersegmental, dorsal epidermis cells, only es neurons and a fraction of their support cells in PNS along with some CNS cells produce BarH1 and BarH2 proteins in the embryo.

Expression of BarH1/BarH2 mRNA

Whole-mount in situ hybridization was carried out using BarH1- and BarH2-specific antisense RNA probes. The
Subtype determination of ES organs

Figure 3. (See facing page for legend.)
expression of mRNA was found to be virtually the same as that of proteins, thus confirming the nervous system-specific expression of BarH1 [Fig. 1H] and BarH2 (data not shown).

Requirement of BarH1/BarH2 proteins for es organ development

BarH1 and BarH2 were found to be quite similar in structure (Higashijima et al. 1992) and expression (Fig. 1), and thus could reasonably be expected to be a pair of redundant homeo box genes. The inactivation of either may cause no distinctive change in phenotype, whereas significant phenotypic changes may be induced by their simultaneous deficiency. An attempt was thus made to determine the possible roles of BarH1/BarH2 proteins in es organ development using a deficiency chromosome Df(1)B263-2° (Sutton 1943), which lacked a 210-kb-long region that included both BarH1 and BarH2 [Fig. 5A; Higashijima et al. 1992]. Df(1)B263-2° is not embryonic lethal, but the individual hemizygous for this deficiency dies in an early larval stage (see Materials and methods). It is thus possible to isolate and examine Bar− first-instar larvae using yellow marker (for details, see Materials and methods). The PNS of the mutant embryo at 12–14 hr of development was initially examined using several markers. No defects could be detected when visualized by mAb22C10 (Fig. 6A–C) or anti-HRP (Jan and Jan 1982), another neuron-specific antibody [data not shown]. Axon projections and dendrite innervations thus cannot be considered abnormal. Outer support cells of the antenna part of the antennomaxillary complex, a typical dome structure was deformed and had a single dome with two humps or a pair of small domes (arrowheads labeled An in Fig. 6D,E). In the maxillary part of the same complex, two large sensory clusters were seen situated apart from each other (arrowheads labeled Mx in Fig. 6D,E). One of three hairs, found in a wild-type Keilin’s organ (Fig. 6F), disappeared completely, and another was extensively reduced in size (Fig. 6G). The shape of basiconical sensilla, the second class of complex es organs in thoracic segments, was changed from a dome-like to a trichoid-like structure [Fig. 6H,I].

Two types of simple es organs are distributed in thoracic and abdominal segments in wild-type larvae [Dambly-Chaudiere and Ghysen 1986; Bodmer and Jan 1987; Hartenstein 1988]. Some simple es organs showed affected morphology in Df(1)B263-2°. The wild-type embryo has two bristles and two papillae on the lateral region of each abdominal segment [A1–A7] [Fig. 6L]. The former correspond to trichoid sensilla innervated by lesA and lesC neurons, the latter campaniform-like sensilla are innervated by lesB and v’es2 neurons. Note that the v’es2 sensillum is doubly innervated. In the Bar− mutant, structural changes were apparent in two campaniform-like sensilla, whereas little or no change could be detected in trichoid sensilla [Fig. 6M]. In mutant v’es2 and lesB organs, tormogens secreted structures similar to sockets of trichoid sensilla, and trichogens protruded hairs, though relatively short, instead of papillae. The mutant-type lesB hair, somewhat longer than that of v’es2, was one-third as long as the wild-type hair.

Figure 8, A and B, shows dorsal and lateral clusters of es organs in the thoracic segment of Df(1)B263-2° and wild-type larvae, respectively. As with abdominal lateral sensilla, three trichoid sensilla, desE, desB, and lesB, were not affected by the Bar− mutation, whereas all campaniform-like sensilla [desC, desA, lesC, and lesA], except for desD, were morphologically changed to trichoid-type sensilla. The degree of morphological change varied considerably according to the organ. In desA, the change in socket structure was noted to be almost complete, but the extent of bristle change was very slight. In desC, an incomplete socket with very short hair was found. Unlike other campaniform-like sensilla, desD was hardly changed in morphology. lesA and lesC had relatively long hairs, but scanning electron micrographs indicated incomplete change in socket structure [Fig. 6K].

Deficiency of the entire Bar region would thus appear to give rise not only to various morphological changes in complex es organs but to partial conversion as well of simple es organs from campaniform-like sensilla to trichoid sensilla without the neuronal network being affected.

Reverse morphological change by ectopic expression of BarH1 and BarH2

Assuming the absence of Bar homeo box genes to result in conversion from campaniform-like sensilla to trichoid sensilla in simple es organs of the Bar− mutant, reverse morphological change should thus occur by the overexpression of either BarH1 or BarH2 or both. For confirmation of this point, transgenic flies with the BarH1 mini-gene associated with the heat shock promoter were examined (Kojima et al. 1991). With heat treatment at 37°C for 15 min in 10- to 11-hr embryos, significant reverse morphological change occurred. Figure 8C shows almost complete conversion from trichoid to campaniform-like sensilla in thoracic desE, lesB, and partial change in thoracic desB. No appreciable morphological change was de-
Subtype determination of es organs

Figure 4. Expression of BarH1/BarH2 proteins in simple es organs [A–E], complex es organs [H, I], and the CNS [F, G]. [A] Relative positions of eight cells belonging to abdominal desC and desD organs. (n) Neuron; (th) thecogen; (tr) trichogen; (to) tormogen. (Solid circles) BarH1/BarH2-positive cell nuclei; (stippled circles) lacZ-positive cell nuclei. Staining patterns of embryonic abdominal desC and desD cells of an enhancer-trap line, A1-2nd-29, with the S12 [B] and anti-lacZ [C] antibodies, and a mixture of the S12 and anti-lacZ antibodies [D]. The lacZ gene is expressed in two outer support cells of each es organ of the A1-2nd-29 embryo. Note that the expression of BarH1/BarH2 proteins in the desD neuron is almost negligible. [E] Anti-lacZ staining of A1-2nd-29 desC and desD cells under a Df(1)B 263-2° background. Mutant (hemi-zygous) embryos were identified as the ones unstained with the S12 antibody. [F] Ventral view of a 12- to 14-hr embryo stained with the S12 antibody. [G] Ventral view of a dissected nerve cord derived from a ryxo25 embryo double-stained with the S12 and anti-lacZ antibodies. In ryxo25, lacZ is expressed in the cytoplasm of en-gailexpressing cells (Hama et al. 1990). Three abdominal segments are shown. Arrows indicate BarH1/BarH2-positive cell nuclei; white stars show en-expressing cells. [H] Dorsal view of anterior BarH1/BarH2-positive clusters. [AnMx] Antennomaxillary complex; [br] brain (supraesophageal ganglion). [J] Dorsolateral view of the posterior half of a 12- to 14-hr embryo stained with the S12 antibody. [tl] Telson.

ected in campaniform-like sensilla or complex es organs such as Keilin’s organs and basiconical sensilla.

The extent of this reverse change in morphology was shown to depend on the time of heat treatment. Little reverse change occurred before 9 hr. Maximum effect was noted at 10–11 hr. There was only partial change at ~12 hr. That the expression of BarH1/BarH2 proteins in simple es organs becomes prominent in 10- to 11-hr embryos [see below] appears to be a significant finding. The effect of the overexpression of the BarH2 gene was also examined by a similar strategy. As shown in Figure 8D, conversion from trichoid to campaniform-like sensilla, similar to that in the BarH1 overexpression, was observed. BarH1 and BarH2 may thus have almost the same functions in the morphogenesis of es organs.

Reverse morphological change may require endoge-

Figure 5. [A] A physical map of the Bar region. An ~210-kb-long region [from ~80 to 130 on the map] is deleted in Df(1)B 263-2° (Higashijima et al. 1992). Sizes and locations of BarH1 and BarH2 are indicated by horizontal solid lines; dotted lines exhibit those of XI [forked], X2, and a putative transposon, T1/T2 [Higashijima et al. 1992]. Arrows labeled M1, B, bd, and M2 indicate the positions of Bar breakpoints in B°M, B, B° and B°M mutants, respectively (Tsubota et al. 1989; Higashijima et al. 1992). [B] Construction of a BarH2 null mutant, Df(1)BH2. Bp1 and Bd1 show the proximal end of the first B repeat and distal end of the second B repeat, respectively.
Figure 6.  (A) Whole-mount mAb22C10-staining pattern of a 12- to 14-hr Df(1)B263-2° embryo. Enlarged figures of two abdominal clusters are shown in C. B exhibits a wild-type lateral cluster. Mutant (hemizygous) embryos were identified as those unstained with the S12 antibody upon double staining. [D–M] Scanning electron micrographs of various es organs. [D,E] Antennomaxillary complexes of wild-type and Df(1)B263-2° first-instar larvae, respectively. [An] Antenna; [Mx] maxillary complex. [F] Wild-type Keilin’s organ; [G] Df(1)B263-2° Keilin’s organ; [H] wild-type basiconical sensillum; [I] Df(1)B263-2° basiconical sensillum; [J] thoracic es organs (wild type); [K] thoracic es organs [Df(1)B263-2°]; [L] abdominal es organs (wild type); [M] abdominal es organs [Df(1)B263-2°]. Partial deformation of bristle structures in K and M are the result of the deficiency of the forked gene in Df(1)B263-2° (see Fig. 5A). Bar, 5 μm in D and E, 1.7 μm in F–I, and 3 μm in J–M.

nous BarH1 and/or BarH2 genes, which may be activated by overexpressed exogenous BarH1 or BarH2. For clarification of this point, examination was made as to whether there was reverse morphological change under a Df(1)B263-2° background. Similar conversion from trichoid to campaniform-like sensilla was noted following the heat induction of BarH1 at ~11 hr (data not shown). However, not only intrinsic trichoid sensilla but Bar−

dependent trichoid-like structures as well were found to be converted to campaniform-like sensilla. Reverse morphological change thus requires no endogenous BarH1 or BarH2. In contrast to simple es organs, no appreciable recovery of morphology could be induced by BarH1 overproduction in complex es organs, in which the BarH1/BarH2 expression occurs at a much earlier stage.

From these observations, BarH1 and BarH2 were con-
cluded to be the paired homeotic genes required for the fate determination of es organs. Their presence promotes the induction of campaniform-like structures, whereas their absence facilitates trichoid-like structure formation.

**Mutual redundancy of BarH1 and BarH2**

So far, neither viable mutations affecting the structure and/or function of sensory organs nor lethal point mutations have been mapped in the Bar region, where both BarH1 and BarH2 are located. Our previous attempt to isolate the lethal mutation in this region was not successful. About 2300 X-linked lethal mutations were examined, but none could be found within the deleted region in Df(1)B^263-2°. A BarH2 null mutation could, however, be generated by an unequal meiotic recombination between B, having a BarH1 duplication, and Df(1)B^263-2° chromosomes (Fig. 5B). In contrast to Df(1)B^263-2° associated with a larval lethal mutation, the resultant Df(1)BH2 flies (forked- BarH2- BarH1+) were fully viable and possessed almost normal eyes (data not shown).

Using Nomarski optics, the morphology of larval sensilla of this mutant was examined. No significant difference in sensillum structure of larvae could be found between this mutant and wild type, thus, virtually confirming the notion that BarH1 and BarH2 are a pair of homeo box genes that determine the morphology of the es organs of embryos in a completely redundant manner.

**Intimate relation between BarH1/BarH2 protein content in es neurons and trichoid campaniform-like sensillum selection in wild-type es organs**

BarH1/BarH2 proteins are expressed in both neurons and thecogens in two types of simple es organs, campaniform-like and trichoid sensilla. The expression levels of BarH1/BarH2 proteins and morphological features of each es organ are summarized in Figure 3, D and H. Strong expression of BarH1/BarH2 proteins was observed in all thecogens regardless of sensillum type, whereas that in neurons varied according to the organ.

Except for abdominal desD, all campaniform-like sensilla appeared to have neurons expressing BarH1/BarH2 at a relatively high level [M or W]. The BarH1/BarH2 expression in neuron of trichoid sensilla was either null or at a very low level [VW]. Although abdominal desD, whose BarH1/BarH2 expression is almost null (Fig. 3G), has been assigned as a campaniform-like sensillum (Campos-Ortega and Hartenstein 1985; Dambly-Chaudiere and Ghysen 1986), our observations indicated that it had a bristle about one-quarter as long as that of an authentic trichoid (Fig. 8E, F), so that abdominal desD may rather be a hybrid between trichoid and campaniform-like sensilla. The labeling intensity of abdominal lesA [trichoid] is relatively high [W], but reproducibly much weaker than that of the neighboring campaniform-like sensillum, lesB (Fig. 3G).

Based on the discussion above, it is suggested that the amounts of BarH1/BarH2 proteins in neurons are an important factor determining subtype in simple es organs.

**Developmental profiles of BarH1/BarH2 protein expression in simple es organs**

Analysis of DNA replication patterns indicates that each es organ originates from a single ectodermal precursor cell, from which four es cells are produced in a lineage-dependent manner (Bodmer et al. 1989). Cells forming a multiple-innervated es organ are also thought to be related by lineage. Developmental stage dependency of BarH1/BarH2 expression in the gnathocephalic region is described in a previous section. The first signals of BarH1/BarH2 expression in thoracic and abdominal segments appeared in embryos at 6.5-7.5 hr of development (Fig. 2B,G). In thoracic segments, labeling was seen in cells presumably belonging to basiconical sensilla [les3 [l3] and v’es3 [v’3]]. In abdominal segments at the same stage, very weak expression was seen in cells belonging to des2 [arrow in Fig. 2G]. In T2-T3 of an 8- to 9-hr embryo (Fig. 2D,H), strong BarH1/BarH2 expression in the Keilin’s organ and weak BarH1/BarH2 expression in a dorsal simple es organ was evident. In A1-A7 at the same stage, the expression in the cells of des2 (d2) be-
Figure 8. Induction of morphological changes of sensory organs by the altered expression of BarH1 and BarH2 genes. (A–C) Lateral and dorsal regions of a thoracic segment (T3) of the first-instar larva. (A) Df(1)B263-2°; (B) wild type; (C) a wild-type larva in which the exogenous hs-BarH1 fusion gene was heat induced at 11 hr after fertilization; (D) dorsal region of a thoracic segment (T3) of a first-instar larva, in which the exogenous hs-BarH2 fusion gene was heat induced at 11 hr after fertilization. All photographs were taken using Nomarski optics; morphological features of es organs are schematically shown in the right margin of each picture. Dotted rectangles indicate transformed es organs. (E) Dorsal region of an abdominal segment of a wild-type first-instar larva. Note that the desD campaniform has a bristle with a length about one-quarter that of a typical trichoid sensillum. (F) A scanning electron micrograph of the abdominal desD of the wild-type first-instar larva Bar, 10 μm in A–E and 3 μm in F.

comes prominent, and very weak BarH1/BarH2 expression, probably corresponding to v’es2 (v’2), could be seen in the ventrolateral region (arrowhead in Fig. 2H). Staining of most simple es organs was apparent in 10- to 11-hr embryos (Fig. 2E,F,L)].

A previous experiment has shown that DNA replication of most embryonic sensory organs occurs in 6- to 8-hr embryos (Bodmer et al. 1989). In fact, almost all of trichogen–tormogen and dorsal neuron–thecogen precursors finish their DNA replication by 8 hr following egg fertilization. DNA replication of the lateral and ventral neuron–thecogen precursors probably occurs in 8- to 9-hr
embryos. Neural antigen expression is initially apparent in neurons of simple es organs at 11–12 hr of development (Hartenstein 1988). The expression of BarH1/BarH2 would thus appear to occur in simple es organs in thoracic and abdominal segments immediately following the second cycle of DNA replication of es primordial cells and shortly before expression of the neural antigen.

Discussion

The expression of two closely related homeo box genes, BarH1 and BarH2, was examined in Drosophila embryos using antibodies specific to their gene products. Bar homeo domain proteins were coexpressed in a limited number of cells of the CNS and es organs in the PNS. In es organs, BarH1 and BarH2 proteins were found to be expressed at the final stage of development and to be essential to the fate determination of these organs.

Sensillum formation and Bar homeo box genes

Although their morphologies clearly differ, trichoid and campaniform-like sensilla are a pair of sibling, simple es organs in thoracic and abdominal segments in Drosophila embryos. Each consists of four cells, derivatives of two cycles of DNA replication of a single progenitor cell, the first precursor cell (see Fig. 7). One of the second precursor cells produces a neuron and a thecogen, whereas the other produces a trichogen and a tormogen (Bodmer et al. 1989). The neural antigen in these organs is initially expressed in 11- to 12-hr embryos (Hartenstein 1988), whereas axon projection and dendrite innervation are complete for the most part at 12–14 hr after egg fertilization. Massive cuticle secretion occurs thereafter. No significant difference was noted between trichoid and campaniform-like sensilla of 12- to 14-hr embryos using various cell-type-specific markers, including neuron-specific antibodies (see Fig. 3F), suggesting that these simple es organs differentiate in an almost identical fashion and acquire their identity at relatively later or final stages of development. Our finding of the conversion from campaniform-like sensilla to trichoid sensilla by the introduction of a small deficiency in the Bar region (Fig. 5A) shows that the deleted region may contain selector genes responsible for subtype determination.

Our previous study indicated that the region deleted in Df(1)B689 is 210 kb in length and contains at least five transcriptional units, two corresponding to BarH1 and BarH2 (Higashijima et al. 1992). The remaining are the putative forked gene [X1], a possible transposon [T1/T2], and an unknown gene, X2, expressing throughout development (Fig. 5A). No conversion between campaniform-like and trichoid sensilla could be found in embryos with a null mutation of the forked gene (S. Higashijima et al., unpubl.). T1/T2 also may not be related to the morphogenesis of es organs, because the T1 region of T1/T2 was deleted in the B mutant, exhibiting normal sensillum structures (Higashijima et al. 1992).

Both BarH1 and BarH2 proteins have homeo domains and are coexpressed in the nuclei of cells of es organs. This would be an indication of the regulatory function of dual Bar homeo domain proteins as transcriptional regulators, as has been suggested for other homeo domain-containing proteins causing homeotic transformation (for review, see Levine and Hoey 1988). The expression of BarH1/BarH2 proteins in thecogens and neurons of simple es organs becomes apparent at relatively later developmental stages (Fig. 7), and subtype selection was found to be quite closely related to the level of BarH1/BarH2 expression in es neurons. All of these findings are consistent with the notion that dual Bar homeo box genes are responsible for sensillum determination. The altered expression of BarH1 and/or BarH2 could bring about conversion between trichoid and campaniform-like sensilla.

A similar example of sensillum subtype conversion is the mutation of the cut locus. In the absence of cut gene activity, es organs are transformed to ch organs. As with BarH1 and BarH2, the cut gene encodes a homeo domain-containing protein, which is expressed in all cells of es organs, but not in any cell of a ch organ (see Fig. 7). There is thus a fundamental difference in expression between cut and BarH1/BarH2. The cut protein is expressed only in one of two intercompatible organs, whereas BarH1/BarH2 proteins are expressed in both, indicating that, in contrast to most homeotic genes including cut, BarH1 and/or BarH2 function not as digital switches for differentiation but as analog switches. This would also partially explain the presence of various types of intermediates or hybrids for trichoid and campaniform-like sensilla, found in Bar-region deficient flies, flies that underwent reverse morphological change, and even wild-type flies.

Larval campaniform-like sensilla may form a sensillum group different from that of the classical campaniform sensilla (Hodgkin and Bryant 1978). Our scanning electron micrographs in Figure 6, J–M, raise the possibility that the larval campaniform-like sensilla may be a kind of trichoid sensilla with extremely truncated hairs. If so, a straightforward interpretation of our results is that it is a quantitative differentiative change in the hair-forming (trichogen) cell that dual Bar homeo box genes, BarH1 and BarH2, control.

Cuticle structure change induced by BarH1 (and/or BarH2) overexpression also provides a clue as to time of sensillum subtype determination under the control of BarH1 and/or BarH2, because it may take place without any assistance from endogenous dual Bar homeo box genes. Immunostaining showed that the overexpressed BarH1 protein remained for ~2 hr following heat treatment [data not shown]. That 10- to 11-hr heat treatment affected reverse morphological change most strongly indicates that BarH1 (or BarH2) protein possibly determines the sensillum subtype at ~10–13 hr following egg fertilization. This was supported further by immunostaining experiments in wild-type embryos. The expression of BarH1/BarH2 proteins in simple es organs was initially seen at ~10 hr of development, it reached a considerable level at ~11–12 hr, and persisted until at least 14 hr at which time immunostaining became diffi-
cut out owing to cuticle formation. Heat treatment of 11- to 13-hr embryos caused only partial morphological change, suggesting that the fate of most sensilla has already been determined to a certain degree at least at 12 hr following egg fertilization.

The expression of BarH1/BarH2 proteins in simple es organs was restricted to neurons and internal support cells, thecogens, whereas sensillum morphology change was seen in cuticle structures secreted from outer support cells in which BarH1/BarH2 were not expressed (Fig. 7). Thus, neurons, thecogens, or both may play key roles in determining sensillum subtype, possibly by cell–cell interactions among the postmitotic sensillum cells. This finding is of particular importance, because traditionally the emphasis regarding sensillum cell determination has always been on cell lineage and cell autonomy [Bodmer et al. 1989]. The simple es organ consists of only four cells, easily identifiable with various cell type-specific markers. It may be a good model system for analyzing molecular mechanisms of cell–cell communication possibly involved in nervous system differentiation.

Relation between BarH1 and BarH2

Possibly no fundamental differences in biological function exist between BarH1 and BarH2 proteins, at least with respect to es cell formation. They commonly share a homoe domain very similar in sequence and appear capable of recognizing an identical set of DNA sequences as targets [Higashijima et al. 1992]. BarH1 and BarH2 are coexpressed equally in >1000 embryonic cells, most being related to the PNS or the CNS [Fig. 1B–G]. The overexpression of either BarH1 or BarH2 results in conversion from trichoid sensilla to campaniform-like sensilla in thoracic and abdominal segments, whereas their deletion causes the opposite homeotic changes [see Fig. 7]. BarH1 and BarH2 proteins would thus appear capable of functioning as similar homoeotic selectors. The finding that the BarH2 null mutation is not lethal and induces no appreciable transformation in sensillum structure is consistent with the notion that endogenous BarH1 and BarH2 genes are redundant in their functions. This is so because the effect of inactivation of one of the two functionally related genes [redundant genes] should be compensated for by the other, as shown by yeast ras-related genes [Kataoka et al. 1984; Tatchell et al. 1984]. At present, whether the endogenous BarH2 functions as a complete substitute of the endogenous BarH1 is a point yet unclarified owing to the absence of the BarH1 BarH2 mutant. However, our recent finding that Klein's organs in In(1)B M2 flies are immunologically BarH1 BarH2 but normal in sensillum shape [unpub.] would support the possibility of such a function.

BarH1 and BarH2 are strictly coexpressed. No embryonic cell, in which only one of the two is expressed, has so far been detected. This strict coexpression may be the result of the presence of common enhancers in the 80-kb region flanked by BarH1 and BarH2. In the embryonic CNS of In(1)B M2, whose breakpoint is located within this 80-kb region [Fig. 5A], BarH1 protein expression is restricted to three mid-line cells in each segment, whereas that of the BarH2 protein occurs exclusively in the remaining CNS cells that are positive to both BarH1 and BarH2 in the case of wild type [S. Higashijima et al., unpub.].

Materials and methods

Fly strains and genetics

Df(1)B263-20 [Sutton 1943] was obtained from Mid-America Drosophila Stock Center (Bowling Green State University, Bowling Green, OH). Enhancer trap lines, A1-2nd-29 [Hartenstein and Posakony 1990] and ryxho245 [Hama et al. 1990] were obtained from Y.N. Jan (University of California, San Francisco) and C. Hama (National Institute of Neuroscience, Tokyo), respectively. The line Q6, in which lacZ is expressed in all cells of the embryonic CNS, was generated in our laboratory [M. Sone, unpub.]. Yellow was introduced into Df(1)B263-20 to identify larvae hemizygous for this deficiency. y larvae are distinguishable from the wild-type larvae by their yellow mouth-hooks. Virgin females of Df(1)B M2 y/In(1)sc7, In(1)AM sc7 carried in an early larval stage. A BarH2 null mutant [Df(1)BH2] was generated by recombination between B, having a BarH1 duplication, and Df(1)B M2, including forked, as shown schematically in Figure 5B. One forked fly found in 20,000 male progeny was verified to be BarH2 BarH1 + by Southern blotting and immunostaining. First-instar larvae homozygous and/or hemizygous for f35e, an extreme forked allele isolated by us [S. Higashijima et al., unpub.], were used to examine the effect of the forked mutation on larval cuticle structures. Under a forked condition, only subtle changes such as slightly shortened hairs and increased susceptibility to Hoyer's solution were observed, but no campaniform–trichoid transformation could be observed.

Preparation of antibodies

The S12 antibody was prepared as follows. A cDNA fragment encoding the carboxy-terminal three-fifths of the BarH1 protein [amino acid positions 250–543] was inserted into the pET3a vector [Rosenberg et al. 1987], and the T7–gene 10–BarH1 fusion protein was overexpressed in E. coli cells [Studier and Moffatt 1986]. After cells were collected, suspended in 10 mM phosphate buffer [pH 7.2], and disrupted by sonication, the supernatant fluid of a centrifugation at 10,000g for 20 min was loaded on a DE52 [Whatmann] column. After washing with 0.3 M NaCl, the fused BarH1 protein, whose purity was 80–90%, was eluted by 0.5 M NaCl. Dialyzed protein preparations were combined and used for immunization of rabbits. The antisera thus obtained was affinity purified. The extract of E. coli cells expressing the fused BarH1 protein was size-fractionated by SDS–polyacrylamide gel electrophoresis and transferred to PVDV filter [Millipore], and a narrow strip including the fused BarH1 was removed. After blocking, this strip was incubated with the anti-BarH1 antiserum. The bound antibody fraction was eluted by washing the strip with 0.2 M glycine [pH 2.5], quickly neutralized with Tris base, and dialyzed against PBS.

The Y2 antibody was prepared as follows. After a cDNA fragment encoding the BarH2 protein [amino acid positions 196–640] was cloned into pUC19, the fused BarH2 protein was overexpressed in E. coli cells, and partially purified by SDS–poly-
acrylamide gel (purity, ~90%). The combined preparations of the fused BarH2 protein were used for immunization. The resultant antiserum was affinity purified as described above.

The S1-1 antibody was obtained by absorption of the S12 antibody with the extract of E. coli cells expressing the fused BarH2 protein.

Immunohistochemistry and in situ hybridization

Whole-mount staining of embryos was performed essentially as described by Thomas et al. [1987], except the Vecta-stain ABC-HRP kit [Vector] was used for signal detection. Rabbit anti-HRP antibody [Cappell] was affinity purified by the HRP protein [Sigma fraction VI], and used at 1:100 dilution. mAb22C10 [1:4] was kindly supplied by S. Fujita [Mitsubishi-Kasei Institute of Life Sciences, Tokyo], whereas purified rabbit anti-lacZ antibody [1:2000; Cappell], mouse monoclonal anti-lacZ antibody [1:200; Promega], and biotinylated anti-rabbit or anti-mouse antibodies [1:500; Vector] were commercial products. ABC-HRP reaction was carried out according to the manufacturer's protocol. For signal intensification, 0.4 mg/ml of NiCl2 was added during color development (brown color development). The double staining was conducted as follows. Following the first-cycle staining, which is described above (black color development), the second-cycle staining was conducted without adding NiCl2 during color development (brown color development).

Samples were mounted on Canada balsam/xylene after dehydration in series of ethanol or mounted directly on 90% glycerol. Samples were dissected longitudinally in 90% glycerol and mounted on the coverslip. Some samples were dehydrated by 30–100% ethanol. Samples were critical-point-dehydrated by 30–100% ethanol. Samples were critical-point-dehydrated by 30–100% ethanol. Samples were critical-point-dehydrated by 30–100% ethanol. Samples were critical-point-dehydrated by 30–100% ethanol. Samples were critical-point-dehydrated by 30–100% ethanol.

Heat-shock treatment

Transgenic flies having the Barth1 minigene associated with a heat-shock promoter have been described previously [Kojima et al. 1991]. Two independent viable homozygous lines, B27 and BHT102, were used in which the Barth1 minigene is located on the second and first chromosomes, respectively. Similar transgenic flies [hs-BarH2-49] with the BarH2 minigene have also been constructed [details will be described elsewhere]. Transgenic embryos were heat-shocked at 37°C for 15–20 min, and morphological changes found in first-instar larvae were examined. No morphological changes in es organs were detected on heat treatment in the case of wild-type larvae. For the examination of heat shock effects under a Df(1)B270-36 background, virgin females Df(1)B270-36 y/In(l)sc7, In(l)AM se' c' car were crossed to B27 males, and effects on resultant yellow larvae were examined.

Acknowledgments

We thank Y.N. Jan and C. Hama for Drosophila strains A1-2nd-29 and ryxho25, respectively. We also thank R. Ueda, T. Uemura, and T. Kojima for helpful discussions. This work was supported in part by grants from the Ministry of Education, Science, and Culture of Japan to K.S.

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

References

Blochlinger, K., R. Bodmer, J.W. Jack, L.Y. Jan, and Y.N. Jan. 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., R. Bodmer, L.Y. Jan, and Y.N. Jan. 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.

Bodmer, K., L.Y. Jan, and Y.N. Jan. 1991. Transformation of sensory organ identity by ectopic expression of Cut in Drosophila. Genes & Dev. 5: 1124–1135.

Bodmer, R. and Y.N. Jan. 1987. Morphological differentiation of the embryonic peripheral neurons in Drosophila. Wilhelm Roux’s Arch. Dev. Biol. 196: 69–77.

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

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

Campos-Ortega, J.A. and V. Hartenstein. 1985. The embryonic development of Drosophila melanogaster. Springer-Verlag, Berlin, Germany.

Dambly-Chaudiere, C. and A. Ghysen. 1986. The sense organs of the Drosophila larva and their relation to the embryonic pattern of sensory neurons. Wilhelm Roux’s Arch. Dev. Biol. 195: 223–238.

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

Fujita, S., S.L. Zipursky, S. Benzer, A. Ferrus, and S.L. Shotwell. 1982. Monoclonal antibodies against the Drosophila nervous system. Proc. Natl. Acad. Sci. 79: 7929–7933.
Higashijima et al.

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

Grodowitz, V.A., J. Krchma, and A.B. Broce. 1982. A method for preparing soft bodied larval diptera for scanning electron microscopy. J. Kansas Entomol. Soc. 55: 751–753.

Hama, C., Z. Ali, and T.B. Kornberg. 1990. Region-specific recombination and expression are directed by portions of the Drosophila engrailed promoter. Genes & Dev. 4: 1079–1093.

Hartenstein, V. 1988. Development of Drosophila larval sensory organs: Spatiotemporal pattern of sensory neurons, peripheral axonal pathways and sensilla differentiation. Development 102: 869–886.

Hartenstein, V. and J.W. Posakony. 1990. A dual function of the Notch gene in Drosophila sensilla development. Dev. Biol. 142: 13–30.

Higashijima, S., T. Kojima, T. Michiue, S. Ishimaru, Y. Emori, and K. Saigo. 1991. Identification of a different-type homeobox gene, BarH1, possibly causing Bar (B) and Om(1D) mutations in Drosophila. Proc. Natl. Acad. Sci. USA 88: 4343–4347.

Hodgkin, N.M. and P.F. Bryant. 1978. Scanning electron microscopy of the adult of Drosophila melanogaster. In The genetics and biology of Drosophila (ed. M. Ashburner and T.R.F. Wright), vol. 2c, pp. 337–358. Academic Press, London, UK.

Jan, L.Y. and Y.N. Jan. 1982. Antibodies to horseradish peroxidase as specific neuronal markers in Drosophila and grasshopper embryos. Proc. Natl. Acad. Sci. 72: 2700–2704.

Kataoka, T., S. Powers, C. McGill, O. Fasano, J. Strathern, J. Broach, and M. Wigler. 1984. Genetic analysis of yeast RAS1 and RAS2 genes. Cell 37: 437–445.

Kojima, T., S. Ishimaru, S. Higashijima, E. Takayama, H. Aki-maru, M. Sone, Y. Emori, and K. Saigo. 1991. Identification of a different type homeobox gene, BarH1, possibly causing Bar (B) and Om(1D) mutations in Drosophila. Proc. Natl. Acad. Sci. 88: 4343–4347.

Levine, M. and T. Hoey. 1988. Homeobox proteins as sequence-specific transcription factors. Cell 55: 537–540.

Matsuzaki, F., K. Koizumi, C. Hamma, T. Yoshioka, and Y. Nabeshima. 1992. Cloning of the Drosophila prospero gene and its expression in ganglion mother cells. Biochem. Biophys. Res. Commun. 182: 1326–1332.

McVey, S.B. 1985. Mechanoreception. In Comprehensive insect physiology, biochemistry and pharmacology (ed. G.A. Kerkut and L.I. Gilbert), vol. 6, pp. 71–132. Pergamon Press, New York.

Rosenberg, A.I., B.N. Lade, D. Chui, S-W. Lin, J.J. Dunn, and F.W. Studier. 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene 56: 125–135.

Sturtevant, A.H. 1925. The effects of unequal crossing-over at the Bar locus in Drosophila. Genetics 10: 117–147.

Tatchell, K., D.T. Chalfé, D. DeFeo-Jones, and E.M. Scolnick. 1984. Requirement of either of a pair of ras-related genes of Saccharomyces cerevisiae for spore viability. Nature 309: 523–527.
Subtype determination of Drosophila embryonic external sensory organs by redundant homeo box genes BarH1 and BarH2.

S Higashijima, T Michiue, Y Emori, et al.

Genes Dev. 1992, 6:
Access the most recent version at doi:10.1101/gad.6.6.1005

References
This article cites 31 articles, 9 of which can be accessed free at:
http://genesdev.cshlp.org/content/6/6/1005.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.

Boost NGS microRNA profiling. Read about 3 methods tested