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Cloning of DNA Sequences from the *white* Locus of D. melanogaster by a Novel and General Method

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Summary

We describe the isolation of a cloned DNA segment carrying unique sequences from the *white* locus of Drosophila melanogaster. Sequences within the cloned segment are shown to hybridize in situ to the *white* locus region on the polytene chromosomes of both wild-type strains and strains carrying chromosomal rearrangements whose breakpoints bracket the *white* locus. We further show that two small deficiency mutations, deleting *white* locus genetic elements but not those of complementation groups contiguous to *white*, delete the genomic sequences corresponding to a portion of the cloned segment. The strategy we have employed to isolate this cloned segment exploits the existence of an allele at the *white* locus containing a copy of a previously cloned transposable, reiterated DNA sequence element. We describe a simple, rapid method for retrieving cloned segments carrying a copy of the transposable element together with contiguous sequences corresponding to this allele. The strategy described is potentially general and we discuss its application to the cloning of the DNA sequences of other genes in Drosophila, including those identified only by genetic analysis and for which no RNA product is known.

Introduction

The existence of transposable DNA sequence elements suggests a general method for the cloning of DNA sequences corresponding to loci identified by genetic or cytogenetic analysis. If a copy of a transposable element whose DNA sequences have been cloned can be found in or near a particular locus, the DNA sequence interval containing the transposable element, together with sequences adjacent to it and corresponding to the locus in question, can be retrieved from the appropriate clone library by virtue of homology to the transposable element DNA sequences. Transposable elements represent mobile units of DNA sequence homology. Where methods exist for screening or selecting for insertion of transposable elements into a genetic region of interest, these elements can be used to label or tag that region for purposes of retrieving in cloned form its DNA sequences.

While a strategy of this sort may be applicable in many organisms, it would be of particular value to the analysis of Drosophila, where a large array of loci has been identified genetically. For many of these loci, showing profound effects on embryological development or on other processes of interest, no RNA product has been identified, and no previously described cloning strategy is generally applicable to the efficient cloning of the DNA sequences corresponding to these loci.

Recent observations have demonstrated that the *copia*-like elements of Drosophila melanogaster are transposable and may occupy a large array of chromosomal sites. Representatives of many of these families of elements have been or readily may be cloned. Moreover, D. melanogaster strains that have been isolated in laboratory culture for relatively brief periods display largely different arrays of sites of labeling when examined by in situ hybridization with the use of cloned copies of *copia*-like elements as probes. These observations led us to suppose that the development of a general cloning strategy based on the behavior of transposable DNA sequence elements could fruitfully be pursued in this organism (Ilyin et al., 1978; Potter et al., 1979; Young, 1979; Dunsmuir et al., 1980; Levis et al., 1980; Meselson et al., 1980; Bingham and Judd, 1981; Rubin et al., 1981).

We report a successful application of this strategy to the cloning of a specific gene. We have used the method to clone unique DNA sequences from the *white* locus of D. melanogaster. The *white* locus is unusual among characterized metazoan gene loci in possessing mutant alleles that affect the regulation of expression of the locus and that act in both the cis and trans configurations (Bingham, 1980 and references therein). Further, several unstable or mutable alleles at the *white* locus have been identified and characterized (reviewed in Green, 1976; Bingham, 1981); mutable alleles occur widely in multicellular organisms, and their physical nature remains to be fully clarified. The availability of the *white* locus DNA sequences in cloned form should allow the detailed investigation of these and other phenomena.

Results

An Overview of the Cloning Strategy

The *white* locus resides in distal 3C on the polytene chromosome map of the X chromosome of D. melanogaster (Lefevre and Wilkins, 1966; Sorsa et al., 1973; Judd, 1974; Bingham, 1981). Gehring and Paro (1980) recently reported that a cloned segment of DNA from D. melanogaster containing a copy of the *copia* DNA sequence element together with additional Drosophila sequences hybridizes to distal 3C in strains carrying the w^a allele but does not so hybridize

in several strains examined that do not carry the w^a allele. Results available to us at the outset of this work confirmed that the w^a allele is indeed associated with homology to the *copia* transposable element in distal 3C (Bingham and Judd, 1981; Rubin et al., 1981) and further demonstrated that this homology to *copia* resides within the *white* locus (Bingham and Judd, 1981). Thus the first requirement for the application of the cloning strategy was fulfilled; an allele at the *white* locus was identified that contains a copy of a transposable element whose DNA sequences had been cloned.

The strategy we have employed in cloning the DNA sequences surrounding the *copia* element present in the *white* locus DNA sequences may be outlined as follows. A hybrid phage clone library was constructed from the DNA from a w^a strain with a phage vector accepting cloned segment inserts substantially larger than the size of the *copia* element. Hybrid phage clones containing sequences homologous to *copia* were retrieved from the library. To distinguish cloned segments corresponding to *white* locus DNA sequences from those corresponding to any of the approximately 50 other sites occupied by *copia* copies in this w^a strain, we had to determine the cytological location of the *copia*-contiguous DNA sequences in each cloned segment. This was accomplished by hybridizing the DNA of the entire hybrid phage in situ to the salivary gland chromosomes of Drosophila simulans, a sibling species of D. melanogaster (Sturtevant, 1929; Lemeunier and Ashburner, 1976). The choice of D. simulans for this application was suggested by the observation that *copia*-like elements cloned from D. melanogaster usually are present in fewer copies in or entirely are missing from D. simulans (Meselson et al., 1980). This feature of the cloning strategy was expected to reduce confusion produced by the presence of non*copia* reiterated sequences on the hybrid phage clones in question. Lastly, a phage clone identified in this way was shown to carry *copia*-contiguous sequences homologous to unique DNA sequences residing in or very near the *white* locus.

The Isolation of a Cloned Segment Containing Sequences from the *white* Locus

The D. melanogaster strain of genotype w^a *mw mit* is labeled heavily in distal 3C as well as at a number of other sites when probes containing *copia* DNA sequences are used for in situ hybridization (Figure 1). A hybrid phage clone library was constructed from DNA isolated from this strain, and clones showing DNA sequence homology to *copia* were retrieved from the library. A group of 40 of these *copia*-homologous clones was screened in lots of five by in situ hybridi-

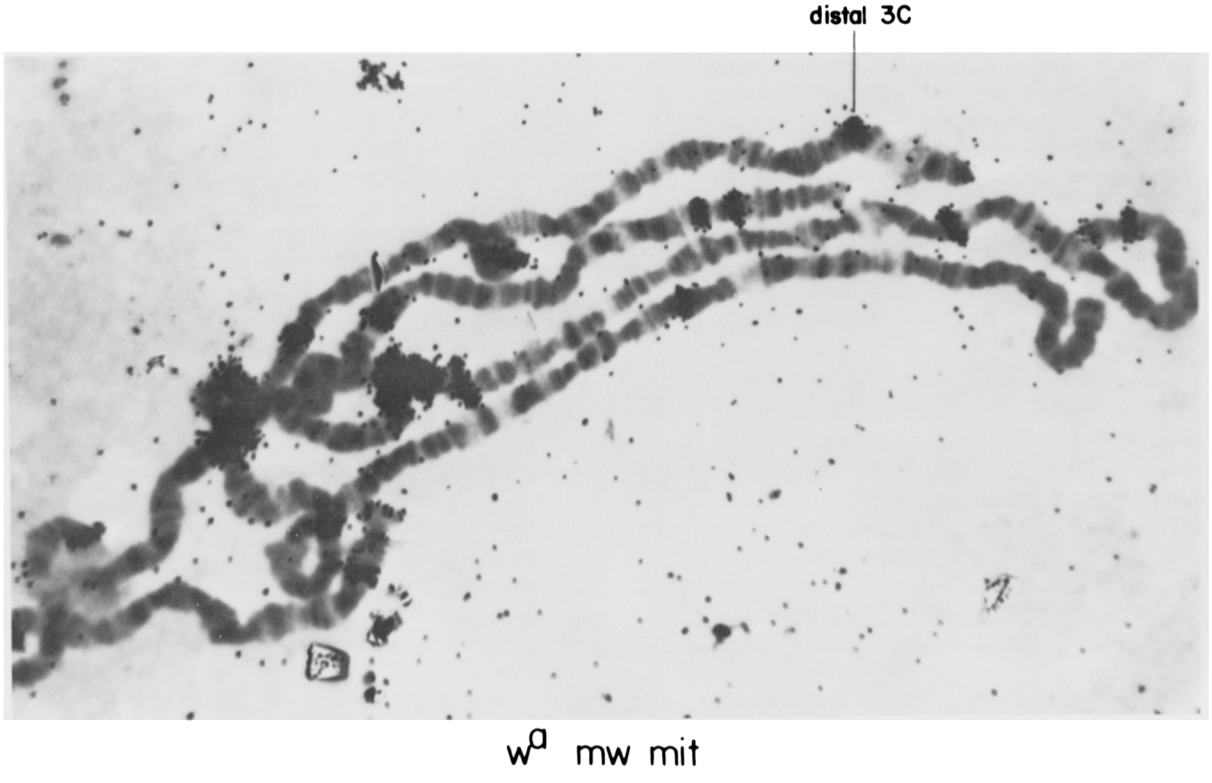

Figure 1. In Situ Hybridization to the Salivary Gland Chromosomes of a w^a *mw mit* Larva with a *copia*-Containing DNA Sequence Probe

The probe in this experiment is the 4.2 kb Hha I fragment from *cDm351* (Potter et al., 1979). The *white* locus region is indicated (distal 3C) and several other *copia* labeling sites are visible.

zation to D. simulans and one set of five showed detectable labeling in distal 3C (results not shown). Of the five members of this set, one, designated λw^a*5.9*, showed strong labeling in distal 3C in D. simulans on subsequent analysis (Figure 2). This hybrid phage, in addition to distal 3C, labels only those sites in D. simulans labeled by *copia* sequences alone. These *copia* labeling sites in D. simulans are the chromocenter and three autosomal, euchromatic sites (Figure 2). In the w^{DZL} strain of D. melanogaster (Bingham, 1980 and 1981) λw^a*5.9* labels distal 3C as well as a number of other chromosomal sites (results not shown). As in D. simulans, these additional sites correspond to those labeled by *copia* sequences alone. A second member of the set of five phages of which λw^a*5.9* is a member, designated λw^a*6.6*, labels distal 3C in the w^{DZL} strain of D. melanogaster but not in D. simulans. The λw^a*6.6* phage labels several sites on the X chromosome of D. melanogaster (w^{DZL}) in addition to those labeled by *copia* DNA sequences (results not shown) and thus presumably carries a reiterated sequence in addition to the *copia* sequence. Such behavior of λw^a*6.6* suggests that the use of D. simulans for screening hybrid phage clones in this way may not be merely a simplification but rather may be crucial to the efficient application of this cloning strategy.

λw^a*5.9* Contains a Copia Element

A restriction map of λw^a*5.9* is shown in Figure 3. An array of two Eco RI sites and one Hind III site indistinguishable from an array found in the central position of the *copia* element (Potter et al., 1979) is observed within the Drosophila DNA segment of λw^a*5.9*. The presence of a *copia* element in this position is further corroborated by the observation of approximately 5.0 kb of DNA sequence homology between this region of the cloned segment and the *copia*-containing plasmid *cDm2056* (Potter et al., 1979) as assessed by electron microscopic examination of heteroduplexes between the two DNA molecules (Figure 4). These results demonstrate that λw^a*5.9* contains a copy of the *copia* element together with additional DNA sequences that do not have extensive homology to the *copia* DNA sequences.

λw^a*5.9* Originates from the *white* Locus Region

To demonstrate that the non*copia* sequences on λw^a*5.9* are derived from the *white* locus region it is sufficient to show that a segment of these sequences has strong homology to the *white* locus region DNA sequences and to show that this same element is without significant homology to any other regions of the genome. The following series of experiments demonstrates the fulfillment of these criteria. The DNA sequence probe used for this series of experiments is the 3.1 kb Bam HI fragment represented by the solid bar on the restriction map of λw^a*5.9* shown in Figure 3. This fragment (henceforth called the 3.1 kb Bam

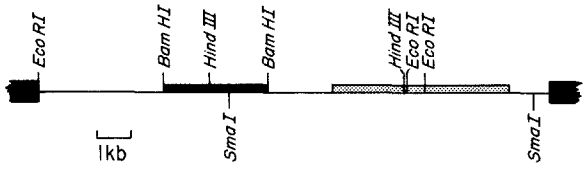

Figure 3. A Restriction Map of the D. melanogaster (w^a *mw mit*) DNA Segment in the λw^a*5.9* Hybrid Phage Clone

The stippled bar indicates the *copia* element in this cloned segment (see Figure 4) and the central solid bar indicates the 3.1 kb Bam HI fragment employed in subsequent analyses (see Figures 6 and 7).

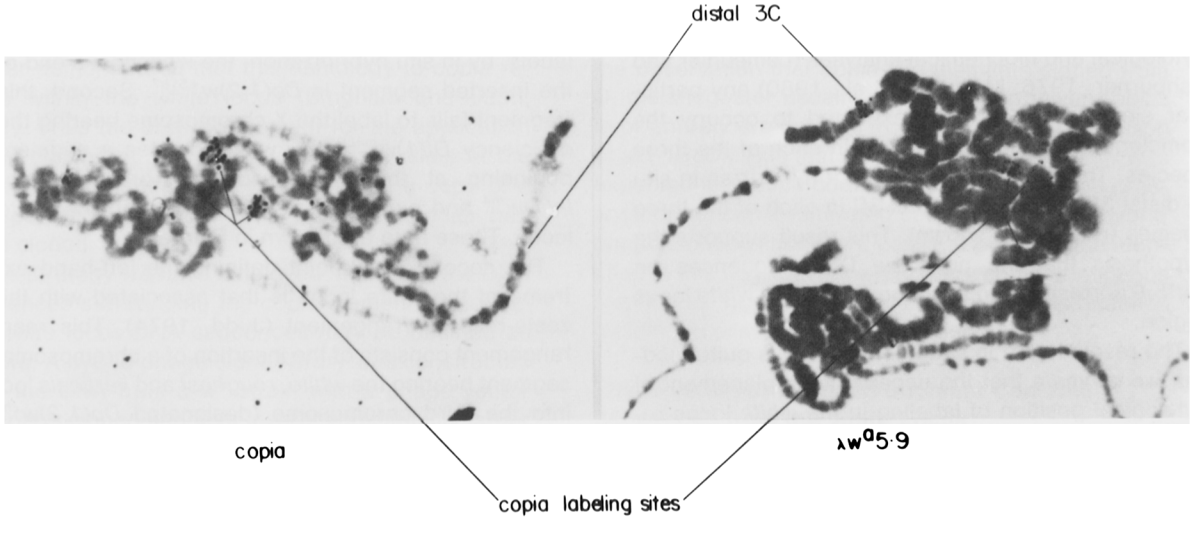

Figure 2. In Situ Hybridization to the Salivary Gland Chromosomes of D. simulans with *copia* Alone (left) and with the *copia*-Containing Hybrid Phage Clone λw^a*5.9* (right)

The *white* locus region (distal 3C) and the sites labeled by *copia* in this strain of D. simulans are indicated. The *copia* probe (left) is the 4.2 kb Hha I fragment from *cDm351* (Potter et al., 1979). A restriction map of λw^a*5.9* (the sequence probe in the right) is in Figure 3.

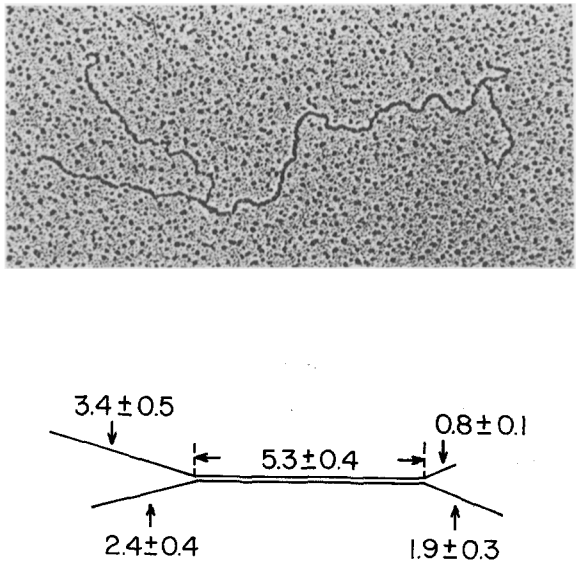

Figure 4. Electron Micrograph of a Heteroduplex Molecule Formed between the 9 kb Sma I Fragment of $\lambda w^a 5.9$ and a Bam HI Fragment of *cDm2056* that Contains a Typical *copia* Element

(Top) An electron micrograph of such a heteroduplex; the 5 kb region of homology observed between the $\lambda w^a 5.9$ (see Figure 3) and *cDm2056* fragments corresponds to the known size and position of the *copia* element within *cDm2056* (Potter et al., 1979). (Bottom) Diagram summarizing the data obtained from measurement of ten such heteroduplex molecules.

fragment) is approximately 2.0 kb from the *copia* element in $\lambda w^a 5.9$ and has no detectable DNA sequence homology to *copia* as assessed by Southern gel analysis (results not shown).

D. simulans and D. mauritiana are very closely related to D. melanogaster, and genetic analysis has demonstrated that the *white* locus in D. simulans occupies a genetic map position on the X chromosome similar to that occupied by *white* in D. melanogaster (Sturtevant, 1929). Further, on the basis of detailed cytological and biochemical analyses (Lemeunier and Ashburner, 1976; Meselson et al., 1980) any particular single copy locus is expected to occupy the homologous cytological position in each of the three species. The 3.1 kb Bam fragment hybridizes in situ to distal 3C and only to distal 3C in each of the three species (results not shown). This result supports the hypothesis that the non*copia* DNA sequences on $\lambda w^a 5.9$ correspond to sequences from the *white* locus region.

The resolution of in situ hybridization is quite modest; we estimate that the uncertainty in placement of cytological position of labeling in the *white* locus region is on the order of 1–2 large polytene chromosome bands, or approximately 50–200 kb. The resolution of this technique may materially be enhanced by employment in conjunction with fine scale genetic and cytogenetic analysis. These last two techniques have allowed other investigators to identify and characterize several chromosomal rearrangement mutations whose breakpoints define the left-hand (distal or telomere-oriented) and right-hand (proximal or centromere-oriented) extremes of the *white* locus.

The left-hand extreme of the *white* locus DNA sequences is defined by two chromosomal rearrangement breakpoints. The meiotic map of the region beginning at the *white* locus and extending to the left is very well characterized and densely populated (Judd et al., 1972), and the map position of the two breakpoints can be inferred from genetic analysis (Figure 5).

The first of these two breakpoints is the *white*-associated breakpoint for the variegating *white* allele in $In(1)w^{m4}$. This breakpoint resides between the *white* locus and the closest known complementation group to its left, *zw9*, as assessed by the behavior of two derivatives of $In(1)w^{m4}$ (Lefevre and Wilkins, 1966). The first derivative, $Df(1)w^{m4L}w^{mJR}$, deletes a segment beginning at the *white*-associated breakpoint of $In(1)w^{m4}$ and extending rightward through the *white* locus. Organisms carrying this deficiency are fully viable and fertile (Lefevre and Wilkins, 1966) and this deficiency is presumed, therefore, not to disturb the function of the *zw9* complementation group; all known point mutant alleles at *zw9* are recessive semi-lethals exhibiting reduced aristae and greatly reduced fertility among individuals surviving to adulthood (Rayle and Green, 1968; Judd et al., 1972). The second derivative is the insertional translocation, $Dp(1,2)w^{+51b7}$, which carries a segment beginning at the *white*-associated breakpoint of $In(1)w^{m4}$ and extending rightward through the *white* locus inserted into the second chromosome. This inserted segment complements alleles at the *white* locus but not those at the *zw9* locus.

The sequences homologous to the 3.1 kb Bam fragment from $\lambda w^a 5.9$ reside to the right of the *white*-associated breakpoint of $In(1)w^{m4}$, as demonstrated by the following observations. First, this fragment labels, by in situ hybridization, the *white* locus end of the inserted segment in $Dp(1,2)w^{+51b7}$. Second, this fragment fails to label the X chromosome bearing the deficiency $Df(1)w^{m4L}rst^{3R}$, which deletes a segment beginning at the *white*-associated breakpoint of $In(1)w^{m4}$ and extending rightward through the *white* locus. These data are shown in Figure 6.

The second breakpoint defining the left-hand extreme of the *white* locus is that associated with the zeste-halo rearrangement (Judd, 1974). This rearrangement consists of the insertion of a chromosomal segment bearing the *white*, *roughest* and *verticals* loci into the third chromosome (designated $Dp(1,3)w^{zh}$) and the possibly corresponding deletion of the *white*, *roughest* and *verticals* loci on the X chromosome (designated $Df(1)w^{zh}$). The insertional translocation complements mutant alleles at the *white* locus but not those at the *zw9* locus, and the deficiency complements alleles at the *zw9* locus but not those at the *white* locus (Judd, 1974 and personal communica-

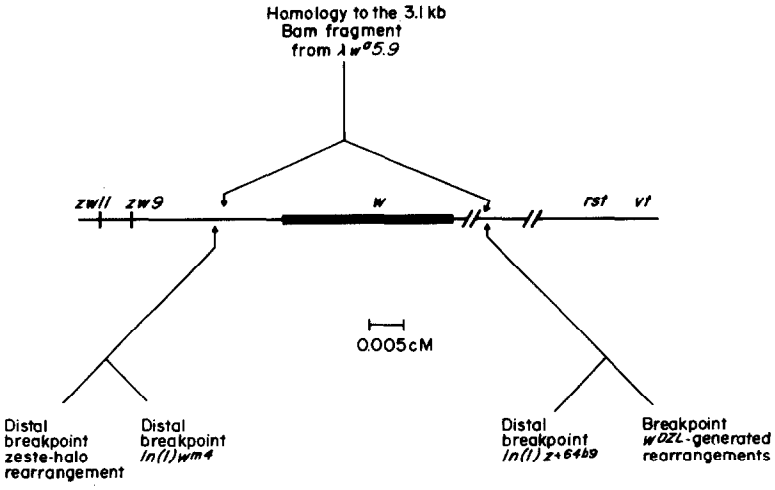

Figure 5. Schematic Summary of the Results of In Situ Hybridization with the 3.1 kb Bam Fragment from $\lambda w^a 5.9$ to D. melanogaster Strains Bearing Chromosomal Rearrangements Whose Breakpoints Bracket the *white* Locus

The central horizontal line represents the genetic map of the *white* locus region and the solid bar on this line represents the approximate extent of the *white* locus. $Df(1)w^{DZL}2$, $Df(1)w^{DZL}14$ and $In(1)z^{+64b9}$ have breakpoints near and to the right of the *white* locus; the 3.1 kb Bam fragment from $\lambda w^a 5.9$ is homologous to sequences to the left of these breakpoints. $In(1)w^{m4}$ and the zeste-halo rearrangement have breakpoints near and to the left of the *white* locus; the 3.1 kb Bam fragment from $\lambda w^a 5.9$ is homologous to sequences to the right of these breakpoints.

tion). The behavior of the zeste-halo rearrangement is consistent with but does not directly demonstrate that the *white*-associated breakpoint for the insertional translocation and for the deletion are the same breakpoint.

The sequences homologous to the 3.1 kb Bam fragment from $\lambda w^a 5.9$ reside to the right of the *white*-associated breakpoint(s) of the zeste-halo rearrangement, as this fragment labels the $Dp(1,3)w^{zh}$ insertional translocation (at 62D on the third chromosome) but does not label the X chromosome bearing $Df(1)w^{zh}$ (Figure 6). Unlike any other strains that we have examined, the $Df(1)w^{zh}$; $Dp(1,3)w^{zh}$ strain in our possession shows an unexpected site of labeling with the 3.1 kb Bam fragment. This additional site resides at 89A on the third chromosome (Figure 6) in 12 of 12 individuals of this genotype that we have examined. The *white* allele associated with $Dp(1,3)w^{zh}$ is unstable (Judd, 1974) as assessed by genetic analysis, and we suggest that this second site of labeling may represent a second copy of the information on $Dp(1,3)w^{zh}$. This second copy could, on this hypothesis, represent the product of a transposition event that occurred after the event giving rise to $Dp(1,3)w^{zh}$.

The right-hand extreme of the *white* locus is likewise defined by two independently derived chromosomal breakpoints (Figure 5). The meiotic map beginning at *white* and extending to the right is much less densely populated than is the map to the left of the *white* locus. The closest clearly defined point mutation to the right of *white* (rst^{CT}) resides approximately 100-fold further from *white* (Lefevre and Green, 1972) than does the closest known point mutation to the left of *white* (Judd et al., 1972). The *white* locus and the closest clearly defined locus to its right (the *roughest* locus) apparently are separated by several polytene chromosome bands (Lefevre and Green, 1972). As a result of these properties of the interval to the right of *white*, fine scale cytological analysis is superior to complementation analysis in defining the relationship between breakpoints to the right of the *white* locus and the locus itself.

The *white*-associated breakpoints of $In(1)w^{m4}$ and the zeste-halo rearrangement have been placed in or very near the interband region between polytene chromosome bands 3C1 and 3C2 (Lefevre and Wilkins, 1966; Judd, 1974). The breakpoints of several rearrangements generated by the mutable w^{DZL} allele (Bingham, 1981) and the breakpoint of $In(1)z^{+64b9}$ (Sorsa et al., 1973) have been well defined as residing to the right of the *white* locus but likewise in or very near the 3C1, 3C2 interband. These breakpoints to the right of *white* are removed from the *white*-associated breakpoints (to the left of *white*) of $In(1)w^{m4}$ and the zeste-halo rearrangement by less than one polytene chromosome band width (Sorsa et al., 1973; Bingham, 1981).

The sequences homologous to the 3.1 kb Bam fragment reside to the left of the breakpoint(s) for two w^{DZL}-generated rearrangements as demonstrated by the following results. $Df(1)w^{DZL}2$ and $Df(1)w^{DZL}14$ are deficiency mutations whose deleted segments begin near the right end of the *white* locus and extend to the right through the *roughest*, *verticals* and *Notch* loci (Bingham, 1981). These deficiency chromosomes are labeled in distal 3C by in situ hybridization with the 3.1 kb Bam fragment. An example is shown in Figure 6.

The sequences homologous to the 3.1 Bam fragment from $\lambda w^a 5.9$ likewise reside to the left of the *white*-associated breakpoint for $In(1)z^{+64b9}$, as demonstrated by the following result. $In(1)z^{+64b9}$ contains an inverted segment beginning near the right-hand end of the *white* locus and extending rightward to 12BC (Sorsa et al., 1973). The *white* locus resides distal to the inverted segment, and the 3.1 kb Bam fragment labels the distal terminus of the inverted segment (Figure 6).

In summary of these results, the 3.1 kb Bam fragment from $\lambda w^a 5.9$ labels, by in situ hybridization, distal

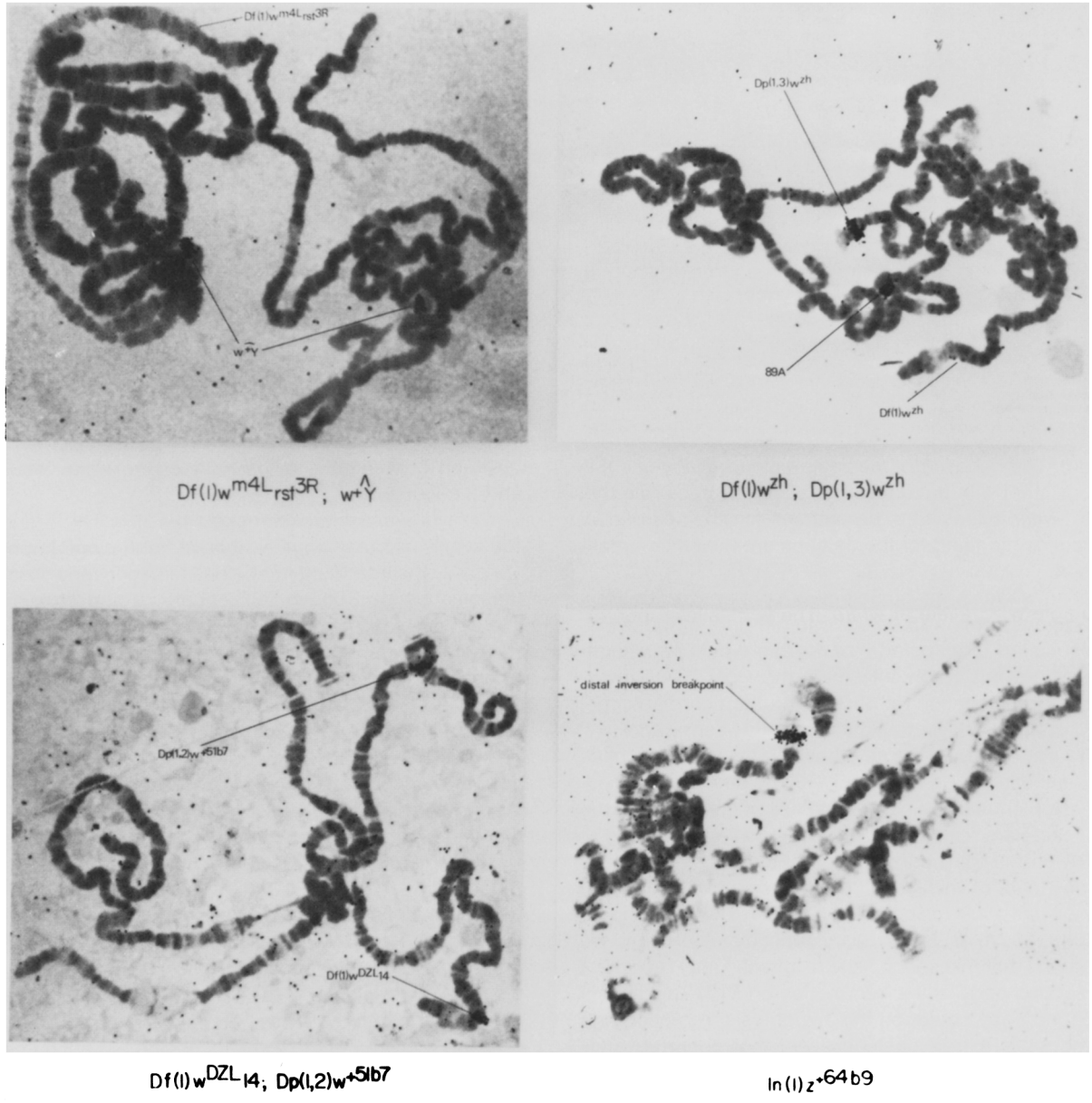

Figure 6. In Situ Hybridization with the 3.1 kb Bam Fragment from $\lambda w^a 5.9$ to Various D. melanogaster Strains Bearing Chromosomal Rearrangements Whose Breakpoints Bracket the *white* Locus

The genotypes of the larvae donating the polytene chromosomes in each panel are given below the panel. The upper left-hand panel shows that the *white* locus region on the w^+Y translocation, but not that of $Df(1)w^{m4L}rst^{3R}$, is labeled by the probe fragment. The upper right-hand panel shows that the *white*-bearing $Dp(1,3)w^{zh}$ insertional translocation, but not the $Df(1)w^{zh}$ *white* deletion, is labeled by the probe fragment; the additional site of labeling at 89A in our $Df(1)w^{zh}$; $Dp(1,3)w^{zh}$ stock is also indicated. The lower left-hand panel shows that the *white* locus region of both $Df(1)w^{DZL}14$ and the *white*-bearing insertional translocation $Dp(1,2)w^{+51b7}$ are labeled by the probe fragment. The lower right-hand panel shows that the distal (*white*-associated) breakpoint of $In(1)z^{+64b9}$ is labeled by the probe fragment.

3C and only distal 3C in all strains not bearing chromosomal rearrangements that we have examined. These include D. simulans, D. mauritiana and the w^{DZL} strain of D. melanogaster. In addition, this fragment is homologous to sequences residing to the left of chromosomal breakpoints defining the right end of the *white* locus and to the right of chromosomal breakpoints defining the left end of the *white* locus. With the exception of the single additional site in our zestehalo rearrangement stock, no homology to the 3.1 kb Bam fragment from $\lambda w^a 5.9$ was detected by in situ hybridization at sites other than those predicted by

the hypothesis that this fragment corresponds to sequences residing in or very near the *white* locus. Figure 5 shows a schematic summary of these results.

The 3.1 kb Bam Fragment Does Not Have Substantial Homology to Any Sequences Other Than Those in the *white* Locus Region

Some chromosomal sequences are underreplicated in the salivary gland polytene chromosomes used for in situ hybridization (Dickson et al., 1971; Gall et al., 1971). Further, it recently has been proposed that underreplication of chromosomal sequences in polytene chromosomes may affect a larger portion of the genome than previously had been supposed (Laird, 1980). The possibility must be considered, therefore, that the 3.1 kb Bam fragment is homologous to sequences elsewhere in the genome, in addition to those in the *white* locus region, but that these sequences are underreplicated in salivary gland chromosomes or for some other reason escape detection by in situ hybridization.

A simple test of this possibility is suggested by the behavior of a class of spontaneous derivatives of the mutable w^{DZL} allele at *white*. The w^{DZL} allele spontaneously gives rise to *white* mutant alleles that produce a white eye color phenotype in the hemizygous and homozygous conditions and these hemizygous and homozygous individuals are fully viable and fertile. These derivatives are not associated with obvious cytological abnormality, and genetic analysis suggests that some or all are very small deficiency mutations whose deleted segments begin at the right-hand end of the *white* locus and extend leftward into or through *white* but do not extend into sequences critical to the function of the *white*-contiguous *zw9* complementation group (Bingham, 1981).

We have examined two of these derivatives, designated w^{pD79a} and w^{pD79k}. In contrast to the parental w^{DZL} chromosome, neither of these derivatives shows detectable labeling by the 3.1 kb Bam fragment from $\lambda w^a 5.9$ as assessed by in situ hybridization (results not shown). These observations strongly corroborate the hypothesis that these derivatives are, indeed, small deficiency mutations. If the 3.1 kb Bam fragment has no substantial homology to sequences other than those in the *white* locus region detected by in situ hybridization, the DNA isolated from w^{pD79a} and w^{pD79k} strains should display no substantial homology to this fragment when examined by filter hybridization techniques (Southern, 1975). The results of such a filter hybridization experiment are shown in Figure 7.

The results in Figure 7 have three important features. First, DNAs isolated from either the w^{pD79a} or w^{pD79k} strains show no fragment with substantial homology to the 3.1 kb Bam fragment. Thus the central prediction of the hypothesis that the probe fragment has no substantial homology to sequences elsewhere than in the *white* locus region is fulfilled. However, approximately ten very faint bands may be seen in the original autoradiographs in addition to the major band of hybridization seen in the DNAs from non-deletion strains. These additional bands display intensities of approximately 1% of that of the major band of hybridization and are seen in all strains that we have examined, including the deletion strains. The origin of these minor bands is currently being investigated and we point out that their existence does not contradict the hypothesis that the 3.1 kb Bam fragment corresponds to sequences from the *white* locus region. Second, a Bam fragment of apparently identical molecular weight to the 3.1 kb Bam fragment from $\lambda w^a 5.9$ is present in DNA isolated from Canton-S organisms, and the intensity of labeling of this fragment is consistent with its being present in one copy per haploid genome (approximately 15 pg/μg of fly DNA). Third, DNA from the w^{DZL} (Oregon-R) strain contains a single hybridizing fragment of obviously different molecular weight than the fragment from Canton-S. The fact that these two strains differ from one another by the loss of a fragment and its replacement with a fragment of dif-

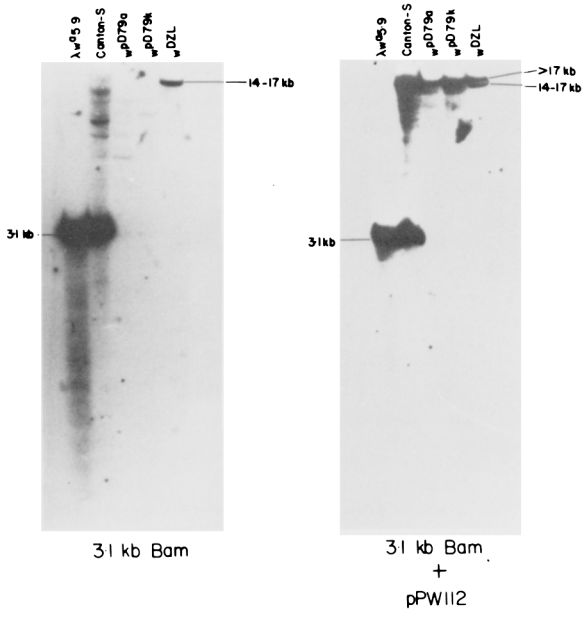

Figure 7. Duplicate Southern Filters Bearing Bam HI Digested DNAs from $\lambda w^a 5.9$ and from D. melanogaster Adults of Various Genotypes

(Left) Filter probed with the 3.1 kb Bam fragment from $\lambda w^a 5.9$; (right) filter probed with this same fragment as well as with the plasmid clone *pPW112*. The *pPW112* clone bears a randomly chosen D. melanogaster DNA segment homologous to DNA sequences present in one copy per haploid genome in D. melanogaster; the sequences homologous to *pPW112* are not cut by Bam HI (P. M. Bingham, unpublished observations). The presence of the expected homology to *pPW112* on the right-hand filter demonstrates that the absence of substantial homology to the 3.1 kb Bam fragment from $\lambda w^a 5.9$ is not due to the failure of transfer of DNA from the deficiency mutant channels to the filter. Each of the two filters bears the following DNAs (from left to right): 0.001 μg $\lambda w^a 5.9$, 5 μg Canton-S (w^+), 5 μg w^{pD79a} (*white* deletion), 5 μg w^{pD79k} (*white* deletion) and 5 μg w^{DZL} (non-deletion mutation in Oregon-R w^+ allele).

ferent molecular weight further corroborates the hypothesis that the 3.1 kb Bam fragment used as probe is homologous to DNA sequences present in only one copy per haploid genome. The argument is as follows. A sequence element present in only one copy per haploid genome will, under the appropriate circumstances, produce a single restriction fragment on Southern gel analysis. Further, base pair substitution mutation or sequence rearrangement mutation can lead to the production of a different size restriction fragment with the concomitant loss of the original restriction fragment, as we observe in comparing the Canton-S and w^{DZL} strains (Figure 7). However, while a sequence element present in more than one copy per haploid genome may, under some circumstances, produce only one size class of restriction fragment on Southern gel analysis, a single mutational event cannot produce a new genotype in which the multiple copies of the element will produce the same size class of restriction fragments now of a different molecular weight than that observed before the mutational event.

In summary, the analysis of these two deletion derivatives of w^{DZL}, w^{pD79a} and w^{pD79k} strongly supports the hypothesis that the only genomic sequences with substantial homology to the 3.1 kb Bam fragment from $\lambda w^a 5.9$ reside in distal 3C. We wish to emphasize that these two independently arising mutations apparently delete both the homology to the 3.1 kb Bam fragment as shown here and at least a portion of the *white* locus DNA sequences as shown by genetic analysis (Bingham, 1981) and do so without the production of an associated mutant phenotype other than that produced by the deletion of *white* locus sequences. This feature of the behavior of these mutants clearly suggests that the sequences homologous to the probe fragment and residing in distal 3C are within or very near the *white* locus DNA sequences.

Discussion

The results described here demonstrate that we have cloned DNA sequences from the *white* locus region of Drosophila melanogaster by a potentially general method. In particular, we have demonstrated that a cloned segment of Drosophila DNA, retrieved from a clone library by virtue of its DNA sequence homology to the *copia* transposable element, contains sequences mapping to the left of chromosomal breakpoints at the right end of the *white* locus and to the right of chromosomal breakpoints at the left end of the *white* locus. Further, small deficiency mutations, deleting *white* locus genetic elements but not those of contiguous complementation groups, delete sequences present on the cloned segment.

Bingham and Judd (1981) have shown that the homology to *copia* associated with the w^a allele resides within the central portion of the *white* locus as assessed by genetic mapping experiments. Moreover, they have shown that the *copia* homology is very tightly linked to and conceivably resides precisely at the w^a site. Lastly, no homology to *copia* was detected in distal 3C in w^a strains other than that mapping in the central portion of the *white* locus. The results of Bingham and Judd, together with those reported here, demonstrate that $\lambda w^a 5.9$ contains sequences from within the *white* locus.

Several comments regarding the application of the gene retrieval strategy described here to the cloning of other genes in Drosophila are warranted. The methods described should allow the cloning of DNA sequences corresponding to any locus at which an allele can be identified containing, in or near the locus, a copy of a transposable element whose DNA sequences have been cloned. The prime difficulty to be overcome in applying this method to any particular case is that of identifying alleles at the locus of interest containing a copy of a transposable element within the required interval. One approach to this problem is that of screening, by in situ hybridization with cloned *copia*-like elements as probes, collections of fly strains to identify strain-probe combinations producing labeling within the required interval. Available observations (Ilyin et al., 1978; Strobel et al., 1979; Young, 1979; Bingham and Judd, 1981; Rubin et al., 1981) suggest that the extent of divergence between readily available strains of D. melanogaster in the cytological locations of members of families of *copia*-like elements is adequately large to allow this approach. We estimate on the basis of these data that the test of approximately 200 probe-strain combinations would be necessary to isolate one usable combination.

An alternative to this procedure for isolating a suitable allele at a locus of interest is suggested by recent studies of hybrid dysgenesis (Bregliano et al., 1980; Kidwell, 1980; Simmons and Lim, 1980; Engels, 1981). We will discuss the approach in the context of the PM hybrid dysgenesis system, but the precisely analogous approach employing the IR system seems equally feasible at this point in the analysis of the two systems. The "P factor hypothesis" (Kidwell, 1980; Engels, 1981; references therein) supposes that the properties of dysgenic hybrids are due in part to the properties of a transposable DNA sequence element (the P factor) supposed to be present in P strains and missing in M strains. This hypothesis further supposes that many of the mutant alleles arising in PM dysgenic hybrids (such hybrids have very high spontaneous mutation rates) result from the insertion of a copy of the P factor into the DNA sequences of the locus of the mutation. If this hypothesis is correct and if the P factor DNA sequences can be cloned, a mutant allele at the locus of interest arising in a dysgenic hybrid would represent a candidate for the application of the gene retrieval strategy described here. This particular strategy, if feasible, would be highly efficient and may

represent the method of choice for cloning many gene sequences from Drosophila.

Experimental Procedures

Drosophila Strains

The Drosophila strains used here are described in the text. Additional information may be found in Lindsley and Grell (1968), Lefevre and Wilkins (1972), Judd (1974), Lemeunier and Ashburner (1976) and Bingham (1980, 1981).

Isolation of Drosophila DNA for Southern Gel Analysis

The DNAs used for Southern gel analysis were isolated as follows. Etherized adults were immersed in liquid nitrogen and ground to a fine powder in a liquid-nitrogen-cooled mortar and pestle. The grindate (1–2 g) was suspended in 30 ml nuclear isolation buffer at 0°C at pH 7.4 containing 10 mM Tris, 60 mM NaCl, 10 mM EDTA, 0.15 mM spermidine, 0.15 mM spermine and 0.5% (v/v) Triton X-100. The dissolved grindate was homogenized with a motor-driven teflon homogenizer, and large particles were removed by centrifugation (one or two times) for 15–20 sec at 1500 rpm on a table top centrifuge. Nuclei were collected by centrifugation of the supernatant from the preceding step for 7 min at 7000 rpm in a Beckman JS13 rotor at 4°C. The pellet from this step was resuspended in 30 ml nuclear isolation buffer, and the nuclei were again pelleted in the Beckman high speed centrifuge as above. The pellet from the preceding step was resuspended in 5 ml nuclear isolation buffer, and 10% (w/v) Sarkosyl (sodium salt of N-Laroyl Sarcosine; Sigma) was added to a final concentration of 2% with vigorous stirring with a glass rod. The lysate from this step was added to 12.1 g CsCl and the final volume of the solution was adjusted to 13 ml with use of nuclear isolation buffer with 2% Sarkosyl as diluent. This solution was centrifuged for 48–72 hr at 55,000 rpms in a Beckman Ty65 fixed angle rotor at 15°C, and the viscous fractions were collected and dialyzed exhaustively against a pH 7.4 buffer containing 10 mM Tris and 1 mM EDTA.

Southern Gel Analysis

Southern gel analysis was performed essentially according to Southern (1975) as modified by Botchan et al. (1976).

Growth of Hybrid Phage Clones in Liquid Culture

To produce the small amounts of DNA required for probes for in situ hybridization experiments the following procedure was used. Fresh plaques (16–24 hr of incubation at 37°C) were removed as a core from soft agar overlay plates whose bottom agar contained 1% (w/v) Trypticase, 0.5% (w/v) NaCl and 0.85% (w/v) agar, and whose top agar contained the same components with the agar concentration reduced to 0.65% (w/v). The cored plaques were discharged into 0.3 ml pH 7.4 buffer containing 20 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$ and 0.01% (w/v) gelatin. This solution was allowed to stand for 30–90 min at room temperature with occasional vortexing. One drop (30–50 μl) of the phage suspension was added to one drop of an overnight culture of bacteria (CSH18 or KH802) grown in a medium containing 2% Tryptone (w/v), 0.5% (w/v) yeast extract, 0.1% (w/v) maltose and 1% (w/v) NaCl. (Variations in cell input of 2 to 4 fold and of phage input of 2 to 10 fold have allowed us to use this procedure for growing hybrid phage clones derived from *Charon 4*, *Charon 28*, *Charon 30* and λ*Sep6*.) The phage-cell mixture was incubated at 37°C for 15 min, and 5 ml of phage growth medium prewarmed to 37°C was added. Phage growth medium contains the following components expressed as w/v percents: 0.7% Na_2HPO_4 (anhydrous), 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 0.36% glucose, 0.4% maltose, 1.0% casamino acids, 0.12% $MgSO_4 \cdot 7H_2O$ and 0.01% $CaCl_2$. Though this medium is somewhat more laborious to make than other phage growth media that have been described (Blattner et al., 1977), we find that it allows greater reproducibility of phage titers and routinely produces titers of $1–4 \times 10^{10}$ pfu/ml under these conditions. The phage stocks were incubated with shaking at 37°C in 18 mm × 150 mm test tubes and stocks were taken that showed lysis at incubation times of 4–7 hr. Growth of these stocks was terminated by the addition of several drops of $CHCl_3$, NaCl to 6% (w/v) and DNAase and RNAase to approximately 0.01% (w/v) followed by incubation on ice for 30 min. This procedure allows the efficient growth of hybrid phage clones in batches of 30–50.

For large scale growth of hybrid phage clones the above procedure was used, with the following modifications: $5 \times 10^6–5 \times 10^8$ pfu were combined with 2.5–4 ml overnight culture and added to 300 ml phage growth medium in a 2 l Erlenmeyer flask after preadsorption of concentrated phage-cell mixture for 15 min at 37°C. While we have found plate stock amplification (see Construction of Hybrid Phage Library) to be more reliable for hybrid phage clones derived from λ*Sep6*, the liquid culture method described above has proven satisfactory for the amplification of hybrid phage clones derived from *Charon 4*, *Charon 28* and *Charon 30*.

Purification of Phage DNA from Liquid Culture Stocks

After the incubation of terminated phage stocks as described under Growth of Hybrid Phage Clones in Liquid Culture the phages were concentrated by polyethylene glycol (PEG) precipitation. The lysate (made 6% [w/v] in NaCl in the preceding step) was centrifuged at 8000 rpm for 20 min at 4°C in either a Beckman JS13 or Beckman JA10 rotor. PEG (Carbowax 6000, Fischer) was added to 7% (w/v) and allowed to dissolve fully. After thorough chilling the solution was centrifuged immediately as above. For purification of DNA from a single hybrid phage clone the entire culture (5 ml) was precipitated. For the purification of DNA from mixed cultures for screening of large numbers of clones by in situ hybridization, one ml from each of five cultures were combined and precipitated. The precipitate from 5 ml of culture was resuspended in 0.2 ml pH 8.0 suspension medium containing 20 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.01% (w/v) DNAase and 0.01% (w/v) RNAase. After incubation for 10–30 min at room temperature the resuspended phage particles were further purified by filtration on a Control Pore Glass Bead column (0.5 cm × 23 cm, PG-240-100, Sigma). Suspension medium without RNAase or DNAase was the developing solvent. The column fractions containing phage particles were pooled and extracted twice with phenol. Nucleic acids were precipitated as described under Nick Translation (below) and the pelleted precipitate was air dried and resuspended in 50 μl of a pH 7.4 buffer containing 10 mM Tris and 1 mM EDTA. RNAase was added to 10 μg/ml and the samples were incubated for 15–30 min at room temperature. The samples were gel filtered on a G-200 column (0.15 cm × 8 cm, Superfine, Sephadex) with a pH 7.4 buffer containing 10 mM Tris and 1 mM EDTA as the developing solvent. An excluded volume of 90–120 μl was collected.

Nick Translation

DNA sequence probes for in situ hybridization were produced by nick translation. One hundred microCuries of ^{3}H-dTTP (dried under forced air from a 50% ethanol solution; 50–100 Ci/mmole, Amersham) was resuspended in 100 μl of a pH 7.4 solution containing 10 mM Tris; 10 mM $MgCl_2$; 0.01% (w/v) gelatin; 0.1 mM each dATP, dCTP and dGTP and 0.2–1.0 μg of DNA. Five units E. coli DNA polymerase I (Grade I, Boehringer Mannheim) and 10 ng pancreatic DNAase I were added. The reaction mixture was incubated for 15 min at 12°–15°C followed by the addition of EDTA to 50 mM and SDS to 5% (w/v). The reaction was fractionated on a G-50 (Sephadex) column (0.5 cm × 23 cm) and the excluded volume was collected. We added 100 μg of sonicated calf thymus DNA (Type I, Sigma) and ethanol precipitated the DNA by the addition of sodium acetate to 0.3 M followed by the addition of three volumes of ethanol and incubation for at least 5 hr at −20°C. The precipitate was collected by centrifugation at 13,000 rpm in a Beckman JS13 rotor for 20 min at 4°C.

DNA sequence probes from mixtures of hybrid phage clones or from small volume cultures were produced by nick translating (as described above) 30 μl of each of four serial 3-fold dilutions of the DNA, purified as described under Purification of Phage DNA from Liquid Culture Stocks. The most dilute sample yielding maximal

incorporation was used to generate the probes for in situ hybridization.

DNA sequence probes for Southern gel analysis were prepared by the same method as described for preparation of probes for in situ hybridization, with the following modifications. ^{32}P-dCTP (2000–3000 Ci/mmole, Amersham) at 0.5 mCi/ml replaced 0.1 mM dCTP, and 0.1 mM dTTP replaced ^{3}H-dTTP.

Heteroduplex Mapping

Sma I–digested λ*w^{a}5.9* and Bam HI–digested cDm 2056 (Potter et al., 1979) were combined in equimolar amounts. Denaturation and renaturation in formamide were exactly as described by Davis, Simon and Davidson (1971). Heteroduplexes were spread from a spreading solution containing 50% formamide on a hypophase containing 20% formamide. ΦX single-stranded and double-stranded DNAs (Sanger et al., 1977) were used as internal length standards to calibrate length measurements of heteroduplex molecules.

Construction of Hybrid Phage Clone Library

λ*Sep6* (E. Meyerowitz, D. Kemp and D. Hogness, personal communication) DNA was phenol extracted from phage purified from confluently lysed lawns of E. coli ED8799 (Murray et al., 1977). Ninety μg of DNA was cut to completion with Eco RI, layered on a 10.5 ml gradient of 5%–25% (w/v) sucrose in a pH 8.0 solution containing 10 mM Tris, 1 mM EDTA, 100 mM NaCl and centrifuged at 39,000 rpm for 4.5 hr at 20°C in a Beckman SW41 rotor. DNA was precipitated by the addition of two volumes of ethanol from fractions enriched in the two vector arm fragments and dissolved in a pH 8.0 solution containing 10 mM Tris and 1 mM EDTA.

Drosophila DNA was purified from embryos of the *w^{a}mw mit* strain of D. melanogaster (Lindsley and Grell, 1968) as described by Wensink et al. (1974). The DNA was methylated with the Eco RI methylase and partially digested with Eco RI under conditions favoring the Eco RI* activity (Polinsky et al., 1975) as described by Kemp et al. (1979). Seventy-five micrograms of DNA were methylated at a concentration of 100 μg/ml with four units of methylase (New England Biolabs) for 2 hr at 37°C and digested with 1250 units of Eco RI (Boehringer) for 4 hr at 37°C. Following sucrose gradient fractionation as described above, DNA in the size range of 10 kb–25 kb was pooled, precipitated by the addition of two volumes of ethanol and redissolved in a pH 8.0 solution containing 10 mM Tris and 1 mM EDTA.

Ten micrograms of each of the enriched vector arms and the 10 kb–25 kb size fraction of Drosophila DNA were combined, precipitated with ethanol and dissolved in a pH 8.0 solution containing 10 mM Tris and 1 mM EDTA. The DNA was adjusted to a final volume of 50 μl with the addition of $MgCl_2$ to 10 mM, $(NH_4)_2SO_4$ to 10 mM and bovine serum albumin to 0.01% (w/v). An excess of E. coli ligase was added, and the mixture was incubated for 9 hr at 8°C. The ligated DNA was packaged in vitro as described by Hohn (1979). Packaging extracts were prepared without irradiation. The yield was approximately 2×10^5 pfu/μg of Drosophila DNA.

Purification of Phage Containing *copia* Sequences

Phage were screened at a density of 2500 plaques per 24 cm × 24 cm dish on lawns of E. coli C600 r^-m^+ NalA$^-$ (from M. Syvanen) bacteria. A nitrocellulose replica, prepared from each dish as described by Benton and Davis (1977), was pretreated with 0.02% (w/v) of each of ficoll, polyvinylpyrolidone and bovine serum albumin in 4× SSCP (Rubin, 1978) for 6–8 hr at 65°C. The replicas were then hybridized in batches of approximately 10/500 ml of the above solution containing 0.5% SDS, 200 μg/ml of sonicated salmon sperm DNA and 4×10^7 cpm of a *copia*-specific probe. The probe used was the 4.2 kb Hha I fragment from cDm351 (Potter et al., 1979), ^{32}P-labeled by nick translation according to Rigby et al. (1977). Phage were eluted from the agar in each area of hybridization and replated at a lower density to produce well separated plaques. Ten plaques from each of the positive phage were transferred with a toothpick onto a grid on a lawn of C600 and a filter replica of this grid was screened as above. Phage, taken from spots giving hybridization, were further plaque purified and retested by hybridization. A total of 190 independent *copia*-containing phage clones were retrieved from the screen of approximately 6×10^4 phage clones. This is in agreement with the number predicted by the known number of copies of *copia* in laboratory strains (Potter et al., 1979).

In Situ Hybridization

We have used a procedure that includes several modifications of previously published procedures and that improves the signal strength. This modified procedure is the unpublished work of P. Bingham and N. Chapman.

Salivary gland chromosome squashes were prepared and denatured according to Atherton and Gall (1972) with the following modifications. Dissection was carried out in 45% (v/v) acetic acid. Slides were pretreated with a bovine serum albumin-ficoll-polyvinylpyrolidone solution according to the procedure described by Brahic and Haase (1978). Coverglasses (No. 1, 18 mm^2, Corning) were used directly from the package. Squashes were treated at 70°C in 2× SSC according to the procedure described by Bonner and Pardue (1976).

Hybridization was carried out with the probe concentrations of 0.1–0.2 μg/ml/kb of probe sequence complexity; half saturation is achieved, under the conditions described here, at approximately 0.04 μg/ml/kb of probe sequence complexity. The hybridization solution consisted of a 1:1 (v/v) mixture of formamide (Fluka) and a pH 6.8 solution containing 0.02 M Pipes; 1 M NaCl; 0.02% (w/v) each of yeast RNA (Type XI, Sigma) and sonicated calf thymus DNA (Type I, Sigma) and 0.001 M each of dATP, dCTP, dGTP and dTTP. Probes at $2–4 \times 10^7$ dpm/μg were ethanol precipitated as described under Nick Translation and resuspended at the appropriate concentration in hybridization solution. Probes prepared in this way and stored at −20°C are usable for at least four months. Two μl of probe solution were denatured per slide to be prepared by placing at 95°C for 4–6 min followed by quenching on an ice-water bath. Denatured and dried squashes were covered with a dry coverglass and 1.2–1.7 μl of denatured probe solution were applied along one edge of the coverglass. When the probe solution was thoroughly dispersed under the coverglass by capillary action, the remaining air bubbles were removed by gentle tapping of the coverglass. The coverglass was sealed by liberal application of rubber cement (Carter's) and the slides were incubated for 24 hr at 25°C. (Hybridization efficiency is approximately 10-fold higher at 25°C than at 37°C under these conditions.) The rubber cement was removed and the coverglass was allowed to float off the slide after immersion in a solution consisting of a 1:1 (v/v) mixture of formamide and a pH 6.8 solution containing 0.02 M Pipes and 1 M NaCl. Slides were washed three more times in this solution (2 hr per wash) at 25°C and dehydrated by treating for 30 sec, with agitation, in 75% ethanol, 4 mM $MgCl_2$ followed by 30 sec in 95% ethanol, 4 mM $MgCl_2$. After air drying, the slides were autoradiographed according to Pardue and Gall (1975) except that approximately 1 g of $CaSO_4$ desiccant (Drierite) per ten slides was included in the autoradiography enclosure. Exposure times of 3–12 days were used for cases involving individual phage or plasmid clones and 10–20 days for mixtures of five phage clones used in the initial screening of *copia*-containing clones.

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