Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 564–568, January 1982
Genetics

Physical map of the *white* locus of *Drosophila melanogaster*

(gene localization/chromosomal rearrangement/*zeste* locus/repetitive DNA)

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Communicated by Donald D. Brown, October 7, 1981

ABSTRACT **The *white* locus of *Drosophila melanogaster* is a genetically well-characterized locus, mutations in which alter the degree or pattern of pigmentation of the eyes. Using a previously cloned DNA segment containing a portion of the *white* locus of a mutant allele, we have cloned and characterized the DNA of a 48-kilobase chromosomal region of the Canton S wild-type strain. We have mapped the positions, relative to restriction endonuclease cleavage sites, of several previously characterized chromosomal rearrangement breakpoints that bracket the *white* locus. These results define a segment of 14 kilobases that contains all of the *white* locus sequences necessary for the production of a wild-type eye color phenotype. By conventional criteria, no repetitive sequences are present within this 14-kilobase segment; however, we have identified an extremely weak DNA sequence homology between a portion of this segment and a chromosomal region in the vicinity of the *zeste* locus.**

The w^1 mutation at the *white* (*w*) locus was the first mutation reported in *Drosophila melanogaster* (1). Null alleles at the locus result in the absence of pigment from the eyes, while other alleles lead to intermediate pigment levels and, in some cases, to an abnormal distribution of eye pigment. Due in part to the easily scored phenotype of mutant alleles at *white*, >100 such alleles are known. Fine-scale genetic analysis has resolved at least seven intralocus mutational sites, separable by recombination, that span a region of ≈0.03 centimorgans. All of the alleles of the *white* locus form a single complementation group, yet there is considerable heterogeneity among them, not only in phenotype but also in their epistatic interactions with the *zeste* locus and in their degree of dosage compensation (for review, see ref. 2; ref. 3). Moreover, several of the alleles of the *white* locus are of particular interest because of their unusual genetic instability (4–6). Neither the gene product(s) of the *white* locus nor their mode of action have been determined.

We have previously isolated, by recombinant DNA techniques, a single-copy DNA segment from the *white* locus of a strain carrying the *white-apricot* (w^a) allele (7). In this report, we describe the molecular cloning of overlapping segments of the Canton S wild-type *white* locus region. This has allowed us to construct a physical map of restriction enzyme cleavage sites in this 48-kilobase (kb) segment, to orient this physical map with respect to the genetic map, and to define a segment of 14 kb that contains all of the *white* locus sequences necessary to allow the production of a w^+ (wild-type eye color) phenotype.

MATERIALS AND METHODS

***Drosophila* Strains and DNA Preparation.** The *Drosophila* strains used have been described (7). DNA was prepared from adult flies that were anesthetized with CO_2, collected on ice, rapidly frozen, and stored at −70°C. Flies were disrupted and a crude nuclear pellet was prepared as described (8) except that all steps subsequent to grinding were done on ice and Nitex 44 filters were used in place of milk filters. The nuclei were suspended in a small volume of homogenization buffer to which was added 10 vol of 30 mM Tris·HCl, pH 8.0/100 mM EDTA containing proteinase K at 0.5 mg/ml followed by *N*-lauroylsarcosine to 1%. After incubation overnight at 37°C, the DNA was banded in CsCl gradients (9).

Recombinant Phage and DNA Preparation. Phage from a Charon 4/Canton S *D. melanogaster* library (10) were screened by plaque hybridization (11) at a density of 2×10^4 plaques per 24 × 24 cm agar plate as described (7). For DNA preparation, phage were grown in 200 ml of liquid culture (7), debris was removed by low-speed centrifugation, and the phage were precipitated with polyethylene glycol (12), suspended in 2 ml of 10 mM Tris·HCl, pH 7.5/10 mM $MgSO_4$ containing 20 μg of DNase (Sigma), extracted with an equal volume of $CHCl_3$, and purified by two cycles of CsCl step-gradient centrifugation (13). DNA was isolated from purified phage by phenol extraction.

Analysis of Cloned DNA. Transfers of DNA from gels to nitrocellulose filters (14) were made as described (15). Filters were hybridized with nick-translated DNA probes (16) as described (17). *In situ* hybridizations were carried out as described (7).

RESULTS

Cloning of a 48-kb Segment from the Wild-Type *White* Locus Region. We have described (7) a hybrid phage, λw^a5.9, that has a *Drosophila* DNA insert containing a copy of the transposable element *copia* and adjacent sequences derived from the *white* locus of flies carrying the w^a allele. A 3.1-kb *Bam*HI fragment from the sequences adjacent to *copia* was shown to have strong homology only to sequences from the *white* locus region as assessed by Southern gel analysis and by *in situ* hybridization to polytene chromosomes (7). We therefore chose this fragment as a hybridization probe to screen a hybrid λ phage library containing DNA segments from the Canton S wild-type strain of *D. melanogaster*. Two distinguishable classes of hybridization signals were observed; among 1.2×10^5 phage screened, seven and six were recovered that hybridized strongly and weakly, respectively (Fig. 1). We describe first the characterization of phage from the strongly hybridizing class.

Restriction endonuclease cleavage maps were prepared from the DNAs of the seven strongly hybridizing phage: λm1.1, λm1.2, λm2.1, λm2.2, λm5.1, λm5.3, and λm6.1. The maps of the seven phage could be aligned in an overlapping array as shown in Fig. 2, defining a composite map of 24 kb. Two of the phage, λm6.1 and λm2.1, appear to carry identical inserts, while each of the others carries a different cloned segment.

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Abbreviation: kb, kilobase(s).

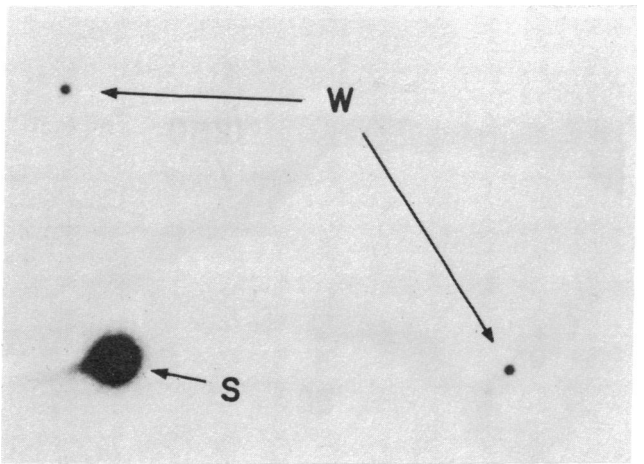

FIG. 1. Example of plaque hybridization to strongly and weakly hybridizing phage. Shown is an autoradiograph of an 8 × 6 cm portion of one of the filter replicas of the hybrid phage library hybridized with the 3.1-kb *Bam*HI fragment of $\lambda w^a 5.9$. Of ≈1600 plaques in this area, one hybridized strongly (s) and two hybridized weakly (w).

Apart from the absence of the *copia* element and two other restriction site differences (due most likely to the different genetic backgrounds of these strains), we have detected no differences between the pattern of restriction sites in the cloned segments from the wild-type library and that of the cloned segment of $\lambda w^a 5.9$ (ref. 7; unpublished data).

The region cloned was extended in both directions by rescreening the wild-type library, using fragments purified from phage λm1.1 and λm1.2. Fig. 2 includes composite restriction maps from the DNA of two phage, λm11B.1 and λm11B.2, extending to the left and from three phage, λm8A.1, λm10A.1, and λ11A.1, extending to the right. The total length of the map deduced from all 12 phage is 48 kb. Coordinates are drawn above the map in Fig. 2 with the position of the *copia* insertion at the w^a site (7, 19) assigned coordinate 0.0. Positions to the right of this point are given positive numbers equal to their distances in kb from this point and positions to the left are given negative numbers.

We have verified, in two independent ways, that the cloned segments accurately reflect the corresponding genomic sequences. First, the restriction maps of the DNAs of overlapping phage are consistent with one another. With the exception of the extremes of the map, all sequences are included within at least two independent hybrid phage. Second, we carried out an extensive series of Southern blot analyses, comparing the restriction fragments of the cloned inserts with homologous fragments from genomic DNA. Restriction digests of hybrid phage DNA and genomic DNA were subjected to electrophoresis in parallel on agarose gels and hybridized with labeled DNA of the cloned phage (see legend to Fig. 2). Comigration of hybridizing cloned and genomic fragments indicated that, within the resolution of the technique, no deletions, insertions, or substitutions occurred during the cloning or propagation of the hybrid phage. The existence of every restriction cleavage site shown in Fig. 2 from coordinate −20 rightward was confirmed by this procedure. The presence of repetitive sequences to the left of coordinate −20 (see below) prevented the use of this method of confirmation for this region.

The Southern blot comparisons of cloned and genomic DNA also provided a means to test for the presence of repetitive sequences within the cloned inserts. We found that labeled DNA from phage λm11B.2 hybridized not only to genomic restriction fragments of mobilities predicted by the cloned sequences but also to several additional fragments with even greater intensity. This suggests that a portion of the sequences of λm11B.2 is repeated elsewhere in the genome in a different arrangement. These repetitive sequences were localized to the left of the

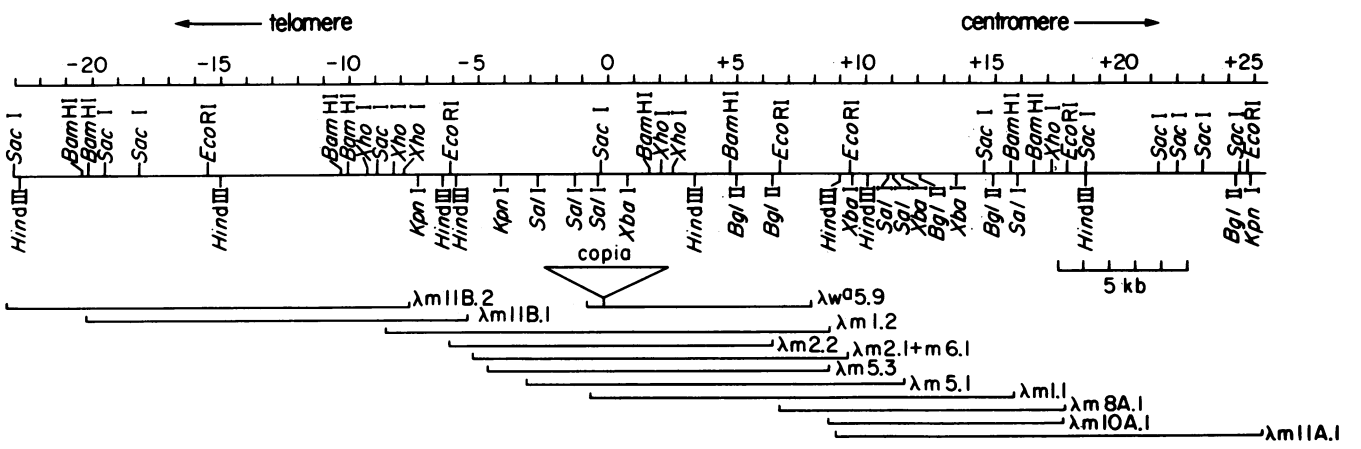

FIG. 2. Restriction site map of the *white* locus region from *D. melanogaster* (Canton S). Restriction maps were initially constructed of the cloned segments of each of the strongly hybridizing phage by examination of the DNA fragments produced by a series of single and double digestions of the phage DNAs or of plasmid subclones derived from them and taking into consideration the map of the vector DNA (18) and its attachment to *Drosophila* DNA by *Eco*RI linkers (10). Multiple cleavage sites for an enzyme within a distance <0.2 kb would not have been resolved. The maps of the overlapping cloned segments were aligned to form the composite map, shown above, with the limits of each of the cloned segments delineated below it. The limits of the corresponding sequences cloned in $\lambda w^a 5.9$ (7) and the point of the *copia* element insertion within it are also indicated. To confirm that this map derived from cloned sequences was the same as that of the genomic DNA, a series of Southern blot analyses was performed. Genomic DNA and a hybrid phage DNA were digested separately with a pair of restriction enzymes, subjected to electrophoresis in parallel on an agarose gel, and hybridized with the labeled DNA of a hybrid phage. The pairs of enzymes used—*Sac* I/*Eco*RI, *Hin*dIII/*Bam*HI, *Bgl* II/*Xba* I, and *Sal* I/*Xho* I—cut the DNA from this region into fragments generally small enough to be resolved from each other in the 0.7% gels used. Hybrid phage λm11B1, λm1.2, λm5.1, and λm11A.1 were used as probes in the initial set of experiments. Shorter purified fragments were used as probes in later experiments in cases in which two or more fragments homologous to a hybrid phage could not be resolved in autoradiographs. Experiments were repeated using 1.4% agarose gels in cases in which fragments were produced that were too small to be resolved on the 0.7% gels; we did not attempt to analyze fragments <0.3 kb.

*Bam*HI site at coordinate −20.1 by hybridization of fragments of λm11B.2 to blots of genomic digests. Because the remainder of the cloned probes hybridized only to fragments of the expected mobilities, we conclude that there are no other large blocks of repeated sequence within this 48-kb region of Canton S DNA. The results of experiments described below in which hybrid phage DNA was hybridized *in situ* to polytene chromosomes are consistent with this conclusion and exclude the possibility that the entire 48-kb region is repeated, in the same arrangement, at other chromosomal sites. We estimate that a 0.5-kb closely conserved repetitive sequence anywhere in the region would have been recognized.

Coordinating the Physical and Genetic Maps. To coordinate the physical and genetic maps, we have determined by *in situ* hybridization the positions, within the cloned interval, of the breakpoints of a series of chromosomal rearrangements. These breakpoints have previously been shown to bracket the white locus by genetic analysis (6, 20, 21) and to bracket the sequences of the 3.1-kb *Bam*HI fragment (coordinates +1.6 to +4.7) (7). (For a more detailed discussion of the rearrangements used, see ref. 7.)

$In(1)z^{+64b9}$ inverts a large chromosomal segment beginning near the proximal end of the *white* locus in 3C and extending toward the centromere to subdivision 12BC (21). The *white*-associated breakpoint of the inversion falls between *white* and *roughest*, the closest known complementation group toward the centromere from *white* (21). The DNA of λm1.1 hybridized to both ends of the inverted segment in $In(1)z^{+64b9}$ (Fig. 3). In contrast, λm1.2 DNA hybridized only or primarily at the distal (*white* locus) end of the inverted segment. These results suggest that the *white*-associated breakpoint of this inversion falls within the segment cloned in λm1.1 and that the chromosomal orientation of the cloned segment is telomere–λm1.2–λm1.1–centromere, as indicated in Fig. 2.

The proximal boundary of the *white* locus is further defined by the deficiency $Df(1)w^{DZL}14$, which deletes a segment extending rightward from a position proximal to (toward the centromere from) the *white* locus (6). Flies carrying this deletion have a fully wild-type eye color (6). This deletion chromosome is labeled strongly by *in situ* hybridization with either λm1.1 or λm1.2. The position of the *white*-associated breakpoint of $Df(1)w^{DZL}14$ was refined by hybridizing fragments purified from λm1.1 *in situ* to a strain homozygous for $Df(1)w^{DZL}14$ and for the insertional translocation, $Dp(1,2)w^{+51b7}$, which also contains the *white* locus. The 3.3- and 2.3-kb *Eco*RI/*Hin*dIII fragments (Fig. 4) at coordinates +3.4 to +6.7 and +6.7 to +9.0 hybridized equally strongly to both the deficiency and insertional translocation chromosomes, showing that most if not all of the sequences homologous to these fragments reside distal to the deficiency breakpoint. However, the 6.0-kb *Hin*dIII (+10.1)/*Eco*RI (+16.1) fragment failed to label the deficiency chromosome detectably but labeled the insertional translocation strongly. The 6.0-kb *Hin*dIII/*Eco*RI fragment labels distal 3C strongly in w^{DZL}, the parent strain of $Df(1)w^{DZL}14$. These observations show that the sequences homologous to the 6.0-kb *Hin*dIII/*Eco*RI fragment are largely or entirely deleted in the deficiency chromosome. Collectively, these experiments place the *white*-associated breakpoint of $Df(1)w^{DZL}14$ within an interval between coordinates +8.0 and +10.6 (Fig. 4). Thus, we conclude that all elements of the *white* locus necessary to allow the production of a w^+ eye color phenotype reside distal to (toward the telomere from) coordinate +10.6.

The maximal limit of the *white* locus at the opposite end was defined by the *zeste–halo* rearrangement (20). This rearrangement consists of the insertion of a chromosomal segment bearing the *white*, *roughest*, and *verticals* loci into the third chromosome, $Dp(1,3)w^{zh}$, and a deletion of the same three loci from the X chromosome, $Df(1)w^{zh}$. Because the inserted and deleted segments bear the same three loci and because they were initially detected in the same individual, it has been suggested that their breakpoints coincide. The *white*-associated breakpoint of $Dp(1,3)w^{zh}$ must, in any case, lie distal to *white*. The λm1.2 probe labeled both the insertional translocation and the deficiency (Fig. 3), while λm1.1 labeled only or primarily the insertional translocation. These data suggest that the segment cloned in λm1.2 crosses the position of the *white*-associated breakpoint of the *zeste–halo* rearrangement and corroborates the orientation of the cloned segment deduced above.

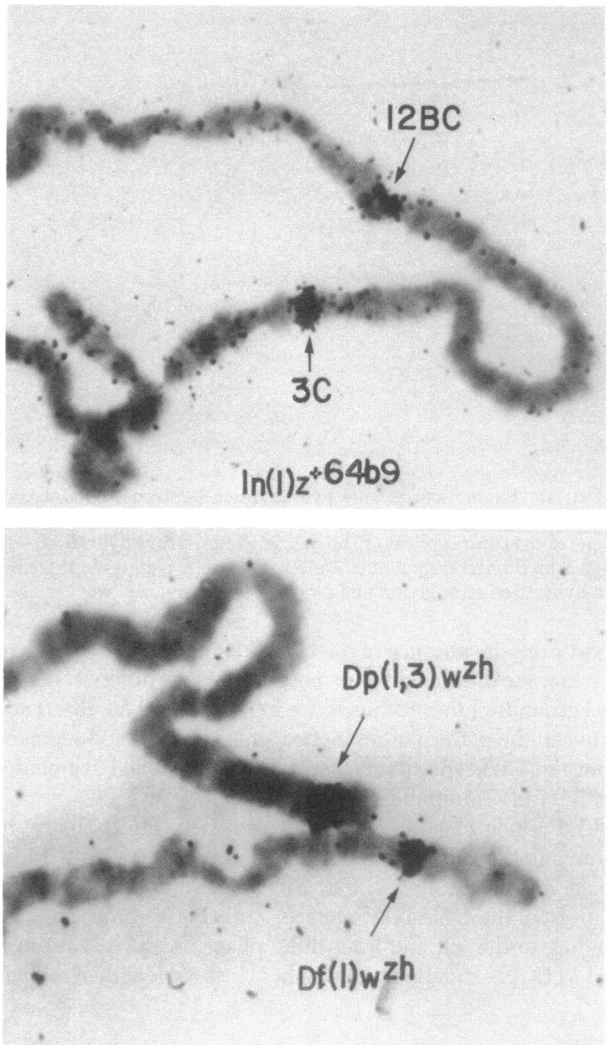

FIG. 3. *In situ* hybridization of the cloned segments to polytene chromosomes with rearrangement breakpoints bracketing the *white* locus. (*Upper*) λm1.1 hybridized to chromosomes homozygous for $In(1)z^{+64b9}$. Arrows, sites of labeling at the ends of the inverted segment. (*Lower*) λm1.2 hybridized to chromosomes homozygous for the *zeste–halo* rearrangement. Arrows, sites of labeling of deficiency and duplication sites.

The breakpoint of the *zeste–halo* rearrangement associated with *white* was more accurately positioned within λm1.2 by *in situ* hybridization of DNA fragments comprising different portions of the cloned insert of this phage. The 3.1-kb *Hin*dIII (−5.8)/*Sal* I (−2.7) fragment labeled $Df(1)w^{zh}$ strongly but did not label $Dp(1,3)w^{zh}$ detectably. On the other hand, the 1.5-kb *Sal* I fragment at coordinates −2.7 to −1.2 labeled the insertional translocation at about one-half the intensity of the defi-

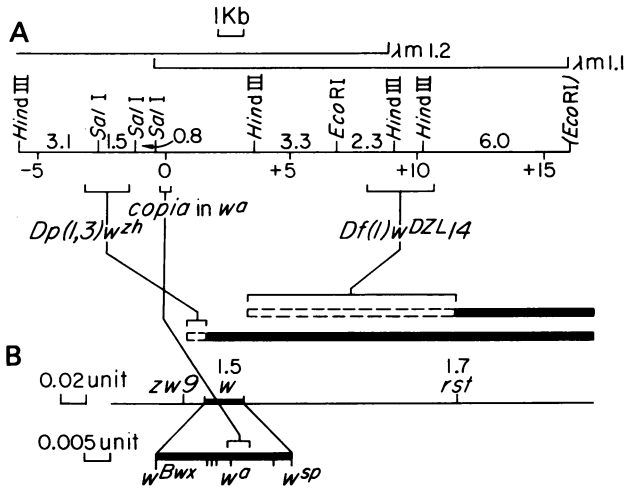

FIG. 4. Coordinating the physical (*A*) and genetic (*B*) maps of the *white* locus region. Below the genetic map, on which the positions of the *zeste–white 9* (*zw9*), *white* (*w*), and *roughest* (*rst*) loci are indicated, is an expanded map on which the positions of seven intralocus sites, including *white–Brownex* (w^{Bwx}), *white–apricot* (w^a), and *white–spotted* (w^{sp}), are indicated. The sources of these data have been described (7). The maps are drawn with the distal direction (toward the telomere) to the left. Solid bars above genetic map show extent of duplication and deficiency, as previously determined by genetic analysis; open, dashed bars to the left of the solid bars show genetic region in which the *white*-associated breakpoint of the rearrangement must lie. The distal breakpoint of $Dp(1,3)w^{zh}$ is between *zw9* and *w* (20) while that of $Df(1)w^{DZL}14$ is between *w* and *rst* (6). Cytogenetic analysis suggests that the distal breakpoint of $Df(1)w^{DZL}14$ is actually much closer to *w* than to *rst* (6). In *A*, the central portion of the restriction map of Fig. 2 is redrawn, including those sites at which cleavages were made to produce the fragments hybridized to the chromosomal rearrangements. The length of each fragment is given above the line and the coordinate scale is below. Our results place the *white*-associated breakpoints of these rearrangements within the intervals spanned by the brackets extending up toward the restriction map. We have assumed that a site that is not detectably labeled by a fragment must contain <0.5 kb of the sequences of that fragment. The position within the Canton S restriction map corresponding to that of the *copia* insertion in w^a was determined by comparing the restriction maps of Canton S and $\lambda w^a 5.9$ (7); the interval in which the *copia* insertion in w^a lies, relative to the genetic map of the *white* locus, is taken from the 95% confidence limits of the determination by Bingham and Judd (19).

ciency chromosome, while the 0.8-kb *Sal* I fragment (coordinates −1.2 to −0.4) labeled both equally. These results show that the $Dp(1,3)w^{zh}$ insertional translocation carries sequences from the cloned region beginning between the coordinates −3.2 and −1.4 and extending rightward (Fig. 4). Since flies carrying $Dp(1,3)w^{zh}$ have a wild-type eye color (20), we conclude that all elements of the *white* locus necessary to produce a w^+ phenotype lie proximal to (toward the centromere from) coordinate −3.2. Our results further indicate that, contrary to the expectation engendered by the origin of the *zeste–halo* rearrangement (7), the *white*-associated breakpoint of $Df(1)w^{zh}$ and that of $Dp(1,3)w^{zh}$ are not identical.

In summary, the results of these *in situ* hybridization experiments allow us to determine the chromosomal orientation of our cloned region and to demonstrate that all *white* locus sequences necessary to produce a w^+ phenotype lie between coordinates −3.2 and +10.6. Thus, the maximum length that can be occupied by the *white* locus, as defined in this way, is 14 kb.

Analysis of Phage with Weak Homology to a Part of the *White* Locus Region. As noted above, during the initial screening of the hybrid phage library, six phage were recovered that hybridized very weakly with the 3.1-kb *Bam*HI fragment of

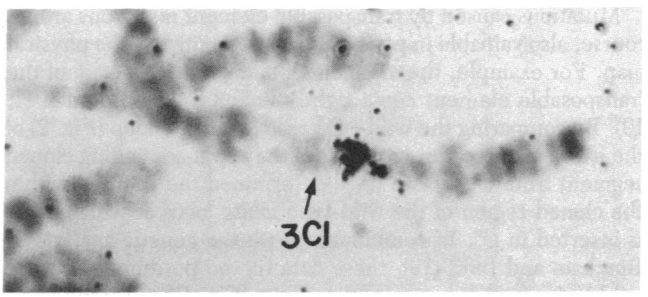

FIG. 5. *In situ* hybridization of the DNA of one of the hybrid phage weakly homologous to the 3.1-kb *Bam*HI fragment to the 3A region on polytene chromosomes of the Oregon R strain of *D. melanogaster*. Arrow points to 3Cl, the site at which the *white* locus resides, which is not labeled.

$\lambda w^a 5.9$. The *Drosophila* DNA inserts of at least four of these contain sequences very weakly homologous to the 3.1-kb *Bam*HI fragment, as assayed by Southern blot analysis to purified phage DNA. However, the restriction cleavage maps of these DNAs bear no resemblance to the map of the *white* locus region derived from the strongly hybridizing cloned segments. Moreover, the DNAs of these four phage each hybridized *in situ* primarily to a site within or very near the distal portion of subdivision 3A on polytene salivary gland chromosomes rather than to distal 3C, where the *white* locus resides (Fig. 5). Distal 3A is the location of the *zeste* locus. Mutant alleles of *zeste* interact, at least formally, with the *white* locus as defined by genetic analysis (3, 22, 23). In addition to the strong site of labeling at 3A of each of these four cloned segments, three also labeled one or two additional sites with approximately one-tenth the intensity with which they labeled 3A. One phage labeled 6F and the other two labeled both 6F and 7A.

DISCUSSION

We have cloned and constructed restriction site maps of a series of overlapping DNA segments that define a 48-kb chromosomal region from the wild-type strain Canton S surrounding and including the *white* locus. To draw a correspondence between positions on the physical map of the cloned interval and the genetic map, we determined, by *in situ* hybridization, the positions of cloned segments relative to the breakpoints of several chromosomal rearrangements bracketing the *white* locus. This procedure for gene localization can recognize the requirement for gene function of linked nontranscribed sequences (7, 24, 25). This approach is especially valuable in characterizing regions, such as that which includes the *white* locus, that contain gene(s) having no biochemically defined products. We previously used this combination of techniques to show that a 3.1-kb segment cloned from the w^a strain originated from a region between breakpoints that separated the *white* locus from its nearest known complementation groups (7). Here we have exploited this method of analysis again to show that, within a 48-kb cloned interval from the *white* locus region, there is a 14-kb segment containing all of the *white* locus sequences necessary for the production of a w^+ eye color phenotype.

The 14-kb region to which we have localized *white* is a maximum interval and may be reduced by further analysis. Until cloned DNA sequences can be modified *in vitro* and reintroduced into the *Drosophila* genome, analyses of the sort we describe here are limited to rearrangements generated *in vivo*. The w^c and w^{DZL} mutations at *white* promote chromosomal rearrangements of *white* locus region DNA sequences at high frequency (4, 6), suggesting that they will be useful tools for future fine-scale mapping of the region.

Mutations caused by transposable element insertions are, of course, also valuable in positioning genetic sites on the physical map. For example, the w^a allele of *white* carries a copy of the transposable element *copia* tightly linked to the mutation (7, 19). By comparing the wild-type restriction site map (Fig. 2) of the *white* locus region with that of the *copia*-containing cloned segment from w^a (7), we have determined the position within the cloned region of the wild-type *white* locus at which *copia* is inserted in w^a. In combination with the genetic analysis by Bingham and Judd (19), these data fix the position within the wild-type restriction map of the w^a site (Fig. 4), one of the seven *white* locus sites that have been separated by recombination. As has been shown for other *copia* element insertions (26), the insertion of *copia* within the *white* locus in the w^a strain does not appear to have disrupted the surrounding DNA organization. Our results are consistent with the hypothesis that the w^a mutation was caused by the *copia* insertion (7, 19, 27).

We have focused, to this point, on the chromosomal region at 3C that is required for *white* locus function. One of the interesting aspects of the *white* locus is that its expression can be modulated by mutant alleles at other loci. The best studied of these interactions is that between *white* and certain mutant alleles at the *zeste* locus (3, 22, 23). It is therefore potentially important that we have shown that DNA sequence homology exists between the 3.1-kb *Bam*HI fragment (coordinates +1.6 to +4.7) and the cytogenetic region within which the *zeste* locus resides. Further analysis will be necessary to determine what relationship, if any, this homology has to the *zeste–white* interaction.

Plaque hybridization may be the method of choice for detection of such weakly crosshybridizing sequences. The sensitivity of plaque hybridization is equivalent to that of Southern gel analysis and exceeds that of *in situ* hybridization to polytene chromosomes. Due to the large average distance between hybridizing plaques, weakly homologous sequences can be detected by plaque hybridization despite the presence of strongly homologous sequences in the same genome. Moreover, weakly homologous sequences detected by plaque hybridization are available as cloned segments for further analysis.

We thank J. Lauer and T. Maniatis for generously supplying us with the hybrid phage library and C. Murphy for technical assistance. This work was supported by National Institutes of Health and American Cancer Society grants to G.M.R. and by National Institute of Environmental Health Science Intramural Research Program funds to P.M.B. R.L. was supported by a National Institutes of Health postdoctoral fellowship.

1. Morgan, T. H. (1910) *Science* **32,** 120–122.
2. Judd, B. H. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, London), Vol. 1b, pp. 767–799.
3. Bingham, P. M. (1980) *Genetics* **95,** 341–353.
4. Green, M. M. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, London), Vol. 1b, pp. 929–946.
5. Rasmuson, B., Montell, I., Rasmuson, A., Svahlin, H. & Westerberg, B.-M. (1980) *Mol. Gen. Genet.* **177,** 567–570.
6. Bingham, P. M. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45,** 519–525.
7. Bingham, P. M., Levis, R. & Rubin, G. M. (1981) *Cell* **25,** 693–704.
8. Strausbaugh, L. D. & Kiefer, B. I. (1979) *Genetics* **93,** 411–422.
9. Schachat, F. H. & Hogness, D. S. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **38,** 371–381.
10. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15,** 687–701.
11. Benton, W. D. & Davis, R. W. (1977) *Science* **196,** 180–182.
12. Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L. & Treiber, G. (1970) *Virology* **40,** 734–744.
13. Thomas, M. & Davis, R. W. (1975) *J. Mol. Biol.* **91,** 315–328.
14. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
15. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
16. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
17. Potter, S. S., Brorein, W. J., Dunsmuir, P. & Rubin, G. M. (1979) *Cell* **17,** 415–427.
18. DeWet, J. R., Daniels, D. L., Schroeder, J. L., Williams, B. G., Denniston-Thompson, K., Moore, D. D. & Blattner, F. R. (1980) *J. Virol.* **33,** 401–410.
19. Bingham, P. M. & Judd, B. H. (1981) *Cell* **25,** 705–711.
20. Judd, B. H. (1974) in *The Eukaryotic Chromosome*, eds. Peacock, W. J. & Brock, R. D. (Aust. Natl. Univ. Press, Canberra, Australia), pp. 169–184.
21. Sorsa, V., Green, M. M. & Beermann, W. (1973) *Nature (London) New Biol.* **245,** 34–37.
22. Gans, M. (1953) *Bull. Biol. Fr. Belg. Suppl.* **38,** 1–90.
23. Jack, J. W. & Judd, B. H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1368–1372.
24. McGinnis, W., Farrell, J. & Beckendorf, S. K. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7367–7371.
25. Spradling, A. C. & Rubin, G. M. (1981) *Annu. Rev. Genet.* **15** 219–264.
26. Dunsmuir, P., Brorein, W. J., Simon, M. A. & Rubin, G. M. (1980) *Cell* **21,** 575–579.
27. Gehring, W. J. & Paro, R. (1980) *Cell* **19,** 897–904.