A Genetically Marked *I* Element in *Drosophila melanogaster* Can Be Mobilized When ORF2 Is Provided in *trans*

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ABSTRACT

I factors in *Drosophila melanogaster* are non-LTR retrotransposons similar to mammalian LINEs. They transpose at very high frequencies in the germ line of SF females resulting from crosses between reactive females, devoid of active *I* factors, and inducer males, containing active *I* factors. The *vermilion* marked *IviP2* element was designed to allow easy phenotypical screening for retrotransposition events. It is deleted in ORF2 and therefore cannot produce reverse transcriptase. *IviP2* can be mobilized at very low frequencies by actively transposing *I* factors in the germ line of SF females. This paper shows that *IviP2* can be mobilized more efficiently in the germ line of strongly reactive females in the absence of active *I* factors, when it is *trans*-complemented by the product of ORF2 synthesized from the *hsp70* heat-shock promoter. This represents a promising step toward the use of marked *I* elements to study retrotransposition and as tools for mutagenesis.

NON-LTR retrotransposons or LINEs are widespread in eukaryotes (Eickbush 1992). In general, most of these elements are in a quiescent state within their host genome, but transposition events occur occasionally. For example, in humans some cases of genetic diseases and cancers were shown to be the result of *L*1 element insertions into genes (see Sassaman *et al.* 1997, and references herein).

The *I* factor in *Drosophila melanogaster* is a non-LTR retrotransposon of particular interest because conditions that allow its transposition at high frequencies are provided by *I*-R (Inducer-Reactive) hybrid dysgenesis (Picard *et al.* 1978; Bregliano and Kidwell 1983; for reviews, see Bucheton *et al.* 1992; Busseau *et al.* 1994). Several potentially active *I* factors are present in the genome of inducer strains, where they remain stable (Bucheton *et al.* 1984), whereas reactive strains are devoid of active *I* factors. Transposition of *I* factors occurs at high frequencies in the germ line of hybrid females, called SF females, produced by crosses involving females from a reactive strain and males from an inducer strain (Picard 1976). Transposition is accompanied by a characteristic syndrome of sterility: a proportion of the eggs laid by SF females dies early in development (Picard and L'Héritier 1971; Lavige 1986). The degree of sterility correlates with the frequency of transposition and depends mainly upon the reactivity level of the females used in the cross (Bucheton *et al.* 1976): females from a strongly reactive stock produce highly sterile SF females (hatching percentage close to zero) with high level of *I* factor transposition (up to more than one event/gamete), whereas females from a weakly reactive stock produce less sterile to normally fertile SF females with a lower level of transposition. The reactivity level is controlled by chromosomal determinants but, in the short run (*i.e.*, in the passage from one generation to the next), is essentially maternally transmitted (Bucheton and Picard 1978; Bucheton and Bregliano 1982).

The *I* factor possesses all features typical of non-LTR retrotransposons. It transposes by reverse transcription of an RNA intermediate (Pélisson *et al.* 1991; Jensen and Heidmann 1991). Transcription is initiated from an internal promoter lying within the first 186 bp (McLean *et al.* 1993) to produce a full-length 5.4 kb RNA that is believed to function both as an intermediate for retrotransposition and a bicistronic messenger for the synthesis of the products of the two open reading frames, ORF1 and ORF2 (Chaboissier *et al.* 1990; Bouhidel *et al.* 1994). ORF1 encodes a nucleic acid binding protein containing a central cysteine-rich motif (Dawson *et al.* 1997). ORF2 has the capacity of encoding a polypeptide with reverse transcriptase, RNase H, and endonuclease domains (Fawcett *et al.* 1986; Abad *et al.* 1989; Martin *et al.* 1995; Feng *et al.* 1996). The 3′ end of *I* factors is typically made of several (three to eight) repeats of a TAA triplet instead of the usual poly(A) tail found in most other LINEs. All as-

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pects of *I* factor retrotransposition regulation and tissue specificity are mainly effective at the level of transcription and/or RNA stability (Chaboissier *et al.* 1990; Lachaume *et al.* 1992; McLean *et al.* 1993; Udomkit *et al.* 1996).

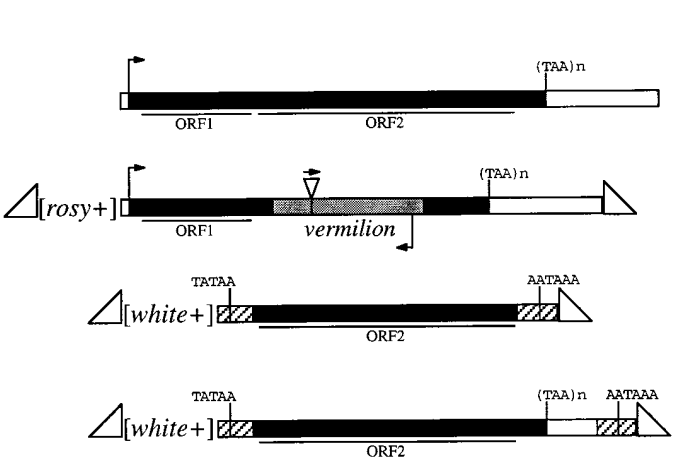
Heidmann *et al.* (1988) have designed a powerful system using a marker gene interrupted by an intron to indicate retrotransposition. This system has been used successfully in the study of various non-LTR retrotransposons (Tchenio *et al.* 1993; Maestre *et al.* 1995; Moran *et al.* 1996) including the *I* factor (Jensen and Heidmann 1991; Jensen *et al.* 1994; Chaboissier *et al.* 1995). However, in the case of the *I* factor, none of the marked elements could be mobilized at high frequency. The *IviP2* element, in which most of the sequences from ORF2 are replaced by the *vermilion* gene disrupted by the second intron of the *P* element, can be complemented in *trans* by actively transposing *I* factors in the germ-line of SF females (Chaboissier *et al.* 1995). Under these conditions, transposition occurs at a very low frequency (3×10^{-4}), rendering this system inefficient for the study of retrotransposition. The data presented in this paper indicate that the efficiency of transposition of the *IviP2* element can be improved by complementing it in *trans* with a construct in which synthesis of the product of ORF2 is controlled by the heat-inducible *hsp70* promoter.

MATERIALS AND METHODS

Fly stocks: All strains used in the experiments are M in the PM system of hybrid dysgenesis (see Engels 1989). The genetic nomenclature follows Lindsley and Zimm (1992). w^{1118} is an inducer strain. The strong reactive w^{K} strain was obtained from Lüning (1981). Strong reactive strains *JA*, carrying *y* and *w* mutations, *yv*, carrying *y* and *v* mutations, and *misy*, carrying *v* and ry^{506} mutations, are from our laboratory. *v*; *Cy/Bl*; ry^{506} has a balancer second chromosome marked with *Cy*.

7.51R is a transgenic line, homozygous for an *X* chromosome containing the *IviP2* element, that derives from the weak reactive *O/O* strain carrying *v* and ry^{506} mutations (Chaboissier *et al.* 1995). Establishment of the strong reactive *7.51RF#6* subline was done by several successive backcrosses of *7.51R* to the strong reactive *misy* strain as follows: females obtained by crossing *misy* females with *7.51R* males were mated to *misy* males to produce males carrying the transgene [scored by the (ry^{+}) phenotype] that were backcrossed to *misy* females. This scheme was repeated three times in succession, after which single matings were performed to establish sublines. The reactivity level of these sublines was determined by dysgenic crosses using the w^{1118} inducer strain. Females from the *7.51RF#6* subline, when crossed to w^{1118} males, constantly produced highly sterile *SF* females (no hatched eggs) over more than 22 generations.

Plasmid constructions and transgenic lines: All cloned sequences from the *I* factor derive from pI407 (Bucheton *et al.* 1984) which contains a fully active *I* factor (Pélisson 1981; Pritchard *et al.* 1988). Site positions are given according to the sequence published by Fawcett *et al.* (1986). Construction of pIviP2 (Figure 1) was previously described (Chaboissier *et al.* 1995). phsORF2HR (Figure 1) was obtained in two steps. First, a *Kpn*I-*Xba*I fragment (positions 4849–5108) and a *Xba*I-*Rsa*I fragment (positions 5108–5213) from pI407 were ligated together to the *Kpn*I-*Sma*I-cut pBluescript KS+ vector, producing clone pIK2. Then, a *Bam*HI-*Kpn*I fragment from pIK2 and a *Hpa*I-*Kpn*I fragment from pI407 (positions 1489–4849) were ligated together to the *Bgl*II/*Hpa*I-cut pCaSpeR-*hs* vector (Thummel and Pirotta 1991). phsORF2HN (Figure 1) was obtained by ligation of the *Hpa*I-*Nru*I fragment from



	Transgenic lines	Chromosomal location	Recipient strain
pI407			
pIviP2	*7.51R*	*X*	*O/O*
phsORF2HR	*HR136*	*II*	*JA*
	HR133	*II*	
	HR95	*III*	
phsORF2HN	*HN27*	*II*	w^{K}
	HN10	*II*	
	HN9	*III*	

Figure 1.—Constructs and transgenic lines. For clarity the vectors are not drawn, but the positions of the transformation markers *white* and *rosy* are indicated. Triangles at the extremities symbolize the *P* element ends. Black boxes represent sequences from the *I* factor with the positions of open reading frames indicated as lines below the boxes. The position of the TAA repeats at the 3′ end of *I* is shown. White boxes represent sequences from the *white* gene flanking the complete *I* factor cloned in pI407. The *vermilion* gene is shown as a shaded box and the position of the intron from the *P* element is indicated by a triangle with an arrow on top showing its orientation. Sequences from the *hsp70* gene are shown as striped boxes with the positions of the TATA box (TATAA) and the polyadenylation signal (AATAAA) indicated. Directions of transcription from the *I* and *vermilion* promoters are indicated by bent arrows at the start sites.

pI407 (positions 1489–5572), that contains all ORF2 as well as the 3′ end of the *I* factor flanked by 200 bp of *white* gene DNA, into the *Hpa*I site of the pCaSpeR-*hs* vector.

P element based transformations were essentially as described by Spradling and Rubin (1982) except that puchsπΔ2–3 (Flybase ID: FBmc0002087) was co-injected as the source of transposase. Transformation recipient strains were w^K for phsORF2HN and *JA* for phsORF2HR. Three independent homozygous transgenic lines were established for each construct (Figure 1) by selecting dark orange-eyed flies, and chromosome localizations of the transgenes were determined using balancer stocks. The *v*; *Cy*/*Bl*; ry^{506} balancer stock was used to introduce *hsORF2HR* and *hsORF2HN* from transgenic lines *HR136* and *HN27*, respectively, into a *v*; ry^{506} background.

Crosses: All crosses were performed on standard fly medium (Gans *et al.* 1975) at 23° unless otherwise stated. Typically, several samples of 10–13 virgin females were mated to 8–10 males in vials. They were transferred on fresh medium when necessary, until the age of 8–10 days when they were discarded. Heat-shock treatments were applied by placing the vials at 37° for one hour once a day, from the embryonic stage to egg-laying adults.

Inverse PCR and sequencing: Inverse PCR on *Nde*II-cut DNA extracted from single flies was performed as described by Gloor *et al.* (1993) and Gloor and Engels (1995). Positions of primers within the *IviP2* sequences are shown in Figure 4. The first round of amplification was with primers RI270 (5′CAACCCTCTAGACCTTCTTAGC3′) and v996 (5′TTGATAATTCCCAGCCTATCTGGC3′). Following 35 amplification cycles (94° for 45 seconds, 60° for 45 seconds, 72° for 45 seconds), an aliquot of the PCR product was subjected to a second round of 30 amplification cycles under the same conditions with primers RI160 (5′GTACATAACAAGCCAGCAATAG3′) and v1003 (5′ATCTGGCAGTGCCCATCGCC3′). PCR products were purified on MicroSpin™ S-400 HR Columns (Pharmacia, Piscataway, NJ) and sequenced with the OmniBase™ DNA Cycle Sequencing System (Promega, Madison, WI) using primer RI80 (5′GTAAGCCCCGTAGCTAATGCTATAC3′).

RESULTS

The low frequency of transposition of the *IviP2* element observed in the germline of SF females (Chaboissier *et al.* 1995) might be due in part to competition for the use of the reverse transcriptase between the *IviP2* transcript and RNA transposition intermediates synthesized from the actively transposing *I* factors. If this is true, the *IviP2* element might be mobilized more efficiently when the reverse trancriptase is supplied in *trans* in the absence of active *I* factors. In order to address this question, the *I* factor ORF2 was placed under the control of the heat-inducible *hsp70* promoter in constructs phsORF2HR and phsORF2HN (Figure 1). These two constructs differ in that phsORF2HR contains the termination signal from the *hsp70* gene, whereas phsORF2HN contains the native 3′ end of the *I* factor followed by the termination signal from the *hsp70* gene.

To exclude position effects, three independent transgenic lines were established for each construct (Figure 1) and used in the experiments described below. Only one line transgenic for *IviP2* was used because previous work showed that, in SF females, mobilization of the *IviP2* element from different independent transgenic lines occurred at similar rates (Chaboissier *et al.* 1995; I. Busseau, unpublished results).

Genetic screens were done in a *v*;*ry* mutant background. Therefore, flies carrying the *IviP2* donor element are [v; ry^+], because this element is associated with the wild-type *rosy* gene that was used as the transformation marker and with an inactive *vermilion* gene, disrupted by the P intron (Figure 1). Flies carrying a transposed copy of *IviP2*, in which the intron was spliced out, are [v^+; ry^+] if they contain also the donor *IviP2* element, and [v^+ry] if they do not.

***IviP2* can be complemented by the product of ORF2 under heat-shock control:** The general scheme of crosses is depicted in Figure 2A. Females from the 7.51*R* stock, homozygous for *IviP2* on the *X* chromosome, were crossed with males from *HR* or *HN* stocks, homozygous for *hsORF2HR* or *hsORF2HN*, respectively, on autosomes. In G1, [v] males carrying both *IviP2* and *hsORF2* transgenes were recovered. In the standard experiments, these males were crossed with females from the strongly reactive [v;ry] *misy* strain, and the resulting G2 females were submitted to heat-shock treatments and mated with their brothers. *Trans*-complementation of *IviP2* might occur in the germ line of half of these fe-

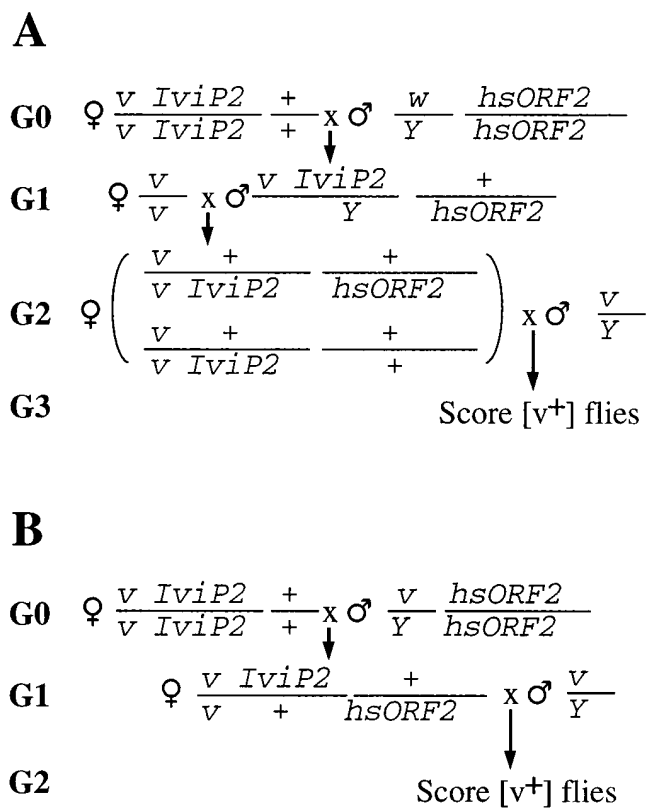


Figure 2.—Schemes to detect complementation of *IviP2* by *hsORF2HR* and *hsORF2HN*. (A) Using the *7.51R* weakly reactive transgenic line. (B) Using the *7.51RF#6* strongly reactive transgenic line. (See text for details.)

males, which receive both the *IviP2* and *hsORF2* transgenes. Retrotransposition events should result in the occurrence of [v$^+$] progeny in G3.

The results are shown in Table 1. In the first set of experiments (P1, S1–2, T3, M1–2), three independent *HR* lines were used. In all cases [v$^+$] flies were recovered at low frequencies, ranging from 0.6 to 1.8 $\times$ 10^{-3}. In experiment T1, in which the heat shock treatments were omitted, no [v$^+$] fly was found among more than 9000 flies observed, suggesting that *trans*-complementation of *IviP2* observed in the other experiments requires heat induction of the synthesis of the product of ORF2. Experiments M3–5 were done using three independent *HN* lines. [v$^+$] flies were again recovered at low frequencies ranging from 1.8 to 2.8 $\times$ 10^{-3}. Interestingly, these frequencies seem to be constantly somewhat higher (roughly twofold) than those obtained when using *HR* (compare M3–5 with M1–2). However, sample sizes are too small to allow statistical analysis. Experiments S3 and S4 were done using G1 females from another strongly reactive strain, *y v*, instead of *misy*. In this case the frequency of transposition of *IviP2* was found to be around 0.5 $\times$ 10^{-3}, which is about two- to threefold lower than when the *misy* strain was used (compare S3 with S1, S4 with S2). No obvious correlation can be made with the reactivity level which was roughly the same in both strains *y v* and *misy*.

Altogether, these results show that transposition of *IviP2* can occur in the absence of transposing *I* factors when the product of ORF2 is available for *trans*-complementation, and, in these conditions (after heat-shock induction), is more efficient than in SF females where the frequency of transposition of *IviP2* was around 3 $\times$ 10^{-4} (Chaboissier *et al.* 1995). However, this system still remains inefficient to be useful in the study of *I* factor transposition; additionally, it is laborious as it requires three generations of flies.

Toward an efficient system of transposition of the marked *IviP2* element: To improve the efficiency of transposition of *IviP2*, the two-generation scheme of crosses shown in Figure 2B was designed. In this scheme, females homozygous for *IviP2* are mated with males from *HR* or *HN* stocks, homozygous for an autosomal insertion of *hsORF2* in a *v;ry* background. Transposition is expected to occur in the germ-line of G1 females, which contain both *IviP2* and *hsORF2*. These females are submitted to heat-shock treatments and mated with their brothers. In this case, the frequency of transposition is equal to the frequency of [v$^+$] flies recovered in the next generation (G2).

This scheme of crosses requires that strongly reactive females are used in G0 to ensure efficient transcription of *IviP2* in the germ line of G1 females. The *7.51R* transgenic line containing the *IviP2* element was derived from the weakly reactive *O/O* strain (Chaboissier *et al.* 1995) and is therefore weakly reactive. The strongly reactive subline *7.51RF#6* was thus established, following several backcrosses of line *7.51R* to the strongly reactive *misy* strain (see materials and methods). The experiments described in Figure 2B were done using females from this subline in G0.

The results are shown in Table 2. A transposition frequency of 1.9 $\times$ 10^{-3} was found in experiment M7 using *HR136*. Sample sizes are too small to allow statisti-

TABLE 1

Results of the experiments described in Figure 2A

Exp.	G0♂	Conditions	Number of [v$^+$]/total flies	[v$^+$] flies frequency	Transposition frequency
P1	*HR136*	Standard	3/3287	0.9×10^{-3}	1.8×10^{-3}
S1	*HR133*	Standard	4/4915	0.8×10^{-3}	1.6×10^{-3}
S2	*HR95*	Standard	1/1958	0.5×10^{-3}	1.0×10^{-3}
S3	*HR133*	G1♀=*y v*	3/9319	0.3×10^{-3}	0.6×10^{-3}
S4	*HR95*	G1♀=*y v*	2/10410	0.2×10^{-3}	0.4×10^{-3}
T1	*HR133*	No heat shock	0/9314	$<0.1 \times 10^{-3}$	$<0.2 \times 10^{-3}$
T2	*HR133*	Dev. 29°	4/3891	1.0×10^{-3}	2.0×10^{-3}
T3	*HR133*	Standard	4/7248	0.6×10^{-3}	1.1×10^{-3}
M1	*HR133*	Standard	6/8529	0.7×10^{-3}	1.4×10^{-3}
M2	*HR133*	Standard	3/9509	0.3×10^{-3}	0.6×10^{-3}
M3	*HN27*	Standard	13/9243	1.4×10^{-3}	2.8×10^{-3}
M4	*HN10*	Standard	9/9926	0.9×10^{-3}	1.8×10^{-3}
M5	*HN9*	Standard	6/5029	1.2×10^{-3}	2.4×10^{-3}

All crosses were carried out following the general scheme depicted in Figure 2A. Standard conditions were as described in the text. In experiment T1, heat-shock treatments were omitted. In experiment T2, development of G2 females was at 29° from embryonic to late pupal stages. In experiments S3 and S4, G1 females were from the *y v* strain instead of from the *misy* strain. Experiments named with the same letter were done simultaneously. As transposition does not occur in half of the G2 females, transposition frequencies were estimated by multiplying by two the frequencies of [v$^+$] flies recovered in G3.

TABLE 2

Results of the experiments described in Figure 2B.

Exp.	G0♂	Conditions	Number of [v$^+$] / total flies	[v$^+$] flies frequency (=transposition frequency)
M7	*HR136*	Standard	7/3779	1.9×10^{-3}
N1	*HR136*	Reciprocal G0	1/10457	0.1×10^{-3}
M61	*HN27*	Standard	3/1025	2.9×10^{-3}
M62	*HN27*	Standard	15/2235	6.7×10^{-3}
M63	*HN27*	Standard	14/3032	5.0×10^{-3}
M64	*HN27*	No heat shock	4/6687	0.6×10^{-3}

All crosses were carried out following the general scheme depicted in Figure 2B. Standard conditions were as described in the text. Experiment N1 was started from reciprocal crosses in G0. Experiments M7, N1, M61 and M62 were done at the same time as experiments M1–5 shown in Table 1, experiments M63 and M64 were done four months later. In experiment M64, heat-shock treatments were omitted.

cal analysis, but this result does not seem significantly different from that obtained in experiment P1 (1.8×10^{-3}, Table 1). Results were better when *HN27* was used: transposition frequencies obtained in experiments M62 and M63 were found to be 6.7×10^{-3} and 5.0×10^{-3}, respectively. Some transposition events occurred, at a tenfold lower level, in experiment M64 in which heat-shock treatments were omitted, reflecting a low level of basal activity of the *hsp70* promoter.

The efficiency of transposition of *IviP2* is affected by the reactivity level: As mentioned earlier, the frequency of *I* factor transposition in the germ line of SF females is modulated by the reactivity level (Picard 1976; 1978), which influences the efficiency of transcription from the internal promoter of the *I* factor (McLean *et al.* 1993; Lachaume and Pinon 1993). The reactivity level is affected by nongenetic factors such as aging and temperature of development (Bucheton 1978; 1979a,b). Indeed, we noticed during the course of this work that, in most cases, [v$^+$] flies were recovered more frequently in the progeny of young females than in the progeny of older females. Moreover, in experiment T2, G2 females developed at 29° from embryonic to late pupal stages. This treatment is known to increase the reactivity level. Heat-shock treatments were performed as usual. The frequency of transposition of *IviP2* was 2.0×10^{-3} in this experiment, whereas it was 1.1×10^{-3} in experiment T3, which was performed in parallel following standard conditions. These various observations, although not statistically significant, suggest that the reactivity level might influence the frequency of mobilization of *IviP2*.

Experiment N1 was designed to definitively address the issue of whether the reactivity level influences the frequency of transposition of *IviP2*. This experiment was done in the same way as experiment M7, except that it was started from the reciprocal cross in G0: females from the *v*; *HR133*; *ry* stock were mated to males from the *7.51RF#6* subline. The reactivity level of the *v*; *HR133*; *ry* stock was rather weak because the hatchability of eggs laid by SF females produced by crossing females from this stock by males from the w^{1118} inducer strain was 32%. Thus, G1 females obtained in both experiments M7 and N1 differ only by their maternally inherited components: they have exactly the same genotype and, therefore, produce the same amount of reverse transcriptase for *trans*-complementation. 10457 G2 flies were observed in experiment N1, among which only one was [v$^+$]: this represents a frequency of transposition of 0.1×10^{-3}, which is 20-fold lower than the frequency observed in experiment M7. Therefore, as expected, *IviP2* is mobilized more efficiently in a strongly reactive background than in a weakly reactive background.

Analysis of transposition events: A total of 102 [v$^+$] flies were recovered in these experiments, 10 of which died before any further analysis. To detect at the molecular level the presence of a spliced copy of *IviP2*, about half of these flies were submitted to PCR analysis as described previously (Chaboissier *et al.* 1995). In all cases, the presence of a spliced copy could be detected, indicating that the observation of a [v$^+$] phenotype is likely to be the result of a retrotransposition event of *IviP2*.

Some of the recovered flies were [v$^+$; ry] and therefore did not contain the parental *IviP2* donor element: this excludes the possibility that splicing of the intron would be an artifact that occurred at the DNA level on the parental *IviP2* element. The flies that were [v$^+$; ry$^+$] were backcrossed to flies from the *misy* strain in order to check that [v$^+$; ry] flies could be recovered in the next generation, indicating that the spliced copy can be separated from the parental *IviP2* donor element. In only two cases was it impossible to separate the [v$^+$] and [ry$^+$] phenotypes, both determinants being localized on the *X* chromosome: in these two cases, the transposed copy of *IviP2* probably inserted very close to the parental *IviP2* element, but the possibility that the [v$^+$] phenotype could be the result of an event other than retrotransposition cannot be excluded.

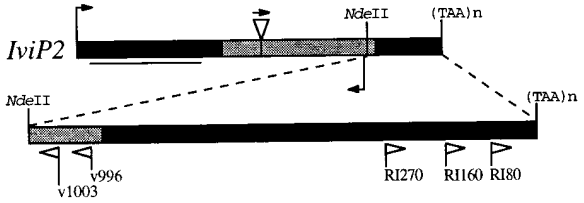


Figure 3.—Positions of primers used in inverse PCR and sequencing experiments. Legend is the same as in Figure 1. The position of the *Nde*II site relevant to this work is shown. Arrowheads indicate the orientations of the primers: RI270, RI160 and RI80 are from the sense strand with respect to *I* factor transcription, v1003 and v996 are from the antisense strand.

Most of the [v$^+$] flies recovered in this work were found independently, but some of them appeared in clusters of two or three in the progeny of the same parents. The experiments described below were conducted to study the 3′ ends and 3′ flanking sequences of the transposed copies of *IviP2*, and to determine whether the ones that appeared in clusters result from a single retrotransposition event or not. Inverse PCR experiments were performed on ligated *Nde*II-cut DNA from 72 single [v$^+$] flies using pairs of backward-oriented primers within the *vermilion* and the *I* factor sequences as shown in Figure 3. Amplification reactions were designed with an elongation time of 45 seconds, therefore, limiting the size of amplified fragments to around 1–1.5 kb. A single fragment of variable size (from 200 bp to 1.5 kb, depending on the couple of primers used) was amplified in 61 out of 72 cases. No amplification was detected in the 11 remaining cases, probably due to distance of the nearest *Nde*II site flanking the element. Sequences at the 3′ ends of some of the transposed copies of *IviP2* are shown in Figure 4. As expected, transposed copies terminate at their 3′ ends by a variable number of TAA repeats: the same number (five) as the donor element, or either less (four) or more (six to eight). Flanking sequences at the 3′ ends of the TAA repeats are all different from one another as well as from sequences that lie at the 3′ end of the donor *IviP2* element. This means that all transposed copies for which sequence information was available have inserted independently, even though some of them (for example, M3-12a and b, M4-10a and b, M7-7a and b) were recovered from the same parents. This corroborates previous observations suggesting that transposition of *I* elements is not a premeiotic event (Picard *et al.* 1978). Each of the sequences localized 3′ to the transposed copies was used to search in databases. None of them corresponds to any known sequences ex-

IviP2: **TCATAATAATAATAATAA**TTAATATGCAAATGTATTCTAAACAAGACTTACATTTATCGTGGCAAAGACGTTTTGAAAGGTCATGT
S1-3a: **TCATAATAATAATAATAATAA**TACAAATGCTTTTATAAATAAATAAATTTACTTAGCTATAAGCCAGTGTTTTTGTCATTAACATG
S1-4a: **TCATAATAATAATAATAATAATAA**TCTATCATACACCTCGGGCCTTACCATAAAGTAGAAATGAAATTTATTAAATGAATATGCAA
S3-3a: **TCATAATAATAATAATAATAATAA**TATAAGCCAGCACTCATGACAAACACGTACCACTCACGACTAAGTCCAATATTTGCACAACA
S4-6a: **TCATAATAATAATAATAATAATAA**TTAATTAATTAATGAAATAGAATATATAGCTATTTTCTATTTTCATTCGATTTCCCAATGTC
T2-3a: **TCATAATAATAATAATAATAA**TATTCTCTGTATAATCGGATTCTCCCGCTCTCCGCACGTTCCTCTCCGGGGAACTGGTTTTGGTT
T2-6a: **TCATAATAATAATAATAA**TTTATAAAAGGTAAGGAATAACAATGTTAATGATTCTTAACCTGCTGATAAACCTTATTTTATAATTT
T3-2a: **TCATAATAATAATAATAATAA**TATTTCAAGCAGGCATTGGTTTACCTGGTGACGTGGCAATTTCGATTTCGATTGCATTACCTGGC
T3-10a:**TCATAATAATAATAATAATAATAA**TACCAGTGGCTGCAGCTAGCGCAACATCTAAACCCAAACAATTTGCAGCATCTTGCCAAGAA
N1-5a: **TCATAATAATAATAA**TATATAGTTTGTTTTTTTTTGTTTTAAATAAATTCTTTCCTTCACTCCTGGAGACTACAAAAAATATTTATA
M1-3a: **TCATAATAATAATAATAATAA**TTTTCCAAGGTGCACCTTTCCGCAGTGGCTTTTTCTAGCGTCCAACGGCTCAGCTGTGGGCCATT
M1-19a:**TCATAATAATAATAATAATAATAATAA**AGTCTTCACAATCAAGCAAAGTGCCAATTACATTAGACGAGACGGCCCTACAGTCTTTT
M2-3a: **TCATAATAATAATAATAATAATAA**AATTAGAATATCTATCTTTTAGTCGAACGACACAATATCACACGGAATTATTGTATTTTTAG
M2-12a:**TCATAATAATAATAATAATAATAA**TATTAAGCATTTAGCGTAGATTTTGTATTAAGGCAAATCGTGAGTGTGGCTTGGTGCACTGT
M3-3a: **TCATAATAATAATAATAATAATAA**TGTTACAGCCAATTAGCCCATCGAAATAGTTGTGAAAATTATACTCTAGAAATTCACAAATA
M3-12a:**TCATAATAATAATAATAATAATAATAA**TTGTATGTCGTATTTAAGCCTTGATC
M3-12b:**TCATAATAATAATAATAATAA**ATATATGTATATATGTATATAAAGAGGACGTGCATAACTGGTCCTGAAGTGCATGGTGAAAATTC
M3-13a:**TCATAATAATAATAATAATAA**TATTCGATGGAAAGTGTATAGCTGGTAGAAGAAGCGTTTCCAACGCCCCATCCGTCCGTCTGTCC
M3-14a:**TCATAATAATAATAATAA**TATACCGTTAAAAATAATTTATTAAATTCGATTTTAAATTCTTCCAGGTCTAATTAATAATTAAATAA
M3-20a:**TCATAATAATAATAATAATAATAATAA**ATCATATGCAGTCGCACACCTTCCTGTTTCTTTTCGTTTTTTTTTTTATTTTTTTATTG
M4-3a: **TCATAATAATAATAATAATAATAA**ATTTGCAGTGGATTTTACCTTACGTCTGTGATTTCTACCAAATTTAAGTCTTGCTGCTGATA
M4-5a: **TCATAATAATAATAATAATAATAA**TTTTTATTATCGAATGTAATTGACAAAGTTGTTTGTTTAAAGAAAATATCCTTTTCTAG
M4-10a:**TCATAATAATAATAATAATAATAA**ATCGATTAGCTTCTGGATC
M4-10b:**TCATAATAATAATAATAATAA**TTGTTGCATCTATAAACGCAATATAAAAATCTTAAACAATGTTTTTTTTTTCGTTATCATACT
M4-11b:**TCATAATAATAATAATAATAA**TTTACCGACTTGCACAGTGAAATGAATAAATATTTGTAGTTTAATTACAAATTGAACGGGCG
M4-16a:**TCATAATAATAATAATAATAA**TAGTATTGCCTTCATAAGATC
M5-4b: **TCATAATAATAATAATAATAATAA**TATTTTTATCATAAGAGCGGCTCTCATTAAATTTAAGTCATTGTTCAAATGAATGGAAATTA
M5-9a: **TCATAATAATAATAATAATAATAA**TGGTAACTTAAAACTGAAATGTATTTAAAACACTTGTCGGTATTTTTTTCATGGCAAATGCG
M7-3a: **TCATAATAATAATAA**TATCATTTCAAAATGTATACGGAAAATGCAATTCCATTTGCATATTTGCCGTTTTATTATTGTTATTGAAT
M7-7a: **TCATAATAATAATAATAATAA**TCACTACCACTGCCACTGATGATGCCGTTCTGGCTGTTGATGTTGATATTGTGGTTATTATTATT
M7-7b: **TCATAATAATAATAATAATAATAA**GAATGTTACCTATTTAACAATTTACACATCCTCTTCTTTCAGGTACTGAATGTACTGCATCA

Figure 4.—Sequences at the 3′ ends of some transposed copies of *IviP2*. Sequences from the 3′ end of the *I* factor are indicated in bold, including all TAA repeats. Transposed copies are named as "E-Xn" where E represents the name of the experiment (see Tables 1 and 2), X represents the sample of parents from which they were recovered and n (a or b) is the order of recovery when more than one copy were found in the progeny of the same parents.

cept those flanking T2-6a that are identical to sequences present in the vicinity of the gene *phyllopod* (Chang *et al.* 1995).

DISCUSSION

Use of the *hsp70* promoter to drive ORF2 expression: The *IviP2* element is likely to behave as an active *I* factor regarding RNA synthesis from the internal promoter of the *I* factor and production of the protein encoded by ORF1, but it cannot produce the protein normally encoded by ORF2. Therefore, this protein has to be provided in *trans* at the right place and the right time to allow retrotransposition of the *IviP2* element to occur. In the experiments described here, the *hsp70* promoter was used to drive ORF2 expression. This promoter is heat-inducible in nurse cells from early stages to stage 9 of oogenesis (Zimmerman *et al.* 1983; Lis *et al.* 1983; Bonner *et al.* 1984), which overlaps with the pattern of *I* factor expression (Lachaume *et al.* 1992; Udomkit *et al.* 1996; M. C. Seleme and D. Teninges, unpublished data). As shown by experiment M64, *trans*-complementation of *IviP2* can occur, at low frequency, in the absence of heat shock, indicating that some product of ORF2 is synthesized probably because of a weak basal activity of the *hsp70* promoter at 23° in ovaries (Zimmerman *et al.* 1983; Kurtz *et al.* 1986), but is tenfold more efficient upon heat induction. This order of magnitude agrees with values obtained in other studies of reporter sequences whose transcription is driven by the *hsp70* promoter (Klemenz *et al.* 1985; Hunt *et al.* 1992). Therefore, the rate of transposition of *IviP2* in the experiments described here depends upon the quantity of reverse transcriptase (the product of ORF2) available for *trans*-complementation, and also depends upon the quantity of the transposition intermediary, *i.e.*, the RNA synthesized from the *I* factor promoter in the *IviP2* element: this is indicated by the observation that mobilization of *IviP2* is more efficient in a strongly reactive background, allowing high activity of the *I* factor promoter (Lachaume and Pinon 1993), than in a weakly reactive background.

Trans-complementation of *IviP2* is more efficient with *hsORF2HN* than it is with *hsORF2HR*. This might appear surprising if one assumes that the reverse transcriptase recognizes some specific sequences at the 3′ end of the *I* factor RNA: then one would expect that the 3′ end of *I* present in *hsORF2HN RNA*s would compete with the 3′ end of *I* present in *IviP2 RNA*s. However, such *cis*-required sequences have not been identified so far. Possibly, the difference between *hsORF2HN* and *hsORF2HR* could be a result of RNA stability. *hsp70* termination sequences are known to activate rapid RNA degradation after heat-shock (Petersen and Lindquist 1988–1989): RNAs synthesized from *hsORF2HR*, terminating with these sequences, might be less stable than RNAs synthesized from *hsORF2HN* that presumably terminate at the 3′ end of *I*, but this has to be checked.

Use of marked *I* elements to study retrotransposition: The use of marked *I* elements is crucial to study most aspects of the retrotransposition process. Several marked elements have been described in the past (Pélisson *et al.* 1991; Jensen and Heidmann 1991; Jensen et al. 1994; Chaboissier *et al.* 1995), but none could be mobilized at frequencies high enough to be useful for this purpose. Transposition of the *IviP2* element is up to 20 times more efficient when the product of ORF2 is provided in *trans* by a construct in which this ORF is transcribed from the *hsp70* promoter, after heat-shock induction, than when it is supplied by transposing *I* factors in the germline of SF females. The *vermilion* retrotransposition indicator provides a powerful and easy way to screen for retrotransposition events simply by observing flies. Transposed copies can easily be analyzed at the molecular level, with the use of probes homologous to sequences of the *vermilion* gene, or more rapidly by inverse PCR as illustrated in the present work. This will allow one to obtain unbiased information on insertion sites generally used by the *I* factor; most insertion sites identified so far were selected on the basis of mutant phenotypes and were therefore in genes, which may not represent preferential targets of *I* factors (Bucheton 1990).

Even in the best experimental conditions presented here, transposition of *IviP2* still occurs at low frequencies compared to transposition of autonomous wild-type *I* factors. This is consistent with the *cis*-mechanism of LINE retrotransposition recently proposed by Boeke (1997), suggesting that the reverse transcriptase would preferentially mobilize the RNA molecule that encoded it. It is possible, however, that other marked *I* elements with the *vermilion* or another reporter gene inserted at different locations (within ORF1 or outside any ORF) would transpose more efficiently. However, retrotransposition of a complete *I* factor into which a reporter gene was inserted within the 3′ UTR, outside from any coding sequences, was found to be inefficient (Jensen and Heidmann 1991).

Use of marked *I* elements for mutagenesis: The use of transposable elements as mutagens is useful because it provides an easy way to clone the mutated gene. In *Drosophila melanogaster*, *P* element mutagenesis has become a routine technique of invaluable help in developmental studies. A limitation of the use of *P* elements is that presumably one third of Drosophila genes cannot be mutated this way (Engels 1989). The use of transposable elements other than *P* offers the hope that they might have other target site preferences. Recently, Dimitri *et al.* (1997) have shown that high mutation rates of genes located in heterochromatin are produced during *I*-R hybrid dysgenesis, suggesting that *I* factors transpose frequently in these regions. A mutagenesis system based on marked *I* elements could be

particularly useful to clone these genes. The work presented here is a promising first step toward this goal. The rate of transposition of *IviP2* in the best conditions is still ten times less than the rate of transposition of defective marked *P* elements mobilized in *trans* by the transposase under the control of the *hsp70* promoter, which is usually around 1–5% (Engels 1989). Other marked *I* elements might be more efficient in transposition and provide powerful new tools for Drosophila mutagenesis.

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