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The identification of the B^S breakpoint and of two possible *Bar* genes

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Summary. Two coding regions were identified within a 110 kb region which includes all mapped *Bar* breakpoints. Both lie proximal to the identified *Bar* breakpoints. The first coding region, designated *BarA*, is 5 kb from the most proximal *Bar* mutation, B^{581}, and 66 kb from the B^S breakpoint. It encodes a 1.3 kb transcript, which is found in late third instar larvae but is absent in 1–3-day-old pupae. B^i, $R(B)^{hd3}$, B^{85c15}, and B^S result in overproduction of this transcript in late third instar larvae. A second coding region, which was previously identified as *BarH1*, maps 18 kb from B^{581} and 79 kb from the B^S breakpoint. In third instar larvae, the abundance of the *BarH1* transcript is very low in both wild type and various *Bar* mutatants, with the exception of $R(B)^{hd3}$. In 1–3-day-old pupae, the level of the *BarH1* transcript is higher. *BarH1* was previously identified as the *Bar* gene. However, this report raises the possibility that *BarA* rather than *BarH1* is the *Bar* gene or that more than one gene may be involved in *Bar* position effects.

Key words: *Drosophila melanogaster* – *Bar* – *BarA* – *BarH1* – Position effects

Introduction

The expression of a gene is dependent on its position in the genome. This phenomenon was first demonstrated by chromosomal rearrangements which changed the positions of genes and resulted in mutant phenotypes (Sturtevant 1925; Lewis 1950). Because of the central role of chromosomal position in the manifestation of the mutant phenotype, these mutations are called position-effect mutations. Position-effect mutations were classically identified because of their association with visible chromosomal rearrangements (Lindsley and Grell 1968; Keppy and Welshons 1977). However, with the advent of molecular genetics, other cytologically invisible position-effect mutations have been identified. These include the insertion of transgenes into novel positions in the genome (Spradling and Rubin 1983; Hazelrigg et al. 1984; Grosschedl et al. 1984; Swift et al. 1984).

In *Drosophila melanogaster*, mutations at the *Bar* locus represent a unique set of euchromatic position-effect mutations. All of these mutations are associated with chromosomal breakpoints within the 16A1–2 region of the X chromosome and are gain-of-function mutations which result in a reduction in the number of eye facets. Previously, 85 kb of the 16A1–2 region were cloned and four of the *Bar* breakpoints were mapped to a 37 kb region within the cloned DNA (Tsubota et al. 1989). The large region defined by the *Bar* breakpoints suggests either that the *Bar* gene is very large or that the position effects of the *Bar* breakpoints can act over very large distances.

The original *Bar* mutation, *B* (Tice 1914), is associated with a tandem duplication of 16A1–7 (Bridges 1936; Muller et al. 1936). However, the *Bar* phenotype is not caused by the extra dose of the *Bar* gene but rather by the breakpoint of the tandem duplication (Sutton 1943; Tsubota et al. 1989). Between the two members of the tandem duplication is a transposable element, *B104*, which most probably was instrumental in the generation of the *B* breakpoint and the subsequent tandem duplication (Tsubota et al. 1989).

Many derivatives of *B* have been isolated which result in either a decrease or an increase in the number of eye facets with respect to that of *B* (Lindsley and Grell 1968; Tsubota et al. 1989). Four of these derivatives, $R(B)^{hd1-3}$ and B^3, are associated with additional insertions of another repetitive element near the *B* breakpoint and result in an increase in the number of eye facets. Another derivative, B^S, is associated with an additional breakpoint near the original *B* breakpoint (Griffin 1941). This breakpoint is part of a reciprocal translocation with chromosome 4 and results in a more severe reduction in the number of eye facets. The available genetic data reveal that the translocation separates the two components of the *B* tandem duplication and that the eye

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phenotype of B^S is associated with the copy in the X-proximal half (4^DX^P) of the translocation. However, the genetic data cannot determine the position of the B^S breakpoint with respect to the *B* breakpoint and whether or not the *B* breakpoint is maintained in B^S.

In *D. ananassae* a gene called *Om(1D)* has been identified and cloned. This gene may be the homologue of *Bar*. Mutations in the *Om(1D)* gene produce a small-eye phenotype very similar to that of *Bar* mutations in *D. melanogaster* (Tanda et al. 1989). Both sets of mutations are semi-dominant and map to analogous positions on the X chromosome. A partial sequence of the *Om(1D)* gene reveals that it encodes a homeobox domain similar to that of *even-skipped* in *D. melanogaster* (Tanda and Corces 1991).

Clones of *Om(1D)* were used to isolate the homologous gene in *D. melanogaster* which has been called *BarH1* (Kojima et al. 1991). The sequence of this gene shows that it, like *Om(1D)*, contains a homeodomain. Evidence was presented to suggest that *BarH1* may be the *Bar* gene. First, *BarH1* localizes to the 16A region of the polytene chromosomes, the approximate location of *Bar*. However, the position of *BarH1* within the cloned *Bar* region was not determined. Second, a construct with the *hsp70* promoter and the *BarH1* coding region can produce a reduction in the number of eye facets when a transformant carrying the construct is heat-shocked during the third instar larval stage. Third, the *BarH1* transcript is overproduced in the pupal stage of the *B* mutant. However, a convincing overproduction of *BarH1* transcript in late third instar of the *B* mutant was not seen.

In this paper, we extend the limits of the *Bar* region by isolating an additional 25 kb and adding two mutations, B^S and B^{581}, to the DNA map. In addition, we localize two genes within the DNA map, *BarA* and *BarH1*, both of which may be involved in *Bar* position effects.

Materials and methods

Drosophila *stocks*. *B* is the original *Bar* mutation associated with a duplication of 16A1–7 (Lindsley and Grell 1968). With the exception of B^{581}, the other *Bar* mutations used in this study were either isolated as more severe or less severe derivatives of *B* and still contain the *B* duplication. B^i, B^{581}, and B^S have been previously described (Lindsley and Grell 1968), as has $R(B)^{hd3}$ (Tsubota et al. 1989). B^{85c15} was isolated by M. M. Green and was kindly provided by him.

DNA sequencing. All sequencing was performed by the dideoxy method (Sanger et al. 1977).

In situ hybridization to polytene chromosomes. Salivary glands from either $T(1;4)B^S$ or $T(1;3)B^{581}$ were isolated and squashed in 45% acetic acid and were prepared for hybridization (Bingham et al. 1981). DNA probes were labeled with ^3H with the Boehringer Mannheim random-priming kit.

Genomic libraries. All lambda clones, with the exception of CS–16A7.2 and CS–16A7.5, were obtained from a previously described library (Tsubota et al. 1989). CS–16A7.2 and CS–16A7.5 were isolated from a Canton S library (Maniatis et al. 1978).

Northern blot analysis. Poly(A)$^+$ RNA was isolated from late third instar larvae and 1–3-day-old pupae from the appropriate strains. For each lane, 7.5 µg of RNA was used in a 0.9% agarose-formaldehyde gel. The gel was blotted onto a Nytran filter (Schleicher and Schuell) and prehybridized and hybridized in a solution containing 5 × SSPE, 1.0% SDS, 10 × Denhardt's solution, 150 µg/ml denatured salmon sperm DNA, and 50% formamide at 52° C. Probes were labeled with ^{32}P-dCTP (ICN Biochemicals) by random priming.

Densitometry. A LKB Ultrascan XL laser densitometer was used to scan autoradiograms. The areas of the peaks for either *BarA* or *BarH1* were divided by the areas of the peaks for *ras* for each genotype. These adjusted values were then divided by the value for Oregon R to obtain values relative to that of Oregon R.

Results

Location of the B^S *breakpoint*

Previous genetic studies had determined that B^S was the result of an additional breakpoint superimposed on the *B* allele (Griffin 1941). The breakpoint resulted in the separation of the two members of the *B* tandem duplication. The B^S phenotype was shown to be associated with the X-proximal half (4^DX^P) of the translocation. The X-proximal half of the B^S translocation was later used to produce the B^SY chromosome (Brosseau et al. 1961; Lindsley and Grell 1968). These genetic studies indicated that the B^S breakpoint was very close to the *B* breakpoint, but could not determine if the B^S breakpoint was proximal or distal to the *B* breakpoint. To localize the B^S breakpoint in the vicinity of the *B* breakpoint, we utilized a combination of genomic Southern blots and in situ hybridizations to the polytene chromosomes. Initially, DNA from B^SY males was probed with DNA from the 16A1 region in the vicinity of the *B* breakpoint (See Fig. 1). These experiments showed that the *B* breakpoint was still present in the B^SY males and, therefore, the B^S breakpoint had to be distal to the *B* breakpoint in the 16A7 region (S.I. Tsubota, unpublished results). To localize the B^S breakpoint, probes from our walk in the 16A7 region (Fig. 2) were used as probes for in situ hybridization to chromosomes from $T(1;4)B^S$ males. Because the B^S breakpoint has separated the two copies of the 16A region associated with *B*, both the 4-proximal half (4^PX^D) and X-proximal half (4^DX^P) will contain the 16A7 region. However, any probe from 16A7 which is proximal to the B^S breakpoint will hybridize only to 4^DX^P. On the other hand, any probe from 16A7 which spans the B^S breakpoint or is distal to the B^S breakpoint will hybridize to both 4^PX^D and 4^DX^P (Fig. 3A). A 3.0 kb

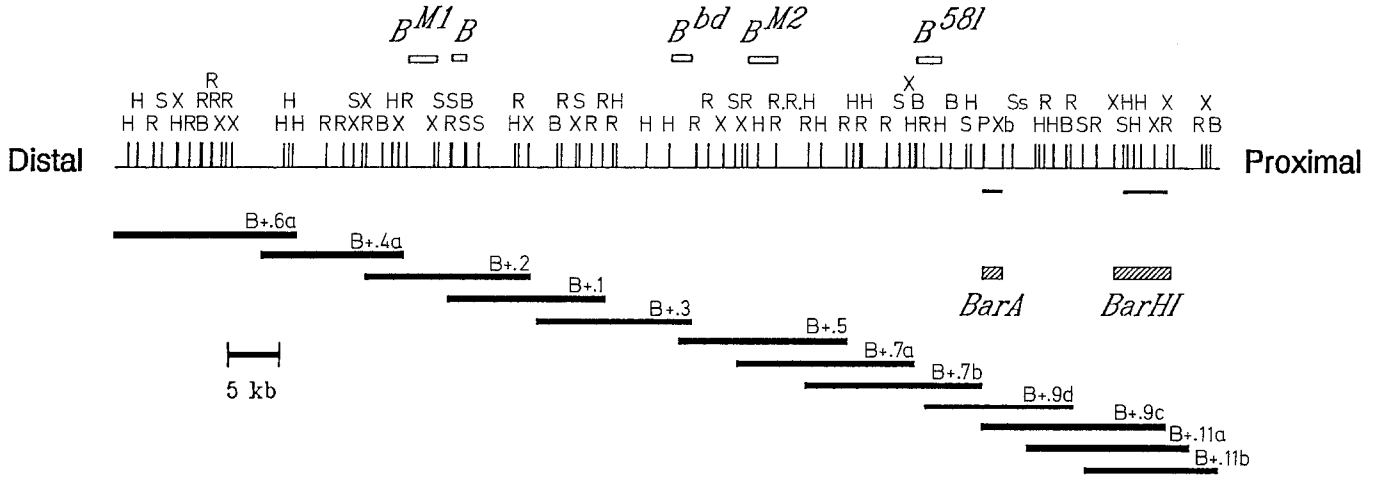

Fig. 1. 16A1–2 region containing the *Bar* breakpoints. The lambda clones that define the region are indicated as *black boxes* below the restriction map. The positions of the *Bar* breakpoints are indicated as *open boxes* above the restriction map. Reading from left to right, the clones B+.6a-B+.7b were previously reported (Tsubota et al. 1989). Clones B+.9d-B+.11b are from this report. The positions of the *BarA* and *BarH1* genes are indicated with *striped boxes*, and the corresponding restriction fragments that were used as probes in the Northern blots (Fig. 5) are indicated with *heavy* lines. B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I; Ss, *Sac*I; X, *Xho*I; Xb, *Xba*I. Only the *Pst*I, *Sac*I, and *Xba*I sites in the region of *BarA* have been mapped

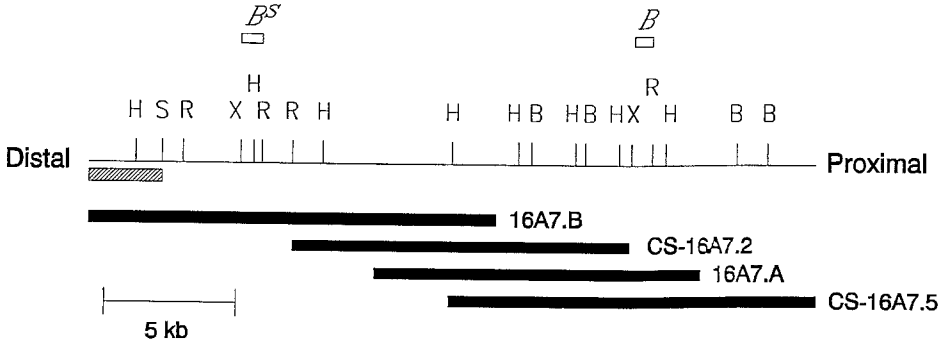

Fig. 2. 16A7 region containing the B^S and B breakpoints. Four wild-type lambda clones are diagrammed as *black boxes* beneath the restriction map. Two of the clones, CS–16A7.2 and CS–16A7.5, were previously reported (Tsubota et al. 1989). The others are from this report. The position of the B and B^S breakpoints are indicated as *open boxes* above the restriction map. The fragment used for the in situ hybridization (Fig. 3B) is indicated as a *striped box*. B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; X, *Xho*I. There are no *Sal*I sites in this region

fragment from the distal end of the 16A7.A clone (Fig. 2) hybridized only to $4^D X^P$, indicating that the B^S breakpoint is distal to this clone (S.I. Tsubota, unpublished results). On the other hand, a 2.8 kb fragment from the distal end of the 16A7.B clone (Fig. 2) hybridized to both $4^D X^P$ and $4^P X^D$, indicating that it either spans the B^S breakpoint or is distal to the B^S breakpoint (Fig. 3B). These data mapped the B^S breakpoint to between the distal end of 16A7.B and the distal end of 16A7.A. Southern blots confirmed these results and further localized the breakpoint to within a 0.8 kb *Xho*I-*Eco*RI fragment in 16A7.B (Fig. 2). This places the B^S breakpoint approximately 15 kb distal to the B breakpoint.

Localization of the B^{581} *breakpoint*

$T(1;3)B^{581}$ is a very severe *Bar* mutation with a phenotype similar to that of B^S. It was *X*-ray induced in a wild-type X chromosome and therefore, unlike B^S, is not associated with the B breakpoint or the duplication. Because B^{581} had been shown to contain a breakpoint in the 16A region, clones from the *Bar* region (Fig. 1) were used for in situ hybridization experiments to localize the breakpoint. Chromosomes from $T(1;3)B^{581}/+$ females were used. Clones B+.6a and B+.1 hybridized distal to the B^{581} breakpoint (S.I. Tsubota, unpublished results). A clone which spans a translocation breakpoint should give three bands of hybridization. One band corresponds to the site on the normal chromosome, while the other two bands correspond to the sites on the distal and proximal halves of the translocated chromosome. A more proximal clone, B+.7b, gave this characteristic pattern (Fig. 4). Genomic Southern blotting localized the B^{581} breakpoint to within a 2.4 kb *Eco*RI-*Hin*dIII fragment in B+.7b (Fig. 1). These experiments, together with the previous mapping data (Tsubota et al. 1989), demonstrate that the *Bar* breakpoints within 16A1–2 span a 50 kb region, with B^{M1} being the most distal and B^{581} being the most proximal.

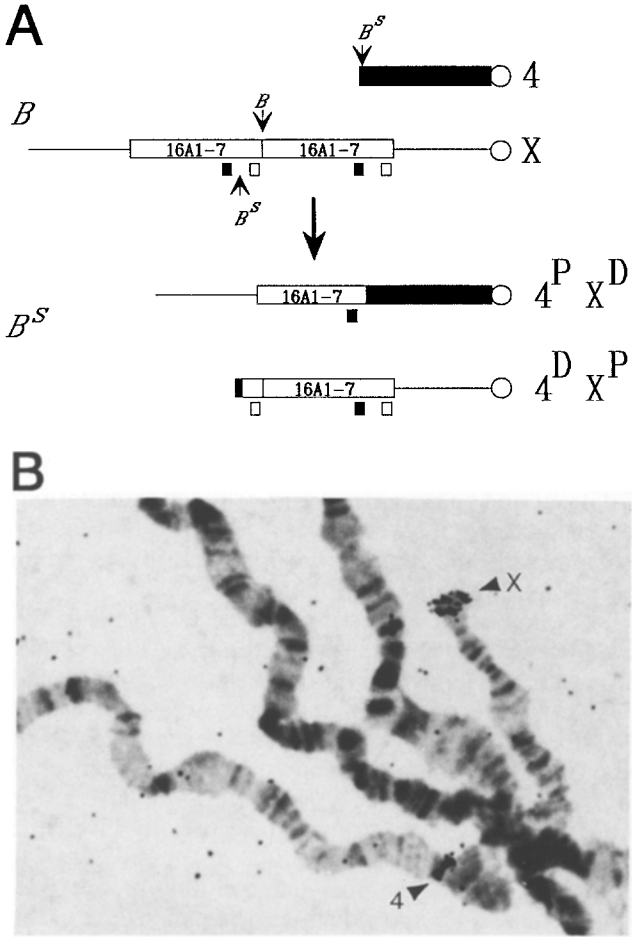

Fig. 3A, B. In situ hybridization to the polytene chromosomes of *T(1;4)B^S*. **A** Cartoon of hybridization to the polytene chromosomes of *B^S*. The X and 4 chromosome of *B* and *B^S* are drawn. The *small open box* represents a clone that is proximal to the *B^S* breakpoint and the *small black box* represents a clone that is distal to the *BS* breakpoint. A proximal clone will only hybridize to 4^DX^P, whereas a distal clone or one that spans the breakpoint will hybridize to both 4^DX^P and 4^PX^D. **B** *T(1;4)B^S* male probed with the distal 2.8 kb fragment from the lambda clone 16A7.B (Fig. 2). This clone, which is just distal to the *B^S* breakpoint, hybridizes to both halves of the *B^S* translocation. The *arrows* designate the hybridizations to 4^DX^P (X) and 4^PX^D (4)

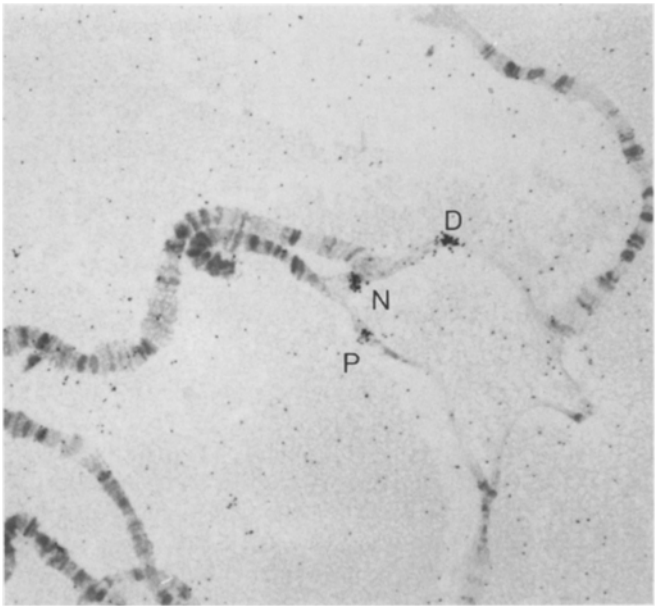

Fig. 4. In situ hybridization to the polytene chromosomes *B^{581}*. Chromosomes from a *T(1;3)B^{581}*/+female were probed with clone B$^+$.7b (Fig. 1). In this heterozygous translocation, the clone will hybridize to three distinct places, the normal X chromosome (N), and the proximal (P) and distal (D) components of the translocated X chromosome

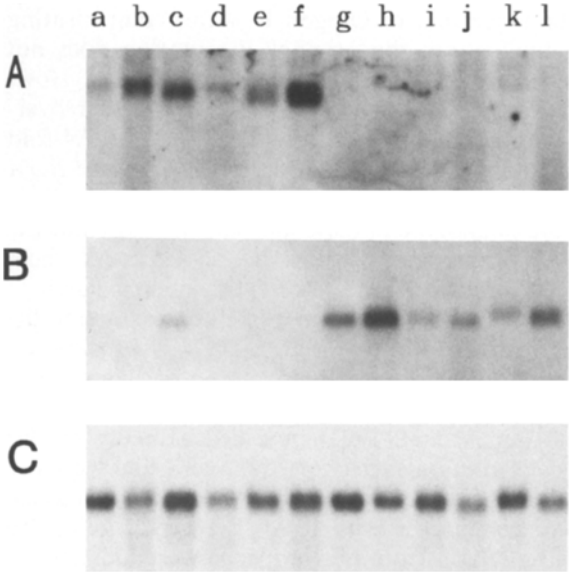

Fig. 5A–C. Northern blot of *BarA*, *BarH1*, and *ras* with 7.5 μg of poly(A)$^+$ RNA loaded per lane. The same filter was probed with **(A)** *BarA* – a 1.9 kb *Pst*I-*Xba*I fragment (Fig. 1); **(B)** *BarH1* – a 4.4 kb *Sal*I fragment (Fig. 1; one of the *Sal*I sites is from the lambda arm.); and **(C)** *ras* (Mozer et al. 1985). RNA in lanes a-f was from late third instar larvae and RNA in lanes g-l was from 1–3-day-old pupae. a, g, Oregon R; b, h, *B^i*; c, i, *R(B)^{hd3}*; d, j, *B*; e, k, *B^{85c15}*; f, l, *B^S*

Identification of two coding regions proximal to the B^{581} *breakpoint*

Genetic and molecular analyses of the *Bar* mutations indicated that the *Bar* gene had to be proximal to the *B* breakpoint. With this in mind, we searched for transcribed regions that were proximal to the most proximal *Bar* breakpoint, *B^{581}*. Using Northern blots and poly(A)$^+$ RNA from late third instar larvae and 1–3-day-old pupae, two coding regions were identified within 30 kb of the *B^{581}* breakpoint.

The first coding region was localized to a 1.9 kb *Pst*I-*Xba*I fragment, approximately 5 kb from the *B^{581}* breakpoint (Fig. 1). We have provisionally named this gene *BarA*. This gene encodes a 1.3 kb transcript which is expressed more highly in late third instar larvae, the time of cell death in the eye imaginal discs of the *B* mutant (Fristrom 1969; 1972), than in 1–3-day-old pupae (Fig. 5A). To determine if this transcript was affected in *Bar* mutants, a Northern blot of poly(A)$^+$ RNA from late third instar and 1–3-day-old pupae was probed with a *BarA* probe. The Northern filter was reprobed with *ras*

Table 1. *BarA* expression in late third instar

	Oregon R	B^i	$R(B)^{hd3}$	B	B^{85c15}	B^s
BarA/ras[a]	0.24	1.43	0.87	0.49	0.93	1.81
Standardized[b]	1.0	5.9	3.6	2.0	3.8	7.4

[a] The autoradiograms in Fig. 5 were scanned with a laser densitometer. The area for each band was taken as a measure of the transcript level. For each stock, the area for *BarA* was divided by the area for *ras* to give a relative expression value
[b] For each stock, the relative expression value *(BarA/ras)* was divided by the corresponding value for Oregon R

to control for differences in the amount of poly(A)$^+$ RNA in each lane (Fig. 5C). The genotypes examined were wild type (Oregon R), *B*, and four derivatives of *B*. Two of these derivatives, B^i and $R(B)^{hd3}$, are partial revertants of *B*, while B^{85c15} and B^S are more extreme derivatives of *B*. To evaluate the levels of *BarA* transcript in these mutants, the autoradiograms were scanned with a laser densitometer and normalized to the values of the *ras* transcript. In each case, there was an overproduction of the *BarA* transcript in the *Bar* mutants with respect to *ras* and Oregon R (Table 1). In evaluating the extent of the overproduction, one has to consider that all of these mutants contain a duplication for 16A1–7 and, thus, contain a duplication for *BarA*. The baseline level, assuming an equal contribution of the two copies of *BarA*, should be twice that of Oregon R. After compensating for the duplication, the original *B* mutation does not show an overproduction of the *BarA* transcript. However, all of the derivatives of *B* display a further overproduction ranging from 3.6-fold for $R(B)^{hd3}$ to 7.4-fold for B^S. Although these changes in the level of the *BarA* transcript do not correlate well with the severity of the mutant eye phenotype in these mutants, they do indicate that the chromosomal lesions associated with these mutations can affect the level of expression of *BarA*.

The second coding region maps 11 kb proximal to the *BarA* gene and 18 kb proximal to the B^{581} breakpoint. An examination of the restriction map of this gene in comparison with that of a previously identified gene, *BarH1* (Kojima et al. 1991), revealed a highly similar pattern of restriction sites. A partial nucleotide sequence of this region (Fig. 6) proved to be almost identical to the published sequence of *BarH1*. All 161 bp of the amino acid coding region from our sequence were identical to those of *BarH1*. The only difference was in the length of the intron. Our sequencing gave an intron of 134 bp whereas the previous report, which did not include the entire sequence of the intron, indicated an intron of 126 bp. These data identify the second coding region as *BarH1* and place the *BarH1* gene within the *Bar* region.

The *BarH1* gene is of interest because it is the *D. melanogaster* homologue of a gene in *D. ananassae* called *Om(1D)*. The *Om(1D)* gene has been postulated to be the homologue to the *Bar* gene (Tanda et al. 1989; Tanda and Corces 1991) and, consequently, the *BarH1* gene has been postulated to be the *Bar* gene (Kojima et al. 1991). The most compelling evidence that *BarH1* may be involved in the *Bar* position effects is provided by the results of heat shocks on a transformant carrying a *hsp70–BarH1* construct. This construct carries the promoter of *hsp70* and the coding region of *BarH1*. When transformants are heat shocked during the late third instar larval stage, *B* phenocopies are produced. Because *B* results in cell death in the eye imaginal disc during late third instar and because *B* is a gain-of-function mutation, the heat shock results were taken as evidence that *BarH1* is the *Bar* gene and that the *BarH1* transcript should be overproduced in third instar larvae.

We examined the expression of the *BarH1* gene in late third instar larvae and 1–3-day-old pupae (Fig. 5B). In third instar larvae, the *BarH1* transcript was not detected in wild type, *B*, or any of the derivatives of *B* except $R(B)^{hd3}$, a P element-induced partial revertant of *B*. In 1–3-day-old pupae, only B^i shows an overproduction of the *BarH1* transcript. $R(B)^{hd3}$, *B*, and B^{85c15} have lower amounts of the *BarH1* transcript, especially given the fact that *BarH1* is duplicated in these stocks (Table 2).

Given that *B* is a gain-of-function mutation, we expected to observe an overproduction or an alteration in the structure of the putative *Bar* transcript during the third instar larval stage. Of the two genes that we have identified, only *BarA* is significantly overexpressed in late third instar larvae. Given these results and the fact that *BarA* is located between the *Bar* breakpoints and *BarH1*, we propose that the *BarA* gene may actually be the *Bar* gene.

Table 2. *BarH1* expression in 1–3-day-old pupae

	Oregon R	B^i	$R(B)^{hd3}$	B	B^{85c15}	B^s
BarH1/ras[a]	0.45	1.36	0.18	0.41	0.19	0.96
Standardized[b]	1.0	3.0	0.4	0.9	0.4	2.2

[a] The autoradiograms in Fig. 5 were scanned with a laser densitometer. The area for each band was taken as a measure of the transcript level. For each stock, the area for *BarH1* was divided by the area for *ras* to give a relative expression value
[b] For each stock, the relative expression value *(BarH1/ras)* was divided by the corresponding value for Oregon R

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CTCGAGAAGTCCTTCGAGCGGCAGAAGTACCTTAGCGTTCAGGAGCGCCAGGAGCTGGCCCACAAGTTGGATCTGAGCGATTGCCAGGTGAAAACTTGGTACCAAAACCG  110

CAGgtgggttttaactttaatgatttttttaatttgtattagttttcaaagatgaacttaagaaaatacaataaaatctatacacatatattcttattgaaagtatactaa  220

agtttggtatctattatcattttgcagAACCAAATGGAAGCGCCAAACAGCAGTGGCCTGGAACTCCTCGCAGAG                                       295
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Fig. 6. Partial sequence of *BarH1*. The nucleotide sequence from the distal *Xho*I site in the *BarH1* probe (see Fig. 1) toward the proximal end of *BarH1* is shown. The amino acid coding regions are in *upper case* letters and the intron is in *lower case* letters. The *Xho*I site is *underlined*

Discussion

The *BarH1* gene has been proposed to be the *Bar* gene (Kojima et al. 1991). The major data to support this contention are the overproduction of the *BarH1* transcript in *B* pupae and the production of a *B* phenocopy by a *hsp70–BarH1* construct. We did not see the same overproduction of the *BarH1* transcript in pupae that was reported by Kojima et al. (1991), and cannot explain the differences between the two sets of results. Disregarding this discrepancy, it should be pointed out that an overproduction of *BarH1* in the pupal stage cannot explain the effect of *B* on eye development, because the time of action of *B* on the development of the eye imaginal disc is during late third instar (Fristrom 1969; 1972). If the *Bar* phenotype is caused by an overproduction of a transcript, one would expect to see this effect during the third instar larval stage when the *B* mutation is having its effect. During this stage, an overproduction of *BarH1* is not seen. Nevertheless, the experiments with the *hsp70–BarH1* construct suggest that overproduction of *BarH1* during the third instar larval stage can reduce the number of ommatidia.

Our results and earlier results (Kojima et al. 1991) do not demonstrate a clear overproduction of the *BarH1* transcript in third instar larvae, yet the heat shock results indicate that overproduction of the *BarH1* transcript can produce a reduction in the number of eye facets. One possible explanation for this discrepancy is that the *B* breakpoint may result in overproduction of the *BarH1* transcript only in the eye-antenna imaginal discs and that this overproduction cannot be detected in RNA from whole larvae. Also, because *B* is associated with cell death, the cells that are overproducing *BarH1* may be dying. This would make detecting the overproduction very difficult. In situ hybridization to the *BarH1* transcript in tissue sections may be required to see the effects of the *B* breakpoints on the *BarH1* transcript.

The large distance of *BarH1* from the *Bar* breakpoints and the location of the *BarA* gene between it and the *Bar* breakpoints raise the possibility that *BarH1* may not be the *Bar* gene. Evidence in favor of *BarA* as the *Bar* gene are its position with respect to the breakpoints and *BarH1* and its pattern of expression. Unlike *BarH1*, *BarA* is expressed at high levels in late third instar larvae, the time of action of the *B* mutation. Also, the data indicate that the *BarA* transcript is overproduced in various *Bar* mutations. If *BarH1* is the *Bar* gene, then the results with the *BarA* gene need to be explained.

The existing data indicate that the expression of *BarA* and *BarH1* are both affected by the *Bar* breakpoints and present the possibility that the *Bar* breakpoints affect the expression of many genes in the region of the breakpoints. If indeed there is only one *Bar* gene, then the effects of the *Bar* breakpoints on many genes may make the identification of the *Bar* gene very difficult. On the other hand, the *Bar*-eye phenotype may be much more complex then we previously envisioned and may involve the altered regulation of many different genes in the region.

What is clear, however, is that the *Bar* breakpoints can act over very large distances. The breakpoints of B^S and B^{581} span a distance of approximately 60 kb. There are several possible explanations for these phenomena. First, the breakpoints may be removing a distant "silencer," allowing the *Bar* gene to be expressed at higher levels and thus resulting in the *Bar* mutant phenotype. Alternatively, the breakpoints may be introducing enhancer sequences which increase *Bar* expression. We favor the second explanation over the first, based on the existence of secondary mutations near the *B* breakpoint. Four independent insertions of transposable elements near the *B* breakpoint result in a decrease in the mutant eye phenotype (Tsubota et al. 1989). If the original *B* breakpoint has removed a silencer, then the insertions of transposable elements in the modifiers of *B* would have to introduce silencers to produce their effects. Three of the insertions are P elements, which have not been shown to contain long-range silencer or enhancer sequences. We think that it is more likely that the *B* breakpoint has introduced an enhancer sequence, whose action is being dampened by the insertion of transposable elements.

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