Mechanism of induction of Bar-like eye malformation by transient overexpression of Bar homeobox genes in Drosophila melanogaster

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Abstract

The Bar locus of Drosophila is known to be a small complex consisting of two similar homeobox genes, BarH1 and BarH2. Using egr as an ommatidium marker, possible mechanisms of formation of malformed eyes were examined. As in the case of BarH1, overexpression of BarH2 was found to be capable of inducing Bar-like eye malformation. It was suggested that suppression of the anterior progression of the morphogenetic furrow and inhibition of reinitiation of normal ommatidial differentiation were mandatory to formation of the reduced eye morphology in Bar mutants.

Introduction

Bar is a classical mutation in which ommatidial differentiation is suppressed in the anterior portion of the eye (Sturtevant, 1925). All Bar mutations so far identified have been shown to be associated with a DNA rearrangement at or near the 16A region on the X chromosome, where a small chromosomal segment is duplicated in the Bar mutation, B (Bridges, 1936; Sutton, 1943). The Bar locus is a small complex consisting of paired homeobox genes, BarH1 and BarH2, both of which encode polypeptides similar in size and possessing a homeodomain virtually identical in sequence (Kojima et al., 1991; Higashijima et al., 1992a). BarH1 and BarH2 are co-expressed in many different types of cells and appear to be functionally redundant to each other (Higashijima et al., 1992a and b, and our unpublished observation). They are required for normal eye morphogenesis (Kojima et al., 1991; Higashijima et al., 1992a) and subtype specification of external sensory organs in embryo (Higashijima et al., 1992b).

Our structural analysis revealed all Bar breakpoints examined so far to be in a narrow region terminated by BarH1 and BarH2 (Higashijima et al., 1992a). Considerably more BarH1 RNA was detected in B than wildtype (Kojima et al., 1991). Furthermore, P-mediated transformation showed Bar-like eye malformation to be inducible by ubiquitous, transient overexpression of BarH1 (Kojima et al., 1991). Thus, reduced eye morphology in strains carrying Bar mutations is reasonably considered to be due to abnormal activation of Bar homeobox genes by DNA rearrangements (Kojima et al., 1991).

In the present paper, we will describe a line of evidence supporting and extending the above notion.

Materials and methods

Fly strains

A fly strain exhibiting the egr (eye-pigmentation gradient) phenotype was obtained during enhancer-trap experiments. In egr flies with wildtype eye morphology, ommatidia near the posterior edge

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Fig. 1. Scanning electron micrographs of malformed eyes obtained after overexpression of Bar homeodomain proteins. (a) A wild type eye (control); (b) A hetero Bar (B/+ ) eye; (c-i) Malformed eyes induced by BarH1 overexpression at late-third-instar (c and d), early-third-instar (e-h) and end of second-instar (i). (d and f) Enlargements of boxed regions in (c) and (e). Note that there are many trichoid and campaniform-like sensilla. Thick arrows show scars. (j-l) Malformed eyes induced by BarH2 overexpression. Anterior is to the right.

Fig. 2. Developmental stage dependency of induction of malformed eyes by BarH1 overexpression. Ratios of malformed eyes induced by a 30 min single heat shock at 37 °C are shown as a function of induction time.
show no appreciable pigmentation, while those in the anterior edge and internal region, respectively, show full and intermediate levels of pigmentation (see Fig. 3b), making it possible to conjecture the origin of each ommatidium by examining coloration. Detailed properties of this line will be described elsewhere (M.S., unpublished data). \( B^{MI} \) and \( B^{M2} \) were obtained from Umeå Drosophila stock center (Sweden), while origins of other strains were described previously (Kojima et al., 1991; Higashijima et al., 1992a and b).

**Immunostaining, electronmicroscopy and molecular cloning**

All experimental procedures used in this paper were essentially as described previously (Kojima et al., 1991; Higashijima et al., 1992a and b).

**Results and discussion**

*Prevention of the anterior progression of the morphogenetic furrow by transient, ubiquitous overexpression of BarH1*

In a previous experiment, using a fusion construct of hsp70 and BarH1 genes (hsp-BH1), transient, ubiquitous overexpression of BarH1 was shown to frequently induce Bar-like eye malformation or reduced eye morphology (Kojima et al., 1991; Fig. 1c). As shown in Fig. 2, effective induction occurred during the period from the late-second instar (60 h after egg laying) to the end of the third instar (120 h after egg laying). In contrast, little or no induction of malformed eyes was observed at other developmental stages. Note that, in the wild-type, non-heat-shocked eye disc, ommatidium formation begins during the mid-third instar (Ready, Hanson & Benzer, 1976).

To determine which portion of ommatidia is eventually removed from the malformed eyes, the effect of BarH1 overexpression was examined under a background of egr, showing a gradient eye pigmentation (Fig. 3b). Three examples of malformed eyes, induced by a 30 min single heat shock at the late-third instar, are shown in Fig. 3c, e and g. In all cases, anterior regions of various sizes, based on the comparison with the coloration in non-heat-shocked control eye, appear to be elimi-
nated as shown in Fig. 3d, f and h. The average number of eliminated ommatidia appeared to decrease with progression of developmental stages when larvae were subjected to heat induction, suggesting that BarH1 overexpression induces the cessation of the anterior progression of the morphogenetic furrow in the eye disc (Ready, Hanson & Benzer, 1976; Tomlinson, 1985; Tomlinson & Ready, 1987) and, thereby, suppresses the differentiation of unpatterned cells to ommatidial cells. Similar cessation of the anterior progression of the morphogenetic furrow could be seen in $B^{+}$ eyes (Fig. 3i) and $B/B$ eyes (data not shown), although the anterior edge of male eyes hemizygous for $B$ was found to induce cells unusually abundant in pigment granules (Fig. 3j).

At the anterior edge of malformed eyes, many isolated sensilla could be seen (Fig. 1 c-d), possibly suggesting that differentiation suppression due to BarH1 overexpression is less severe, if any, on sensillum primordial cells than other ommatidial precursor cells. The presence of campaniform-like sensilla, usually absent in wild-type eyes (Ready, Hanson & Benzer, 1976), may suggest that, as in the case of embryonic sensilla (Higashijima et al., 1992), homeotic transformation of trichoid to

Fig. 4. Tangential sections of malformed eyes. Anterior is to the right. (a) Non-scarred eye. Numbers in the upper and lower margins, respectively, show the distance in column number from the stopped morphogenetic furrow and ratios of ommatidia abnormal in appearance. Arrowheads, examples of abnormal ommatidia; (b) Scarred eye. Arrowheads show the scarred region. Note the presence of unusually heavy pigmentation in the scarred region.
campaniform-like sensilla occurs in BarH1-overexpressed eye discs.

**Overexpression of BarH1 at the early-third-instar-larval stage results in scarred eyes**

Two examples of malformed eyes induced by BarH1 overexpression at the early-third-instar-larval stage are shown in Fig. 3k and m. Unlike the malformed eyes generated by heat induction at the late-third-instar (see Fig. 3c, e and g), the ommatidial region is divided into two or three areas by scars. As can be seen in Fig. 3k and m, levels of pigmentation suddenly or discontinuously change at the scarred region, suggesting an internal ommatidial region, about 10 columns wide, to be eliminated in scarred eyes as shown by a short black belt in Fig. 3l and n. This coupled with the fact that the scar perpendicular to the A-P axis is rich in both trichoid and campaniform-like sensilla (Fig. 1f) may suggest that scar formation is brought about by temporal interruption of anterior movement of the morphogenetic furrow by BarH1 overexpression, elimination of internal unpatterned cells and subsequent reinitiation of ommatidium formation in the area corresponding to the anterior region of the wild-type eye disc. Since ommatidium reinitiation is suppressed by repeated overexpression of BarH1 (data not shown), it is feasible that ommatidium formation is reinitiated only when the concentration of the BarH1 protein is lower than a threshold value. As shown in Fig. 3l and j, no scarred eye formation was observed in B mutant individuals, thus suggesting that all B cells that are eventually eliminated express the BarH1 protein at a level higher than the threshold value.

**Effect of BarH1 overexpression on photoreceptor differentiation**

Fig. 4a and b, respectively, show thin sections of malformed eyes induced at late- and early-third-instar-larval stages. In both cases, heavy pigmentation was found to occur in the region corresponding to the stopped morphogenetic furrow. In the region 1-6 columns posterior to the stopped morphogen-

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**Fig. 5.** Effects of BarH1 overexpression on anti-Bar staining patterns in eye discs. Larvae carrying hsp-BH1 were heat shocked for 30 min at 37 °C and discs were stained with S12 after 1 h (b), 6 h (c and d), 12 h (e), 24 h (f) and 48 h (g) of recovery at 25 °C. (a) Control (no heat shock). Large arrowheads show the morphogenetic furrow (a-f) or the scar (g), while small arrowheads, ommatidia abnormal in staining pattern. Large arrows in (g) indicate the location of a new morphogenetic furrow. Anterior is to the right for (a-c) and (g), or the top for (d-f).
Fig. 6. Bar homeodomain protein expression in eye discs mutant for B (b), B\textsuperscript{M1} (c) and B\textsuperscript{M2} (d). (a) Wild type. Arrowheads, morphogenetic furrow; arrows, ectopic expression of Bar proteins found in (c) and (d).

etic furrow, 5-50% ommatidia exhibited morphological abnormality, including reduction in photoreceptor number, conversion of photoreceptor types and aberrant ommatidial orientation (Fig. 4a). Similar defects were also found in the reinitiated region (Fig. 4b). On the other hand, little or no change in morphology was detected in ommatidia in the region from column 7 to the posterior edge of the eye, suggesting only early stages of photoreceptor differentiation to be sensitive to BarH1 overexpression.

Changes in distribution patterns of the BarH1 protein in eye imaginal discs subjected to BarH1 overexpression

Larvae homozygous for P[hsp-BH1] insertion were subjected to a 30-min single heat shock at 37 °C and eye discs dissected from third-instar larvae were examined by staining with the Bar-specific antibody, S12 (Higashijima et al., 1992b), after 1, 6, 12, 24 or 48 h of recovery at 25 °C. One hour after heat shock, high levels of BarH1 protein were de-

Fig. 7. Structure of hsp-BH2-containing P element. BarH2 cDNA, containing the entire coding sequence, was inserted into the BamHI site of pHST14, a pUC plasmid having a partial P sequence along with the 5' and 3' portions of the hsp70 gene (see Kojima et al., 1992). Then, a ry\textsuperscript{+} marker was inserted as a selective marker. The hsp-BH2 encodes a hsp70-BarH2 fusion protein.
tected in every cell in the eye-antenna disc (Fig. 5b). BarH1 protein was substantially reduced 6 h after heat shock (Fig. 5c), and the wild-type BarH1 expression pattern (R1/R6-specific expression (Higashijima et al., 1992a); Fig. 5a) began to stand out. However, the normally intense expression of BarH1 protein in the morphogenetic furrow was greatly reduced (Fig. 5a and c). The absence of BarH1 expression in the morphogenetic furrow persisted at least until 24 h after heat shock (Fig. 5d-f). Observation of refractive bodies at the base of the disc under the light microscopy may suggest cell death to occur around the stopped morphogenetic furrow. In contrast, ommatidial preclusters just posterior to the stopped morphogenetic furrow continued to develop so that the majority of them could acquire eight prephotoreceptor cells 24 h after heat shock (Figs. 4 and 5f).

Although ubiquitous BarH1 expression caused the morphogenetic furrow to stop its anterior progression for at least 24 h (Fig. 5f), examination of discs 48 h after heat shock (Fig. 5g) demonstrated that the ommatidial assembly process could reinitiate. A new morphogenetic furrow is shown by large arrows in Fig. 5g. Posterior to this new furrow, new ommatidial clusters have begun to assemble.

Expression of the BarH1 protein in Bar mutants

Fig. 6b, c and d, respectively, show expression patterns of the BarH1 protein in three Bar mutants, B, B\textsuperscript{M1} and B\textsuperscript{M2}. In the posterior region of the B disc, stained pairs, somehow irregularly situated and presumed to be R1 and R6 prephotoreceptor cells, could be seen in each ommatidium precursor. However, as in the case of the wild type eye disc 6-24 h after BarH1 overexpression, little or no BarH1 protein was detected in the region presumably corresponding to the morphogenetic furrow. In B\textsuperscript{M1} and B\textsuperscript{M2} eye discs, ectopic expression of BarH1 was frequently observed as indicated by small arrows in Fig. 6c and d. We presume ectopic BarH1 products to cause misdirected differentiation of ommatidia in these mutant eyes.

Induction of eye malformation by BarH2 overexpression

To examine whether BarH2 overexpression causes eye malformation, a hsp-BH2 construct (Fig. 7) was made and introduced into flies by P-mediated transformation and the third instar larvae were subjected to a 30 min single heat shock (37 °C). BarH2 was found to serve as a substitute for BarH1 in all experiments described above (Fig. 1f), possibly suggesting that either BarH1 or BarH2 or both are activated in Bar mutants and cause the reduced eye morphology.

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References