The Cloning of the *Bar* Region and the *B* Breakpoint in *Drosophila melanogaster*: Evidence for a Transposon-Induced Rearrangement

Stuart I. Tsubota,*[,1] Daniel Rosenberg,* Helen Szostak,* Deborah Rubin[†,2] and Paul Schedl[†]

**Department of Biology, University of Michigan, Ann Arbor, Michigan 48109 and [†]Department of Biology, Princeton University, Princeton, New Jersey 08544*

Manuscript received July 15, 1988
Accepted for publication April 27, 1989

ABSTRACT

We have cloned the *B* breakpoint in *Drosophila melanogaster* using DNA from a P-M-induced revertant of *B*, which has a *P* element inserted at the *B* breakpoint. The analysis of the *B* DNA reveals that there is a transposable element, *B104*, right at the breakpoint. This suggests that this element may have been involved in the generation of the *B* breakpoint and the associated tandem duplication. One possible mechanism to generate the *B* duplication is a recombination event between two *B104* elements, one at 16A1 and the other at 16A7. DNA sequencing data of the junctions of the *B104* element support this model. Four partial revertants of *B* are the result of insertions of transposable elements very close to the *B* breakpoint. This supports the hypothesis that the breakpoint is the cause of the *B* mutation. The clones from *B* were used to isolate wild-type clones from 16A1, the location of the *Bar* gene. Four rearrangement breakpoints associated with various *Bar* mutations map within a 37-kb region, suggesting that the *Bar* gene is very large.

MUTATIONS which have been isolated at the *Bar* locus of *Drosophila melanogaster* are all associated with a chromosomal rearrangement with one breakpoint in the 16A1-2 region of the *X* chromosome. All of these mutations are gain-of-function mutations and, with the exception of B^{bd}, are semidominant (LINDSLEY and GRELL 1968). No point mutations have ever been isolated at the *Bar* locus. This statement is strengthened by the fact that a large number of mutation screens utilizing a variety of mutagens have been performed on *D. melanogaster*, and the fact that *Bar* mutations are semidominant, easily seen, and viable.

The first *Bar* mutation, *B*, was isolated as a single male (TICE 1914). Most of the information available on the *Bar* gene is based on studies of this allele. Homozygous or hemizygous *B* flies have narrow eyes in which the facet number has been reduced from the wild-type number of approximately 780 to 70–80. The *B* mutation is associated with a tandem duplication of 16A1-16A7 (BRIDGES 1936, MULLER, BROKOFYEVA-BELGOVSKAYA and KOSSIKOV 1936) although it is not the duplication of the DNA *per se* that is responsible for the *B* mutation (SUTTON 1943). This conclusion is supported by the fact that *B* is the only *Bar* mutation associated with a duplication of 16A1-16A7. The other mutations are inversions and translocations with one breakpoint in the region 16A1-2. Thus all of the *Bar* mutations have in common a breakpoint within the 16A1-2 region. This led Sutton to postulate that the *Bar* mutations were position-effect mutations caused by the chromosomal breakpoints. While the extra dose of 16A1-16A7 is not responsible for the mutant phenotype of *B*, it has been involved in many of the unusual aspects of this allele. *B* is an unstable mutation, reverting to wild type at a frequency of 1 in 1000 to 2000 and mutating to a more extreme variant at a similar frequency (ZELENY 1919, 1921). The instability was shown to be restricted to females and to be associated with the recombination of flanking markers (STURTEVANT 1925). To explain his findings, STURTEVANT proposed that unequal crossing-over was responsible for *B* instability. *B* contained a mutant allele, whereas wild type contained no mutation. Unequal crossing-over either resulted in a loss of the mutant allele and thus a wild-type phenotype or a duplication of the mutant allele and a more severe mutant phenotype. *B* was later shown to be a tandem duplication of 16A1-16A7 and it was proposed that the unequal crossing-over was occurring within the duplicated region (BRIDGES 1936; MULLER, PROKOFYEVA-BELGOVSKAYA and KOSSIKOV 1936). Wild-type revertants would lose the duplication while the more severe variants would gain another copy of the duplication.

These features of the *Bar* gene, dominant *cis*-acting position-effect mutations associated with chromosomal rearrangements and the lack of point mutations argue that a disruption of the chromosome in the

[1] To whom correspondence should be addressed.
[2] Present address: University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104.

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region of 16A1 is necessary for the production of the *Bar* mutant phenotype. It was expected that these genetic features would be reflected by some unusual features at the DNA level. With this in mind and with the hope that a molecular characterization of *Bar* would help explain its genetic properties, we proceeded to clone the mutant *B* allele and the wild-type *Bar* gene. The approach that was used is as follows. Since *B* is a dominant gain-of-function mutation, it can be reverted by mutations which eliminate *Bar* function (SUTTON 1943). Sutton used this approach to isolate X-ray-induced revertants of *B*. It should also be possible to use hybrid dysgenesis to revert *B*. P-M hybrid-dysgenesis-induced revertants of *B* should contain a *P* element at the mutant *B* allele, thus enabling the cloning of the *Bar* gene by transposon-tagging (BINGHAM, LEVIS and RUBIN 1981). We used this approach to clone the *B* breakpoint and then, using subclones from the breakpoint clones as probes, isolated the wild-type 16A1 and 16A7 regions. We found a transposable element at the *B* breakpoint and give evidence that it generated the *B* breakpoint via unequal crossing-over. These results and our initial analysis of the wild-type *Bar* region are presented in this paper.

MATERIALS AND METHODS

Stocks:

1. *B* = *Dp(1;1), B*. Two different *B* strains were used. The first strain, *Basc* (also known as Muller-5), was the M strain used in the hybrid-dysgenesis crosses and the progenitor chromosome for the P-M hybrid-dysgenesis-induced revertants of *B*. The second strain, *FM7*, was used to construct a *B* DNA library to jump from 16A7 into 16A1.
2. $\pi 2$. This is the P strain that was used in the hybrid-dysgenesis crosses to isolate *B* revertants.
3. *C(1)RM*, y^2. This is a compound-*X* chromosome which allows for the recovery in males of mutations which were induced in their fathers.
4. $R(B)^{hd1}$, $R(B)^{hd2}$, $R(B)^{hd3}$. These are three hybrid-dysgenesis-induced revertants of the *B* allele in *Basc* that were isolated in the present study.
5. B^3. This is a spontaneous partial revertant of *B* (LINDSLEY and GRELL 1968).
6. B^{M1} = *In(1)*, B^{M1}. B^{M2} = *In(1)*,B^{M2}. B^{bd} = *T(1;2)*,B^{bd} (LINDSLEY and GRELL 1968).
7. *Rev-r*$^{16-4}$ (TSUBOTA and FRISTROM 1981). This strain was used to construct a "wild-type" DNA library.

For a more complete description of the *Bar* mutations, see LINDSLEY and GRELL (1968).

Mutation scheme: *B* is a dominant gain-of-function mutation. It can be reverted by mutations that inactivate it. This rationale was used to isolate hybrid-dysgenesis-induced revertants of *B*. *Basc* females were mated to π-2 males. The *Basc* male progeny were then crossed to *C(1)RM*, y^2 females and the male progeny were examined for *B* revertants. Any *B* reversions generated in the germ line of the F_1 males would be seen among the F_2 males.

***In situ* hybridization:** Salivary glands were dissected and squashed in 45% acetic acid and were prepared for hybridization (BINGHAM, LEVIS and RUBIN 1981). Both ^{3}H-labeled probes and biotin-labeled probes were used. Biotin-labeled probes were synthesized and processed as described in the manual from Enzo Biochem. ^{3}H-Labeled nucleotide triphosphates were obtained from ICN.

Southern analysis: Agarose and all restriction endonucleases were obtained from Boehringer Mannheim; nitrocellulose and nylon filters were obtained from Schleicher & Schuell; ^{32}P-labeled nucleotide triphosphates were obtained from ICN. Southern analysis was performed as previously described (TSUBOTA and SCHEDL 1986).

Genomic libraries: The initial library, which was used to clone the *B* allele, was made from DNA from $R(B)^{hd3}$ one of the hybrid-dysgenesis-induced revertants of *B*. Southern analyses had shown that there were very few *P* elements in this strain and that they were all contained in large *Bam*HI fragments, which could be easily cloned into a λ vector. A genomic library was made from *Bam*HI fragments cloned into the *Bam*HI cloning site of EMBL3. This library was screened for *P*-element-containing phage using the plasmid $\pi 25.1$ as a probe (O'HARE and RUBIN 1983). *Bam*HI libraries were also made from *Basc* and B^3. These libraries were screened with probe *a* (Figure 1) to isolate the respective clones. A wild-type *Bar* library was made from *Rev-r*$^{16-4}$ DNA partially digested with *Mbo*I. While this strain contains certain mutations, it is wild type for the *Bar* region. Large molecular weight fragments were isolated from a NaCl gradient (PIRROTTA 1986) and cloned into the *Bam*HI cloning site of EMBL3. The library was amplified and used for the cloning of the wild-type 16A1 region. The same procedure was used to construct a library from *FM7,B*. In addition, some wild-type clones from the 16A7 region were obtained from a Canton S library (MANIATIS *et al.* 1978).

DNA sequencing: DNAs for sequencing were subcloned into pBluescript SK(+/−). Single-stranded DNA was prepared for dideoxy sequencing. DNA was sequenced using Sequenase and the protocol from United States Biochemicals.

RESULTS

Revertants of *B*: We decided to clone the *Bar* gene by first isolating *P*-element insertions in *Bar* and then using a *P*-element probe to isolate *Bar* clones. To do this, crosses were performed to isolate hybrid-dysgenesis-induced revertants of *B*. The rationale was that each revertant should contain a *P* element in the mutant copy of the *Bar* gene in the *Basc* stock. The crosses were performed such that all of the mutations would be generated in males. This eliminated the isolation of revertants due to unequal crossing over, the major cause of *B* reversion (STURTEVANT 1925), since this type of event normally occurs very rarely in males.

No complete revertants of *B* were isolated. However, four partial revertants were isolated from 2122 chromosomes. This is approximately 1 mutation per 500 chromosomes, which is a very high mutation rate for insertional mutagenesis. It is possible that the *B* allele in the *Basc* chromosome is a hot spot for *P*-element insertions. It is also interesting that all of the revertants were partial revertants. These males had eyes that were about three times the size of eyes of *B*

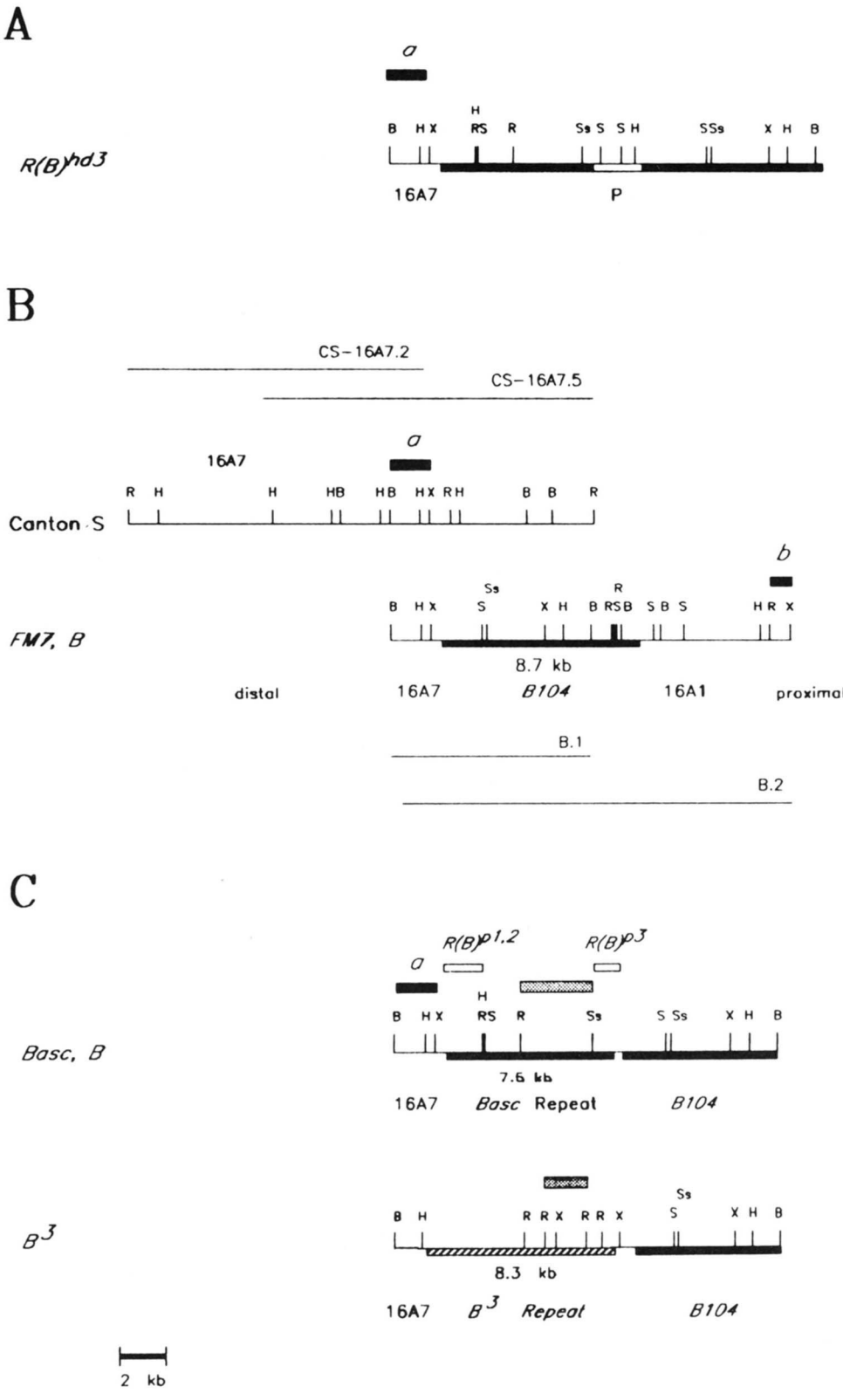

FIGURE 1.—Clones of the *B* breakpoint and wild-type 16A7 region. In all of the restriction maps, the regions designated by probe *a* are aligned to show the homology between the various phage DNAs. (A) $R(B)^{hd3}$. This is the restriction map of the *Bam*HI clone which was isolated in the initial *B* cloning experiment. The solid bar represents repetitive DNA. The open bar is the *P* element in this clone. The single line is the single-copy region. Probe *a* is shown as a solid bar above the map. B = *Bam*HI, H = *Hin*dIII, R = *Eco*RI, S = *Sal*I, Ss = *Sst*I, X = *Xho*I. (B) Wild-type and *FM7,B*. The restriction map of the wild-type 16A7 region from Canton S is shown represented by two phages, CS-16A7.2 and CS-16A7.5. The map of the *B* breakpoint is drawn underneath the wild-type map with the 16A7 regions of each clone aligned vertically. The *FM7* map is represented by two λ clones, B.1 and B.2. The *B104* element at the *B* breakpoint is drawn as a solid bar. Probe *a* from 16A7 and probe *b* from 16A1 are drawn as solid bars above the maps. The distal and proximal orientation of the clones on the *X* chromosome is indicated. (C) *Basc, B* and B^3. The restriction maps of the *Bam*HI clone of the *B* breakpoint in *Basc* and in B^3 are shown. The *B104* element is drawn as a solid bar. The *Nijinski* element in *Basc* is drawn as a solid bar and the *Isadora* element in *B* is drawn as a striped bar. Probe *a* is the same as in A and B. The positions of the insertions in $R(B)^{hd1}$, $R(B)^{hd2}$, and $R(B)^{hd3}$ are indicated by open bars above the map of *Basc*. The fragments that were used as probes for the *Nijinski* and *Isadora* elements in Figure 3 are drawn as stippled bars above the restriction maps.

males, but still much smaller than normal eyes.

Cloning of the *B* breakpoint: One of the four revertants was lost. Of the remaining three, $R(B)^{hd3}$ was shown to contain very few *P* elements, one of which had inserted into the *Bar* region of the polytene chromosomes. A genomic library was made from a total *Bam*HI digest of $R(B)^{hd3}$ DNA. Each clone was examined by *in situ* hybridization to Canton S salivary gland chromosomes to determine its cytogenetic position. One clone showed hybridization to the *Bar* region, 16A, however, this clone also hybridized to 50 to 100 additional sites due to the presence of a

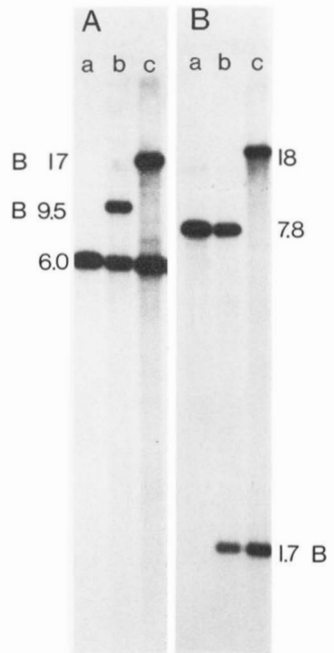

FIGURE 2.—Southern blots with probes from 16A7 and 16A1. DNA was digested with *Bam*HI, electrophoresed and transferred to nylon filters. In each blot the DNA samples are the following: (a) Oregon R, (b) *FM7, B,* (c) *Basc, B.* The bands that correspond to the *B* bands are labeled with a B. (A) Probe *a* from 16A7 (Figure 1). (B) probe *c* from 16A1 (Figure 5).

repeated, dispersed element within the clone. It was assumed that part of the clone would be single-copy DNA from *Bar*. To test this hypothesis, regions of the recombinant phage were subcloned into the plasmid vector, pUC 18, and each subclone was used as a probe for genomic Southern blots to determine its copy number. Almost all of the Drosophila DNA in the phage was repetitive. However, a 1.8-kb *Bam*HI-*Xho*I fragment was isolated which was entirely single-copy DNA (Figure 1A).

This fragment, which will be referrred to as probe *a*, was used to examine DNA from a wild-type strain, Oregon R, and two *B* strains, *FM7* and *Basc.* In Southern blots, Oregon R DNA gives a *Bam*HI fragment of 6.0 kb (Figure 2A, lane a). This band by definition is the wild-type band. Both *FM7-B* (Figure 2A, lane b) and *Basc-B* (Figure 2A, lane c) give the wild-type band, but each gives a second larger band of 9.5 kb and 17 kb, respectively. The fact that two bands are seen in *Basc* and *FM7* indicates that probe *a* is from DNA contained within the *B* duplication. One of the bands is from one copy of the duplication, while the other band is from the other copy. Since our λ clone should contain part of the mutant *B* allele, and since it has been hypothesized that the breakpoint of the *B* duplication is responsible for the mutation, we speculated that the second bands seen in *Basc* and *FM7* were fusion fragments of 16A1 and 16A7 from the *B* breakpoint. If this were true, then the original λ clone of $R(B)^{hd3}$ must contain DNA from the *B* breakpoint and probe *a* must be from either 16A1 or 16A7.

In order to test this hypothesis and to examine more closely the molecular differences between the wild-type and mutant bands that were seen in the Southern blot, probe *a* was used to isolate recombinant λ clones from *Basc-B* and *FM7-B,* the two *B* stocks used in Figure 2, and from a Canton S library (MANIATIS *et al.* 1978). In addition probe *a* was used to isolate clones from a library made from B^3, a spontaneous partial revertant of *B* (LINDSLEY and GRELL 1968). The clones were restriction mapped and compared to each other (Figure 1, B and C). First of all it can be seen that the DNA from *FM7* contains a repetitive element (Figure 1B). From the restriction map, this element has been identified as a *roo* or *B104* element (SCHERER *et al.* 1982). The orientation of the *B104* element as we have drawn it is 5′ to the left and 3′ to the right. The presence of this element explains the second band of *FM7* that was seen in the genomic Southern blot (Figure 2A, lane b). This band is created by cutting at the *Bam*HI site outside of the *B104* element and at a *Bam*HI site within the *B104* element. The homologous DNA from *Basc* also contains the *B104* element, but has acquired a second insertion very near the left end of the *B104* element (Figure 1C). This second insertion has been shown to be a repetitive element by genomic Southern blots (data not shown) and *in situ* hybridization to polytene chromosomes (Figure 3A). This element is present in the chromocenter and in about 20 copies in the euchromatic arms of the wild-type strain, Oregon R. The restriction map of this element does not coincide with the maps in a recent compilation of transposable elements in *Drosophila melanogaster* (FINNEGAN and FAWCETT 1986). We have named this element, *Nijinski.* This second insertion is the reason for the 17-kb fragment seen in the genomic Southern blot of *Basc* DNA (Figure 2A, lane c). The presence of this element does not affect the *Bar* phenotype, as the size of the eyes of *FM7* and *Basc* flies is the same.

B^3 also contains the *B104* element that is present in *FM7* and *Basc.* It does not contain the *Nijinski* element found in *Basc,* but it does contain a second insertion which, like the *Nijinski* element, is inserted outside of the 5′ end of the *B104* element. This element is present at eight sites in the euchromatic chromosomal arms but is not present in the chromocenter of Oregon R (Figure 3B). Since this element also does not appear to be related at the level of restriction mapping to any previously identified elements, we have given it the name *Isadora.* While it cannot be proven at this time, we believe that this *Isadora* element is responsible for the partially revertant phenotype of B^3.

All three of the hybrid-dysgenesis-induced partial revertants of *B* were generated in the *Basc* chromo-

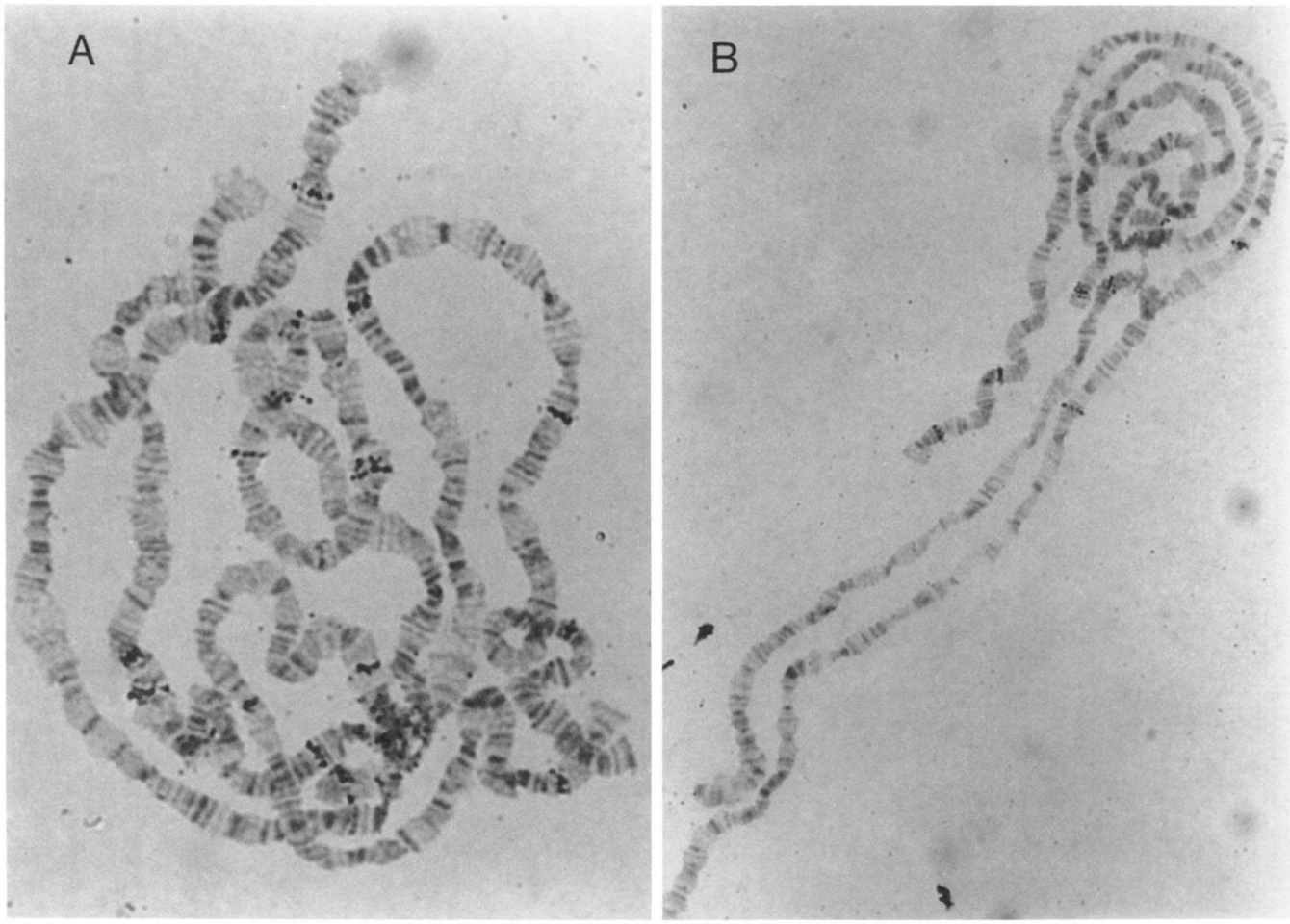

FIGURE 3.—*In situ* hybridization of *Nijinski* and *Isadora* elements. ^{3}H-Labeled probes for each element were hybridized to salivary gland polytene chromosomes of the strain, Oregon R. The DNA fragments that were labeled are designated as stippled bars in Figure 1C. (A) *Nijinski* element. (B) *Isadora* element.

some. All of them contain insertions into the *Nijinski* element next to the *B104* element (Figure 1C). Given the fact that the revertants were all generated by hybrid dysgenesis, the insertions are most likely *P* elements. Since four partial revertants of *B* (B^3, $R(B)^{hd1}$, $R(B)^{hd2}$, and $R(B)^{hd3}$) are all associated with insertions of transposable elements into the same region, the clones of *FM7* and *Basc* must contain part of the *B* allele.

We had previously speculated from the results of the Southern blots (Figure 2A) that not only had we cloned part of the *B* allele, but that we had cloned the *B* breakpoint itself. A comparison of the restriction maps of the Canton S and *FM7* clones supports this contention (Figure 1B). If the *B104* element were a simple insertion within 16A7, then the DNA on either side of it would normally be contiguous. However, only the DNA to the left of the *B104* element matches the wild-type DNA. The restriction map of the DNA to the right of the *B104* element in *FM7* is different from what should be the corresponding region in the Canton S DNA. This implies that the sequences on either side of the *B104* element are from different positions in the genome. A plausible explanation for this observation is that the *B104* element is right at the *B* breakpoint, that is the junction of 16A7 and 16A1.

To test this hypothesis, DNA from either side of the *B104* element was hybridized to the polytene chromosomes to localize their positions. One probe should hybridize to 16A1, whereas the other probe should hybridize to 16A7. This turns out to be the case. DNA to the left of the *B104* element hybridizes to the proximal end of 16A or 16A7 (Figure 4A), while DNA to the right of the *B104* element hybridizes to the distal end of 16A or 16A1 (Figure 4B).

Structure of the *B* breakpoint and the 16A1 and 16A7 regions: The data indicate that the structure of the *B* breakpoint is as diagrammed in Figure 1B. A *B104* element separates the two copies of the duplication such that the order on the chromosome is telomere . . . 16A1-16A7/*B104*/16A1-16A7 . . . centromere. As we have diagrammed the chromosome, the *B104* element is between the duplicated regions

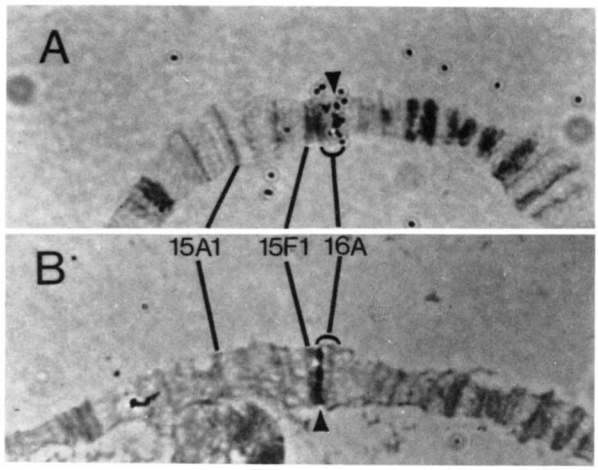

FIGURE 4.—*In situ* hybridization of 16A7 and 16A1 clones to wild-type polytene chromosomes. Positions of hybridization are marked with arrowheads. (A) Probe *a*. This picture shows hybridization to the proximal side of the 16A region, which localizes probe *a* to 16A7. A ^{3}H-labeled probe was used, so the hybridization appears as black silver grains. 16A begins with a heavy doublet band and ends with a lighter but distinct dotted band. The positions of the 16A region, and bands 15A1 and 15F1 are indicated. (B) Clone B^{+}.4a. In this *in situ* hybridization a biotin-labeled probe was used, so the hybridization appears as a dark band. Hybridization is seen on the distal side of 16A, localizing B^{+}.4a to 16A1.

and is not part of the region that is duplicated. However, it is possible that the *B104* element is part of the duplicated DNA. If this were the case then the order on the chromosome would be either *B104* 16A1-16A7/*B104* 16A1-16A7 or 16A1-16A7 *B104* 16A1-16A7 *B104* or *B104* 16A1-16A7/*B104*/ 16A1-16A7 *B104*. We examined these possibilities with genomic Southern blots, using a probe from 16A7, probe *a*, for one blot (Figure 2A) and a probe for 16A1, probe *c* (Figure 5), for another blot (Figure 2B). The DNA was digested with *Bam*HI, which cuts within the *B104* element and, therefore, allows us to assay for its presence. If the *B104* element is present at the normal 16A1 region (*B104* 16A1-16A7/*B104* 16A1-16A7), then only one band will be seen when probe *c* is used. Likewise, if the *B104* element is present at the normal 16A7 region (16A1-16A7 *B104*/16A1-16A7 *B104*), then a single band will be seen when probe *a* is used. On the contrary, if the *B104* element is absent from the wild-type 16A1 or 16A7 region, then two bands will be seen, a wild-type band and a *B* band. Probe *a* hybridized to two bands in the Southern blot of the *B* DNAs (Figure 2A, lanes b and c), the wild-type 6.0-kb band and a fusion *B* band. This result indicates that the *B104* element is not present in the wild-type 16A7 region. This is also the case for the 16A1 region (Figure 2B, lanes b and c). *FM7* contains the wild-type band and the smaller 1.8-kb fusion band. *Basc* also contains the fusion band but in this case contains a larger wild-type band. The cause of this polymorphism in the wild-type 16A1 region has not yet been determined. Together these data show that the *B104* element is present at the *B* breakpoint and is absent in the corresponding positions in the normal 16A1 and 16A7 regions.

Origin of the *B* breakpoint: We have shown that the *B* mutation has a *B104* element at or very near the duplication breakpoint. The presence of this element suggests that it may have had a role in the generation of the breakpoint. One possible mechanism is a recombination event between a *B104* element at 16A7 in one homolog and one at 16A1 in the other homolog (Figure 6A). This produces a tandem duplication in one chromosome and the corresponding deletion in the second chromosome. This mechanism for unequal exchange has been shown to occur within the *white* region (GOLDBERG *et al.* 1983; DAVIS, SHEN and JUDD 1987) and has actually been proposed to explain the origin of the *B* duplication (GOLDBERG *et al.* 1983, GREEN 1985).

Another possibility, however, is that the *B104* element at the *B* breakpoint may represent an insertion event independent of the generation of the breakpoint. At the DNA level, this model can be distinguished from the unequal crossing-over model. If the *B104* element inserted near the breakpoint, it would be unlikely that the site of insertion would be exactly at the junction of 16A7 and 16A1. Also, if its presence is the result of an insertion event, the *B104* element would be flanked by the characteristic 5-bp site duplication (SCHERER *et al.* 1982). On the other hand, if the presence of the *B104* element is the result of a recombination event, the element will be exactly at the junction of 16A7 and 16A1 and, more important, it will not be flanked by a 5-bp site duplication. Instead, one end will contain a sequence from 16A7 and the other end will contain a sequence from 16A1. To test these models, the left and right junctions of the *B104* element at the breakpoint were sequenced, as were the corresponding wild-type regions in 16A7 and 16A1 (Figure 6, B and C). The sequences at the junctions were then compared to the published *B104* sequence and to the sequences of the corresponding wild-type regions to determine the exact positions of the junctions. The right end of the *B104* element, which corresponds to the 3′ end of the element, is joined to 16A1. There is one difference between the *B B104* sequence and the published sequence. The sequence CA is found instead of TT at positions 423 and 424 of the published sequence. These basepairs are boxed. The sequence at the left junction is more complicated. The 5′ end of the *B104* element is joined to a 9-bp sequence which is not present in the wild-type 16A1 and 16A7 sequences that are in the immediate vicinity of the *B* breakpoint. These 9 bp separate the *B104* element and the 16A7 sequence. The same sequence at the 5′ junction is seen in both

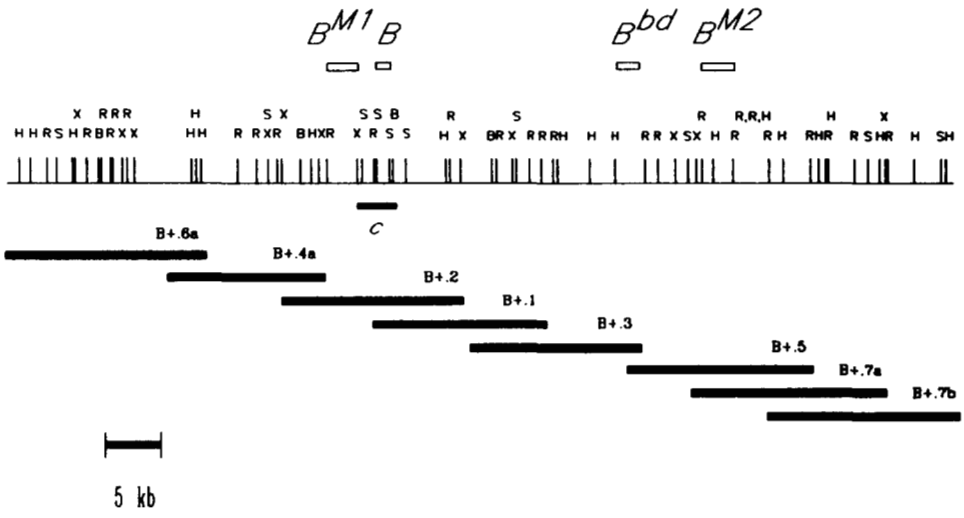

FIGURE 5.—16A1 chromosome walk. The λ clones isolated in the walk are indicated as solid bars under the restriction map. Probe *c* used for the Southern blot (Figure 2B) is also shown as a solid bar under the map. The positions of *Bar* breakpoints within the cloned region are indicated as open bars above the map. B = *Bam*HI, H = *Hin*dIII, R = *Eco*RI, S = *Sal*I, X = *Xho*I.

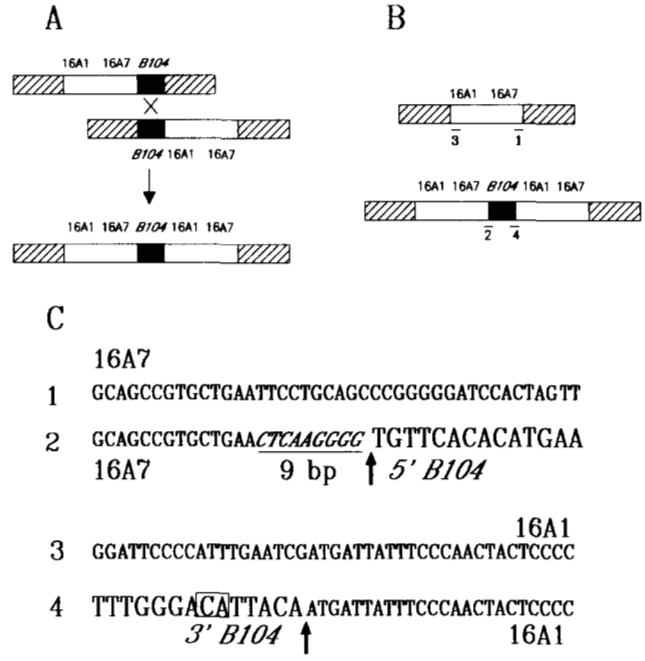

FIGURE 6.—Recombination model for the origin of the *B* breakpoint. (A) Recombination between two *B104* elements. In this model a recombination event has occurred between a *B104* element at 16A1 and one at 16A7 to generate the *B* duplication. (B) Diagram of the chromosomal regions that were sequenced. (C) Sequence data. The junctions of the *B104* element and the corresponding wild-type regions were sequenced. Arrows mark the junctions of the *B104* element and either 16A7 or 16A1. The 9 bp of unknown origin are underlined and italicized. The two nucleotides in the 3′ end of the *B104* element which differ from the published sequence are boxed. In the published sequence these positions are occupied by Ts.

FM7 and *Basc*. The origin of these 9 bp is not known. It is possible that they represent a polymorphism in the 5′ end of *B104* elements or a polymorphism in the 16A7 region. Whatever the origin of these 9 bp, two facts are clear from the sequences. First, the *B104* element is at the *B* breakpoint. The order of the DNA sequence at the *B* breakpoint is "16A7-9-bp of unknown origin-*B104*-16A1." If not for the ambiguity caused by the 9 bp, the *B104* element would be exactly at the breakpoint separating 16A7 from 16A1. Second, the *B104* element at the breakpoint is not flanked by a 5-bp site duplication which is characteristic of a *B104* transposition event. The lack of the site duplication supports the supposition that the presence of the *B104* element is not the result of a normal transposition event. Together the data strongly support the hypothesis that a recombination event between two *B104* elements generated the *B* breakpoint and the *B* duplication.

Cloning of the wild-type 16A1 region: Since it had been demonstrated genetically that *Bar* mapped to 16A1 (SUTTON 1943), we decided to perform a chromosomal walk in a wild-type 16A1 region. The walk was initiated with probe *b*, a DNA fragment to the right of the *B104* element at *B* (Figure 1B). Approximately 70 kb have been cloned (Figure 5). If this DNA contained the *Bar* gene, we expected that the other *Bar* mutations would map within the region. DNA from the walk was used as probes for genomic Southern blots to identify the positions of the breakpoints of the *Bar* mutations. To date, the breakpoints of four mutations have been localized, *B*, B^{M1}, B^{M2}, and B^{bd}. The breakpoints span a region of approximately 37 kb, indicating that the *Bar* gene may be very large.

DISCUSSION

The origin of the *B* mutation: The analyses of clones of the *B* breakpoints from *FM7* and *Basc* show that there is a *B104* element at the breakpoint. The *Basc* chromosome contains a second element, a *Nijinski* element, inserted in 16A7 very close to the 5′ end of the *B104* element (Figure 1C). Without obtaining the progenitor chromosome to *B*, it is impossible to determine if the *Nijinski* element was present in the original *B* chromosome and was lost in the progenitor chromosome of *FM7* or if the *Basc* chromosome obtained the element subsequent to the formation of the

B breakpoint. Since *B* was isolated in 1914, the identity of its progenitor is uncertain. However it should be mentioned that we have examined other *B* chromosomes with Southern blots and have never seen evidence for the presence of the *Nijinski* element next to the *B104* element (data not shown). These *B* chromosomes include an uninverted *B* chromosome, *BB, Binsc, C1B, FM3, FM4, FM6,* B^{i}, B^{3}, and B^{36d}. Three of these mutations *BB,* B^{i}, and B^{3} were isolated very shortly after the isolation of *B* and therefore represent three very early isolates of chromosomes containing the *B* breakpoint. While these data do not prove that the progenitor chromosome to *B* did not have the *Nijinski* element next to the *B104* element, the simplest explanation is that the *Nijinski* element was not present in the original *B* chromosome and was most likely obtained by the *Basc* chromosome either shortly before or after its construction. Whatever the origin of the *Nijinski* element in *Basc,* its presence is not necessary for the *Bar*-eye phenotype, nor does its presence affect the mutant phenotype. The *Bar*-eye phenotype of *Basc* flies is identical to that of *FM7* flies.

The DNA sequence data of the *B* breakpoint support the hypothesis that the *B104* element generated the *B* breakpoint. One possible model for the involvement of the *B104* element in the generation of the *B* breakpoint is a recombination model (Figure 6A). In this model recombination between two *B104* elements, one at 16A1 and one at 16A7, generated the *B* breakpoint and the *B* duplication. This recombination model for the origin of *B* had been suggested previously, based on data on unequal exchange between *BEL* elements at the *white* locus (GOLDBERG *et al.* 1983; GREEN 1985). In this case a recombination event between one *BEL* element in one position in one chromosome and another *BEL* element in a different position in the other homolog is responsible for the production of a deficiency and the complementary duplication in the *white* region (GREEN 1959, 1963). In a similar situation within the *white* region (JUDD 1959, 1961a), unequal recombination between different *B104* elements has produced the reciprocal duplications and deficiencies (DAVIS, SHEN and JUDD 1987). In the case of the *BEL* elements the distance separating them was greater than 60 kb and in the case of the *B104* elements the distance was 30 kb.

While these duplications and deficiencies are on the order of one or two chromosomal bands in length and involve only one gene, unequal crossing-over has been shown to generate much larger duplications and deficiencies (JUDD 1961b; GELBART and CHOVNICK 1979). In the former case the duplication/deficiency products were on the order of 15 chromosomal bands. These products were repeatedly produced by unequal exchange and, importantly, by only certain chromosomal combinations. These data were taken to indicate that these exchange products were also the result of recombination between repetitive elements (DAVIS, SHEN and JUDD 1987). When the products of unequal exchange in the *rosy* region could be selected for, very large duplications of one to two numbered units on the polytene map were isolated (GELBART and CHOVNICK 1979).

The data on the generation of duplication/deficiency products by unequal exchange and the data on the role of transposable elements at *white* and *Bar* in unequal exchange suggest that repetitive elements may play an important role in the duplication and deletion of large chromosomal segments. While the role of transposable elements in unequal crossing-over has not been demonstrated in all cases, it is clear that unequal crossing-over is a constant source of tandem duplications and deficiencies. In the studies on unequal exchange in the *rosy* region, the authors extrapolated their data to include the whole genome (GELBART and CHOVNICK 1979). They concluded that one unequal crossing-over event occurs once per genome in approximately every 500 meioses.

The importance and frequency of these events in chromosomal evolution is difficult to assess, since many of the events will go undetected because they do not produce a visible phenotype or because they are lethal and may be lost from the population. However, in some cases a dominant phenotype may be produced which will allow for the detection of the deficiency or the duplication. In *D. melanogaster* many dominant mutations are associated with a loss-of-function, *e.g., Notch, Ultrabithorax, Plexate, Lyra, Star,* whereas others are associated with a gain-of-function, *e.g., Stubble, Curly, Beadex, Hairy wing, Bar* (LINDSLEY and GRELL 1968). Some of the alleles of *Beadex, Hairy wing,* and *Bar* are associated with duplications and it has been proposed that unequal crossing-over between repetitive elements may have generated them (GOLDBERG *et al.* 1983; GREEN 1985). Similarly, recombination between repetitive elements may explain the spontaneous origin of many of the dominant loss-of-function mutations that are associated with deficiencies.

Nature of the *B* mutation and the *Bar* gene: SUTTON (1943) concluded from her examination of the cytogenetics of X-ray-induced revertants of *B* and of other *Bar* mutations that the *Bar* gene mapped to the 16A1-2 region. With respect to the *B* mutant, this means that the *Bar* phenotype is not the result of a dose effect caused by the duplication, but is most likely the result of a position effect caused by the juxtaposition of 16A7 to 16A1. The hybrid-dysgenesis-induced revertants confirm this interpretation. All three of the revertants are insertions very close to the breakpoint. Another partial revertant of *B,* B^{3}, also contains an additional insertion near the breakpoint

(Figure 1). All of these insertions are probably alleviating the position effect caused by the breakpoint. The fact that all of these insertions result in only a partial reversion of the *Bar*-eye phenotype indicates that they reduce the mutant *B* expression but do not totally eliminate it. A total inactivation of the *B* mutation would result in a complete reversion. These results are consistent with the interpretation that the *P* elements and the *Isadora* element are not inserting within a coding region of the *Bar* gene, but are inserting within a noncoding region, possibly involved in the control of *Bar* expression. This interpretation is supported by the fact that *P* elements often insert within 5′ control regions of genes (VOELKER *et al.* 1984; TSUBOTA, ASHBURNER and SCHEDL 1985; CHIA *et al.* 1986).

All of the *Bar* mutations that have been examined are caused by rearrangement breakpoints which occur within at least a 37-kb interval. Given that they are not loss-of-function mutations, it is unlikely that the breakpoints are all within coding regions of the *Bar* gene. It is more likely that they are within a control region or within an intron. If the breakpoints are within a control region, then it is possible that all of the *Bar* mutations result in the abnormal regulation of a coding region that is proximal to the breakpoints. This would mean that mutations within a very large region can alter the normal expression of *Bar*. Another possibility is that the breakpoints occur within a very large intron. This could result in a truncated *Bar* protein which functions abnormally. Alternatively, the breakpoints may create fusion proteins, which produce the mutant phenotype. Rearrangement-induced dominant gain-of-function mutations have been found associated with human oncogene activation (HALUSKA, TSUJIMOTO and CROCE 1987) and with mutations at the *Antennapedia* locus in *D. melanogaster*. One of these mutations, $Antp^{73b}$, has been shown to result in the fusion of the 5′ end of one gene to the 3′ end of the *Antennapedia* gene (FRISCHER, HAGEN and GARBER 1986). This fusion gene most likely expresses a "normal" *Antennapedia* protein in an abnormal location, the eye-antennal imaginal disc, resulting in the transformation of the antenna into leg structures. The possibility that 16A1 does not contain a *Bar* coding region at all, but only a control region must be considered. The various *Bar* rearrangements may be introducing coding regions into the *Bar* control region. The similar phenotypes produced by all of the mutations could be explained by the similar abnormal patterns of expression that have been given to each coding region by the *Bar* control region.

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Communicating editor: W. M. GELBART