Deletions and Amplifications of Tandemly Arranged Ribosomal 5S Genes Internal to a *P* Element Occur at a High Rate in a Dysgenic Context

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ABSTRACT

We observed unusual kinds of rearrangements within tandemly clustered 5S genes internal to a *P* element in dysgenic context. Rearranged *P* transposons, initially containing eight 5S genes, were found to display discrete numbers of 5S genes, from 4 up to 17 units. Precise deletions and amplifications occurred at a high rate (40%), at both original and new insertion sites. These events can be explained by a "cut and paste" transposition model. Possible links between rearrangements due to dysgenic-like processes and concerted evolution are discussed.

P transposable elements are responsible for *P-M* hybrid dysgenesis in *Drosophila melanogaster* (ENGELS 1989). Complete *P* elements are 2,907 bp long and contain 31-bp terminal and 11-bp subterminal inverted repeats. A 20-bp direct repeat is also present in the third exon. At least two coding sequences overlap in the transposon. One codes for a 87-kD transposase protein and the other for a 66-kD protein which represses the transposase activity (MISRA and RIO 1990). Many defective *P* elements that result from internal deletions are present in *P* strains. Sizes and locations of these deletions are variable but do not overlap the terminal repeats. The deleted *P* elements are unable to transpose autonomously, but can be *trans*-complemented by a complete *P* element, or any sequence encoding a functional transposase, such as the *P*[*ry*$^{+}$Δ2-3](99B) element (ROBERTSON *et al.* 1988).

Internal deletions arise in dysgenic context during the excision/transposition process (ENGELS 1989). Small as well as large portions of the transposon can be removed during these rearrangements. Breakpoints frequently occur within short direct repeats, from 2 up to 6 bp (O'HARE and RUBIN 1983). Repeated sequences are also implicated during insertion of the *P* element, since the 8-bp target genomic sequence is finally duplicated to surround the transposon.

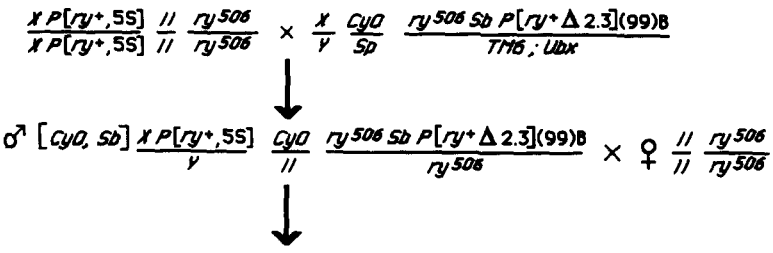
Recently, high rate of precise excision of the *P* element has been shown to require a wild-type homolog of the insertion site (ENGELS *et al.* 1990). A model for transposition has been proposed that combines a "cut-and-paste" mechanism for transposition, followed by a double-strand gap repair. In the first step, the *P* element would be excised, before transposing to a new site. The gap left by the excision would be repaired by a conversion-like event that would generate a new complete *P* element, or a defective *P* element if the gap repair process is interrupted (ENGELS *et al.* 1990). This two-step model is not *per se* a replicative mechanism for transposition. An interesting application of this mode of transposition would be to induce targeted gene replacement in any gene into which *P* insertion has occurred. GLOOR *et al.* (1991) obtained such a gene replacement for the *white* gene.

We have previously observed that a *P* element containing a tandem array of 20 5S genes of *Drosophila teissieri* was rearranged when coinjected with a helper plasmid in *D. melanogaster* embryos (SAMSON and WEGNEZ 1989). Two among eight transformed strains were found to possess *D. teissieri* 5S gene clusters containing only eight and eleven gene units, respectively. In this report, we show that rearrangements occur within a *P* element carrying a 5S gene cluster when it is placed in a dysgenic context. Precise deletions as well as amplifications of several 5S gene units were observed at high frequency. Similar results were obtained in the analysis of *P* elements either at the original site or at new insertion sites. We discuss the implications of these results in relation to the general model of *P* element transposition proposed by ENGELS *et al.* (1990).

MATERIALS AND METHODS

***Drosophila* strains:** We used the transformed *D. melanogaster* 35/11 strain that carries a *P* element on the *X* chromosome bearing a cluster of *D. teissieri* 5S genes (SAMSON and WEGNEZ 1989). This cluster contains eight 5S units, and not nine as was previously determined. We also used the *ry*^{506} (COTÉ *et al.* 1986) and the *ry*^{506}; *Sb P*[*ry*$^{+}$Δ2-3](99B)/*TM6* (ROBERTSON *et al.* 1988) strains.

DNA preparation and molecular analyses: Individual adult flies or populations of about 50 flies were homogenized in 500 μl of extraction buffer (100 mM Tris-HCl, pH 7.8, 50 mM EDTA, 200 mM sucrose, 0.5% sodium dodecyl



♂	*Sb+ ry+*	transposition *P[ry+,5S]*	39
	Sb+ ry	normal	197
	Sb ry+	normal • transposition *P[ry+,5S]*	185
	Sb ry	excision *P[ry+Δ2.3](99)B*	0
♀	*Sb+ ry+*	normal • transposition *P[ry+,5S]*	280
	Sb+ ry	excision *P[ry+,5S]*	95
	Sb ry+	normal • transposition + deletion *P[ry+,5S]*	328
	Sb ry	excision *P[ry+,5S]* and *P[ry+Δ2.3](99)B*	0

FIGURE 1.—Experimental design to study rearrangements within 5S genes internal to a *P* element. *P* elements inserted at original and new insertion sites were selected after a two generation crossing. Females of the strain 35/11 (SAMSON and WEGNEZ 1989), homozygous for a *P* transposon carrying 8 *D. teissieri* 5S genes (Figure 3), were crossed with males carrying the *P*Δ2-3 element (ROBERTSON *et al.* 1988). Sixteen *CyO, Sb* males were recovered in G_1 and crossed with ry^{506} females. Among the G_2 progeny, 33 Sb^+ry^+ males and 26 Sb^+ry^+ females were selected. These males carry a $P(ry^+, 5S)$ element mobilized in G_1. In Sb^+ry^+ females, not mobilized as well as mobilized transposons can be recovered. The Sb^+ry^+ flies were crossed with ry^{506} to establish strains.

sulfate). The mixture was incubated at 65° for 10 min. After addition of 120 μl of 3 M potassium acetate, pH 5.4, DNA was extracted with chloroform and precipitated with 0.6 volume of isopropanol after addition of 10 μl of 5 M NaCl. The precipitate was dissolved in 400 μl H_2O and precipitated again with ethanol. Restriction digests were fractionated on 0.6% or 1% agarose gels and DNA was transferred to Hybond-N membranes (Amersham). We also used 5.2% polyacrylamide gels to analyze 5S gene polymorphism (SAMSON and WEGNEZ 1984).

RESULTS

P elements containing a 5S gene cluster are rearranged at a high rate in dysgenic context: The strain 35/11 has been obtained by transformation of ry^{506} with a *P* element vector containing a cluster of 20 *D. teissieri* 5S genes, but was found to possess only eight *D. teissieri* 5S genes (SAMSON and WEGNEZ 1989). The number of *D. teissieri* 5S genes in this strain was found to be stable, in a nondysgenic context, during a 4-year survey.

We crossed 35/11 females to males carrying the $P[ry^+\Delta 2\text{-}3](99B)$ element and selected transposition events in G_2 males as described in Figure 1. Sixteen G_1 fertile males were crossed individually to ry^{506} females. We recovered 39 Sb^+ ry^+ males out of 236 Sb^+ males. Since the *X* chromosome is available for a new insertion, the approximate transposition rate is 20% (39/236 × 5/4). Thirty-three of the Sb^+ ry^+ males produced progeny which were molecularly analyzed (Figure 2).

The *D. teissieri* 5S genes, within the integrated *P* element of the 35/11 strain, are flanked by two *Hin*dIII restriction sites (Figure 3). DNA of the 33 strains with newly inserted *P* elements was extracted, digested with *Hin*dIII and analyzed by Southern blotting as shown in Figure 4. We used the *D. teissieri* 5S gene spacer as a probe since it hybridizes only with *D. teissieri* and not with *D. melanogaster* 5S genes (SAMSON and WEGNEZ 1984). Rearrangements within the 5S gene cluster, corresponding to increases as well as to decreases, clearly occurred in 14 strains. As shown in Figure 2, these strains derive from only eight among the 16 G_1 males, and thus do not probably reflect 14 independent events. The C5.2 and C5.5 strains, for example, have the same G_1 male progenitor and share an identical 5S restriction pattern, suggesting a clonal inheritance. It is interesting to note that the inserted *P* element in the C2.1 strain (Figure 2) segregates with males, that most likely implicates an insertion on the *Y* chromosome. Such insertions are rare as reported by ENGELS (1989).

As shown in Figure 4B, no rearrangements of 5S genes were observed in transposase-free control flies obtained by crossing Sb^+ ry^+ G_1 males to ry^{506} females. Similar results were obtained when analyzing DNA from 60 individual Sb^+ ry^+ G_2 females and from 60 individual 35/11 flies (data not shown). Dysgenic context thus is required for the high rate rearrangements we observed.

Rearrangements are precise deletions or amplifications of the 5S gene units: The rearrangements observed in Figure 4 could result from different molecular events. They could for example correspond to small deletions including 5S genes and contiguous genomic sequences. It was thus important to check for the presence/absence of the *Hin*dIII sites flanking

C2.1 C2.2	
C4.1	
C5.2 11 **C5.3* 13** **C5.5* 11**	
C7.1	
C8.1* 9 **C8.2 3**	
C9.1* 6	
D3.1 D3.2 **D3.3* 16**	D3.A D3.B
D5.1 D5.2	**D5.A* 13** D5.B D5.C D5.D D5.E D5.F
D7.1 **D7.3* 3**	D7.A D7.B **D7.C* 17** **D7.D* 11** D7.E
D8.1 D8.2	D8.A **D8.B* 10**
D9.1* 4 **D9.2* 6** **D9.3* 6** **D9.5* 5**	D9.A D9.B
D10.1 D10.2	**D10.A* 17** **D10.B* 6** D10.C
D11.1	
D13.1 **D13.2* 6**	D13.A D13.B
D16.1* 7 D16.2	**D16.A* 7**
D17.1 D17.2 D17.3	**D17.A* 10** **D17.B* 12** **D17.C 13**

FIGURE 2.—Redundancy of 5S genes within *P* elements in the dysgenic progeny of the 35/11 strain. A two symbol code (C2, C4, D3 . . .) has been used to name strains derived from a single G_1 dysgenic male (Figure 1). Strains derived from Sb^+ry^+ G_2 males are characterized by numbers (C2.1, C2.2, . . . , left column) while strains derived from Sb^+ry^+ G2 females are characterized by letters (D3.A, D3.B, . . . , right column). All molecularly tested strains are listed. Strains with rearranged *D. teissieri* 5S clusters are indicated with boldface characters, and the determined number of 5S genes is given. Strains which have been tested for a putative rearrangement of the *P* element outside of the 5S cluster are marked by an asterisk. Insertion in C2.1 is on the *Y* chromosome.

the 5S genes (Figure 3) in the rearranged *P* elements. The results shown in Figure 5 clearly demonstrate that both *Hin*dIII sites present in the original 35/11 strain are still present in the strains with rearranged 5S clusters. The rearrangements thus are due to deletion and amplification events within the 5S gene minilocus.

The close analysis of our data strongly suggests that the rearrangements are very precise, *i.e.*, they correspond to deletions or amplifications of discrete numbers of 5S gene units. The sizes of the *Hin*dIII *D. teissieri* 5S fragments of the rearranged strains [1.60 kb (C8.2), 1.95 kb (D9.1), 2.30 kb (D9.5), 2.65 kb (C9.1, D9.2, D9.3, D13.2), 3.00 kb (D16.1), 3.70 kb (C8.1), 4.40 kb (C5.2, C5.5) and 5.10 kb (C5.3), respectively] differ by 0.35-kb increments, the size of one *D. teissieri* 5S gene unit (Figure 4 and data not shown).

Rearrangements of the *D. teissieri* 5S cluster occur also in *P* elements at the original site: The strains analyzed above derive from males selected for a transposition event. In dysgenic context, rearrangements have been observed more frequently in *P* elements at original sites than at new insertion sites (ENGELS 1989). We thus crossed 26 among 280 Sb^+ ry^+ females (Figure 1) from nine independent G_2 progenies and analyzed their offspring. Ten among the 26 progenies are characterized by a modification of the *P* element structure (Figures 2 and 6A). These rearrangements are internal to the *D. teissieri* 5S cluster and do not affect adjacent sequences (Figure 6B and data not shown). As precedently observed for transposed *P* elements, the *Hin*dIII fragments differ in size by 350-bp increments [2.65 kb (D10.B), 3.00 kb (D16.A), 3.35 kb (35/11), 3.70 kb (D17.A, D8.B), 4.40 kb (D7.D), 4.75 kb (D17.B) and 5.10 kb (D5.A), respectively].

Some of the *P* elements in these strains could be located at a new insertion site (Figure 1). A rate of about 20% can be expected from the rate found for males selected for transposition (see above). We thus tested the strains analyzed in Figure 6 to determine if the rearranged *P* element was at the original site or at a new insertion site. As shown in Figure 7, two of these strains (D5.A and D17.A) were found to carry rearranged *P* elements at a new insertion site, the other strains being rearranged at the original site. The absence of a 2.75-kb *Eco*RI fragment in D5.A and D17.A lanes (Figure 7) is not due to a dysgenesis induced excision of the original *P* element, but to the loss of the chromosome bearing this element when establishing the two strains (data not shown).

Size polymorphism within the *D. teissieri* 5S gene clusters: Amplifications and deletions within *D. teissieri* 5S gene clusters involve discrete numbers of gene units (Figures 4 and 6, and data not shown). To refine the analysis of these rearranged gene clusters, we analyzed their structure by using *Sal*I, a restriction enzyme cutting once in each 5S unit (Figure 3). As shown in Figure 8, the eight *D. teissieri* 5S genes of the 35/11 strain differ by their sizes. Four size classes,

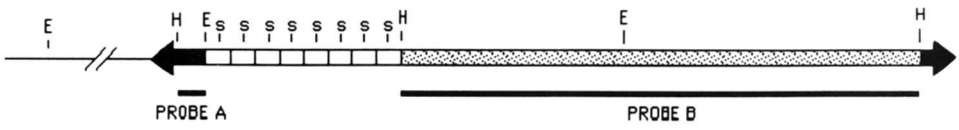


FIGURE 3.—Structure of the 35/11 strain *P* element. Eight *D. teissieri* 5S genes tandemly arranged (open boxes) and the ry^+ marker gene (spotted box) are inserted into the transposon (SAMSON and WEGNEZ 1989). Arrows correspond to the short terminal inverted repeats and filled boxes to *P* element sequences. Probe A (550 bp) and probe B (7,200 bp) were used to control integrity of the transposons. The thin interrupted line at the left side corresponds to genomic DNA flanking integrated *P* elements that was tested for integration sites. Restriction sites: E, *Eco*RI; H, *Hin*dIII; S, *Sal*I.

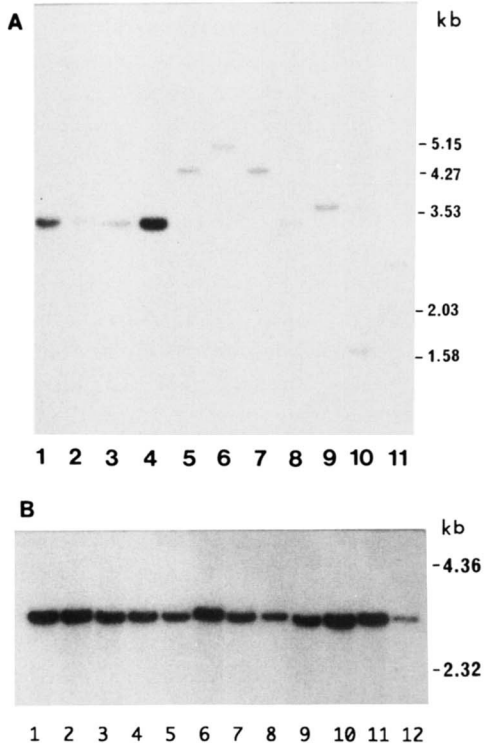


FIGURE 4.—Rearrangements of 5S gene clusters within newly inserted *P* elements. (A) Analysis of Sb^+ry^+ G_2 males (Figures 1 and 2). Autoradiogram of genomic DNA digested with *Hin*dIII, fractionated on a 0.6% agarose gel, and hybridized to a *D. teissieri* 5S spacer probe. *Hin*dIII digestion releases a fragment including all *D. teissieri* 5S units and 550-bp adjacent sequences (Figure 3). DNA lanes: (1) 35/11, (2) C2.1, (3) C2.2, (4) C4.1, (5) C5.2, (6) C5.3, (7) C5.5, (8) C7.1, (9) C8.1, (10) C8.2, (11) C9.1. (B) Control of 5S gene cluster stability in a nondysgenic context. Sb^+ry^+ G_1 males (Figure 1) were crossed with ry^{506} females. Twenty strains were then obtained by crossing ry^+ G_2 females with ry^{506} males (Str.C5S1 to Str.C5S20). Analysis of 5S gene clusters was performed as described in A. DNA lanes: (1) 35/11, (2 to 12) C5S1 to C5S11. M_r markers are indicated on the right.

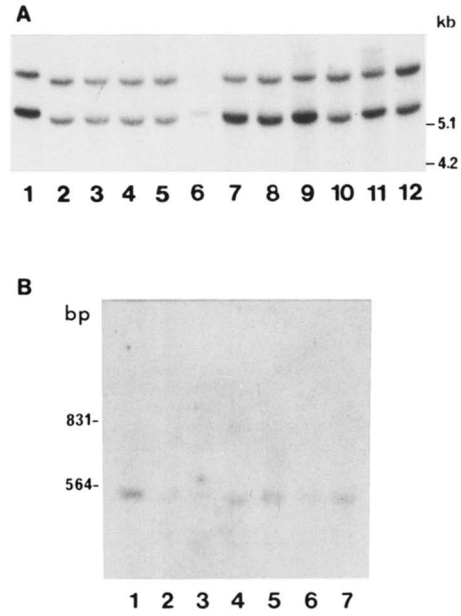


FIGURE 5.—Control of the sequences flanking the *D. teissieri* 5S genes within the newly inserted *P* elements. (A) Control of the *Hin*dIII sites flanking the 5S genes and the ry^+ marker (Figure 3). DNA of 11 strains was digested with *Hin*dIII and analyzed as in Figure 4. The probe was the 7,200 bp *Hin*dIII-*Hin*dIII fragment corresponding to the ry^+ marker gene (probe B, Figure 3). The ± 10-kb fragment corresponds to the genomic ry^{506} sequence. DNA lanes: (1) 35/11, (2) C5.5, (3) C8.1, (4) C9.1, (5) D3.3, (6) D7.3, (7) D9.1, (8) D9.2, (9) D9.3, (10) D9.5, (11) D13.2, (12) D16.1. M_r markers are indicated on the right. (B) Control of the *Hin*dIII site between *D. teissieri* and *P* element sequences (Figure 3). DNA of six strains was tested as in (A), except that digestion was with *Hin*dIII and *Eco*RI. The probe was the 550-bp *Hin*dIII-*Eco*RI fragment corresponding to the extremity of the *P* element (probe A, Figure 3). DNA lanes: (1) 35/11, (2) C5.3, (3) C5.5, (4) C9.1, (5) D3.3, (6) C8.1, (7) D9.1. M_r markers are indicated on the left.

ranging from 340 to 360 bp, can be discriminated by polyacrylamide gel electrophoresis. The same size classes are present in all strains with rearranged *P* elements deriving from Sb^+ry^+ G_2 males or Sb^+ry^+ G_2 females (Figure 8). Differences between strains thus are due to changes in the relative proportions between these 5S types.

DISCUSSION

In this report, we analyze the molecular events affecting a *P* element carrying a mini-cluster of ribosomal 5S genes when placed in a dysgenic context. Fourteen out of 33 *P* elements were found to be

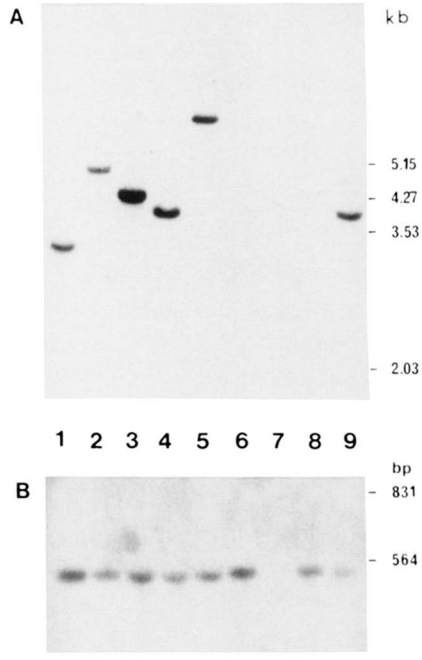


FIGURE 6.—Analysis of strains carrying *P* elements at original and new insertion sites. Eight strains derived from Sb^+ry^+ G2 females (Figures 1 and 2) carrying rearranged 5S clusters were analyzed as in Figure 4. (A) Analysis of genomic DNA digested with *Hin*dIII. (B) Control for the presence of *Eco*RI and *Hin*dIII sites in the sequence adjacent to the 5S cluster (probe A, Figure 3). Absence of a signal in lane 7 is due to an artifact: no DNA loaded. DNA lanes: (1) 35/11, (2) D5.A, (3) D7.D, (4) D8.B, (5) D10.A, (6) D10.B, (7) D16.A, (8) D17.B, (9) D17.A. M_r markers are indicated on the right.

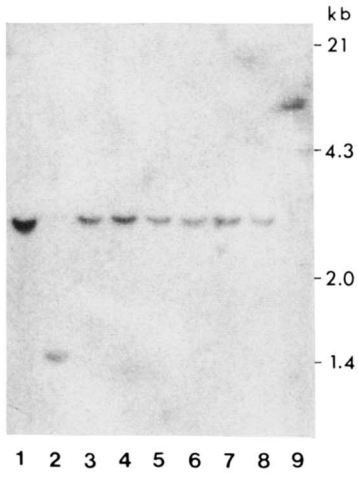


FIGURE 7.—Determination of the *P* element insertion status (*i.e.*, original or new insertion site) in Sb^+ry^+ G_2 females (Figures 1 and 2). DNA of the eight strains analyzed in Figure 6 was digested with *Eco*RI and hybridized with probe A (Figure 3). The presence of a 2.75-kb fragment is indicative of a *P* element located at an original site (as in strain 35/11), while other sized fragments are indicative of a *P* element located at a new site. DNA lanes: (1) 35/11, (2) D5.A, (3) D7.D, (4) D8.B, (5) D10.A, (6) D10.B, (7) D16.A, (8) D17.B, (9) D17.A. M_r markers are indicated on the right.

rearranged at new insertion sites. Roughly the same rate of rearrangement (10/26) was observed when analyzing *P* elements at original sites. Rearrangements

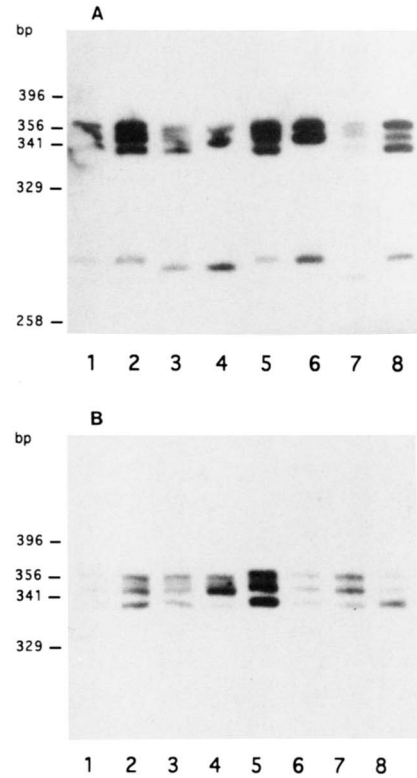


FIGURE 8.—Size polymorphism of *D. teissieri* 5S genes within original and newly inserted *P* elements. Autoradiogram of DNA digested with *Sal*I and *Hin*dIII, fractionated on a 5.2% polyacrylamide gel, and hybridized to a *D. teissieri* 5S spacer probe. *Sal*I digestion releases 5S gene units. The ± 290-bp band in each lane of (A) corresponds to the *Sal*I-*Hin*dIII fragment of the 5S unit adjacent to the ry^+ marker (Figure 3). This fragment ran out of the gel in B. The *Sal*I-*Hin*dIII fragment including some bp of one 5S unit and adjacent *P* element sequences (±800 bp, Figure 3) is not shown. (A) Analysis of strains derived from Sb^+ry^+ G_2 males. DNA lanes: (1) 35/11, (2) C5.5, (3) C8.1, (4) C9.1, (5) D3.3, (6) D7.3, (7) D9.5, (8) D13.2. (B) Analysis of strains derived from Sb^+ry^+ G_2 females. DNA lanes: (1) 35/11, (2) D5.A, (3) D7.D, (4) D8.B, (5) D10.A, (6) D16.A, (7) D17.A, (8) D17.B. M_r markers are indicated on the left.

then occur at a high rate (about 40%) within 5S gene clusters internal to a *P* element in dysgenic flies. As shown in Figures 4 and 6, rearrangements affecting *P* elements at original as well at new insertion sites are of the same type. In all cases, the *Hin*dIII fragments of such *P* elements carrying the 5S genes range within a discrete number of sizes. The size differences are multiples of 350 bp, *i.e.*, the size of one *D. teissieri* 5S gene unit (SAMSON and WEGNEZ 1984). These results strongly suggest that precise additions or deletions of 5S units occurred in all rearranged transposons. Similar results, *i.e.*, deletions and amplifications of tandemly arranged sequences internal to a *P* element, have been obtained by D. COEN and D. ANXOLABEHERE (personal communication).

The three main features characterizing the molecular rearrangements of the 5S gene clusters are the following. (1) They occur at original and new insertion sites at approximately the same rate. (2) They include

deletions as well as amplifications at similar frequencies. (3) They occur at a high frequency (40% per gamete).

Deletions within *P* elements have been observed mainly at original sites, new transposed elements being generally intact (ENGELS 1989). LAURIE-AHLBERG and STAM (1987) for example selected transposed *P* elements carrying initially both Adh^+ and ry^+ markers on the ry^+ phenotype. The Adh^+ marker was recovered in 65 among 66 ry^+ strains. However, this result stems from a certain bias. Transposed *P* elements are usually selected on a phenotype conferred by a marker gene (ry^+, Adh^+ . . .), and any event that would inactivate this gene would be undetected. Frequent deletions including parts of both ry^+ and Adh^+ markers in the experiments of LAURIE-AHLBERG and STAM (1987) thus would have not been recovered. On the contrary, the rate of *P* element rearrangements at the original sites can be estimated with good accuracy, since they are detected on the basis of a marker gene loss. In this way, almost all the imprecise excisions are recovered. A systematic study in a dysgenic context of all *P* transposons thus should be performed in order to determine whether or not rearrangements occur at significantly different rates at original and new insertion sites. For the present time, the simplest interpretation of our results would be to postulate that the excision/repair process involving the *P* element was very frequent. In these conditions, any new insertion site would immediately become a donor site with a high probability for the *P* element 5S cluster to be rearranged.

Direct repeats seem to be very important in excision events. For example, mismatches in the direct 8-bp repeats flanking inserted *P* elements lead to imprecise excision (RIO, LASKI and RUBIN 1986). Analysis of the excision properties conferred by a double insertion of two defective *P* elements in reverse orientation at the *singed* locus (sn^w allele) gives in this respect interesting insights. In dysgenic context, reversion toward wild type (sn^+) or a more extreme phenotype (sn^e) occurs at a very high frequency, up to 50%. The two insertions are separated by a 8-bp sequence, that is also present at the other end of both elements. The inverted 31 bp repeats present at the extremities of each *P* element are, in the sn^w strain, direct repeats relative to the inverted repeats of the other element. ROIHA, RUBIN and O'HARE (1988) suggested that the high rate of excision observed in this strain could be due to the abnormally large size of the direct repeats present at these *P* ends: 39-bp direct repeats (8 + 31 bp) relative to 8 bp, the normal size for direct repeats. Excisions internal to *P* elements could also be initiated at the sites of repeated sequences: breakpoints frequently occur at short directly repeated sequences (2–6 bp) within the transposon (ENGELS 1989). The transposase, and/or the other enzyme(s) presumably involved in generating double-strand breaks at the original site, recognize(s) some part of the *P* element sequence. Repetitions within or adjacent to *P* elements could play some role in the specificity/efficiency of DNA cutting. A large repetitive structure such as a 5S gene tandem repeat could thus induce high frequency cutting.

The molecular processes involved in *P* element transposition are not yet well characterized. ENGELS *et al.* (1990) demonstrated that *P* element excision was homolog dependent. They then proposed a model ("cut and paste" type) predicting that rearrangements could occur at the site of excision (original site) after imperfect correction on the sister chromatid or homologous chromosome. This model, based on very strong arguments, is suitable for integrating all the data related to *P* transposition. A targeted gene replacement predicted by this model has recently been performed with the *white* gene (GLOOR *et al.* 1991). The types of events we obtained do not contradict the model (Figure 9). Deletions or amplifications at the original site could occur, depending on the extent of the repair and on the 5S units involved in the pairing (Figure 9, B and C).

Internal deletions generated within *P* elements in dysgenic crosses are usually characterized by random endpoints. Our results demonstrate that deletions as well as amplifications of 5S genes are all in increments of ± 350 bp, *i.e.*, the size of one 5S unit (Figures 4 and 6). This most probably is a consequence of the repetitive structure of the *P* element insert : perfect matching of 5S gene units could occur during the repair process (Figure 9). Analysis of the size of individual 5S gene units is also interesting to consider. Some length heterogeneity has been demonstrated within the 5S gene family of *D. melanogaster*. It is due to variable numbers of a repetitive heptamer that follows the coding region (TSCHUDI and PIRROTTA 1980). Size heterogeneity among the *D. teissieri* 5S genes is also evident when analyzing the 35/11 strain and all strains with rearranged 5S clusters (Figure 8). These differences could be used to map all rearranged 5S clusters. This would give a clue to decipher the molecular mechanisms involved during the transposition process.

One last point deserves consideration: is there any link between the kinds of rearrangements we observed within *P* elements bearing 5S genes in a dysgenic context, and concerted evolution of 5S genes during their "normal" evolution? As pointed out by ENGELS *et al.* (1990), the process of repair following *P* transposition is in some way similar to a conversion event. Conversions as well as unequal crossovers are frequently proposed "mechanisms" to explain concerted evolution (SMITH 1976; NAGYLAKI and PETES 1982).

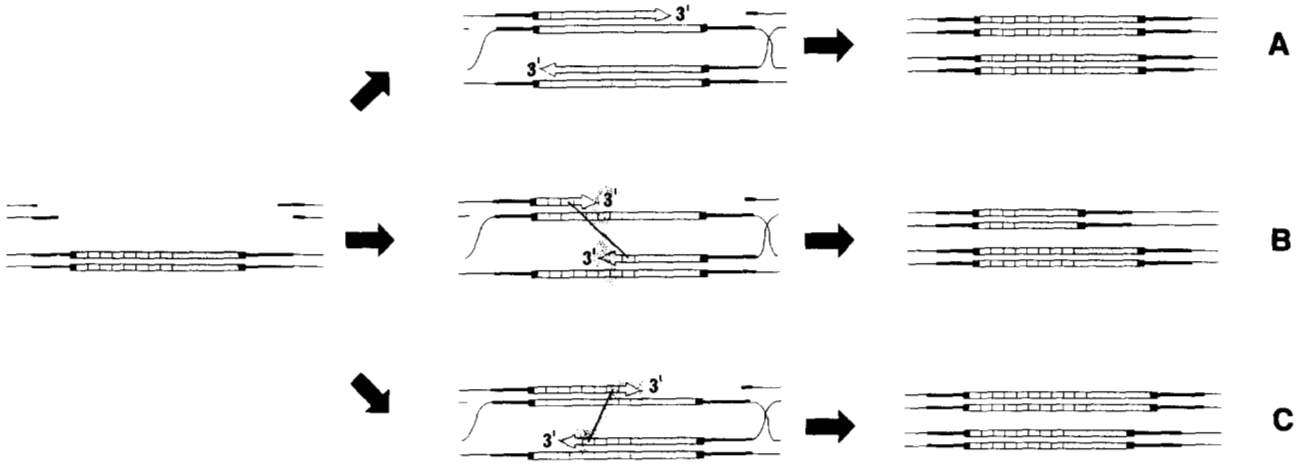


FIGURE 9.—Model for rearrangements in a dysgenic context within 5S genes internal to a *P* element. This is an adaptation for repeated sequences of the model of ENGELS *et al.* (1990). The first step involves a double-strand cut followed by a widening of the gap by exonucleases. A conversion-like process then allows repair on the sister chromatid or homologous chromosome. The result either is a restitution of the initial structure (A), or rearranged structures (B, deletions; C, amplifications). These rearrangements arise when repair is interrupted during the conversion process. In this case, one needs to postulate that pairing occurs between the 3′-extending ends. Unequal single-strand annealing could account for deletion and amplification events. This process could require some ssDNA binding protein with renaturase activity as the products of the SF1 and RAD10 genes that promote renaturation of DNA *in vitro* (NORRIS and KOLODNER 1990; SUNG, PRAKASH and PRAKASH 1992). These postulated proteins are symbolized by the shadows around the extending ends. Replication fork slippage could lead to the same result.

A dysgenic context, either due to *P* elements or to other transposable elements, conceivably could induce high rates of rearrangements within clustered 5S genes. TSCHUDI, PIRROTTA and JUNAKOVIC (1982) have shown that the transposable B104 element was able to induce important rearrangements within the *D. melanogaster* 5S gene cluster. The *Rex* element also promotes high frequency recombinations within the rDNA locus (WILLIAMS and ROBBINS 1992). Any deregulation in the expression of genes involved in DNA metabolism could conceptually lead to some kind of dysgenesis. Overexpression of a DNase gene for example could induce DNA breaks at abnormally high rates, followed by rearrangements. Such deregulations, due to different background genotypes of flies, could be frequent in natural populations. Our observations on rearrangements occurring within a 5S minicluster internal to a *P* element thus are perhaps more closely related to "normal" processes that could be thought at first sight.

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