The Molecular Structure of *TE146* and Its Derivatives in *Drosophila melanogaster*

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ABSTRACT

TE146 is a giant transposon of *Drosophila melanogaster*. It carries two copies of the *white* and *roughest* genes, normally found on the *X* chromosome. The structure of this transposon has been studied at the molecular level. *TE146* may transpose to new chromosome positions, excise and be lost from the genome or undergo internal rearrangements. The termini of *TE146* are foldback DNA elements (*FB*); the transposon also carries two internal *FB* elements. Loss or internal rearrangement of *TE146* involves recombination between different *FB* elements. These events have been mapped molecularly, by taking advantage of the fact that the *FB* sequences are composed largely of a regular 155-bp repeat sequence that is cut by the restriction enzyme *Taq*I, and are shown to be nonrandom. We suggest that these *FB-FB* exchange events occur by mitotic sister-chromatid exchange in the premeiotic germ line.

A family of very large transposons was discovered in *Drosophila melanogaster* by ISING and RAMEL over 25 years ago (RAMEL 1966; ISING and RAMEL 1976). This family originated by the transposition of two genes, *white* and *roughest* from a *Basc (Muller-5) X* chromosome to chromosome arm *2R*, and was first detected because the transposon acted as a dominant suppressor of *white* (ISING 1964). The unusual property of this suppressor was first evident when its genetic position jumped from a wild-type sequence chromosome arm *2R* to a *Cy* balancer chromosome. Since then, this *TE* has been hopping around the genome of *D. melanogaster* and over 200 different transpositions have now been mapped (ISING and BLOCK 1984; G. ISING, personal communication; D. GUBB and J. ROOTE, unpublished data). These *TE*s show two other properties, each indicative of their unstable nature. First, they may excise from a site and be lost from the genome. Because the *TE* carries a functional allele of *white* (either w^a or a reversion of this allele to an almost wild-type form), losses are very easily detected as long as the *TE* is kept on a genotypically w^- background. Second, the *TE* can undergo internal duplications or deletions. This can be seen by the changing reaction of the *white* genes carried by the *TE* to the *zeste*[1] mutation, since the yellow eye-color of z^1 requires two contiguous copies of w^+ for its expression (PIRROTTA 1991, for a recent review). Originally, the *TE* carried a single copy of *white* and gave a red eye-color on a z^1 w^- background. Sometimes, its *white* genes become suppressible by z^1. These new forms of the *TE* have duplicated their *white* gene (and, usually, their *roughest* gene, too). Such duplicated forms of the *TE* can subsequently lose one copy of *white* and revert to a form that is red-eyed (with z^1). In fact, this process of duplication, deletion, subsequent duplication and then deletion can be followed *ad infinitum*, with the *TE* remaining at the same insertion site (GUBB *et al.* 1985, 1986).

The first clue to the molecular nature of the sequences that cause the *TE* to be transposable and unstable came with the discovery that the termini of the element are foldback sequences (GOLDBERG, PARO and GEHRING 1982). POTTER (1982a) had characterized a family of foldback DNA that was repetitive in the genome of all strains of *D. melanogaster*, usually being found at about 20–30 different chromosomal locations. Sequences of this family (*FB*) were found at the junction of the *TE* with sequences at its insertion site. They were also found to be associated with transposons of independent origin, *e.g.*, w^c and w^{+IV} (GOLDBERG, PARO and GEHRING 1982; LEVIS, COLLINS and RUBIN 1982, PARO, GOLDBERG and GEHRING 1983) and with the unstable mutation w^{DZL} (LEVIS, COLLINS and RUBIN 1982; LEVIS and RUBIN 1982; ZACHAR and BINGHAM 1982). *FB* elements are often associated with other DNA sequences (TRUETT, JONES and POTTER 1981). One class of *FB* elements is associated with a particular sequence with coding potential, known as the *NOF* sequence. The composite *FB-NOF* element was first found at an end of *TE28* by GOLDBERG, PARO and GEHRING (1982) and then at the unstable w^c mutation (COLLINS and RUBIN 1982; LEVIS, COLLINS

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and RUBIN 1982). *FB-NOF* sequences are normally rare in the genome (0–2 copies) although many strains carrying ISING's transposon, as well as *Basc* and w^{DZL} strains, may have 10–20 copies (HARDEN and ASHBURNER 1990; LOVERING 1988). Two *FB-NOF* elements have been sequenced (TEMPLETON and POTTER 1989; HARDEN and ASHBURNER 1990) and the 4-kb *NOF* sequence has the potential to code for a 120-kD polypeptide. Although circumstantial, the evidence is that the *NOF* sequence encodes a function required for *FB* mediated transposition.

The structure of *FB* elements suggests that recombination between different *FB*s may be the mechanism for their excision and transposition. In two cases there is evidence that this is indeed so. The unstable *FB-NOF* associated w^c allele frequently mutates to stable, male-viable, w^- derivatives. Many of these are 14-kb deletions that result from exchange between the *FB-NOF* element within w^c and a *FB* element 14-kb more distal (COLLINS and RUBIN 1984). Several "complete" losses of one *TE, TE146(Z),* inserted at 35B1.2 within the *no-ocelli* (*noc*) gene (GUBB *et al.* 1985), have been analyzed molecularly by CHIA *et al.* (1985a). These losses of the *TE* revert the strong mutant *noc* allele associated with the insertion to noc^+. Their important feature is that they are all imprecise excisions of the *TE* and leave between 3 and 10 kb of DNA, which is largely, if not solely, *FB* DNA, at the insertion site. They must have resulted from an exchange between the *FB* elements that flank *TE146(Z).*

Despite the evidence for *FB/FB* mediated exchange being important for the instability of the *TE,* the precise structures of the *FB* elements, and more especially of their junctions with non-*FB* sequences, are not known. In this paper we describe these junctions and provide further evidence for the nature of *FB* mediated recombination.

MATERIALS AND METHODS

Stocks: The original stock of *TE146(Z)/CyO* was from G. ISING. The *SR* series of spontaneous derivatives of *TE146(Z)* was recovered as red-eyed z^1 w^{11E4}; *TE146(Z)/CyO* flies from zeste-eyed parents. They have been characterized genetically by GUBB *et al.* (1986).

Probes: *noc sequences: TE146(Z)* is inserted at coordinate −107.5 kb of the *Adh* region chromosome walk, within DNA covered by the phage λob9.04 (CHIA *et al.* 1985b). Two clones, described by CHIA *et al.* (1985a), were used to study the junctions between *noc* and *TE146(Z)* sequences. One (*noc*:D) is a 0.7-kb *Hin*dIII-*Sal*I fragment from coordinates −107.5 to −108.2. The *TE* is inserted 50 bp 5′ of the *Sal*I site. This probe is, in practice, specific for sequences immediately distal to the insertion site. For sequences immediately proximal to this site the clone *noc*:P was used. This is the 0.65-kb *Sal*I-*Eco*RI fragment that, in wild-type DNA, is immediately proximal to *noc*:D.

white sequences: The distal end of the *white* sequences carried by *TE146(Z)* is included in the phage λm11B.2 of LEVIS, BINGHAM and RUBIN (1982). This phage wholly includes the 5-kb *Hin*dIII-*Bam*H1 fragment D of pΔ of GOLDBERG, PARO and GEHRING (1982), identified as being close to the distal end of *white* sequences carried by *TE28* (Figure 1a). Two fragments of λm11B.2 (provided by K. O'HARE) were subcloned in pBR322, the 2.6-kb *Sst*I-*Eco*RI fragment that corresponds to coordinates −15.5 to −18.2 kb of the *white* walk (LEVIS, BINGHAM and RUBIN 1982) (we will call this *w:SR*2.6) and the 450-bp *Hin*cI-*Eco*RI fragment that includes sequences from −15.5 to −16 kb (the *w:HR*0.45 clone). In addition, as a probe to the *white* gene sequence itself, we used the 11-kb *Kpn*I-*Eco*RI fragment carrying DNA from coordinates −4 to +7 (see Figure 5b). This clone was made by K. MOSES from λm2.1 of LEVIS, BINGHAM and RUBIN (1982) (provided by K. O'HARE).

rst sequences: The rst^+ gene has not been characterized at the molecular level. However, PARO, GOLDBERG and GEHRING (1983) isolated several clones from *TE*s which they mapped to sequences proximal to *rst.* One of these, T2, a 6.0-kb subclone in pBR325 from the phage λ98/2 derived from a *TE98* library, was the gift of M. GOLDBERG (see Figure 1b). A genomically unique 1-kb *Sal*I-*Eco*RI fragment of T2 (in pBR325) was used as a probe for sequences normally proximal to the *rst* gene (*rst:SR*0.8; this fragment is the same as PARO, GOLDBERG and GEHRING's T2B). *TE98* carries a *NEB* element between its *rst* gene and the adjacent *FB* DNA, within the *Eco*RI-*Sal*I fragment. *rst:SR*0.8 does not include these *NEB* sequences.

NOF sequences: The *NOF* probe has been described by HARDEN and ASHBURNER (1990), it is the *Eco*RI-*Sal*I fragment of pΔ1 of GOLDBERG, PARO and GEHRING (1982) and was the gift of M. GOLDBERG.

Molecular techniques: Routine techniques for the isolation and analysis of DNA are described in MANIATIS, FRITSCH and SAMBROOK (1982). The extraction of DNA from single flies used the method described by JOWETT (1986). For the partial digestion of DNA with *Taq*I the enzyme concentration was 0.3 unit/μg DNA at 65° for 15–30 min. Denaturing conditions were used for the Southern transfers, as described by REED and MANN (1985).

***In situ* hybridization:** *In situ* hybridization to polytene chromosomes was done with either [^{3}H]thymidine or bio-UTP-labeled probes as described in ASHBURNER (1989).

RESULTS

A summary of the gross organization of *TE146(Z)* is shown in Figure 2. The basic features of this structure, that this *TE* includes a tandem repeat of w^+ and rst^+ genes bounded by elements of POTTER's *FB* family (TRUETT, JONES and POTTER 1981), have been determined by genetic (GUBB *et al.* 1985, 1986) and previous molecular (CHIA *et al.* 1985a) studies. We identify eight different DNA boundaries of sequences that are contiguous in *TE146(Z)* but not in wild-type DNA. To help the presentation of the data these are labeled A to H, and the adjacent *FB* sequences will be called limbs A to H. An *FB* element consists of two limbs in opposite orientation with a variable amount of non-*FB* DNA separating them. The eight boundaries have been mapped, by probing DNA extracted from flies of an appropriate genotype after digestion with restriction enzymes, electrophoresis and transfer to filters.

The insertion site of *TE146* in the *noc* gene: CHIA

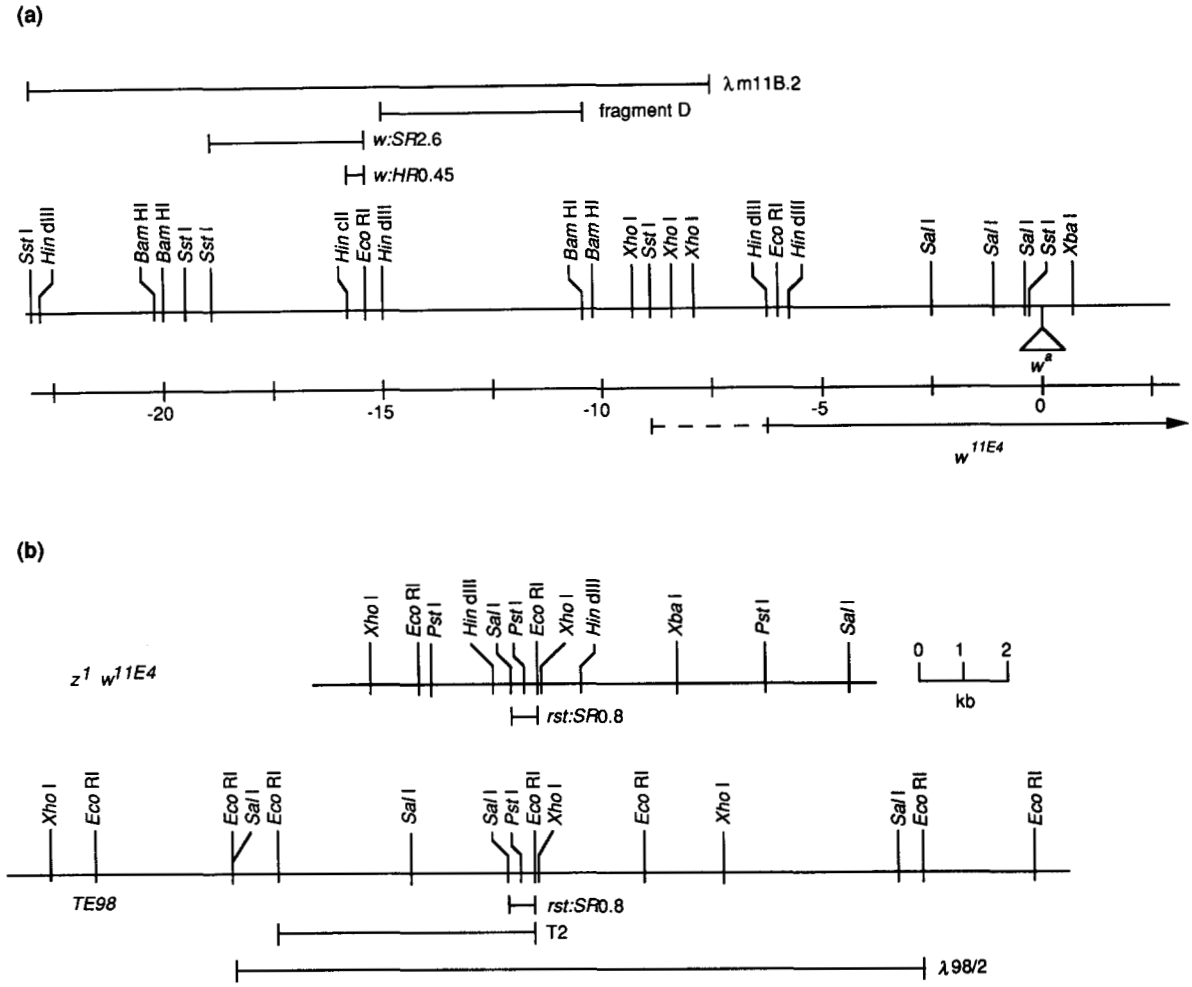

FIGURE 1.—(a) Restriction map of the DNA distal to the *white* gene of *D. melanogaster* (from LEVIS, BINGHAM and RUBIN 1982) showing the positions of the various clones used in this work and the distal limit of the w^{11E4} deletion (mapped by ZACHAR and BINGHAM 1982). Fragment D is that isolated from *TE28* by GOLDBERG, PARO and GEHRING (1982). (b) Restriction maps of the region proximal to the *roughest* gene in z^1 w^{11E4} and *TE98* showing the position of the *rst:SR*0.8 probe and its relationship to the T2 and λ98/2 clones.

et al. (1985a,b) showed that *TE146(Z)* is inserted in sequences of the *noc* gene, as expected since this insertion results in a strong mutant *noc* phenotype (GUBB *et al.* 1985). The insertion site is within the 0.7-kb *Hin*dIII-*Sal*I fragment at coordinate −108.2 to −107.5 of the *Adh* region chromosome walk. Junctions A + B are represented by a 11.4-kb *Pst*I fragment seen when DNA from *TE146(Z)* is probed with *noc:*D. Junctions G + H are represented by a 9.0-kb *Pst*I fragment seen when this DNA is probed with *noc:*P (CHIA *et al.* 1985a).

The orientation of the *white* genes within *TE146:* Genetically, *TE146(Z)* is known to carry two functional *white* genes that are in tandem orientation with respect to each other (GUBB *et al.* 1986). These genetic data also strongly suggested that the most distal gene carried by the *TE* was *white* (rather than *rst*), that is to say the map of the *TE* could be represented as $noc^D|white\ rst|white\ rst|noc^P$, where noc^D and noc^P represent the distal and proximal regions of the *noc* gene relative to the *TE*'s insertion site. To verify this structure at the molecular level we used spontaneous derivatives of *TE146(Z)* that carry only a single *white* gene. As suggested by GUBB *et al.* (1986), and as will be proven below, some of these *SR* derivatives have lost the distal copy of *white* and some the proximal copy. When DNA from *TE146(Z)* is digested with *Pst*I and probed with *w:HR*0.45 two fragments from *TE* DNA hybridize, of 11.4 and 7.0 kb (Figure 3, tracks 1–3). DNA from the spontaneous red-eyed derivative *SR5* lacks the 11.4-kb fragment while DNA from another derivative, *SR35,* lacks the 7.0-kb fragment. These data suggest that *SR5* has lost one of the copies of *white* and *SR35* the other.

The 11.4-kb *Pst*I fragment comes from junctions A + B, since it is also hybridized by the *noc:*D probe, but not by the *noc:*P probe. The 7.0-kb *Pst* fragment is from junction F since it fails to hybridize to either of the two *noc* probes (Figure 3). *SR5* retains junction F (the 7.0-kb fragment) but not junctions A + B, the 11.4-kb fragment being replaced by a novel fragment of 17-kb (Figure 3, track 4). We conclude that *SR5*

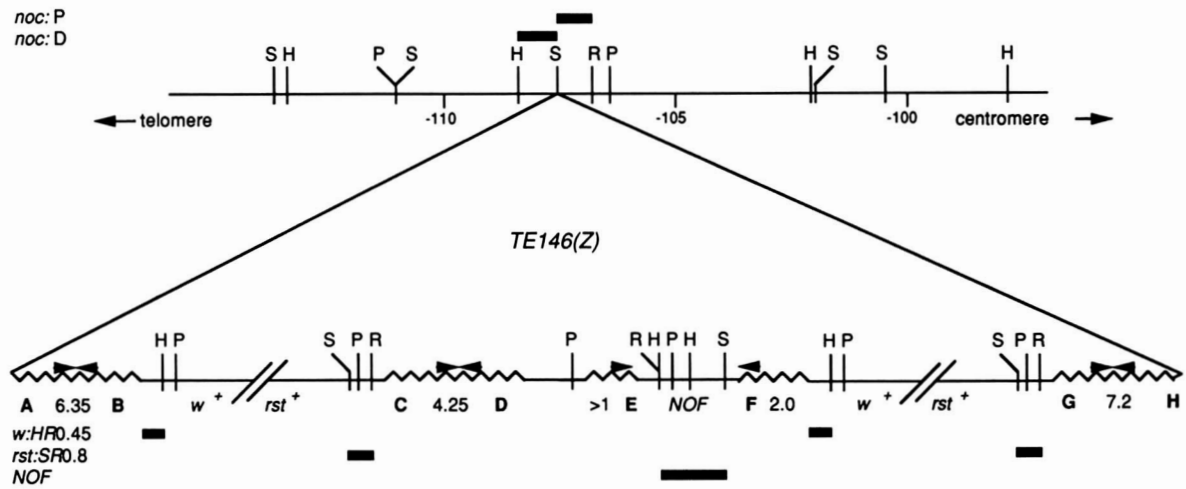

FIGURE 2.—A summary of the molecular map of the *noc* region (coordinates are from the *Eco*RI site 5′ to *Adh*) showing the insertion site of *TE146(Z)* (CHIA *et al.* 1985b). *TE146(Z)* is shown inserted at coordinate −107.5. The positions of the two *noc* probes used to map sites from this insertion site are indicated. Some restriction enzyme sites within the body of the *TE* are indicated. Some of these had also been mapped by CHIA *et al.* (1985a) but data for them all are given in this paper. The letters A to H indicate the eight junctions between *FB* (as wriggly lines) and non-*FB* (as straight lines) DNA. The lengths of *FB* sequences are not to scale, but their total lengths are indicated (in kb). The positions of probes to *white, roughest* and *NOF* sequences within the *TE* are shown below the diagram. Abbreviations for restriction enzyme sites used are: H, *Hin*dIII; P, *Pst*I; R, *Eco*RI and S, *Sal*I.

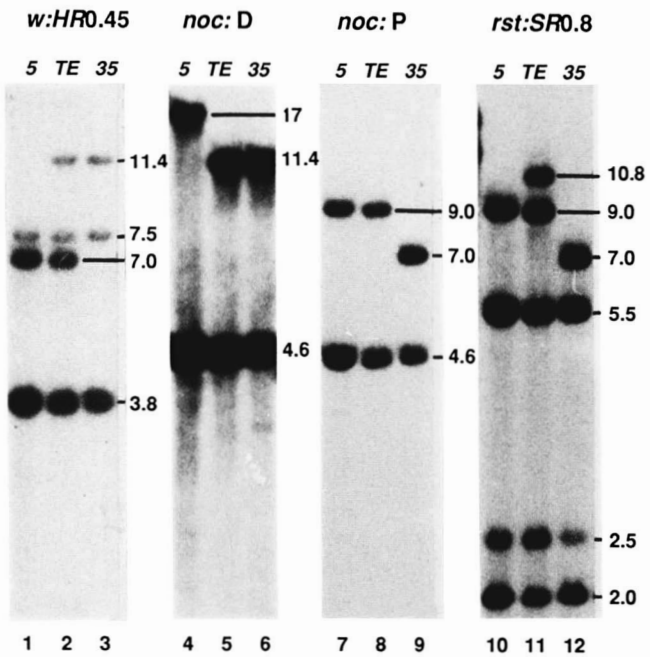

FIGURE 3.—Genomic DNA from z^1 w^{11E4}; *TE146(Z)/CyO* and from two spontaneous red-eyed derivatives of this stock, *SR5* and *SR35*, was digested with *Pst*I and then hybridized in turn with *w:HR*0.45 (tracks 1–3), *noc:*D (tracks 4–6), *noc:*P (tracks 7–9) and *rst:SR*0.8 (tracks 10–12). With the *white* gene probe the 7.5- and 3.8-kb fragments are from the z^1 w^{11E4} chromosome; with the *noc* gene probes the 4.6-kb fragment is from the *CyO* chromosome; with the *rst* probe the 5.5- and 2.0-kb fragments are from the *X* chromosome. (We would expect the intensity of hybridization of the *w:HR*0.45 probe to be similar to the 7.0- and 11.4-kb fragments in track 2 (as in Figure 7a). Presumably, the higher molecular weight fragment was poorly transferred to the filter here).

has lost the distal copy of *white*. SR35 retains junctions A + B (the 11.4-kb fragment) but has lost junction F (the 7.0-kb fragment that is hybrized by *w:HR*0.45). We conclude that this derivative has lost the proximal *white* gene. These conclusions were confirmed by probing the same filter with *noc:*P (Figure 3, tracks 7–9). This hybridizes to a 9.0-kb *Pst*I fragment in *TE146(Z)* and *SR5* (junctions G + H) but to a novel, 7.0-kb, fragment in *SR35*.

The interpretation of these data is that a *white* gene is close to junctions A + B but distant from junctions G + H, *i.e.*, that the structure summarized in Figure 2, deduced from genetic data, is correct.

The position of *rst* sequences: Although the *rst* gene has not been characterized molecularly, a probe to DNA sequences proximal to *rst* was available from the experiments of PARO, GOLDBERG and GEHRING (1983). This probe, *rst:SR*0.8, was used with the same filters of *Pst*I digested DNA. With DNA from *TE146(Z)* three *Pst*I fragments hybridize, of 10.8, 9.0 and 2.5 kb. Both *SR5* and *SR35* lack one of these (figure 3, tracks 10 and 12). The 10.8-kb fragment is absent from *SR5* and is not replaced by any novel fragment; both the 10.8-kb and 9.0-kb fragments are absent from *SR35* and are replaced by a novel 7.0-kb fragment. Which of these fragments represents the fusion with *noc* sequences? Only the proximal *noc* probe, *noc:*P hybridizes to these bands, in fact to the 9.0-kb band of *TE146(Z)* and *SR5* and to the 7.0-kb fusion band of *SR35*. Therefore, the 9.0-kb *Pst*I fragment represents junction G + H and the 10.8-kb fragment represents junction C. These data confirm the model of *TE146(Z)*'s structure shown in Figure 2.

The position of *NEB* sequences in *TE146(Z):* By *in situ* hybridization to polytene chromosomes *TE146(Z)*

carries two sites of *NEB* sequence (McGILL 1985). *NEB* is a transposable element of the retroviral class that was mapped between the *rst* and *FB* sequences of *TE98* by PARO, GOLDBERG and GEHRING (1983). A comparison of the restriction maps of *TE98* and of junctions G + H of *TE146(Z)* shows them to be very similar (see below). This suggests that a *NEB* element is located between *rst* and *FB* sequences at junction G.

When *Pst*I digested DNA of *TE146(Z)* was probed with *rst:SR*0.8 there is a 2.5-kb fragment that hybridizes (Figure 3, track 11). This fragment results from the insertion of *NEB* into a 2.0-kb *Pst*I region of the wild-type DNA. Its size is unchanged in both *SR5* and *SR35* (Figure 3, tracks 10 and 12), but note that in these its intensity relative to the 2.0-kb fragment is reduced, as expected since these *SR*s should have one and not two *rst* genes. These data suggest that both of the *rst* genes of *TE146(Z)* have a *NEB* element inserted at a similar site. Interestingly, the *Basc* chromosome, from which this family of *TE*s was derived, does not have a *NEB* sequence at the homologous position proximal to *rst*, a conclusion drawn from the *Pst*I fragment sizes of this chromosome and confirmed by digestion with *Eco*RI and *Xho*I (LOVERING 1988).

Are the two *white* junctions in *TE146(Z)* the same? ISING's family of *TE*s originated carrying one copy of the *white* and *roughest* genes. *TE146(Z)*, and many other members of this family have duplicated these, by an unknown mechanism (see DISCUSSION). In *TE146(Z)*, we have already shown that the distal limit of the proximal copy of the transposed *white* sequences (*i.e.*, junction F) is about 16.4-kb distal to the site of *copia* insertion in w^a (HARDEN and ASHBURNER 1990; see also GOLDBERG, PARO and GEHRING 1982). Is junction B the same? To study this, DNA was digested with *Dra*I and probed with *w:SR*2.6, a wild-type sequence that crosses the sequenced *FB/white* junction. With w^{11E4} (a deletion of *w* that does not extend this far distal, see Figure 1a) and *TE146(Z)SW*, derivatives of *TE146(Z)* that have lost their *w* genes (CHIA *et al.* 1985a), four hybridizing *Dra*I fragments are seen (Figure 4a, tracks 2 and 3; their origin is indicated on the *Dra*I map, Figure 4b). With *TE146(Z)* itself there is an extra 0.46-kb band (track 1); this must represent the *FB/w* junction. Since only one band is seen, this suggests that this junction is the same for both *w* genes. Note that the intensity of hybridization of the probe to this band is about equal to that of the 0.56-kb fragment from the w^{11E4} chromosome.

Five different *SR* derivatives, each containing only one *w* gene, have been analyzed in a similar manner (Figure 4a, tracks 4–8). All show the same fragments as *TE146(Z)*, but the 0.46-kb band hybridizes less intensively than the 0.56-kb band (Figure 4a, track 1). It so happens that all of the *SR*s used in this experiment were of the *SR35* class, *i.e.*, have lost the proximal copy of *white* and, therefore, junction F. These hybridization data, together with the sequence of junction F (HARDEN and ASHBURNER 1990), suggest that junctions B and F are very similar to each other, if not identical.

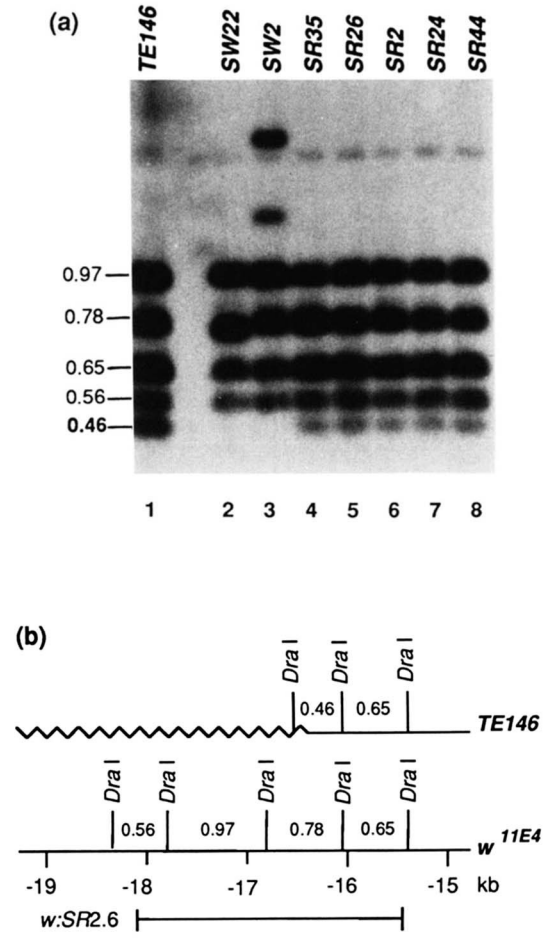

FIGURE 4.—(a) Restriction enzyme mapping of the *white* genes of *TE146*. *Dra*I-digested DNA was probed with *w:SR*2.6 (see Figure 1a). The DNA sample from *TE146(Z)SW2* was only partially digested. DNA was extracted from z^1 w^{11E4}; *TE/CyO* flies. (b) *Dra*I restriction maps of the *white* gene of w^{11E4} (coordinates with respect to the *copia* insertion of w^a) and of a junction between *FB* sequences (wriggly line) and *white* sequences in *TE146(Z)*, showing the origin of the fragments seen by hybridization.

The *white* genes of *TE146*: The *white* gene of *Basc*, the precursor of this family of *TE*s, is a typical w^a allele, resulting from the insertion of a *copia* element (ISING and RAMEL 1976; GEHRING and PARO 1980; BINGHAM and JUDD 1985). Indeed many of these *TE*s remain white-apricot in phenotype. Many others, however, carry revertants of w^a and give a more nearly wild-type eye color. When *Hinc*II-digested DNA from *TE146(Z)* and Canton-S were compared, after probing with an 11-kb *Kpn*I-*Eco*RI clone that includes all of the *white* gene, only a single difference is seen–the replacement of a 0.79-kb fragment of the wild type by one of 1.05 kb in *TE145(Z)* (Figure 5). This is the

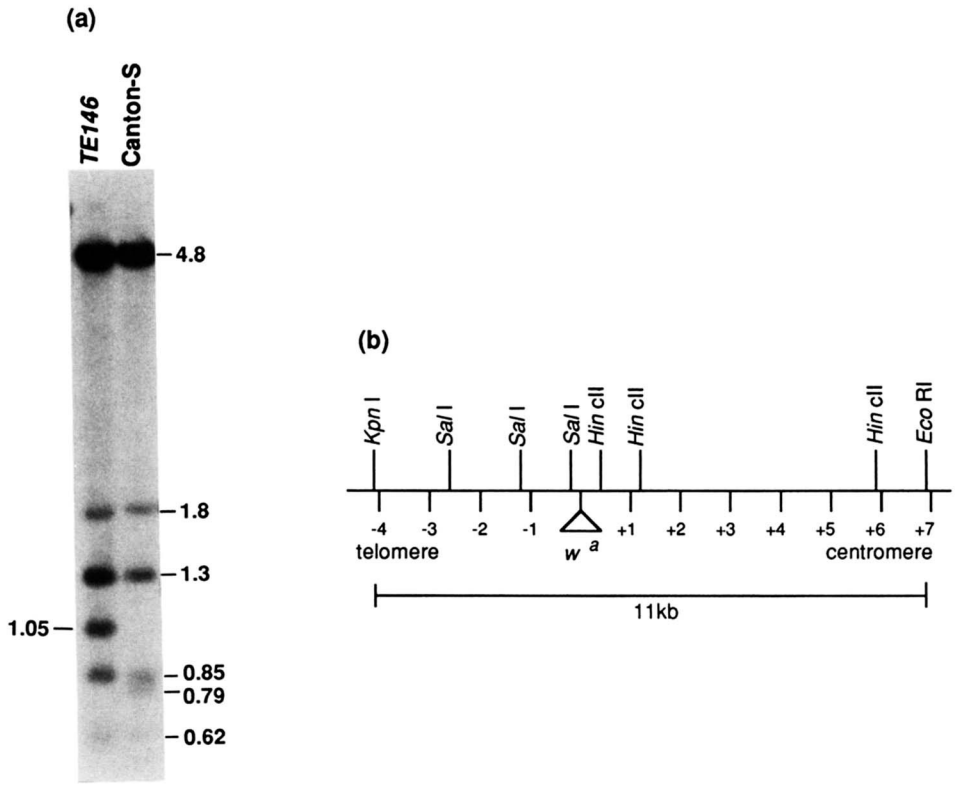

FIGURE 5.—(a) DNA from *TE146(Z)* and Canton-S digested with *Hin*cII and then probed with a 11-kb *Kpn*I-*Eco*RI region of the *white* gene (b). The only difference between these DNAs is the increase in size of the 0.79-kb *Hin*cII fragment to 1.05 kb, due to a remnant of the *copia* inserted in the *white* genes of the *TE*. This is, presumably, a solo *copia* LTR (see text).

fragment that spans the *copia* insertion site in w^a. These data suggest that the *w* genes of *TE146(Z)* still carry a small (*ca.* 250 bp) insertion in *w*, presumably a *copia* LTR (see CARBONARE and GEHRING 1985).

The structure of *TE146(Z):* The data discussed so far are summarized in Figure 6. We have determined the gross structure of junctions A to H by probing suitable restriction digests with a variety of clones whose positions in their corresponding wild-type genes are known. These data have been confirmed by digests with two other enzymes (*Hin*dIII and *Sal*I) (LOVERING 1988). The region of greatest uncertainty is that of limbs D and E; these will be discussed below.

The structure of half-losses of *TE146(Z):* One of the first indications that *TE146(Z)* carried two copies of w^+ was the phenotype of z^1 w^-; *TE146(Z)/+* flies. These are zeste-colored, and not red (GUBB *et al.* 1985). However, a stock of z^1 w^{11E4}; *TE146(Z)/CyO* frequently gives red-eyed derivatives and many of these have been selected (GUBB *et al.* 1986). The majority, though not all, of these *SR*s have a single copy of *white* by a number of criteria: unlike the original form of *TE146* they have three, and not six, polytene chromosome bands; they show only one, and not two, *in situ* hybridization sites with a *white* gene probe and they produce only half as much red-pigment as does *TE146(Z)* (GUBB *et al.* 1986).

Two of these *SR*s have been used in the analysis of the junctions between *TE146* and *noc* sequences, *SR5* and *SR35*. In *SR5* junctions A and B are changed in size but junctions G and H remain as in the original form of this *TE*. In *SR35* junctions A and B are unchanged but junctions G and H are novel. We concluded (above) that *SR5* had lost the distal copy of *white* and retained the proximal and that *SR35* had lost the proximal copy and retained the distal. A further 15 *SR*s have been studied in the same way: six are similar to *SR5* and eight to *SR35*, although the precise sizes of the novel junction fragments may differ between *SR*s (Figure 7 and Table 1). One, *SR47*, differs from these in that both the distal and proximal junctions (*i.e.*, both A + B and G + H) are novel. This may have been a consequence of the primary event which generated this derivative or of some event occurring subsequently. Two of the *SR*s, *SR33* and *SR64*, have polymorphic fragments representing junctions A + B and E + F, respectively (Figures 7, a and b). These are true polymorphisms since DNA from single flies of either of these strains show only one of the two forms. These polymorphisms are evidence for secondary changes occurring to junctions.

***NOF* in *TE146(Z)* half-losses:** An interesting correlation is seen when the half-loss derivatives of *TE146(Z)* are probed for *NOF* sequences. All of the distal half-losses retain a 7.0-kb *Pst*I fragment that hybridizes with *NOF* (Figure 7e) (except the unusual *SR47*). None of the proximal losses retain this fragment. The first conclusion drawn from these data is that loss of the distal half of the element occurs by an event involving sequences distal to *NOF*, *i.e.*, distal to

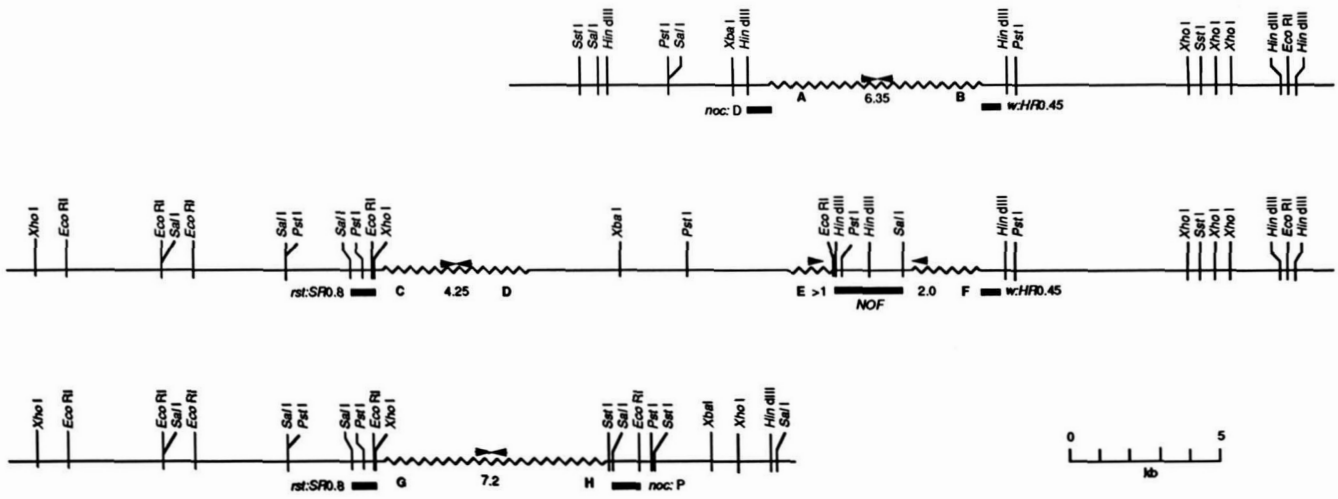

FIGURE 6.—Restriction enzyme map of the junctions of *FB* and non-*FB* DNA in *TE146(Z)*. *FB* DNA is shown, to scale, as wriggly lines, except for limb E which is estimated to be >1 kb. The nature of sequences between limbs D and E is unknown. The positions of the probes used to deduce these maps are indicated by the solid bars.

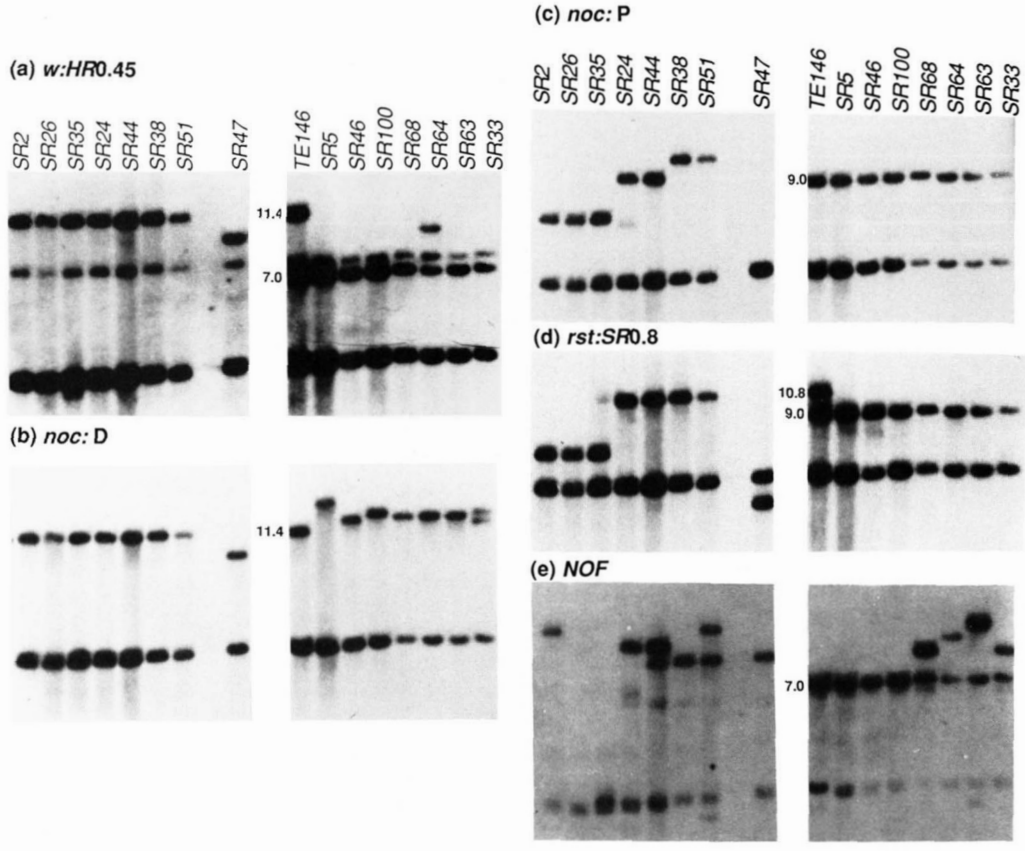

FIGURE 7.—Genomic DNA from *TE146(Z)* and several different spontaneous half-losses (*SRs*) was digested with *Pst*I and the filter hybridized in turn with (a) *w:HR*0.45, (b) *noc:*D, (c) *noc:*P, (d) *rst:*SR0.8 and (e) *NOF* probes. Fragments common to all tracks, and those whose sizes are not indicated alongside the *TE146(Z)* track, are from the z^1 w^{11E4} or *CyO* chromosomes.

junction E. The interpretation of the proximal half-losses is not as straightforward.

As can be seen from Figure 7e and Table 1 two of the proximal half-losses (*SR26* and *SR35*) show only the small 3.7-kb *Pst*I fragment hybridizing with *NOF*. This fragment is present in *TE146(Z)* and all of its derivatives. It is from a site at 66AB (Figure 8a and HARDEN 1989). The conclusion is that *SR26* and *SR35* lack *NOF* sequences within the *TE*. The events which generated these derivatives presumably involved sequences distal to *NOF*, *i.e.* limbs C, D or E.

All of the other proximal half-losses possess novel *Pst*I fragments hybridizing with *NOF* and, in many cases, more than one such fragment. These originate in two quite different ways. In *SR24* and *SR44* the 10.5-kb *Pst*I fragment also hybridizes to the *noc:*P probe. It is presumably a consequence of a novel junction between the *FB-NOF* sequences and *noc* DNA, *i.e.*, a novel junction "G + H." The other *NOF* fragments in these *SR*s do not hybridize to *noc* probes:

TABLE 1

Sizes of *Pst*I fragments of *TE146* and its derivatives detected by the five different probes used in these experiments

Chromosome	*w:HR*0.45	*noc*:D	*noc*:P	*NOF*	*rst:SR*0.8
+	3.8, 7.5	4.6	4.6	3.7	2.0, 5.5
TE146(Z)	7.0, 11.4	11.4	9.0	7.0	2.5, 9.0, 10.8
Distal half-losses:					
SR5	7.0	*17*	9.0	7.0	2.5, 9.0
SR33	7.0	*11, 13.5*	9.0	7.0, *8.0*	2.5, 9.0
SR46	7.0	*12*	9.0	7.0	2.5, 9.0
SR47	*8.4*	*8.4*	*4.6*	*8.0*	2.5, *4.6*
SR63	7.0	*13*	9.0	7.0, *11.5*	2.5, 9.0
SR64	7.0, *8.8*	*13*	9.0	7.0, *8.8*	2.5, 9.0
SR68	7.0	*12*	9.0	7.0, *8.0*	2.5, 9.0
SR100	7.0	*14*	9.0	7.0, *>20*	2.5, 9.0
Proximal half-losses:					
SR2	11.4	11.4	*7.0*	*11.5*	2.5, *7.0*
SR24	11.4	11.4	*10.5, 6.5*	*10.5, 6.5*	*2.4, 10.8*
SR26	11.4	11.4	*7.0*	—	2.5, *7.0*
SR35	11.4	11.4	*7.0*	—	2.5, *7.0*
SR38	11.4	11.4	*11.0*	*8.0*	2.5, *11.0*
SR44	11.4	11.4	*10.5*	*10.5, 8.0*	2.5, *10.8*
SR51	11.4	11.4	*11.0*	*11.5, 8.0*	2.5, *11.0*

No hybridization is indicated by —. Fragments from the *X* or *CyO* chromosomes are only listed in the "+" row. Numbers in italics represent novel fragments.

in fact they are not from the *TE* at all but represent *amplified NOF* sequences that appear to have been generated coincidentally with the *SR*. For three derivatives this has been confirmed by *in situ* hybridization to polytene chromosomes with a *NOF* probe: *SR2* and *SR51* do not show a *NOF in situ* site in region 35 but have a site at 94A (Figure 8b). The third derivative is *SR48,* a deletion of the proximal-half of *TE146(Z)* extending across the adjacent *osp* and *Adh* loci (GUBB *et al.* 1986). This chromosome has no *NOF* sequence in region 35 but does have one in region 37. None of these sites have been seen to be occupied by *NOF* in *TE146(Z)* nor have *NOF* hybridizing restriction fragments of an appropriate size ever been seen in this stock. These *SR*s, therefore, have been generated by an event involving sequences distal to *FB-NOF*. This interpretation is confirmed by probing these half-losses with *rst:SR*0.8. *TE146(Z)* shows two *Pst*I fragments, of 10.8 and 9.0 kb, that hybridize with this probe. The 9.0-kb fragment extends from the *Pst*I site near the proximal copy of *rst* to that in *noc* DNA proximal to the *TE*'s insertion site. This fragment is retained in all of the distal half-losses and is lost from all of the proximal half-losses. The 10.8-kb *Pst*I fragment extends from the *Pst*I site near the distal copy of *rst* to a *Pst*I site that maps between limbs D and E. This fragment is only retained by *SR24* and *SR44,* the two losses of the proximal half of the *TE* that retain *NOF* DNA. In *SR38* and *SR51* this 10.8-kb fragment is replaced by one of 11.0 kb, in *SR2, SR26* and *SR35* it is replaced by a 7.0-kb *Pst*I fragment. These novel fragments indicate the sizes of the DNA remaining between *rst* and proximal-*noc* sequences, a fact confirmed by their hybridization with the *noc*:P probe (Table 1).

The complete loss of *TE146(Z), i.e.,* to a *white*-eyed derivative, involves exchange between the flanking *FB* elements, that is between junctions A + B and G + H. These losses leave a few kilobases of *FB* DNA within the *noc* gene (CHIA *et al.* 1985a). The data from the half-losses indicate that these originate by a similar class of event, that is exchange between flanking *FB* sequences (Figure 9).

Mapping the *FB* elements of *TE146*: Restriction enzyme digestion data has already indicated that there is considerable DNA between adjacent pairs of junctions within *TE146(Z)*. We have made the implicit assumption, so far, that this includes sequences of the *FB* family (POTTER *et al.* 1980; TRUETT, JONES and POTTER 1981). The bases for this assumption are: the earlier studies of GOLDBERG, PARO and GEHRING (1982) and PARO, GOLDBERG and GEHRING (1983) on other members of the *TE* family, *in situ* hybridization with ^{3}H-labeled probes showing at least two *FB* sites within *TE146(Z)* (GUBB *et al.* 1985), the evidence from the mapping of junctions A and H, *in situ* evidence from the *SW* derivatives (CHIA *et al.* 1985a) and, finally, the cloning and sequencing of junction F (HARDEN and ASHBURNER 1990). In the following series of experiments we not only show that this assumption is justified for all of the boundaries within *TE146(Z),* but also provide more detailed structural data for these boundaries.

The experiments take advantage of the fact that much of the *FB* DNA is cut by the enzyme *Taq*I into a 155-bp unit (TRUETT, JONES and POTTER 1981). If

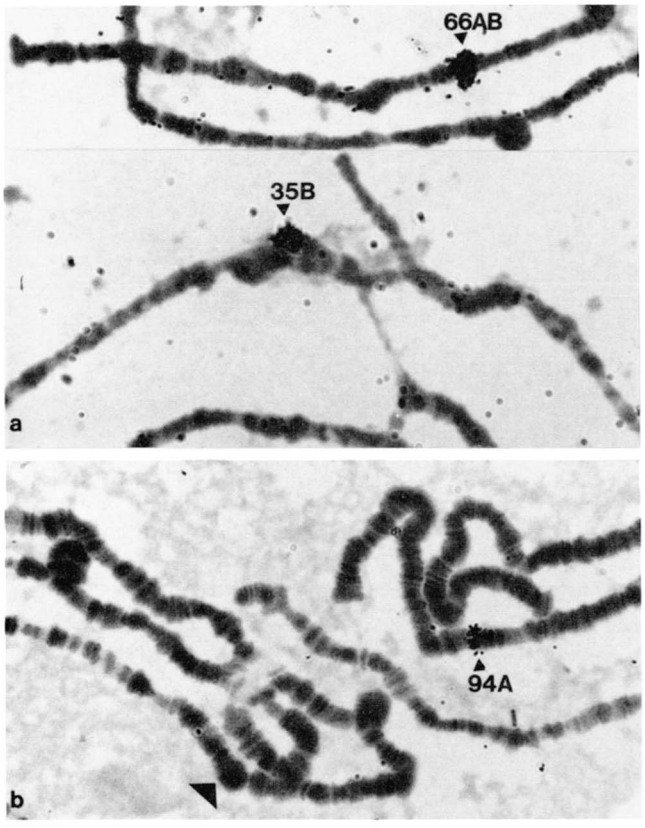

FIGURE 8.—(a) *In situ* hybridization to the polytene chromosomes of z^1 w^{11E4}; *TE146(Z)/CyO* with a tritiated *NOF* probe, showing two sites of hybridization, at 35B within the *TE* and at 66AB on chromosome arm *3L*. (b) *In situ* hybridization to the polytene chromosomes of z^1 w^{11E4}; *TE146(Z)SR2/CyO* with a tritiated *NOF* probe showing hybridization to 94A on chromosome arm *3R* but not to the site of the *TE* (large arrow).

FB sequences are only partially digested with this enzyme then a ladder of fragments, with a 155-bp periodicity, is seen. The method used here was simply to partially digest DNA from *TE146(Z)* and its derivatives with *Taq*I and then hybridize the DNA with a unique probe from a flanking region, *i.e.*, *noc*:D or *noc*:P. Then, the size of a labeled fragment will represent its distance from the probe, *i.e.*, from the junction of *noc* and *FB* sequences. DNA from both distal and proximal half-losses were used to study the internal junctions. If the *Taq*I sites are interrupted within the repetitive region of *FB* DNA then the position and size of sequences that lack *Taq*I sites will be seen from the break in the *Taq*I ladder (see LEVIS, COLLINS and RUBIN 1982).

Figure 10 is a more detailed view of the external side of an *FB* element [after TRUETT, JONES and POTTER (1981) and POTTER (1982a)]. Two features are important for our analysis: the first is the cluster of three *Taq*I sites between bases 134 and 154, the second is the *Hin*fI site at base 53.

Junctions A + B: When DNA from *TE146(Z)* is completely digested with *Eco*RI and *Hin*dIII and then probed with *noc*:D there is an 8.5-kb hybridizing fragment. Partial *Taq*I digestion of this fragment gives an additional 7.0-kb band and a ladder of *Taq*I fragments. This ladder is interrupted between 3.4 and 3.85 kb (Figure 11, track 2). With a *Hin*fI partial digestion of the *Hin*dIII fragment there is a 7.05-kb band. These fragments from partial digestion, the 7.0-kb *Taq*I band and the 7.05-kb *Hin*fI band, originate by cutting the *Hin*dIII fragment at the end representing junction B (see Figure 11, track 3). (The smaller fragments from junction A have run off this gel.) More extensive *Taq*I digestion simply leads to a *Taq*I ladder down to a size of about 1.2 kb; this ladder is interrupted between 3.4 and 3.85 kb due to an absence of *Taq*I sites this distance from the probe (Figure 11, track 1). Below 1.2 kb the ladder becomes irregular and the stoichiometry between the bands changes. This results from the irregular distribution of *Taq*I sites at the end of an *FB* element and, in particular, the cluster of three *Taq*I sites about 0.95 kb from the probe (Figure 10). Our interpretation of

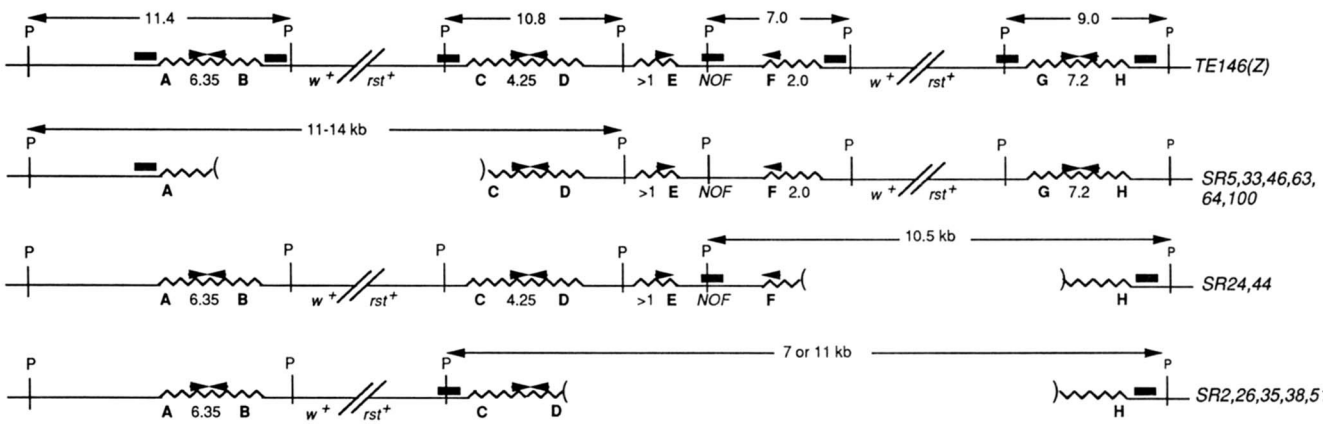

FIGURE 9.—A summary of the mapping of *SR* derivatives of *TE146(Z)*. The top line is a partial *Pst*I (P) restriction map of *TE146(Z)* (not to scale) showing the sizes of the fragments seen with the various probes (which are shown as bars). The structure of seven distal half-losses and of seven proximal half-losses are drawn below, with the DNA deleted indicated as lying between the brackets. *FB* DNA is indicated by wriggly lines.

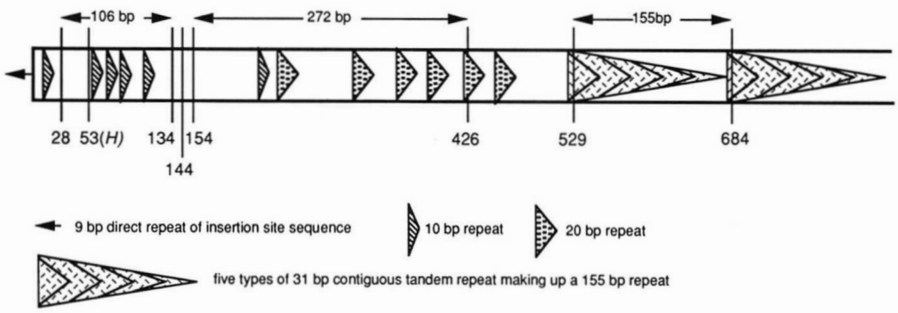

FIGURE 10.—A molecular map of the end of a *FB* element (after TRUETT, JONES and POTTER 1981) showing the repeat structure and the positions (in bp from the junction to non-*FB* DNA) of *Taq*I restriction sites. The single *Hin*fI site at position 53 is indicated as H.

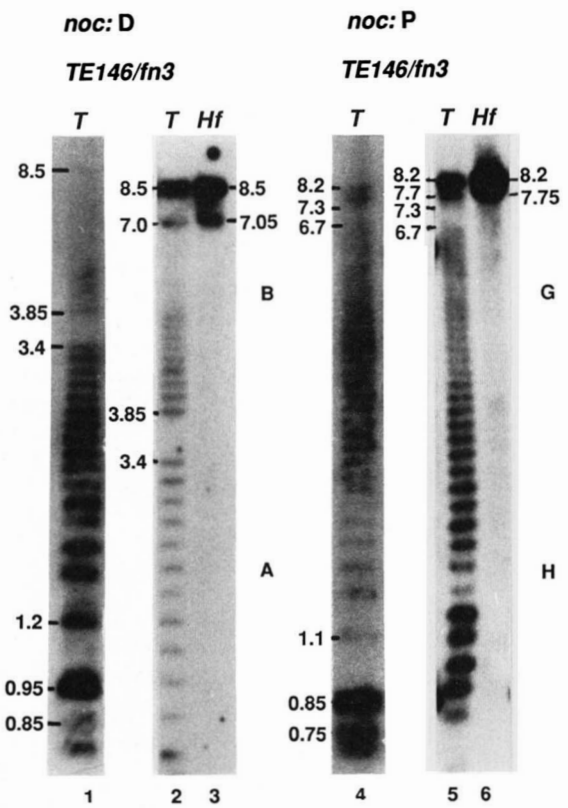

FIGURE 11.—*Taq*I analysis of the *FB* sequences at junctions A, B, G and H of *TE146(Z)* and its *SR* derivatives. Genomic DNA was digested to completion with *Eco*RI and *Hin*dIII and then partially digested with either *Taq*I (T) or *Hin*fI (Hf). The filters were hybridized with either the *noc:*D or *noc:*P probes, as indicated. The ends of the *FB* regions are labelled alongside the tracks, as A to H. Tracks 1 and 2, and 4 and 5 differ in the time of *Taq*I digestion, tracks 1 and 4 being longer than tracks 2 and 5.

these data, that is to say a restriction map of junctions A + B, is given in Figure 12a.

Junctions G + H: The filters used in the analysis of junctions A + B were washed free of label and then rehybridized with *noc:*P so as to map the proximal junctions of *TE146(Z)*. The data are shown in Figure 11, tracks 4, 5 and 6. The initial products of partial *Taq*I or *Hin*fI digestion are 7.7- or 7.75-kb bands. Further *Taq*I digestion yields a *Taq*I ladder down to about 1.1 kb and then a few smaller bands, including an intense band at 0.85 kb (due to the *Taq*I cluster at junction H). The *Taq*I ladder shows an asymmetric interruption, between 6.7 and 7.3 kb. These data are interpreted in Figure 12b.

Junctions C, D and E: Five of the *SR*s (*SR2, SR26, SR35, SR38* and *SR51*) were interpreted as being the consequence of exchange between limbs C or D and G or H. If so, the pattern of *Taq*I partial digestion products, seen after hybridization with the *noc:*P probe, should be identical, at least at the proximal end of limb H. If the events generating these half-losses had been exchanges between limbs C and G, then limb H should be identical to that seen in *TE146(Z)*. On the other hand, had the exchange events been between limbs D and H then the lengths of the *Taq*I ladder at junction H may differ from that seen in the parental element. Although the small *Taq*I fragments at junction H of *SR2, SR26* and *SR35* are the same size as those in *TE146(Z)* (*i.e.*, 0.75, 0.85 and 1.1 kb) the 155-bp ladder is not. It extends only 4.0 kb, not 6.7 kb as in *TE146(Z)*. After a 0.45-kb region without *Taq*I sites the ladder restarts, representing the 155-bp repeat of limb C (Figures 13 and 14).

Junction D was analysed with *Eco*RI-*Hin*dIII digested DNA from *SR64*, a loss of the distal copies of *white* and *roughest* (Figure 13, tracks 3 and 4). The pattern of small *Taq*I fragments (0.85–1.2 kb), at the distal end of limb A is identical in *TE146(Z)* and *SR64*. The 155-bp ladder, presumably the consequence of an exchange between limbs A and C, is uniform to 3.5 kb from the *noc:*D probes. From 3.5 to 3.9 kb there are three irregularly spaced *Taq*I sites, followed by 0.4-kb lacking *Taq*I sites. Thereafter the pattern of sites is regular for 2.15 kb; these are the sites within limb D. The higher molecular weight *Taq*I sites are not regular and must lie in non-*FB* sequences between limbs D and E. The complexity of this region is also evident from its pattern of *Hin*fI sites (Figure 13, track 4) and has been confirmed by digestion with other enzymes (see Figure 14a). The resolution of the high molecular weight *Taq*I fragments is insufficient to allow any detailed analysis of the *FB* sequences of limb E. Figure 14a is an interpretation of these data.

Junction F: Junction F has been cloned and sequenced (HARDEN and ASHBURNER 1990). The sequence of junction F indicates the presence of nine *Taq*I sites within the *NOF* sequence that lies between

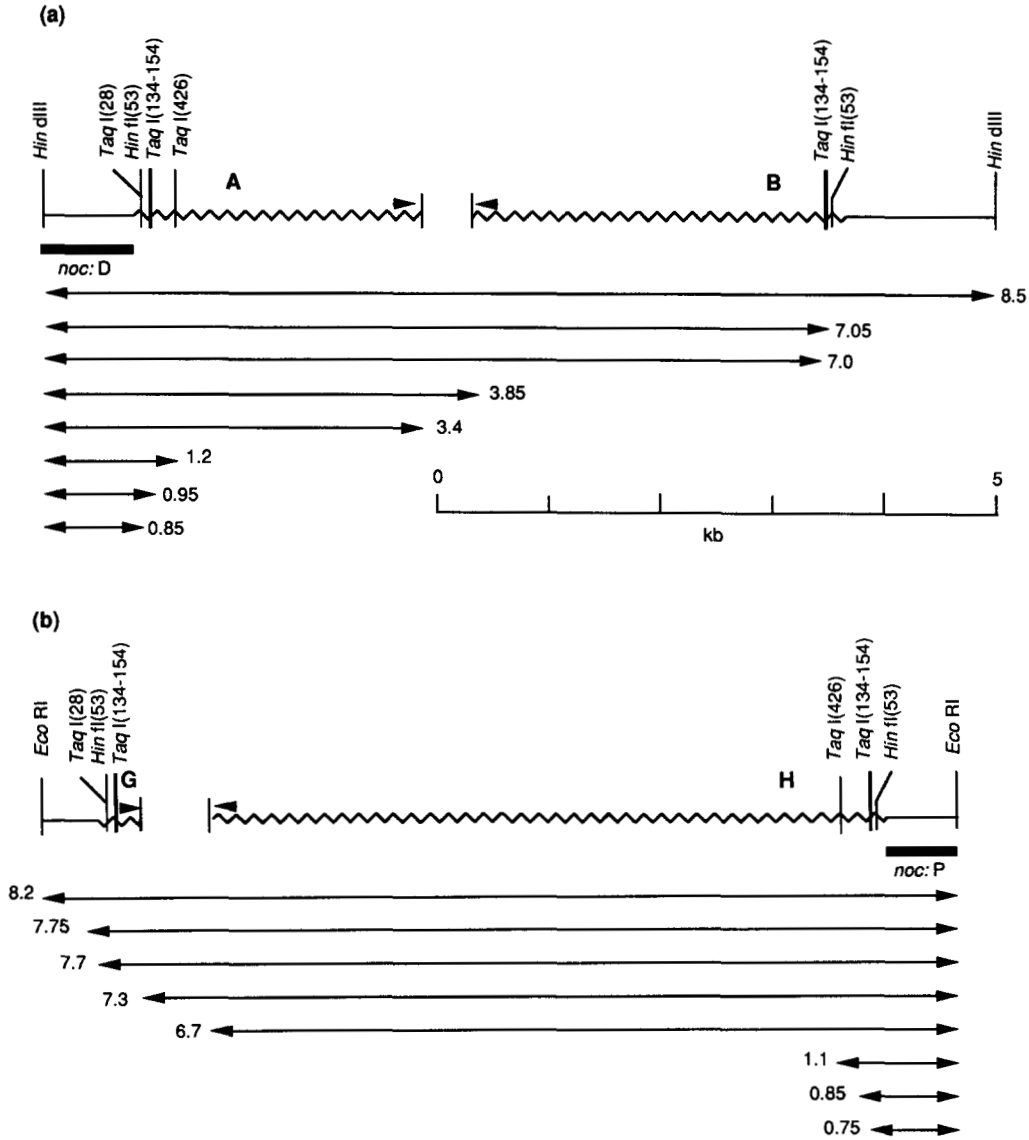

FIGURE 12.—An interpretation of the structures of junctions A and B and G and H in *TE146(Z)*. The solid bars represent the *noc*:D and *noc*:P probes to *Hin*dIII-*Eco*RI fragments from these junctions and to their *Taq*I and *Hin*fI partial digestion products. The sizes of the major fragments (but not those of the internal 155-bp *Taq*I ladder) are indicated. The *FB* limbs are shown as wriggly lines, the gaps between them representing the sequences that lack *Taq*I sites. The positions of the *Hin*fI and *Taq*I sites at the ends of the *FB* DNA are from Figure 10.

the *Hin*dIII site and *FB* DNA (Figure 14c). These generate a number of *Taq*I partial restriction fragments between 6.9 and 9.4 kb in size which have only been poorly resolved by electrophoresis (see Figure 15). Nevertheless the map of this junction deduced from *Taq*I mapping is wholly consistent with its DNA sequence.

In summary, *Taq*I restriction enzyme mapping, using probes adjacent to the insertion site of *TE146*, have allowed a detailed analysis of all of the *FB*/non-*FB* DNA junctions within this element. This allows a more detailed analysis of the events that led to loss of parts of this element when the *SR* derivatives originated.

Recombination generating the *SR* derivatives of *TE146(Z)*: The frequent occurrence of spontaneous red-eyed derivatives of *TE146(Z)* by the loss of one copy of *white* suggests a rôle of *FB* elements in their origin (GUBB *et al.* 1986). If so, then the *SR*s should have novel junctions between *FB* and non-*FB* sequences. At a gross level we have already shown this to be true (see above). Moreover, the precise organization of *FB* repeats should also be novel in these derivatives, since the *FB* limb itself will have been generated by a recombination event between two *FB* limbs. These features of the *SR*s have been studied by *Taq*I partial restriction enzyme mapping.

Proximal half-losses: The proximal half-losses of *TE146(Z)* are of two types, as seen from the size of their fusion fragments with *noc* DNA and by the fact that only some retain *NOF* sequences (see above). We have concluded (above) that *SR2*, a proximal half-loss that lacks *NOF* DNA, resulted from an exchange between limbs D and H (see Figure 14a). The restriction patterns of *SR26* and *SR35* are very similar (if not identical) to that of *SR2* (Figure 15). The two proximal half-losses that retain *NOF* sequences have very different restriction patterns when probed with

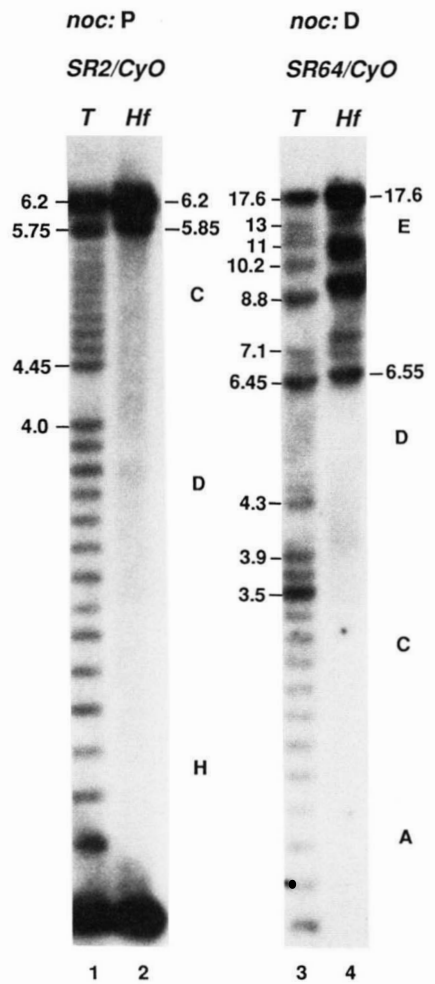

FIGURE 13.—A *Taq*I restriction enzyme analysis of *SR2/CyO* (tracks 1 and 2) and of *SR64/CyO* (tracks 3 and 4). DNA was digested to completion with *Hin*dIII and *Eco*RI and then partially digested with either *Taq*I (T) (tracks 1 and 3) or *Hin*fI (Hf) (tracks 2 and 4). The lower molecular weight *Taq*I fragments are not shown, as these were partly obscured by a heavy fragment from the *CyO* chromosome.

noc:D (Figure 15, lanes 1 and 2). Although the junction of limb H and proximal *noc* DNA is the same in these *SR*s and *TE146(Z)* the 155-bp repeat regions are much longer. The data are consistent with the model that these derivatives originated from an exchange between limbs F and H.

Distal half-losses: All of the losses of the distal *white* gene from *TE146(Z)* retain a *FB-NOF* element (see above), suggesting that they all result from exchange events between junctions A and C or B and D. Our analysis of *SR64* (see Figure 14a) led to the model that this derivative originated by an exchange between limbs A and C. *SR63* is a very similar derivative, but *SR46* and *SR68* differ in the lengths of the region occupied by 155-bp *Taq*I repeats, and in the positions of sequences that lack *Taq*I sites (Figure 16). This variation is largely due to the length of the most distal *FB* limb of these *SR*s. This variation could well arise if the exchanges between limbs A and C were unequal.

All of these distal half-loss *SR*s show a number of high molecular weight *Taq*I partial fragments, in each case, however, the size range of these is the same, about 10.5 kb. This is just the pattern of partials expected from the region between limbs D and E, with the absolute sizes of these fragments varying in accordance with the length of *Taq*I repeats within the body of the *FB* limbs.

DISCUSSION

The outstanding molecular feature of ISING's family of transposons is their intimate association with fold-back DNA sequences (GOLDBERG, PARO and GEHRING 1982). It is the *FB* DNA that gives the *TE* its genetic instability. In principle, we can imagine that *FB* sequences bounding any other DNA sequence could become unstable and form a transposon; it just so happens that a favorable genetic background, and a philosophy of "treasuring one's exceptions," lead to the identification of this *TE* family in which the *white* and *roughest* genes were mobilized. Three important questions follow from the molecular characterization of these *TE*s: (1) what is the mechanism of recombination between *FB* elements that leads to loss or transposition of the *TE*? (2) how is the propensity for recombination seen by *FB* elements that bound a transposon controlled? and (3) given the existence of 20–30 copies of the *FB* sequence in the "average" genome of *D. melanogaster*, how is promiscuous recombination between them, which would result in great genomic instability, avoided? Clearly, these three questions are related, and answering any one would throw light on the other two. As a first step towards an analysis of these problems we have made a detailed molecular characterization of one particular member of ISING's *TE* family, *TE146(Z)*, and of many of its spontaneous derivatives.

There is a strong similarity between the instability of *TE146(Z)* and that found by GABAY and LAUGHNAN (1970) for *Dp(1;1)MNB-8*, an unusual derivative of *Dp(1;1)B*. *Dp(1;1)MNB-8* was highly unstable in the male premeiotic germ-line, giving rise to at least three different classes of product (GABAY and LAUGHNAN 1970). Like some *FB* associated mutations (*i.e.*, w^{c} and w^{DZL}, see BINGHAM and ZACHAR 1989), this duplication showed a tendency to stabilize in stock. Moreover, the genetic basis of this duplication's instability was not separable from the duplication itself (LAUGHNAN, GABAY and MONTGOMERY 1971).

TE146(Z) is an unusual, but by no means unique, member of the ISING family of transposons since it is duplicated for both its *white* and *roughest* genes (GUBB *et al.* 1985, 1986). Three different classes of event can readily be seen to affect *TE146(Z)*: complete excision, "half-excision," where one copy of w^{+} rst^{+} is lost and the other remains, and transposition (GUBB

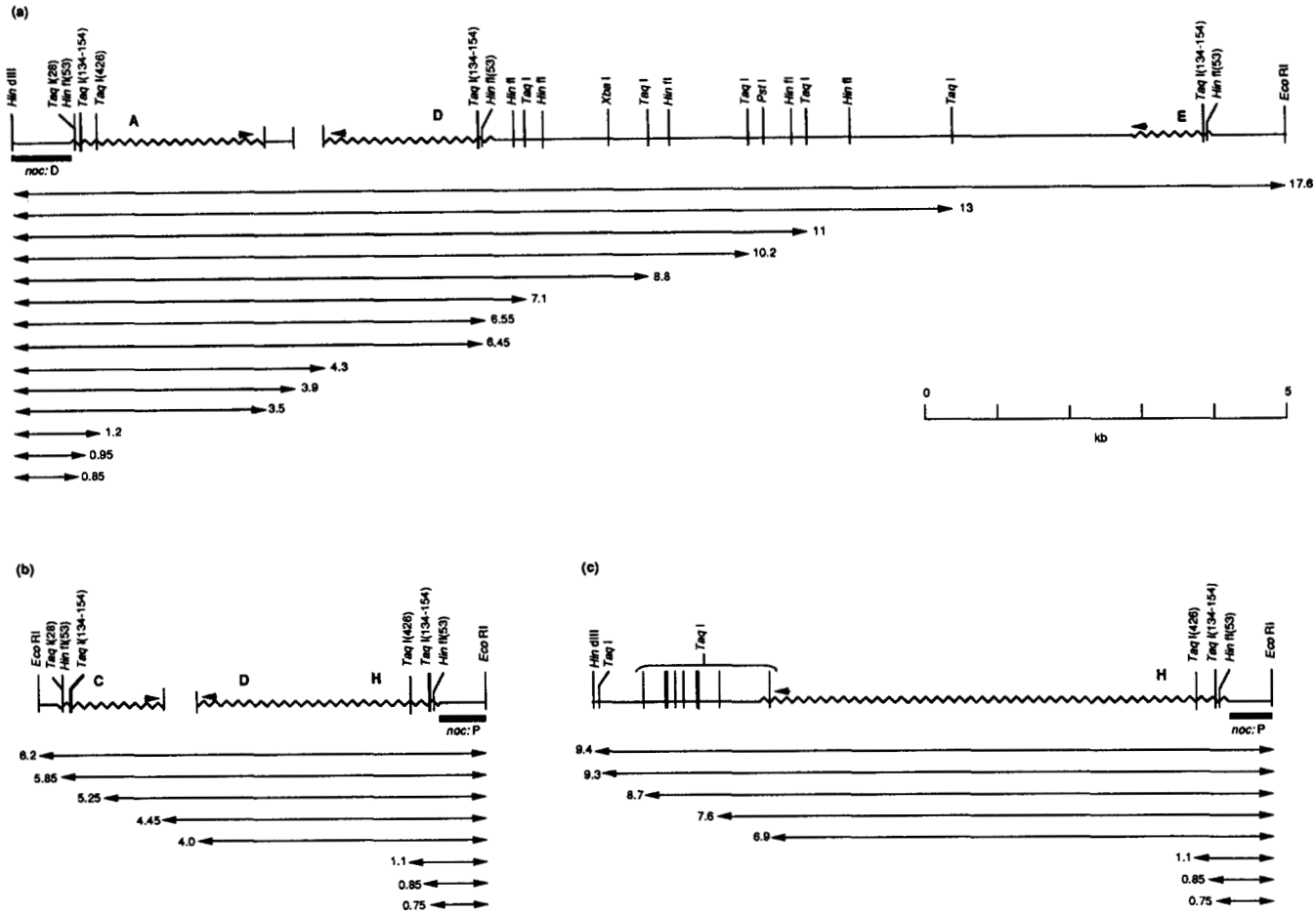

FIGURE 14.—(a) Interpretation of the restriction map of limbs D and E and of the fusion between limbs A and C in *SR64*. The proximal *Eco*RI site is within *NOF* DNA; it is 918 bp 5′ to the junction between *FB* and *NOF* sequences (see TEMPLETON and POTTER 1989). The *FB* sequences of limb E have not been mapped, their representation is schematic. (b) Interpretation of the restriction map of limb C and of the fusion between limbs D and H seen in *SR*s that have lost the proximal half of *TE146*. (c). A restriction map of the fusion of limbs F and H as deduced from restriction enzyme mapping of *SR44*. The *Hin*dIII site is the central such site within *NOF* sequences (at 1030 bp of the sequence of HARDEN and ASHBURNER 1990); the *NOF/FB* junction is at position 3412. The positions of the *Taq*I sites within the *NOF* DNA distal to limb F are from HARDEN (1989). *FB* limbs are represented as wriggly lines, the gaps between them represent DNA lacking *Taq*I sites.

et al. 1985, 1986; CHIA *et al.* 1985a; D. GUBB, M. ASHBURGER and J. ROOTE, unpublished data).

A hallmark of *FB* sequences is their length heterogeneity (TRUETT, JONES and POTTER 1981). Not only are the limbs of *FB* elements heterogeneous in length, both between and within elements (see POTTER 1982b), but also there is great heterogeneity in the length and nature of the sequences that lie between the limbs of an element. Such heterogeneity is evident from the different *FB* elements associated with *TE146*. The lengths of the *FB* limbs vary between 0.6 and 6.0 kb, and the lengths of the loop sequences that separate the limbs from 0.5 to 4 kb. The longest loop is that between junctions E and F, this loop is the *NOF* sequence, first found to be associated with *FB* and *TE* elements by GOLDBERG, PARO and GEHRING (1982) and PARO, GOLDBERG and GEHRING (1983). The *NOF* sequence is also found associated with the *FB* element of the w^c mutation (COLLINS and RUBIN 1982). Two *NOF* sequences have been determined, including the one from *TE146(Z)* (TEMPLETON and POTTER 1989; HARDEN and ASHBURNER 1990). The sequence data indicate that *NOF* may code for a large polypeptide, which, it has been suggested, is required for recombination between or within *FB* elements.

A genetic analysis of spontaneous derivatives of *TE146(Z)* that were no longer phenotypically zeste when on a z^1 background (*i.e.*, the *SR* derivatives) lead to the suggestion that most resulted from the loss of either the distal or proximal copy of w^+ rst^+. We have shown by a molecular analysis that this is indeed so. With one exception (*SR47*) all of these "half-loss" *SR*s change one *noc/FB* junction but leave the other conserved; of 14 *SR*s, seven have lost the distal copy of w^+ rst^+ and seven the proximal. It is clear, however (see Figure 9), that the different *FB* limbs were not

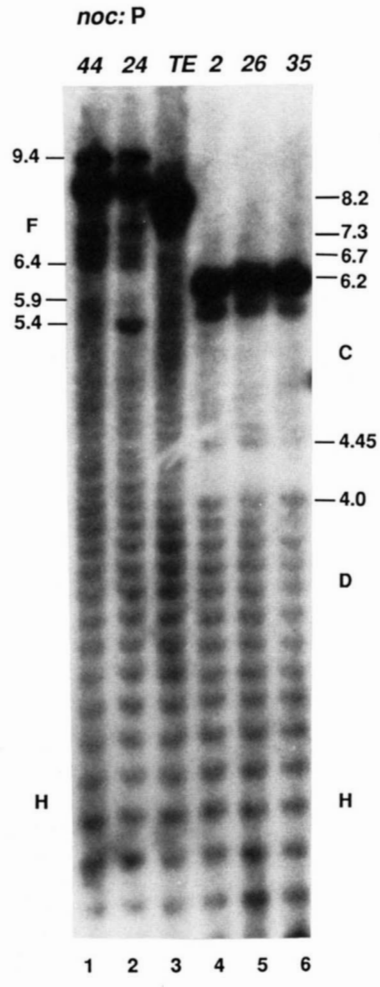

FIGURE 15.—DNA from *TE146(Z)* and five different proximal half-losses (*SR*s) was digested to completion with *Eco*RI and *Hin*dIII and then partially digested with *Taq*I before being probed with *noc*:P.

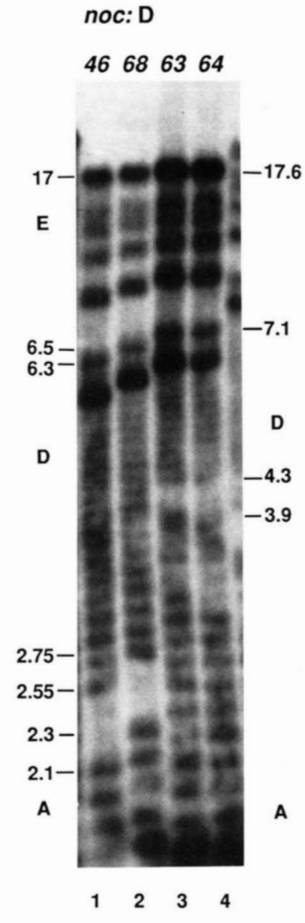

FIGURE 16.—DNA from four different distal half-losses (*SR*s) of *TE146(Z)* was completely digested with *Eco*RI and *Hin*dIII and then partially digested with *Taq*I. The filter was probed with *noc*:D.

involved with equal probability in the exchanges which generated these losses. For example, all of the losses of the distal half of *TE146(Z)* retain the *FB-NOF* element and have novel *FB* elements at junction A whose structures suggest that they resulted from an exchange between limbs A and C. In no case was there an exchange involving limb B, or one involving either limb of the *FB-NOF* element. Most (5/7) of the proximal half-losses have lost *FB-NOF*, and the structure of their junction with *noc* sequences indicates their origin by exchange between limbs D and H. Only *SR24* and *SR44* have originated by an exchange involving the *FB-NOF* element, in fact of its proximal limb (F) with limb H. In no case were limbs E or G involved.

There is an additional nonrandomness in the exchanges that generated a particular class of derivative, the similarity in restriction enzyme sites between derivatives of the same class suggests that there are preferred regions within *FB* limbs where exchanges occur. This is most clearly seen by the similarity of junction D in *SR2, SR26* and *SR35* (see Figure 15). These observations all confirm the suggestion (POTTER 1982b) that heterogeneity in length, and precise structure, of *FB* limbs occurs as a consequence of exchanges between limbs. The repetitive nature of the *FB* sequence (POTTER 1982a) is, of course, an ideal substrate for exchange between sequences that are not precisely aligned.

We have confirmed and extended the data that implicate *FB-FB* exchange events in the genetic instability of ISING's transposon. All of the derivatives we have characterized have *FB* limbs of novel fine structure, in comparison with those of the parental *TE146(Z)*. In particular, the evidence that all of the half-losses have an altered distal or proximal *noc/FB* junction indicates that half-loss does not occur by exchange between the duplicated copies of *w* and *rst*. Were it to do so, then both the distal and proximal junctions would remain unchanged in structure. It is clear, however, that these events are not normal meiotic exchanges. Although the instability of *TE146(Z)* and other members of this family is germline specific it is due to a premeiotic rather than meiotic process (GUBB *et al.* 1985; G. ISING, personal

communication). Moreover it occurs with a similar frequency in males and females (see ASHBURNER 1989).

The obvious alternative models are that the exchanges between *FB* elements occur either as sister- or as iso-chromatid events (PETERSON and LAUGHNAN 1963, 1964; GABAY and LAUGHNAN 1970; GREEN and LEFEVRE 1979). With respect to the half-loss class of spontaneous-red derivatives of *TE146(Z)* discussed in this paper, no distinction can be made between these two types of exchange. There is, however, another class of *SR* derivative that differs from that of the half-loss *SR*s in several respects: these derivatives remain cytologically large insertions and their w^+ gene(s) cannot be suppressed by z^1 in any genotype (GUBB *et al.* 1986). We originally thought that these *SR*s (*e.g.*, *SR36*) possess two copies of *white*, from the evidence of *in situ* hybridization. Higher resolution *in situ* analysis with biotinylated probes shows that these anomolous *SR*s have three *white* genes (D. GUBB and J. TRENEAR, unpublished data) (in retrospect this is even evident from the [^{3}H]thymidine *in situ* analysis, see Figure 3a of GUBB *et al.* 1986). Triplications can result from duplications by sister-chromatid, but not by iso-chromatid, exchange. The simplest interpretation of these data is that loss, half-loss and triplication of *TE146(Z)* occur by premeiotic sister-chromatid exchange that is limited to the *FB* limbs of the transposon.

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