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Molecular lesions associated with white gene mutations induced by I-R hybrid dysgenesis in Drosophila melanogaster

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We have identified molecular lesions associated with six mutations, wIR2 and wIR4–8, of the white gene of Drosophila melanogaster. These mutations arose in flies subject to I-R hybrid dysgenesis. Four of the mutations give rise to coloured eyes and are associated with insertions of 5.4-kb elements indistinguishable from the I factor controlling I-R dysgenesis. The insertion associated with wIR4 is at a site which, within the resolution of these experiments, is identical to that of two previously studied I factors. This appears to be a hot-spot for I factor insertion. We have compared the sites of these insertions with sequences complementary to white gene mRNA identified by Pirrotta and Bröckl. The hot-spot is in the fourth intron. The insertion carried by wIR5 is either within, or just beyond, the last exon. The insertion carried by wIR6 is near the junction of the first exon and first intron. The wIR2 mutation is a derivative of w1. It contains an insertion of I factor DNA within, or immediately adjacent to, the F-like element associated with w1, and results in restoration of some eye colour. This insertion is just upstream of the start of the white mRNA. Mutations wIR7 and wIR8 are deletions removing mRNA coding sequences. Both determine a bleached white phenotype.

Key words: Drosophila melanogaster/hybrid dysgenesis/transposable elements/white gene

Introduction

Hybrid dysgenesis (see reviews by Bregliano and Kidwell, 1983; Kidwell, 1983; Engels, 1983) is the name given to the appearance of a set of unusual characteristics in the progeny of crosses between certain strains of Drosophila melanogaster (Picard and L’Héritier, 1971; Kidwell, 1975; Kidwell et al., 1977). These characteristics include lowered fertility, recombination in males and increased frequencies of mutation and chromosome aberrations. Two independent systems of hybrid dysgenesis, P-M and I-R, are known (Kidwell, 1979). Strains of D. melanogaster may be classified into one or other of two types with respect to each system. In the P-M system the effect is seen in male and female progeny of crosses between M strain females and P strain males. In the I-R system the effect is seen only in the female progeny of crosses between reactive (R) strain females and inducer (I) strain males. These dysgenic females are known as ‘SF’ females. The progeny of all other crosses appear normal.

The characteristics of P strains and inducer strains are controlled by transposable genetic determinants known as P factors and I factors, respectively (Picard, 1976; Bingham et al., 1982). Many P-M induced mutations are unstable in individuals subject to P-M dysgenesis (Engels, 1979; Rubin et al., 1982) and this led to the suggestion that mutations induced by P-M dysgenesis are due to insertion of P factor DNA into the genes in question (Green, 1977; Golubovsky et al., 1977; Simmons and Lim, 1980). This has been tested by Rubin et al., 1982). They compared DNA of six P-M induced white gene mutations with that of the wild-type allele. Each mutation had foreign DNA inserted into the white gene. Two insertions were of members of the copia family of transposable elements (Finnegan et al., 1978). The other four insertions varied in length from 0.5 kb to 1.4 kb, but were related in sequence. Rubin et al. (1982) argued that these insertions were too short to be functional P factors, which probably code for at least a transposase and a regulatory molecule, but that they might be deleted derivatives of P factors. O’Hare and Rubin (1983) have confirmed this. Using one of these putative P elements as a probe to screen a library of recombinant phages containing DNA from a P strain they recovered clones containing a conserved 2.9-kb sequence which has subsequently been shown to have at least some of the properties of a P factor (Spradling and Rubin, 1982).

We have started to investigate the molecular basis of I-R hybrid dysgenesis by examining molecular lesions associated with mutations of the white gene produced in SF females. Two mutations, wIR1 and wIR3, are associated with insertions of indistinguishable 5.4-kb elements at apparently identical sites within the white gene (Bucheton et al., 1984). We believe that these insertions are copies of the I factor, which controls I-R dysgenesis, since both wIR1 and wIR3 are closely linked to I factor activity (Pélisson, 1981; Bucheton et al., 1984). Here we describe the genetical and molecular properties of a further six white gene mutations induced in SF females. Three of these mutations, wIR4, wIR5 and wIR6, determine a coloured eye phenotype and are associated with insertions of 5.4-kb elements which are very similar, if not identical, to the I factor. Two of the remaining mutations, wIR7 and wIR8, determine a bleached white phenotype and are associated with deletions of DNA from the white region. The last mutation, wIR2, contains an insertion of I factor DNA into the white region of a chromosome carrying the w1 allele. This results in a partial restoration of eye colour. We discuss these results in terms of the properties of the I factor and of the white gene.

Results

The mutation wIR2, like wIR1, arose in SF females produced by crossing females of the reactive strain seF8 with males of the inducer strain w1 cf f (Picard et al., 1978; Pélisson, 1981). Its properties have been described briefly by Pélisson (1981) who referred to it as 1a11. Mutations wIR4–8 were found amongst 60 000 male progeny of SF females produced by crossing females of the reactive strain XCha with males of the inducer strain XOre 1. They are the results of independent mutation events. Mutations wIR2 and wIR4–6 determine col-
oured eye phenotypes and all show dosage compensation. We first determined the approximate locations of molecular lesions associated with these mutations by restriction digestion and Southern (1975) transfer experiments. Initially, recombinant lambda phages A26 and M365 (Figure 1) were used to probe digests of mutant and wild-type DNAs. These phages together cover ~30 kb of the white locus. Subclones of appropriate restriction fragments allowed a more detailed analysis.

Properties of the mutation \(w^{R4}\)

The mutation \(w^{R4}\) determines a red-brown eye colour which is lighter at 25°C than at 20°C. This phenotype is identical to that of \(w^{R1}\) and \(w^{R3}\) (Pelisson, 1981, and unpublished). The only detectable difference between wild-type and \(w^{R4}\) DNAs in the region of the white gene is within the 0.86-kb \(SalI\) fragment lying between coordinates −1.4 and −0.6 in Figure 1. This is the location of the 5.4-kb insertion associated with the \(w^{R1}\) and \(w^{R3}\) mutations (Bucheton et al., 1984). Figure 2, tracks a–d, shows the result of a Southern transfer experiment in which wild-type, \(w^{R1}\), \(w^{R3}\), and \(w^{R4}\) DNAs were digested with \(SalI\) and then probed with 32P-labelled pCS155, a plasmid containing this 0.86-kb fragment. The 0.86-kb \(SalI\) fragment of wild-type DNA is replaced by a 6.2-kb fragment in \(w^{R4}\) DNA which co-migrates with the corresponding fragments of \(w^{R1}\) and \(w^{R3}\). This indicates that these mutations are all associated with 5.4-kb insertions in this region.

The 5.4-kb elements present in \(w^{R1}\) and \(w^{R3}\) are apparently identical copies of the I factor. These have been cloned and characterised by Bucheton et al. (1984). They are not cut by BamHI, EcoRI, SalI or XhoI and the same is true of the element present in \(w^{R4}\) (data not shown). The copies of the I factor in \(w^{R1}\) and \(w^{R3}\) have two \(HindIII\) sites 1 kb apart, and in order to compare the element in \(w^{R4}\) with these I factors we digested wild-type, \(w^{R1}\), \(w^{R3}\) and \(w^{R4}\) DNAs with \(HindIII\). The DNAs were then hybridised, in a Southern transfer experiment, with pCS54, a plasmid carrying the \(BamHI-HindIII\) fragment lying between coordinates −6 and 1.5 in Figure 1. The 9.2-kb \(HindIII\) fragment of wild-type DNA which hybridises to this probe is replaced, in the three mutant DNAs, by fragments of 7.4 kb and 6.2 kb (Figure 2, Fig. 1. Restriction map of the white region in chromosomes carrying \(w^{R}\) mutations. This map shows the positions of restriction sites on the parental \(w^{+}\) chromosomes on which \(w^{R}\) mutations were isolated. The positions of the insertions in \(w^{R4}\), \(w^{R5}\) and \(w^{R6}\) are shown offset from the map. The positions of the deletions associated with the \(w^{R7}\) and \(w^{R8}\) mutations are shown below the map. The dashed lines indicate uncertainties regarding the end points of the deletions. Restriction sites marked * are not present on the chromosome carrying the \(w^{R4}\) and \(w^{R5}\) mutations, but are present on the chromosome carrying \(w^{R6}\). The \(HindIII\) site at coordinate 8.9 is not present in chromosomes carrying the \(w^{R}\) or \(w^{R6}\) mutations. The positions of fragments which have been cloned from the white region and used as probes in these experiments are also indicated below the map. The scale above the map is in kilobases. The zero position is the site of the copia element associated with the white-apricot mutation (Levis et al., 1982). Positive coordinates are towards the centromere. The position of the major \(w^{+}\) transcript as determined by Pirrotta and Broccoli (1984) is shown under the restriction map. The boxed regions indicate exons while the lines joining them indicate introns. Transcription is from right to left.

Fig. 2. Southern transfer experiment comparing the \(w^{R1}\), \(w^{R3}\) and \(w^{R4}\) mutations. Genomic DNAs of \(seF_{s}\), \(w^{R1}\), \(w^{R3}\) and \(w^{R4}\) strains were digested with \(SalI\), tracks a–d, or \(HindIII\), tracks e–h. The DNAs were then run on a 1% agarose gel and transferred to nitrocellulose. The DNAs in tracks a–d were hybridised with 32P-labelled pCS155, while DNAs in tracks e–h were hybridised with 32P-labelled pCS54. The strain \(seF_{s}\) has the same restriction map in the white region as does the parental strain \(XCh\). a and e, \(seF_{s}\); b and f, \(w^{R1}\); c and g, \(w^{R3}\); d and h, \(w^{R4}\).
tracks e--h). This suggests that wIR4 contains a copy of the same 5.4-kb element as is present in wIR1 and wIR3, and that it is inserted at the same site and in the same orientation. We refer to this orientation of the I factor with respect to the white locus as being orientation 1, and to the opposite as orientation 2.

Properties of the mutation wIR5

The mutation wIR5 determines a brown eye colour which is similar to that of wIR1 at 20°C, but it is not temperature sensitive. The only detectable difference between the white regions of wild-type and wIR5 DNAs is in the 1.5-kb SalI fragment between coordinates −2.9 and −1.4. Figure 3, tracks a and b, shows the result of hybridising SalI digests of wild-type and wIR5 DNAs with pCS156, a plasmid containing this fragment. The 1.5-kb SalI fragment of wild-type DNA is replaced by a 6.9-kb fragment in wIR5. This indicates that this mutation is also associated with insertion of a 5.4-kb element.

The insertion in wIR5 is not cut by BamHI, EcoRI, SalI or XhoI (data not shown) but is cut by HindIII. Figure 3, tracks c and d, shows the result of digesting wild-type and wIR5 DNAs with both HindIII and BamHI and then hybridising them with pCS156 in a Southern transfer experiment. The 7.8-kb fragment of wild-type DNA which hybridises to this probe is replaced in wIR5 by fragments of 7 kb and 5.2 kb. This indicates that, like the I factor in wIR1, the 5.4-kb element in wIR5 contains at least two HindIII sites −1 kb apart. This suggests that the element present in wIR5 is another copy of the I factor.

To orient and position this element we hybridised a HindIII-BamHI digest of wIR5 DNA separately with plasmid pL769, which contains the HindIII-SalI fragment between coordinates −6 and −2.9, and pCS157, which contains the SalI-XhoI fragment between coordinates −0.6 and 2.1 (Figure 1). The 7-kb HindIII fragment hybridised to pL769 and the 5.2-kb fragment to pCS157 (data not shown). The 7.0-kb fragment is therefore to the left and the 5.2-kb fragment to the right. If the element in wIR5 has the same restriction map as the I factor in wIR1 then it must be inserted at about coordinate −2 and in orientation 2. This would place a PstI site −1.6 kb to the right of the SalI site at coordinate −2.9, and 5.3 kb to the left of the SalI site at coordinate −1.4 (Figure 2). To position the insert more precisely we digested wild-type and wIR5 DNAs with both SalI and PstI and hybridised them with pCS156 (see Figure 3, tracks e and f). The 1.5-kb SalI fragment of wild-type DNA which hybridises to this probe is replaced by 1.4-kb and 5.5-kb fragments in wIR5 DNA, which places the wIR5 insert at about coordinate −2.2.

We have cloned the HindIII fragments containing the left-hand and right-hand ends of the wIR5 insert, using the lambda vector NM1149 (Murray, 1983). The recombinant phages obtained, λ1453 and λ1454, contain the left-hand and right-hand ends of the insert, respectively. Digestion of λ1453 with both HindIII and SalI yields a fragment of 3.7 kb which includes the left-hand end of the insert, while digestion of λ1454 gives a 2.2-kb fragment including the right-hand end. To compare the insert present in wIR5 directly with the I factor in wIR1, we subcloned these SalI-HindIII fragments in pUC8 (Vieira and Messing, 1982) and heteroduplexed them with the corresponding subclones from wIR1. The results of this experiment indicate that the wIR5 insertion is homologous to I factor DNA for 1.4 ± 0.14 kb to the left of the first internal HindIII site of the I factor, as shown in Figure 1, and for 2.8 ± 0.09 kb to the right of the second internal HindIII site. No region of mismatch could be detected. These results are consistent with the wIR5 insertion being an I factor.

Properties of the mutation wIR6

The mutation wIR6 determines a red-brown eye colour which is similar to that of wIR1 at 20°C, but which is not temperature sensitive. The only detectable difference between wIR6 and wild-type DNAs is in the 11.3-kb SalI fragment between coordinates −0.6 and 10.7 (Figure 1). This is replaced in wIR6 DNA by a fragment of −17 kb (data not shown). This is consistent with there being a 5.4-kb I factor inserted within this fragment. We have confirmed that there are I factor sequences associated with the wIR6 mutation by in situ hybridisation experiments (data not shown).

There are differences between the restriction maps of the inducer and reactive parents of wIR6 in this region. The BamHI site at coordinate 4.4 and the EcoRI site at coordinate 6.4 are both present in DNA of the XOre I, but not the XChα parent. The DNA from wIR6 contains both sites indicating that this mutation occurred on an X chromosome from XOre I. We have further located the wIR6 insertion by hybridising HindIII digests of wIR6 and XOre I DNAs with pL52, a plasmid containing the 6.8-kb HindIII fragment lying between coordinates 3.2 and 10. This was cloned from a strain lacking the HindIII site at coordinate 8.9. This probe hybridised to 5.7-kb and 1.1-kb fragments of XOre I DNA, and 7.1-kb and 1.1-kb fragments of wIR6 DNA (Figure 4, tracks a and b). Therefore the insertion must be within the 5.7-kb fragment, and must contain at least one HindIII site. The fact that only one new fragment of wIR6 DNA hybridises to this probe probably indicates that the insert is very close to one end of the HindIII fragment. These results suggest that an I factor is present just to the right of the HindIII site at coordinate 3.2 and in orientation 2, or just to the left of the HindIII site at coordinate 8.9 and in orientation 1.
We have determined the position and orientation of this insertion by hybridising Xhol-PstI and BamHI-PstI digests of XOre I and wIR6 DNAs with pl768, a plasmid carrying the SalI-HindIII fragment lying between coordinates −0.6 and 3.2 (Figure 1). The results are shown in Figure 4, tracks c−f. The 2.3-kb Xhol-PstI (coordinates 2.1−4.4), and the 2.9-kb BamHI (coordinates 1.5−4.4) fragments of XOre I DNA are replaced by 1.7-kb and 2.3-kb fragments, respectively, in wIR6 DNA. These results are consistent with an I factor being present at about coordinate 3.2 and in orientation 2 (Figure 1).

Mutations wIR7 and wIR8 are deletions

Mutations wIR7 and wIR8 determine a bleached white phenotype. Chromosomes carrying the wIR7 mutation do not complement mutations at the roughest gene which lies proximal to white. This suggests that wIR7 is associated with a long deletion. We have confirmed this at the molecular level.

Figure 5, tracks a and b, shows the result of hybridising a SalI digest of wild-type and wIR8 DNAs with pCS54. Both DNAs contain the 1.5-kb SalI fragment between coordinates −2.9 and −1.4, and the large fragment of ~20 kb to the left of it. Only wild-type DNA contains the 0.86-kb fragment between coordinates −1.4 and −0.6 and the 11.3-kb fragment between coordinates −0.6 and 10.7, indicating that wIR8 has lost DNA in this region. A faint fragment of ~12 kb can be seen in wIR7 DNA. This is presumably a fusion fragment generated by the deletion. The fact that it so faint suggests that the left-hand breakpoint of the deletion is close to the SalI site at coordinate −1.4. We cannot detect any homology between wIR7 DNA and λA26 so the deletion must extend proximally beyond the limits of this phase, probably as far as the roughest gene.

The only difference we have detected between wild-type and wIR8 DNAs is in the region between coordinates −2.9 and −0.6. Neither the 1.5-kb nor the 0.86-kb SalI fragments are present in wIR8 indicating that a deletion has removed DNA in this region. This includes the SalI site at coordinate −1.4 (data not shown). We have measured the extent of this deletion by hybridising BamHI-HindIII digests of wild-type and wIR8 DNAs with pCS54. The 7.8-kb fragment of wild-type DNA is replaced by a fragment of ~4.9 kb in wIR8 DNA (Figure 5, tracks c and d). This confirms the presence of a deletion in this region and indicates that it is ~2.5 kb long. The SalI sites at coordinates −2.9 and −0.6 are still present after digestion of wIR8 DNA with both HindIII and SalI yields fragments which co-migrate with the HindIII-SalI fragments of wild-type DNA between coordinates −6.0 and −2.9, and between −0.6 and 3.2, and which hybridise to pCS54 (data not shown).

Properties of the mutation wIR2

The mutation wIR2 arose in an SF female resulting from a cross between seF6 females and w1 ct f males. It determines a brown eye phenotype which is not temperature sensitive. The chromosome initially carrying wIR2 also carried the ct and f markers, suggesting that the wIR2 mutation occurred in an X chromosome from the inducer parent and that it is a partial revertant of w1.

The w1 mutation is associated with insertion of a 5.7-kb element between coordinates 3.1 and 4.4 in Figure 1 (Zachar and Bingham, 1982). This insertion is related to transposable elements known as ‘F’ elements (O'Hare and Rubin, 1983; Di Nocera et al., 1983). We have investigated the structure of the white locus in chromosomes carrying wIR2 by hybridising digests of w1 ct f and wIR2 DNAs with 32P-labelled λA26 and λM365. The results indicated that wIR2 contains all or part of the w1 insertion together with additional DNA. The second insertion is either just within, or just to the right of, the w1 insertion. We have cloned, from wIR2 DNA, the BamHI fragment lying between coordinates 1.5 and 4.4 using the lambda vector EMBL4 (Frischauf et al., 1983). This yielded the recombinant phage A531. Figure 6 is a restriction map of the BamHI fragment carried by this phage. This fragment includes both the w1 and wIR2 insertions. It contains I factor se-
sequences since it hybridises to I factor probes and has a region with a restriction map which corresponds to that of the I factor from \(w^R1\) (Bucheton et al., 1984). This region is indicated in Figure 6. To measure the total length of I factor sequences present in \(w^R2\), we heteroduplexed \(\lambda 531\) with pl407, a plasmid containing the complete I factor from \(w^R3\) (Bucheton et al., 1984). An uninterrupted double-stranded region of 5.4 ± 0.24 kb long was formed. These results are consistent with there being a complete I factor within the \(w^1\) insertion. The restriction map of the region of \(\lambda 531\) corresponding to the \(w^1\) insertion is not identical to that published by Zachar and Bingham (1982) but, so far as we can tell from Southern transfer experiments, it is the same as the equivalent regions of genomic \(w^R2\) and parental \(w^1\) and \(f\) DNAs.

Discussion

All of the white gene mutations described here are associated with DNA rearrangements. We have no formal proof that these rearrangements are responsible for the mutant phenotypes associated with the mutations but this seems very likely. We have compared the molecular lesions associated with the \(w^R\) mutations with information concerning transcription of the white gene. Pirrotta et al. (1983), O’Hare et al. (1983) and Pirrotta and Brockl (1984) have mapped the regions of the white locus which are complementary to mRNA. Transcription is from right to left in Figure 1, that is proximal to distal on the X chromosome, and Pirrotta and Brockl have located five exons between coordinates – 2.1 and 3.6. The positions of these exons are shown in Figure 1. O’Hare et al. (1984) have reached similar conclusions from analysis of the sequence of the white locus.

The two mutations which determine a bleached white phenotype, \(w^R7\) and \(w^R8\), are associated with deletions affecting one or more exons and would not be expected to have any white gene function. The site of the 5.4-kb insertion associated with \(w^R4\) is indistinguishable from that of the insertions associated with \(w^R1\) and \(w^R3\) (Bucheton et al., 1984). We have shown by DNA sequence analysis that the I factor associated with \(w^R1\) is inserted 96 bp to the right of the SalI site at coordinate – 1.4 (D. Fawcett and D.J. Finnegan, unpublished data) and so clearly lies within an intron. These three mutations determine an identical red-brown eye colour, indicating that they do not abolish white gene function completely. They may interfere with RNA processing and reduce the level of full length white mRNA. Pirrotta and Brockl (1984) and Levis et al. (1984) have shown that this is true of the \(w^1\) mutation which is due to insertion of a copia element within the short intron at coordinate 0. Alternatively, white mRNA might terminate within these insertions and code for a partially active white gene product.

We cannot say exactly where the insertions associated with \(w^R2\) and \(w^R6\) are in relation to white mRNA sequences. The \(w^R2\) insertion is either just downstream of the mRNA coding sequence or just within the last exon, and may affect the termination of transcription or the C-terminal end of the white gene product. The \(w^R6\) insertion is either at the 3’ end of the first exon or the beginning of the first intron. It is the first mutation giving an altered eye colour to be mapped between the \(w^1\) and \(w^6\) mutations (O’Hare et al., 1983). The fact that this mutation determines a coloured eye phenotype suggests that it is within the intron and reduces the level of wild-type white mRNA. Interruption of the white gene product near its N-terminal end would probably inactivate it completely. The exact position of these insertions will be determined by DNA sequencing and their effects on white mRNA measured directly.

The coloured phenotype of the \(w^R2\) mutation is particularly interesting since it is a derivative of \(w^1\) which gives a bleached white phenotype. The \(w^1\) mutation is associated with insertion of a 5.7-kb transposable element 0.5 kb to the right of the HindIII site at coordinate 3.2 (Zachar and Bingham, 1982; Pirrotta and Brockl, 1984). This is related to the ‘F’ family of elements (O’Hare et al., 1983; Di Nocera et al., 1983). In \(w^R2\) there is a second insertion just within, or immediately to the right of, this F-like element. Two other derivatives of \(w^1\) give coloured eyes. These are \(w^h\) and \(w^e\) (for references, see Lindsley and Grell, 1968). They are both associated with rearrangements within the F element. The \(w^e\) allele has a 1.1-kb deletion while the \(w^h\) insertion is 0.2 kb longer than that of \(w^1\) and has a slightly altered restriction map.

The \(w^1\), \(w^e\) and \(w^h\) insertions are very close to the start of the white gene mRNA (O’Hare et al. 1983; Pirrotta and Brockl, 1984; Levis et al., 1984). Levis et al. (1984) and O’Hare et al. (1984) believe that these insertions lie just within
a short 5′-untranslated leader sequence. These mutations have been assigned to a regulatory region of the white locus since they suppress the phenotype of the zeste mutation, z1. In addition, the \( w^c \) mutation does not show dosage compensation, unlike \( w^b \) and \( w^{R2} \) (Lindsley and Grell, 1968; Smith and Lucchesi, 1969; Judd, 1976). The \( w^c \) allele directly synthesizes of the major mRNA from the white region, as expected from its coloured phenotype, but the level of this RNA is greatly reduced (Pirrotta and Broccoli, 1984). We expect that the same will be true of \( w^{R2} \).

We have now analysed a total of eight white gene mutations induced in SF females. Six of these are associated with insertion of similar, if not identical, 5.4-kb sequences, and two are deletions. We have presented evidence previously which strongly suggests that the insertions present in \( w^{R3} \) and \( w^{R4} \) are genetically active I factors (Bucheton et al., 1984).

The only other mutation to have been tested for activity is \( w^{R2} \) mutation 1a11 in Pélisson, (1981), and it is not linked to an active I factor. This could be because the I factor present in \( w^{R2} \) differs from functional I factors by one or more base substitutions or small deletions or insertions which would not have been detected in these experiments, or because its activity is prevented by adjacent DNA. The F-like element may inhibit its expression, for example.

The genomes of both inducer and reactive strains contain many I elements, at least some of which are incomplete compared with I factors. The results of whole genome Southern transfer experiments indicate that most I elements are located at very similar chromosomal sites in all strains suggesting that they transpose rarely, if at all (Bucheton et al., 1984; Crozatier, Vaury and Bucheton, unpublished data). This could explain why we have not found any white gene mutations associated with I elements. These results differ from those obtained by Rubin et al. (1982) for P-M induced white mutations. They found that all six mutations they investigated were associated with insertions but that the elements involved were not identical. Four were different deletion derivatives of the P factor, and two were copia elements. This suggests firstly that P elements can transpose more readily than I elements and secondly, that P-M dysgenesis can mobilise copia elements and possibly other transposable sequences. There is no reason to suppose that this is true of I-R dysgenesis.

Mutations \( w^{R1} \), \( w^{R3} \) and \( w^{R4} \) are due to insertion of I factors at apparently identical sites at coordinate -1.3, suggesting that this is a hot-spot for insertion. This may be because I factor insertion is to some extent sequence specific. There is a hot-spot for P element insertion at coordinates -1.9, and P elements can insert at this site in either direction. The I factors at -1.3 are all in the same orientation but the sample is too small to say whether or not this is significant.

The deletions present in \( w^{R3} \) and \( w^{R4} \) are either the direct result of I-R dysgenesis or arose fortuitously in SF females. The majority of I-R induced white mutations are associated with a recessive lethal phenotype and are probably deletions (A. Pélisson, unpublished data). This suggests that I-R dysgenesis can induce deletions at this locus. Chromosome rearrangements induced by P-M dysgenesis often have P elements at their breakpoints (Bingham et al., 1982) but we have been unable to find any I factor sequences associated with \( w^{R3} \) and \( w^{R4} \). In situ hybridisation experiments have given no indication of I factor sequences associated with these mutations, although this does not rule out the presence of up to two or three hundred bases of I DNA. There is no detectable I factor DNA in the wild-type white locus at positions corresponding to the end points of these deletions. Perhaps they were formed by secondary events shortly after insertion of I factors at the white locus.

### Materials and methods

#### Bacterial strains

All plasmids were propagated in Escherichia coli HB101 (Boyer and Roulland-Dussoix, 1969) and recombinant phages in strains C600 (Appleyard, 1954), Q359 (Karn et al., 1980) and NMS14 (Arber et al., 1983).

#### Drosophila strains

All strains of D. melanogaster are from the collection of Laboratoire de Génétique, Université de Clermont-Ferrand.

#### Enzymes and isotopes

Restriction enzymes were purchased from Bethesda Research Laboratories, Amersham International and New England Biolabs and were used as recommended by the manufacturers. T4 DNA ligase was purchased from New England Biolabs. E. coli DNA polymerase was the gift of B.M. Will. [\( ^{3}p \)dCTP (40 Ci/mmol) and \( [\text{H}]dCTP \) were purchased from Amersham International. \( [\text{H}]dTTP \) was purchased from C.E.A. Sacy, France.

#### DNA preparation

Plasmid and phage DNAs were prepared as described by Will et al. (1981) and Maniatis et al. (1982). D. melanogaster DNA was prepared as described by Bucheton et al. (1984).

#### Agarose gel electrophoresis

Horizontal slab gels were run in Tris/acetate buffer (40 mM Tris, 20 mM Na acetate, 1 mM EDTA, pH 8.2) at 0.5 V/cm. DNA was transferred to nitrocellulose by the modification of the method of Southern (1975) described by Smith and Summers (1980). Fragments of \( \lambda \) phage DNA digested with HindIII were used as size markers.

#### In vitro labelling of DNA, hybridisation and autoradiography

These procedures were carried out as described by Will et al. (1981) except that dextran sulphate (mol. wt. 500 000) was present at a concentration of 0.5% during hybridisation. After hybridisation filters were washed for 2 h in 2 x SSC, 0.1% SDS at room temperature, and then for a further 2 h in 1 x SSC, 0.1% SDS at 37°C.

#### Construction of libraries

A library of cloned HindIII fragments of \( w^{R5} \) DNA was constructed by ligating 1 \( \mu \)g HindIII cut ANM1149 DNA with 1 \( \mu \)g HindIII cut \( w^{R5} \) DNA, in a total volume of 1 x SSC. Ligation was carried out overnight at 10°C in 100 mM Tris-HCl pH 7.2, 10 mM EDTA, 100 mM MgCl2, 100 mM dithiothreitol, 10 mM ATP and with 60 units T4 DNA ligase. Recombinant molecules were packed in vitro (Scherer et al., 1981) and the resulting phages were plated on E. coli strain NM514. The library was first screened with \( pcS34 \) to recover phages containing fragments from the appropriate region of the white locus. Phages hybridising to this probe were then tested with \( pcS157 \) and p7697 to detect those containing sequences from the right or left of the \( w^{R5} \) insertion, respectively.

A library of cloned BamHI fragments of \( w^{R2} \) DNA was made as follows. 10 \( \mu \)g of EMBL4 DNA was digested with both BamHI and SalI and the resulting polylinker fragment was eliminated by precipitating once with ethanol (Friaschaf et al., 1983). This vector DNA was dissolved, together with 3 \( \mu \)g BamHI cut \( w^{R2} \) DNA, in 100 \( \mu \)l ligation buffer and ligated overnight at 10°C. The ligated DNA was packaged in vitro and the resulting phages plated on E. coli strains Q359 and C600. Plaques were screened with \( ^{32}p \)-labelled p7678. Phages hybridising to this probe should contain the BamHI fragments immediately to the left or right of coordinate 1.5. Phages carrying the left-hand fragment were identified by screening with \( pcS156 \). One of the phages, \( J531 \), which did not hybridise to the probe was taken for further analysis.

#### In situ hybridisation

Hybridisation to polytenic chromosomes was carried out as described by Pardee and Gall (1975) as modified by Bucheton et al. (1984).

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