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Unequal crossing-over within the *B* duplication of *Drosophila melanogaster*: a molecular analysis

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Summary

The *B* mutation is associated with a tandem duplication of 16A1-16A7. It is unstable, mutating to wild type and to a more extreme form at a frequency of one in 1000 to 3000. The reversion to wild type is associated with the loss of one copy of the duplication, whereas the mutation to extreme *B* is associated with a triplication of the region. The instability of *B* has been attributed to unequal crossing-over between the two copies of the duplication. Recent molecular data show that there is a transposable element, *B104*, between the two copies of the duplication and support the hypothesis that this element generated the duplication *via* a recombination event. These data suggest that unequal crossing-over within the duplication may not be the cause of the instability of *B*. Instead, the instability may be caused by a recombination event involving the *B104* element. This issue was addressed using probes for the DNA on either side of the *B104* element at the *B* breakpoint. All of the data indicate that the *B104* element is not involved in the instability of *B* and support the original unequal crossing-over model.

1. Introduction

The original *Bar* mutation, *B*, of *Drosophila melanogaster* was isolated as a single male (Tice, 1914) with small eyes. It is a semi-dominant sex-linked mutation which results in a large reduction in the number of facets in the compound eye. Homozygous females and hemizygous males have from 70–80 facets per eye as compared with the approximately 780 found in a wild-type eye.

It was noted that *B* is very unstable, reverting to wild-type at a frequency of one in 1600 flies and to a more severe mutant phenotype at a similar frequency (Zeleny, 1919; 1921). Zeleny also noted that the reversion appeared to be occurring in females and not in males.

In an extensive study of the instability of *B*, Sturtevant reported that wild-type and more severe *B* flies were generated primarily in females and that the instability, with few exceptions, was associated with a recombination of flanking markers (Sturtevant, 1925). He also noted that the more severe *B* mutations, which he called double *B*, were also unstable, reverting back to wild-type and *B*. These events were also associated with the recombination of flanking markers.

Sturtevant concluded that the revertants and the double *B* mutations were generated by unequal crossing-over. Wild-type was simply the lack of *B*. *B* had one copy and *BB* had two copies of the mutant allele. In this model, the unequal crossing over in the *B* mutant could generate a chromosome with no copy of *B* (wild type) or two copies of *B* (*BB*). The revertant allele should be identical to the normal wild-type allele and therefore be stable, whereas the *BB* allele should be unstable.

It was later shown that the *B* mutation was a tandem duplication of the chromosomal segment 16A1-16A7 (Bridges, 1936; Muller *et al.* 1936). Wild-type flies and *B* revertants possessed only one copy of the region, whereas *BB* flies were triplicated for the region. These researchers concluded that a reasonable explanation for the instability of *B* was, as Sturtevant had hypothesized, unequal crossing-over, and that the region in which unequal crossing-over had occurred was the duplicated region, 16A1-16A7.

The *B* breakpoint has been cloned (Tsubota *et al.* 1989) and the transposable element, *B104* (Scherer *et al.* 1982), was found to reside at the breakpoint (Fig. 1). DNA sequencing data revealed that the *B104* element is exactly at the junction of 16A7 and 16A1. This element was also shown not to be flanked by a characteristic five-base pair site duplication, making it unlikely that its presence was the result of a transposition. Rather the sequencing data support the

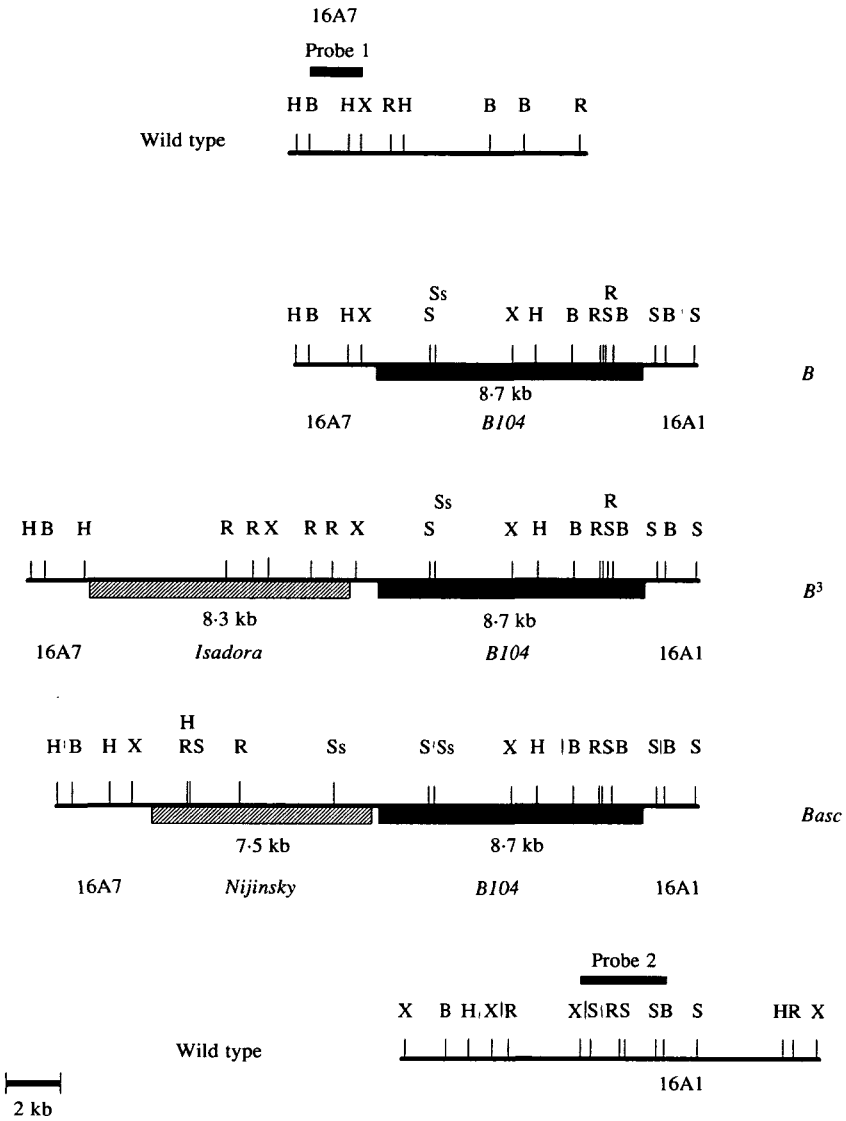

Fig. 1. Restriction maps of the breakpoints of *1B*, B^3, and *Basc-B*. The structure of the *B* breakpoint with the *B104* element is shown. The element is located at the *B* breakpoint, between regions of 16A7 and 16A1. The wild-type 16A1 region is aligned with the homologous sections in the three mutant alleles. The additional insertions of *Isadora* in B^3 and *Ninjinsky* in *Basc-B* are shown. The two probes, probe 1 and probe 2, that were used in the Southern blots in Figs. 3 and 4 are diagrammed as solid bars over the appropriate regions in 16A7 and 16A1. B = *Bam*HI. H = *Hind*III. R = *Eco*RI. S = *Sal*I. Ss = *Sst*I. X = *Xho*I.

hypothesis that a recombination event between two *B104* elements, one in chromosomal position 16A1 and the other in 16A7, generated the *B* breakpoint and the duplication. The role of this element in the origin of the *B* duplication suggests the possibility that the element is also involved in the instability of the *B* mutation. The wild-type revertants and the *BB* mutations might be generated by unequal crossing-over as predicted by Sturtevant, but the crossing-over event may not be occurring between the two copies of the duplicated region, 16A1-16A7. Instead crossing-over may be occurring between the *B104* element at the breakpoint and another element at one end of the duplication. This possibility is supported by the fact that *B104* elements have been shown to recombine with each other (Davis *et al.* 1987). In this study recombination between *B104* elements in the region of the *white* locus resulted in the generation of duplications and deficiencies.

At the DNA level, recombination between *B104* elements and the original unequal crossing-over hypotheses predict two different structures among *B* revertants. In the original hypothesis the *B104* element and the breakpoint are lost (Fig. 2*a*). In the *B104* model the breakpoint is lost but the *B104* element remains (Fig. 2*b*). The existence of either one of these hypothetical structures was examined in *B* revertants with DNA from the 16A1 and 16A7 regions that span the *B* breakpoint.

2. Materials and Methods

(i) *Genetic Stocks*

cv v B; *f* B^3; *Basc-B*. These stocks were used as the

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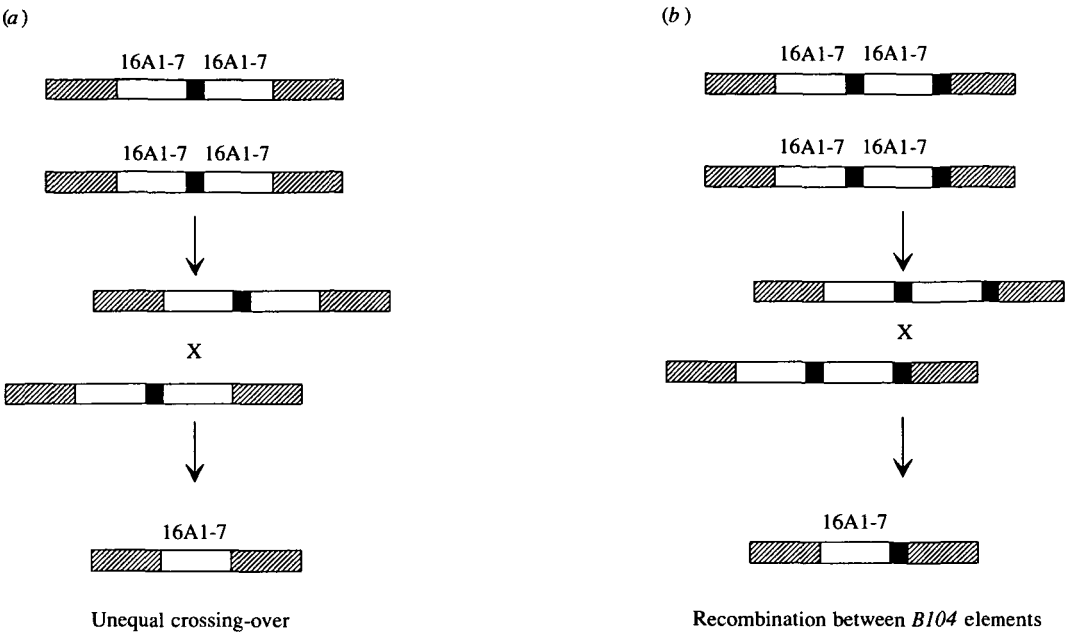

Fig. 2. Models for the reversion of *B*. Sections of the X chromosome are diagrammed. The open bars are the 16A1-7 regions and the hatched bars are the adjoining regions. The small solid bars are *B104* elements. (a) Unequal crossing-over. In this model a recombination event between two obliquely paired 16A regions generates a revertant which has lost both the *B* breakpoint and the *B104* element. (b) Recombination between *B104* elements. This model requires the presence of an additional *B104* element in the wild-type 16A7 or 16A1 region. A recombination event between the two elements produces a wild-type revertant. In this model the breakpoint is lost but the *B104* element remains attached to the 16A7 region.

progenitor stocks for the isolation of spontaneous wild-type revertants. For a more complete description of the mutations see Lindsley & Grell (1968). A few remarks should be made about the *B* alleles in these three stocks. All three alleles contain the *B104* element at the *B* breakpoint (Tsubota *et al.* 1989 and Fig. 1). B^3, a spontaneous partial revertant of *B*, retains the *B* duplication and the *B104* element but contains an extra element, *Isadora*, inserted in 16A7 very close to the *B104* element. This element may be the cause of the partial-revertant phenotype of B^3. The *B* allele in *Basc-B* also contains an extra element, *Nijinsky*, inserted in 16A7 very near the end of the *B104* element. This does not affect the mutant *B* phenotype.

(ii) *Reversion*

The three *B* stocks were first examined to ensure that no wild-type or *BB* alleles existed in the stocks. Bottles were set up for each stock and in the next generation, spontaneous revertants of *B* were collected. Since *B* is on the X chromosome, these flies were identified as males with wild-type eyes or females with eyes characteristic of *B*/+ heterozygotes. Stocks for each revertant were established for DNA analysis. An exact record of the number of flies that were examined was not kept. However, mutations were isolated at frequencies that were similar to those previously reported (Zeleny, 1919, 1921; Sturtevant, 1925).

(iii) *Southern analysis*

Agarose and all restriction endonucleases were purchased from Boehringer-Mannheim; nitrocellulose and nylon filters were obtained from Schleicher and Schuell. ^{32}P-labelled nucleotides were obtained from ICN. *Drosophila* genomic DNA isolation and Southern analysis were performed as previously described (Tsubota & Schedl, 1986).

3. Results

(i) *Isolation of revertants*

The three *Bar* mutations, *B*, *Basc-B*, and B^3, that were used in the study all contain the *B* duplication and the *B104* element at the breakpoint of the duplication (Tsubota *et al.* 1989. Fig. 1). *B* and *Basc-B* produce the same small-eye phenotype. *Basc-B* contains an additional transposable element named *Nijinsky*, inserted in 16A7 very close to the *B104* element. This element does not affect the mutant eye phenotype. B^3 was isolated as a spontaneous partial revertant of *B* and produces eyes that are larger than those of *B* but smaller than those of wild type. In the B^3 mutation there is a second transposable element, *Isadora*, inserted very close to the *B104* element on the 16A7 side of the breakpoint. In our present screens using *B*, eight wild-type revertants were isolated. From B^3, five revertants were isolated and from *Basc-B*, one revertant was isolated. We also obtained a wild-type

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revertant of *B* in the *Basc-B* stock from the *Drosophila* stock centre at Bloomington, Indiana.

(ii) *Models for the generation of wild-type revertants of B*

It has been hypothesized that the wild-type revertants and the extreme *B* mutations are generated by unequal crossing-over within the 16A1-16A7 tandem duplication, since the instability is associated with the recombination of flanking markers (Fig. 2*a*). Recent molecular data suggest another possibility. The transposable element, *B104*, has been shown to have the ability to recombine with other *B104* elements in different chromosomal positions (Davis *et al.* 1987). A copy of this element resides at the *B* breakpoint and most likely generated the *B* duplication *via* a recombination event (Tsubota *et al.* 1989). Given the ability of *B104* elements to recombine with each other, it is possible that recombination between two *B104* elements generates the *B* reversions. This model requires that a *B104* element exists at either the beginning or the end of the tandem duplication in the same orientation as the element at the *B* breakpoint. A recombination event between these two elements would generate the wild-type revertants and the *BB* mutations (Fig. 2*b*).

At the DNA level the original unequal crossing-over model can be unambiguously distinguished from this model. In the unequal crossing-over model, the *B104* element and the 16A7/16A1 junction are lost in the generation of wild-type revertants. In the model involving the *B104* element the 16A7/16A1 junction is lost but the *B104* element remains attached to either the 16A7 or 16A1 region of the breakpoint, depending on the position of the second *B104* element. In Figure

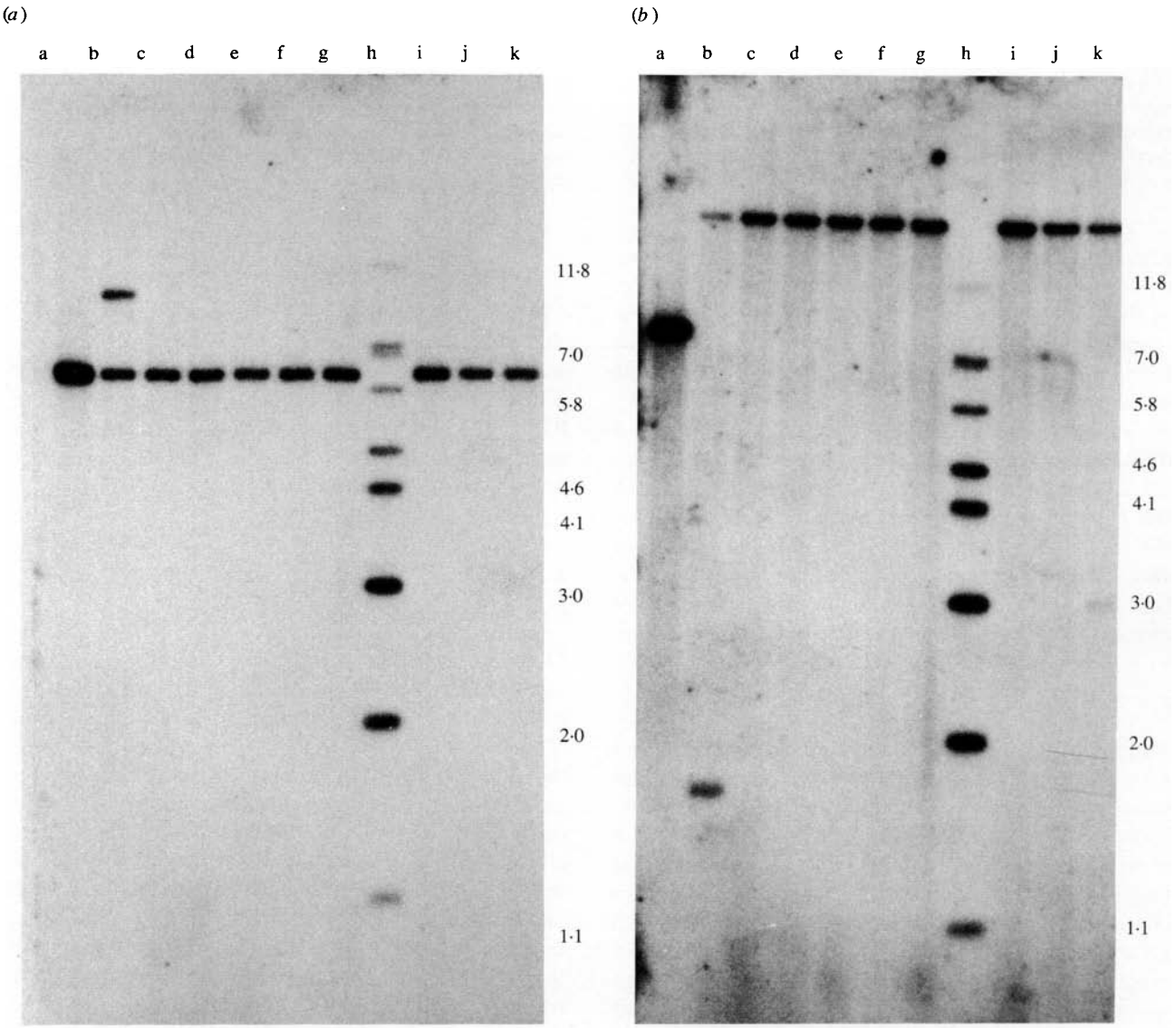

Fig. 3. Southern blots of *B* revertants. Genomic DNA was digested with *Bam*HI, electrophoresed, and transferred to nylon filters. (a) Probe 1 from 16A7 (Figure 1). Lane a, Oregon R; b, *B*; c, $R(B)^1$; d, $R(B)^2$, e, $R(B)^3$; f, $R(B)^4$; g, $R(B)^5$; h, markers; i, $R(B)^6$, j, $R(B)^7$; k, $R(B)^8$. (b) Probe 2 from 16A1 (Figure 1). Lane a, Oregon R; b, *B*; c, $R(B)^1$; d, $R(B)^2$; e, $R(B)^3$; f, $R(B)^4$; g, $R(B)^5$; h, markers; i, $R(B)^6$; j, $R(B)^7$; k, $R(B)^8$.

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2*b* only the case where the *B104* element remains attached to 16A7 is shown. An examination of the 16A1 and 16A7 regions in the wild-type *B* revertants for the presence or absence of the *B104* element will distinguish between the original unequal crossing-over model and the *B104*-element model.

(iii) *Southern analyses of the wild-type revertants of* B

Southern blots were used to analyze the 16A7 and 16A1 regions of the wild-type revertants. Two different probes were used (Fig. 1). Probe 1 is a *Bam*HI–*Xho*I fragment from 16A7, while probe 2 is a *Bam*HI–*Xho*I fragment from 16A1. Since *B* is associated with a duplication of 16A1-7, each probe will hybridize with two *Bam*HI bands in the Southern analysis of *B* DNA. One band is from either the wild-type 16A1 or 16A7 region and the other band is from the *B* breakpoint. The mutant *Bam*HI fragment is generated by a *Bam*HI site in either 16A1 or 16A7 and one within the *B104* element at the breakpoint. Since one of the *Bam*HI sites is within the *B104* element, this analysis will detect the presence of the *B104* element. Oregon-R, a wild-type strain, will give only one band.

The results of the Southern blots of the wild-type revertants of *B* are shown in Fig. 3. The probe for 16A7, probe 1, hybridizes to two bands in *B* as previously discussed (Fig. 3*a*, lane b). The larger 9·5 kb band is the mutant band while the smaller 6·0 kb band which co-migrates with that of Oregon R (lane a) is the wild-type band from 16A7. All of the revertants (lanes c–g, i–k) have lost the mutant band and contain only the wild-type band. Therefore both the 16A7/16A1 junction and the *B104* element have been lost. Similarly, a probe for 16A1, probe 2, shows that all of the revertants have lost the 16A7/16A1 junction and the *B104* element and retain only the wild-type fragment (Fig. 3*b*). In this figure it should be noted that the wild-type 16A1 region in *B* (lane b) contains a larger *Bam*HI fragment than that found in Oregon R (lane a). The reason for this polymorphism has not yet been determined. This wild-type band is retained, while the 1·7 kb mutant band is lost in all of the revertants. These data indicate that in the generation of wild-type revertants of *B*, both the 16A7/16A1 junction and the *B104* element are lost.

The wild-type revertants of B^3 and *Basc-B* give the same results as those of *B* (Fig. 4). In each case the 16A7/16A1 junction and the *B104* element are lost and only the wild-type fragments in both the 16A7

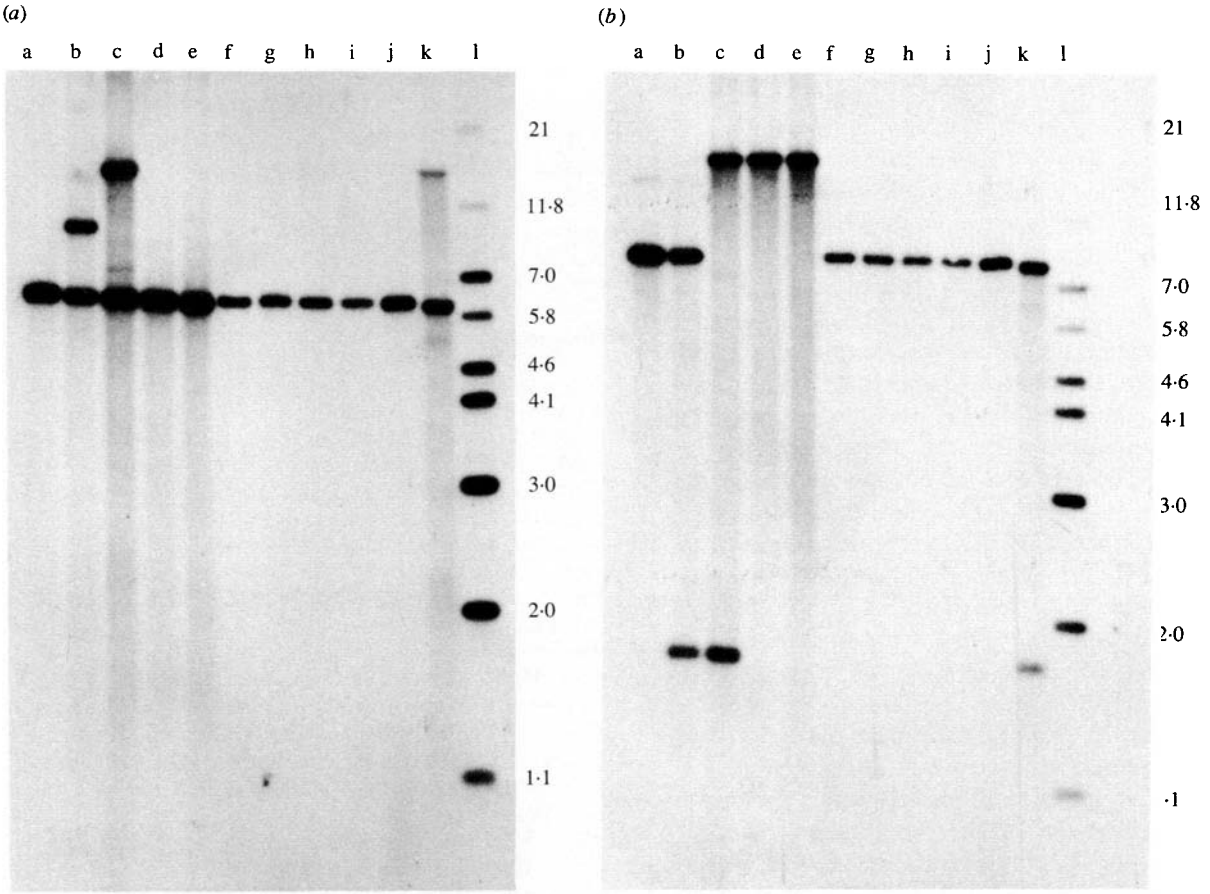

Fig. 4. Southern blots of *Basc-B* and B^3 revertants. Genomic DNA was digested with *Bam*HI, electrophoresed, and transferred to nylon filters. (a) Probe 1 from 16A7 (Figure 1). Lane a, Oregon R; b, *FM7-B*; c, *Basc-B*; d, $R(Basc)^1$; e, $R(Basc)^2$; f, $R(B^3)^1$; g, $R(B^3)^2$; h, $R(B^3)^3$; i, $R(B^3)^4$; j, $R(B^3)^5$; k, B^3; l, markers. (b) Probe 2 from 16A1 (Figure 1). Lane a, Oregon R; b, *FM7-B*; c, *Basc-B*; d, $R(Basc)^1$; e, $R(Basc)^2$; f, $R(B^3)^1$; g, $R(B^3)^2$; h, $R(B^3)^3$; i, $R(B^3)^4$; j, $R(B^3)^5$; k, B^3; l, markers.

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(Fig. 4*a*) and the 16A1 (Fig. 4*b*) regions are retained. In B^3 (Fig. 4*a*, lane k) and *Basc-B* (Fig. 4*a*, lane c), the 16A7 mutant fragment is larger than the 9·5 kb mutant fragment seen in *B* (Fig. 3*a* and 4*a*, lane b). In both cases this larger mutant fragment is caused by the presence of an additional transposable element inserted near the *B104* element (Fig. 1). These extra elements are also lost in the generation of the revertants of *Basc-B* (Fig. 4*a*, lanes d and e) and B^3 (Fig. 4*a*, lanes f–j). Probe 2 indicates that *Basc-B* (*Fig.* 4*b*, lane c) contains the same polymorphism in the wild-type 16A1 region that is seen in *B* (Fig. 3*b*, lane b), while B^3 (Fig. 4*b*, lane k) and another *B* strain, *FM7* (Fig. 4*b*, lane b), contain the same band as Oregon-R. As with the revertants of *B*, the revertants of *Basc-B and* B^3 have lost the 1·7 kb mutant fragment and retain their respective wild-type bands (Fig. 4*b*, lanes d–j).

These data show that both the *B* breakpoint and the *B104* element are lost in the generation of wild-type revertants of *B*, *Basc-B*, and B^3. Thus, they are inconsistent with the model that postulates an involvement of the *B104* element in generation of the wild-type revertants and support the model for unequal crossing-over within the 16A1-16A7 duplication.

4. Discussion

All of the Southern analyses support the original model for the generation of wild-type revertants and *BB* mutations. The *B104*-element-dependent model for the *B* instability predicted different structures at the DNA level than were seen in the revertants. If the *B104* element is involved in the instability of the *B* mutation, it would have to be lost in the process of generating the revertants. Even if the *B104* element induced the generation of the revertants and was lost in the process, we would still predict that the revertants would retain either the 16A1 or 16A7 region from the breakpoint and would lose the corresponding wild-type region. The existence of polymorphisms in the stocks used in the reversion experiments argues against this and strengthens the case for the original unequal crossing-over model. *B* and *Basc-B* contain a marker for the wild-type 16A1 region in the form of a larger *Bam*HI fragment (Fig. 3*b*, lane b and Fig. 4*b*, lane c). In the revertants of *B* and *Basc-B*, this polymorphism in the wild-type 16A1 region is retained. B^3 contains a marker for the 16A7 mutant region in the form of an additional transposable element, *Isadora* (Fig. 4*a*, lane k). Similarly, the 16A7 mutant region in *Basc-B* contains an insertion of the transposable element, *Nijinsky* (*Fig.* 4*a*, lane c). In the revertants of B^3 and *Basc-B*, the polymorphisms in the 16A7 mutant region are lost and the wild-type 16A7 region is retained. Together these data indicate that the wild-type revertants retain the wild-type 16A1 and 16A7 regions and lose the mutant 16A1 and 16A7 regions. Again this is most simply seen as the result of a recombination event within the 16A1-7 duplication and not as the result of a *B104*-induced event.

The *B* revertants that were examined in this study are the most common form of *B* revertants. They are generated in females, are interchromosomal events accompanied by the recombination of flanking markers, and, as is supported by this study, are generated by unequal crossing-over in the 16A region. A second and much less frequent class of *B* revertants has also been observed (Sturtevant, 1925; Peterson & Laughnan, 1963; Green, 1968; Gabay & Laughnan, 1970). This class is composed of revertants that are not associated with the recombination of flanking markers and can be shown to also occur in males. It has been proposed that these events are the result of intrachromosomal exchanges either between sister chromatids or within the same chromatid (Peterson & Laughnan, 1963). The intrachromosomal events also differ from the more common interchromosomal events in that their frequency is independent of the length of the duplication (Green, 1968) and they are often found associated with chromosomal aberrations (Peterson & Laughnan, 1963). There are also data indicating that some of these events may be premeiotic in origin (Peterson & Laughnan, 1964, Gabay & Laughnan, 1970). All of the differences between the interchromosomal and intrachromosomal events argue that different mechanisms are behind the generation of these two classes. While we have shown that the *B104* element at the *B* breakpoint is probably not involved in the interchromosomal events, it remains to be seen if it may be involved in the intrachromosomal events.

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