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Characterization of *MR* (P) strains of *Drosophila melanogaster*: the number of intact P elements and their genetic effect

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Summary

To study the effect of mutagenic/carcinogenic agents on P-element transposition, the P strains used should be defined, especially with respect to the number of intact and functional P elements present. In this investigation, the relation between the number of complete P elements present in dysgenic males and P-insertion mutagenesis was studied in several *MR* (P) strains. The main conclusions from this investigation are: (1) Complete P elements can be present in the genome without genetic activity (even in a 'dysgenic' cross). As a consequence, the number of complete P elements present in particular dysgenic flies, is not necessarily an indication of their dysgenic genetic activity. (2) The *MR-h12/Cy* strain carries two complete P elements, one on the X chromosome without and one on the *MR* chromosome with genetic activity (making this strain most suitable for studies on P-transposition mechanisms).

1. Introduction

Studies involved in the elucidation of the mechanisms of chemically and physically induced mutagenesis/carcinogenesis often use the occurrence of spontaneous mutational events as a convenient frame of reference. In *Drosophila*, where it is evident that many of these spontaneous events are the result of transposition of mobile elements, it seems obvious to investigate the effects that mutagenic/carcinogenic treatments have on the transposition of these elements. One class of mobile elements, the P elements, appear especially useful in this respect, since their transposition can, to a certain degree, be manipulated. However, in order to study interactions of mutagenic/carcinogenic treatments, the P strains used should be defined, especially with respect to the number of intact and functional P elements.

The transposition of P elements is particularly high in specific crosses (P–M hybrid dysgenesis, for reviews see Bregliano & Kidwell, 1983; Engels, 1983, 1989). P elements are heterogeneous, both in their molecular structure and in their properties. Intact elements are 2·9 kb long and are able to transpose themselves and other P elements; other elements lack internal sequences and, although they can be transposed by an intact element, are unable to induce their own transposition (O'Hare & Rubin, 1983; Spradling & Rubin, 1982; Rubin & Spradling, 1982). When males carrying intact P elements (P-strain males) are crossed to females lacking these elements (M-strain females), the P elements transpose at a high frequency in the F_1 (dysgenic) progeny, resulting in a number of traits including a high frequency of mutations. In the reciprocal cross transposition is suppressed, suppression involving cytoplasmic factors present in P strains, a cellular condition called P cytotype. The regulation of P-element transposition, mediated by cytoplasmic factors, could be due to a P-element encoded regulator (O'Hare & Rubin, 1983; Laski *et al.* 1986; Rio *et al.* 1986; Black *et al.* 1987, 1988) or by transposase titration as proposed by Simmons & Bucholz (1985).

Genetic studies showed that the relative magnitude of dysgenic traits (mostly the temperature-dependent female sterility) is a function of the particular P strain involved (Bregliano *et al.* 1980; Bregliano & Kidwell, 1983; Engels, 1983, 1989). However, in no case has the exact number, type and distribution of P elements in the various P strains been studied in relation to mutation induction and reversion capacity.

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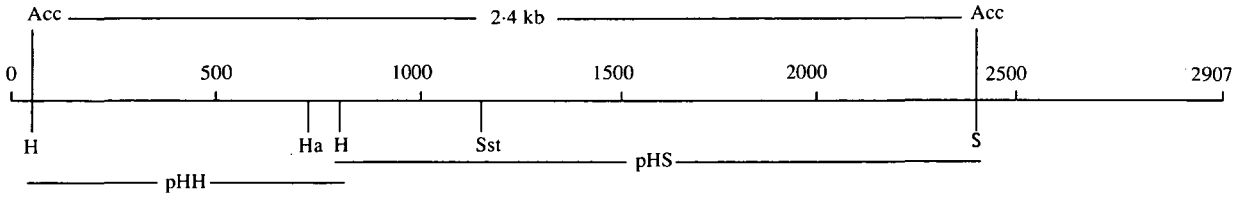

Fig. 1. Physical map of pπ25.1 according to O'Hare & Rubin (1983). Indicated are pHS used to recognize full length P elements. Some restriction sites are indicated: *Eco*R I (E), *Sal* I (S), *Hin*d III (H), *Sst* I (Sst) and *Hae* III (Ha).

In this investigation several *MR* (*Male Recombination*) strains were studied. Each of these strains contains a second chromosome isolated from flies from natural populations (balanced over inversions) in a genetic background derived from laboratory stocks. In addition to recombination in males, the *MR* chromosomes induce high frequencies of P-insertion mutations (Eeken *et al.* 1985), showing that *MR* strains behave as genuine P strains with active P elements located on the *MR* chromosome. The number of intact P elements of several *MR* strains was determined. In addition their ability to induce sex-linked recessive lethal and visible mutations and to revert unstable, P-insertion, visible mutations was measured. The *MR* strains used are well characterized (Green, 1977, 1978; Yannopoulos, 1978) and a comparison with respect to their ability to induce and suppress female sterility has been reported (Yannopoulos *et al.* 1986).

2. Materials and methods

(i) Drosophila *strains*

The *MR* strains used in this study are: *MR-h12/Cy*, *MR-n1*, *MR-GB39/Cy*, *MR-T007/Cy* and *MRF-31.1/CyL*^{4}. All refer to isolated second chromosomes of natural wild-type *Drosophila melanogaster* populations. The first three were isolated by Dr M. M. Green; *MR-h12* from a population in Israel and *MR-n1* and *MR-GB39* from wineries in California (USA). *MR-T007* also obtained from Dr M. M. Green, was originally isolated from a Texas (USA) population by Dr Y. Hiraizumi (1971). *MRF-31.1* was isolated in Greece by Dr G. Yannopoulos (Yannopoulos & Pelecanos, 1977); *MRF-31.1* is kept in stock balanced over a *CyL*^{4} chromosome that in several tests shows nearly as much activity as the *MRF-31.1* chromosome itself. All other stocks are balanced over *Cy* inversions, with the exception of *MR-n1* that is kept as a homozygous stock. The main factor in *MR-h12* and *MR-T007*, responsible for male recombination, has been localized genetically close to the centromere on the left arm of the chromosome, between *Tft* (tuft bristles, 53·2) and *pr* (purple eye colour, 54·4) (Slatko & Green, 1980).

(ii) *Genetic characterization*

The ability of the *MR* chromosomes to induce sex-linked recessive lethals (SLRL) was determined in F_1, *MR*-carrying, males (progeny of crosses between wild-type *Berlin K* females, P-element free, and males from the various *MR* strains). Standard SLRL test procedures were followed using *Basc* females (Würgler *et al.* 1977).

Sex-chromosome-linked visible mutations were scored in the offspring of F_1, *MR*-carrying, males from the same parental cross as described above, after crossing them to *C(1)DX, y f* females. Several stocks were established of unstable *yellow*, *singed* or *raspberry* mutations induced by the various *MR* strains. Following the induction, the second and third chromosome were replaced by chromosomes from a laboratory stock marked with *dumpy* and *ebony*. All these mutations appear stable in these stocks, indicating the absence of intact P elements (see Eeken, 1982).

The ability of the *MR* chromosomes to revert these visible mutations was determined in F_1, *MR*-carrying, males, progeny of a cross between females homozygous for a particular *MR*-induced visible mutation and males from the MR strains. The reversions were detected after crossing these F_1 males to *C(1)DX, y f* females. All crosses were maintained at 25 °C unless stated otherwise.

(iii) *DNA isolation/blot hybridization*

DNA from the *MR* strains was isolated essentially as described by McGinnis *et al.* (1983) and purified by CsCl gradient centrifugation. Plasmid DNA was isolated according to Maniatis *et al.* (1982). Genomic DNA was digested with *Acc* I and *Sal* I and electrophoresed on 0·8% agarose gels in Tris-borate buffer (Maniatis *et al.* 1982). Transfer of DNA to Gene Screen Plus and filter hybridizations were carried out essentially as described by the manufacturers (NEN Research Products). Blot hybridization was performed using the inner sequences of the intact P element (pHS, the *Hin*d III–*Sal* I fragment of pπ25.1, kindly provided by Dr G. Rubin; see Fig. 1). The copy number of the *Acc* I fragments (2·4 kb) of the P elements present in the various *MR* strains was determined using densitometric data of the autoradiographs.

(iv) *Cloning procedure*

P elements were cloned as follows: genomic DNA of the strains *MR-h12/Cy* and *MR-T007/Cy* was isolated and digested with *Bgl* II. A preparative agarose

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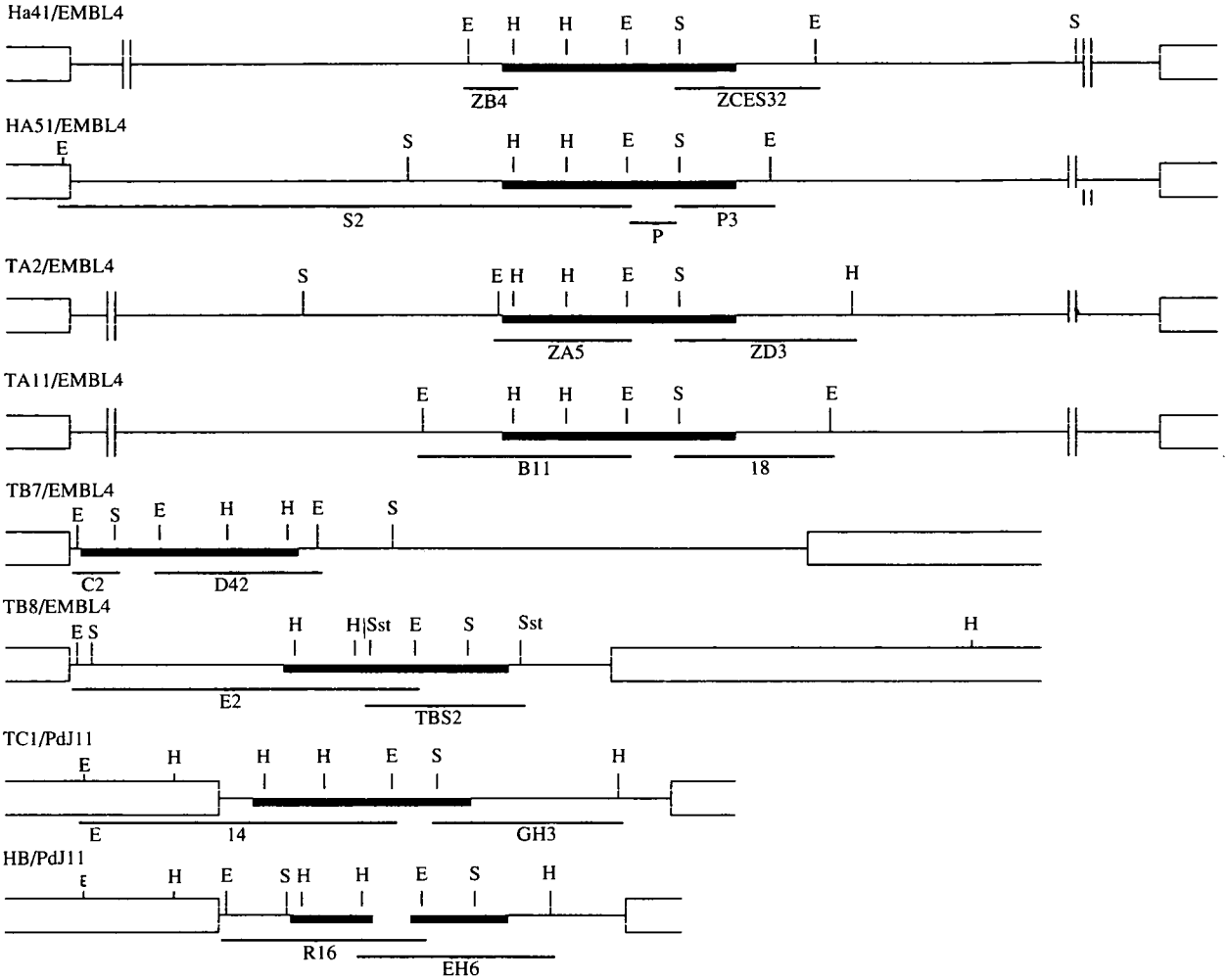

Fig. 2. Physical map of the cloned P elements from the *MR* strains *MR-h12/Cy* and *MR-T007/Cy*. Some restriction sites are shown: *Eco*R I (E), *Sal* I (S), *Hin*d III (H) and *Sst* I (Sst). Underneath each element the subcloned fragments are indicated that were used for DNA sequencing.

gel was loaded with approximately 2 μg of DNA. After separation, part of the gels were blotted and hybridized with pHS as probe. DNA from *MR-h12/Cy* shows two bands of hybridization with sizes of approximately 16 and 4 kb. DNA from *MR-T007/Cy* shows three bands of hybridization, at fragment size of 23, 9 and 6 kb (data not shown). Bands showing hybridization were excised and the DNA was electro-eluted. Depending on the size of the *Bgl* II fragments eluted, this DNA was ligated into the lambda vectors EMBL4 or PdJ11, a derivative of L47.1 (A^{am} B^{am}; P. de Jong, unpublished), cleaved with *Bam*H I. EMBL4- and PdJ11-ligated DNA was packaged *in vitro* and used to infect *E. coli* NM538 (Frischauf *et al.* 1983) and LE392 (Maniatis *et al.* 1982) respectively. Hybrid phages containing P elements were identified by plaque hybridization with pHS as probe as described by Benton & Davis (1977). From *MR-T007/Cy* a total of 25 positive clones were found, 20 of which were tested. These 20 clones contained several copies of 5 different P elements (TA2, TA11, TB7, TB8, TC1). From *MR-h12/Cy* a total of 5 from 14 positive clones were tested. These contained copies of 3 different P elements (HA41, HA51, HB). A simple restriction map of the cloned P elements is given in Fig. 2.

(v) *S1 protection experiments*

To determine small deletions or duplications in the cloned P elements, S1-nuclease protection experiments were performed. As protection fragments, two fragments were subloned from pπwc (O'Hare & Rubin, 1983) into M13mp10. The first fragment contains the left end of P from restriction site *Sst* I (1150) to a *Bam*H I site in the flanking sequence of pπwc, M13mp10–*Bam/Sst*. The second fragment, a *Hae* III fragment from pπwc, includes the right end of P element wc from restriction site *Hae* III (724) to the *Sal* I site at 2882 and some pBR322 sequences. The ends of the *Hae* III fragment were end-filled and cloned into the *Sma* I site of M13mp10, M13mp10–*Hae*. Twenty ng hybrid-phage DNA containing the P elements, non digested, was mixed with 50 ng of M13mp10–*Bam/Sst* (and M13mp10–*Hae*) in 24 μl 10 mM Tris-HCl, pH 7·5; 1 mM-EDTA. The mixture was heated for 5 min at 95 °C and chilled on ice; 1 M-NaCl was added to the final concentration of 0·2 M

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Table 1. *The induction of SLRL mutations by* MR *strains*

	Sex-linked recessive lethal mutations		
MR strain	Chromosomes (*n*)	Lethal (*n*)	% lethal
T-007	1889	52	2·7 ± 0·4
MRF-31.1	3182	51	1·6 ± 0·2
CyL^4	3113	38	1·2 ± 0·2
n1	2529	38	1·5 ± 0·2
GB39	2461	26	1·1 ± 0·2
h-12[a]	6072	69	1·1 ± 0·1
Cy(h-12)[b]	6342	9	0·1 ± 0·1

[a] See Sobels & Eeken (1981).
[b] Pooled data of several Cy controls from various experiments (Eeken, unpublished).

and the samples were incubated for 1·5 h at 37 °C. After chilling, 300 μl nuclease S1 buffer (Maniatis *et al.* 1982) containing 1000 U/ml nuclease S1 was added. Digestions were carried out for 1 h at 37 °C. S1 protection products were analysed by blot hybridization after electrophoresis on 1·5% agarose gels (Maniatis *et al.* 1982). Using this method we were able to detect deletions as small as 6 bp (Pastink *et al.* 1990).

(vi) *Sequencing*

The ends of all the cloned P elements including the inverted repeats and part of the flanking DNA have been sequenced. Appropriate fragments (indicated in Fig. 2), containing flanking sequences, that were smaller than 3 kb were subcloned in M13mp18 and M13mp19 (Messing & Vieira, 1982) for single-strand sequencing. In some cases (HA51, TB8, TC1) only a larger fragment containing the flanking sequence was readily available and in those cases these larger fragments were subcloned in pUC18 and a double-strand DNA-sequencing procedure was applied. In one case, ZB4 (left end of HA41), a universal M13 primer was used, whereas in all other cases primers were used, that were manufactured, based on the known internal P sequence: PL (left) 5′CCTCTCAACAAGCAAACG-3′(5′[90]-3′[73])[2] and PR (right) 5′-TACGACACTCAGAATACT-3′(5′[2807]3′[2824]). Sequencing was carried out using the dideoxy chain termination method (Sanger *et al.* 1977).

(vii) In situ *hybridization*

In situ hybridizations were performed according to Engels *et al.* (1986) using biotin-labelled probes and alkaline phosphatase (BluGENE non-radioactive nucleic acid detection system from BRL).

3. Results

(i) *Induction of sex-linked recessive lethal (SLRL) mutations*

The ability of the *MR* chromosomes *MR-T007*, *MRF-31.1* and its homologue *CyL*^{4}, *MR-n1*, *MR-GB39*, *MR-h12* and the homologue of *MR-h12*, *Cy(h12)* to induce SLRL mutations was determined (Table 1). Although the induction frequency by *MR-T007* is significantly higher (2·7%) than those by *MRF-31.1* and *MR-n1* (1·6 and 1·5%) and of *CyL*^{4}, *MR-GB39* and *MR-h12* (1·2, 1·1 and 1·1% respectively), the differences between the *MR* chromosomes are relatively small. The *Cy*-brothers of the *MR-h12* F_1 males tested only show normal spontaneous background levels of induction. The SLRL frequencies observed are not unlike those reported on P-mutation induction by other investigators; they range from 0·5–2·3% for several *MR-T* (Texas) chromosomes (Slatko & Hiraizumi, 1973), 1·4 and 3·0% for v^6 (Q strain) and π_2 (Simmons *et al.* 1980, 1984), 2·5% for both π_2 and *Harwich* (Zusman *et al.* 1985) and 1·8% for *Cranston* (Kidwell *et al.* 1977). The results with the *Cy*-homologue indicate the absence of active P elements on this and other autosomal chromosomes of the *MR* strains.

(ii) *Induction of sex-linked visible mutations*

Sex-linked visible mutations [predominantly at the *yellow* (*y*), *singed* (*sn*) and *raspberry* (*ras*) loci] were

Table 2. *The induction of X-linked visible mutations by* MR *strains*

		Sex-linked visible mutations at the loci					
MR strain	Chromosomes tested (*n*)	*yellow*	*white*	*singed*	*raspberry*	*miniature*	Frequency/10^4 single events
T-007	8117	0	0	2 × 1[a]	2 × 1	0	5
MRF-31.1	7403	1	0	8 × 1	0	1	14
CyL^4	5151	1 × 5[b]	0	4 × 1, 2 × 2	2 × 1	0	19
n1	8452	1 × 9	0	2 × 1	2 × 1	0	6
GB39	14295	1	1	1	1 × 1, 1 × 5	0	3
h12	40930	0	0	15 × 1, 2 × 3	6 × 1	0	6

[a] 2 × 1, two single events.
[b] 1 × 5, one cluster of 5 mutations.

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Table 3. *The reversion of unstable mutations by* MR *strains at 25 °C* (%)

		T007		*31.1*		*CyL*^{4}		*n1*	*GB39*	*h12*	
n1	*sn 46*	6/40	(15)	2/10	(20)	90/411	(20)	122/1501 (8·1)	76/1652 (4·6)	60/3394	(1·8)
	y 25.2	43/174	(25)	35/170	(20)	44/603	(7·3)	113/3628 (3·1)	127/3422 (3·7)	10/2061	(0·5)
	sn 30	35/472	(7·4)	2/35	(6)	27/361	(7·5)	110/3789 (2·9)	64/2414 (2·7)	17/2933	(0·6)
h12	*sn 17A*	134/1147	(11)	44/326	(13)	69/829	(8·3)	113/2807 (4)	158/4973 (3·2)	16/1944	(0·8)
	sn 39B1	56/670	(8·4)	21/188	(11)	44/514	(8·5)	157/3386 (4·6)	162/4148 (3·9)	179/12664	(1·4)
	sn 25A	61/1768	(3·5)	41/568	(7)	41/1167	(3.5)	111/3099 (3·6)	44/3737 (1·2)	14/3332	(0·4)
T007	*sn 42 GY*	35/643	(5)	105/3763	(2·8)	21/995	(2·1)	n.t.[a]	n.t.	8/3317	(0·2)
	sn 18 GY	3/790	(0·4)	9/686	(1·3)	11/1059	(1·0)	n.t.	n.t.	0/2657	(0)
	sn 50s	sterile		0/78	(0)	3/31	(10)	20/3969 (0·5)	4/3470 (0·1)	1/5532	(0·02)
GB39	*ras 44.2*	1/50	(2)	3/24	(12)	27/483	(5·6)	48/2790 (1·7)	23/3175 (0·7)	1/3461	(0·02)
	ras 44s2	1/154	(0·7)	2/16	(12)	37/1288	(2·9)	59/3036 (1·9)	23/3073 (0·7)	6/3749	(0·2)
	ras 44s1	0/83	(0)	2/27	(7·5)	16/677	(2·4)	50/4169 (1·2)	35/3530 (1·0)	5/5379	(0·1)
F-31.1	*sn 22A*	27/1145	(2·4)	21/598	(3·5)	14/1937	(0·7)	10/8938 (0·1)	8/4712 (0·2)	1/4655	(0·02)
	y 42B	17/4396	(0·4)	8/1271	(0·6)	22/5380	(0·4)	0/4973 (0)	4/5721 (0·07)	0/1242	(0)
	sn 4B	6/6875	(0·1)	1/6675	(0·01)	0/4822	(0)	0/9496 (0)	0/9856 (0)	1/5755	(0·02)
CyL^4	*ras 44A*	4/2088	(0·2)	0/187	(0)	8/1329	(0·6)	0/8315 (0)	28/6555 (0·4)	44/4711	(0·9)
	sn 9A	sterile		1/26	(4)	n.t.		2/4597 (0·04)	2/3910 (0·05)	4/5051	(0·1)
	y 46A	0/103	(0)	0/677	(0)	0/2730	(0)	0/6069 (0)	0/5695 (0)	1/2916	(0·03)

[a] n.t., not tested.

Table 4. *Male sterility induced by* MR *strains at 25 °C* (%)

		T007	*F31.1*	*CyL*^{4}	*n1*	*GB39*	*h12*
n1	*sn 46*	97	95	60	12	19	11
	y 25.2	80	79	60	3	6	55
	sn 30	49	87	86	0	6	29
h12	*sn 17A*	9	40	26	0	0	32
	sn 39B1	33	72	52	11	1	n.t.
	sn 25A	7	60	42	1	0	15
T007	*sn 42 GY*	n.t.[a]	n.t.	n.t.	n.t.	n.t.	n.t.
	sn 18 GY	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	sn 50s	100	95	89	0	17	16
GB39	*ras 44.2*	91	91	71	2	9	4
	ras 44s2	91	92	58	9	7	41
	ras 44s1	99	92	70	1	4	0
F-31.1	*sn 22A*	53	17	39	1	4	13
	y 42B	8	7	3	3	4	20
	sn 4B	12	17	16	4	4	28
CyL^4	*ras 44A*	19	70	54	1	1	6
	sn 9A	100	78	n.t.	4	14	11
	y 46A	89	73	48	6	1	30

[a] n.t., not tested.

induced with all the different *MR* chromosomes used in this study (Table 2). All these mutations were induced in the P-element-free X chromosome of a laboratory wild-type stock, *Berlin K* (also used in earlier investigations, Eeken *et al.* 1985). All *MR* chromosomes are capable of inducing *sn* mutations; 5 of the 6 *MR* chromosomes induced *ras* mutations and only 4 induced *y* mutations. The number of tested chromosomes in these experiments is limited since the objective was only to obtain a small number of visible mutations induced by each of the different *MR* chromosomes. These were then used in the reversion experiments described in the next section. The general distribution of mutations over the loci *sn* (36 independent events), *ras* (14) and *y* (4) is not different from that observed in previous experiments (Green, 1977, 1978). The induction frequencies given in Table 2, based on all the visible mutations recovered in these experiments, can only be regarded as approximate. However, it is obvious that only minor differences exist in the ability of these *MR* chromosomes to induce sex-linked visible mutations. All visible mutations obtained were recovered, brought into stock, and the second and third chromosomes in these stocks were replaced. In the established stocks all the mutations are stable.

(iii) *The ability of the* MR *chromosomes to revert unstable mutations*

In the following experiments, visible X-linked mutations recovered as described in the previous section,

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Table 5. *Male sterility induced by* MRF31.1 *and* CyL^4 *at 25 °C and at 17 °C in combination with reversion frequencies at these temperatures* (%)

		Sterility at 25 °C		Sterility at 17 °C		Reversion at 25 °C		Reversion at 17 °C	
		MRF-31.1	*CyL^4*	*MRF-31.1*	*CyL^4*	*MRF-31.1*	*CyL^4*	*MRF-31.1*	*CyL^4*
n1	*sn 46*	95	60	81	34	20	20	4·6	7·5
	y 25.2	79	60	6	7	20	7·3	3·2	4·4
	sn 30	87	86	4	4	6	7·5	4·5	2·4
h12	*sn 17A*	40	26	3	10	13	8·3	6	3·6
	sn 25A	60	42	8	13	7	3·5	2·7	1·4
T007	*sn 50s*	95	89	7	4	0	10	0·5	0·3
GB39	*ras 44.2*	91	71	1	1	12	5·6	0·8	0·5
	ras 44s2	92	58	0	1	12	2·9	2·8	0·1
31.1	*sn 22A*	17	39	17	12	3·5	0·7	0·4	0·6
	y 42B	7	3	5	25	0·6	0·4	0·1	0·03
	sn 4B	17	16	4	7	0·01	0	0·02	0·07
CyL^4	*ras 44A*	70	54	20	20	0	0·6	0·9	0·4
	y 46A	73	48	12	10	0	0	0	0·04

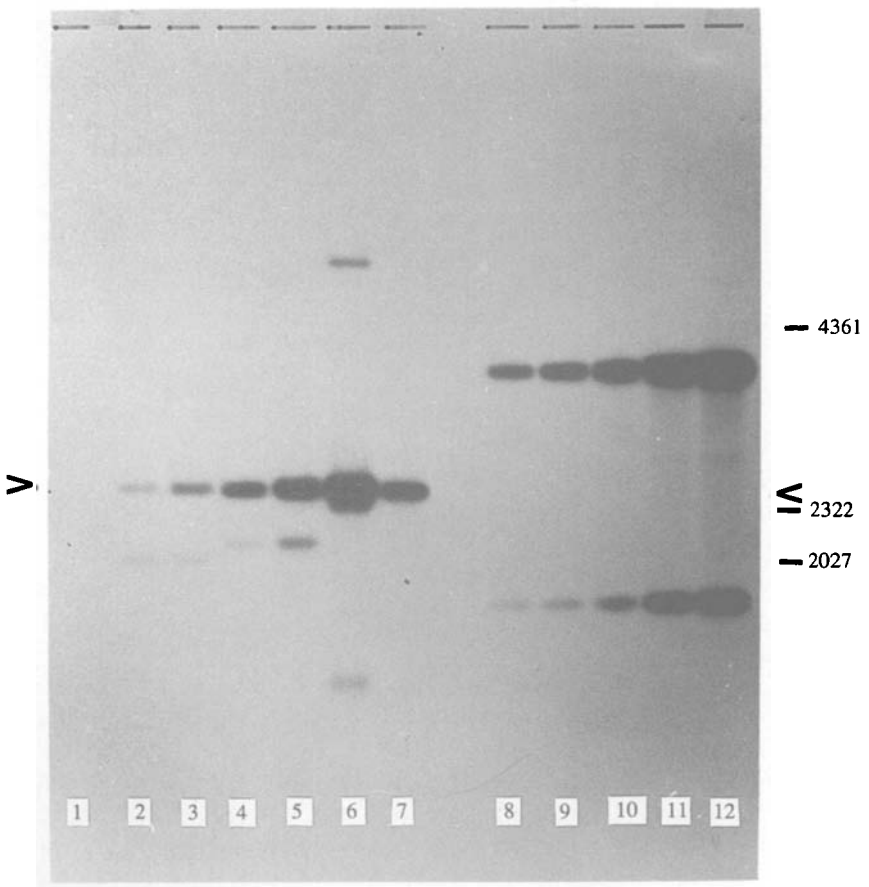

Fig. 3. *Acc* I digest of total genomic DNA of the *MR* strains: *MR-13-1/Cy, MR-h12/Cy, MR-GB39/Cy, MR-n1, MRF-31.1/CyL^4* and *MR-T007/Cy* (lanes 2–7, respectively) and a P-free laboratory strain *Berlin K* (lane 1). Lanes 8–12 contain genomic equivalents of respectively 1·5, 3, 6, 15 and 30 copies of *Acc* I digested pHS. Probe pHS.

were tested to examine whether all the *MR* chromosomes were able to revert these mutations and to ascertain the relative abilities of the *MR* chromosomes to do so. Females homozygous for a given, *MR*-induced, visible mutation (*y*, *sn* or *ras*), were mated to males of the *MR* strains. The F_1 males carrying the *MR* chromosome were selected. For *MR-n1*, kept as a homozygous stock, this implies that all F_1 males could be used. In case of *MRF-31.1/CyL^4*, the *MRF-31.1* as well as the *CyL^4* chromosomes were tested separately. On average 80 F_1 males were subsequently crossed individually to 2–4 *C(1)DX* females and the F_2 male offspring scored for reversion of the mutation to wild type. The results are given in Table 3. The *MR* chromosome that induced a particular mutation is indicated in the first column, followed by the column

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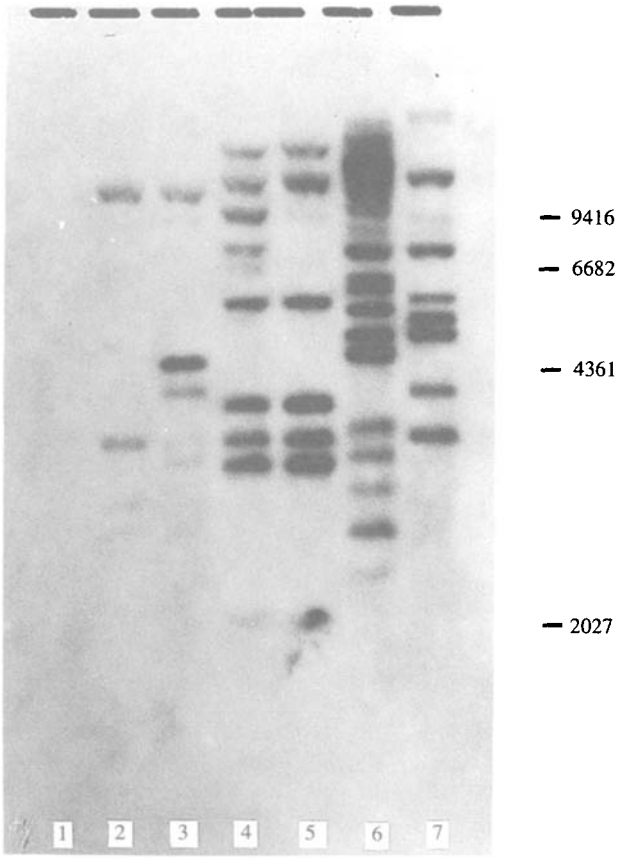

Fig. 4. *Sal* I digest of total genomic DNA of the *MR* strains: *MR-13-1/Cy, MR-h12/Cy, MR-GB39/Cy, MR-n1, MRF-31.1/CyL⁴* and *MR-T007/Cy* (lanes 2–7, respectively) and a P-free laboratory strain *Berlin K* (lane 1). Probe pHS.

indicating the particular visible mutation used in the test. The top line indicates the *MR* chromosome used to revert the mutations. All reversions are included regardless whether they arose in the same male or not.

With respect to the capacity of the various *MR* chromosomes to revert the tested visible mutations it is clear that *MR-T007, MRF-31.1* and the homologue of the *MRF-31.1* chromosome, *CyL⁴*, are very powerful. The capacity of *MR-n1* and *MR-GB39* is moderate whereas *MR-h12* is relatively weak. A rank order test indicates that there are no significant differences between: (1) *MR-T007, MRF-31.1* and the homologue of the *MRF-31.1* chromosome, *CyL⁴* and (2) *MR-n1* and *MR-GB39*. On the other hand there are highly significant differences between the three groups. The range of reversion frequencies for specific visible mutations was at least a factor of about 10 (*sn 46, sn 30, sn 17A*), but can be as much as a factor 100 (*ras 44.2, ras 44sl, sn 22A*). If it is assumed that these ranges reflect the number of intact P elements in the tested *MR* chromosomes, it may be hypothesized that *MR-h12* carries one intact P element, *MR-GB39* and *MR-n1* possibly 4–7 and *MR-T007, MRF-31.1* and *CyL⁴*, about 8–12.

It appears noteworthy that, in general, visible mutations induced by *MR-n1* and *MR-h12* can be reverted easily, whereas, on the other hand mutations induced by *MRF-31.1* and its homologue, *CyL⁴*, revert only at very low frequencies. It remains to be tested whether the type of P insert causing the mutation is dependent on the particular inducing *MR* chromosome.

In all these tests, the *MR*-carrying males were raised at 25 °C. This resulted in male sterility in a number of cases, especially for the *MR* chromosomes *MR-T007, MR-F31.1* and *CyL⁴*. Although no specific test for male sterility was performed, a crude measure was obtained by the number of males that proved totally sterile in the reversion tests (Table 4). The ability to induce male sterility is weakest for the *MR* chromosomes *MR-n1* and *MR-GB39*. Obviously there is no correlation between the ability to induce sterility in males and the ability to induce reversions. The fact that the induction of sterility is minimal by *MR-n1* and *MR-GB39* indicates that the observed male sterility in other cases is not due to activity of the X chromosomes themselves.

To circumvent induced male sterility, the *MR*-carrying males were raised at a lower temperature (17–21 °C). An interesting question is whether the lower temperature that affect male sterility considerably also changes the frequencies of reversion. Table 5 shows male sterility data and reversion frequencies of a number of tested visible mutations in combination with the *MR* chromosomes *MRF-31.1* and its homologue *CyL⁴* at 25 °C (see also Table 3) and at 17–21 °C. It is clear that lowering the temperature decreases the sterility by a factor of 2–10 (or more). The same appears to be true for the reversion frequencies.

(iv) *Determination of intact P elements in* MR *strains*

To estimate the number of intact P elements in the various *MR* strains, we measured the copy number in these strains of the 2·4 kb *Acc* I fragment (see Fig. 1) of the P element. The results are shown in Fig. 3. The first 7 lanes contain *Acc* I-digested genomic DNA of a control (*Berlin K*) and several *MR* strains as indicated in the legend (*MR-13-1* is a re-isolated *MR-h12* chromosome). The *Berlin K* strain clearly is devoid of P elements. It is obvious that although several smaller *Acc* I fragments can be detected, the hybridization of pHS with the *MR* strains is predominantly with *Acc* I fragments of 2·4 kb. Rehybridization with pHH (Fig. 1) indicated the presence of several deleted P elements in addition to the complete elements (data not shown). The number of 2·4 kb *Acc* I fragments from each strain was determined by densitometric scanning of the autoradiograph. Different genomic equivalents of *Acc* I-digested pHS were used as a reference (Fig. 3, lanes 8–12). The *MR-T007/Cy, MRF-31.1/CyL⁴, MR-n1, MR-GB39/Cy* and *MR-h12/Cy* strains contain respectively 7, over 16, 11, 6 and 2 copies of the 2·4 kb fragment. Since upon outcrossing males from

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Table 6. *The reversion of unstable mutations by the* Cy *homologue of* MR *strains at 25 °C*

(total reversions/number of F_2 male progeny scored)

		T007	*Cy-T007*	*GB-39*	*Cy-GB39*	*h12*	*Cy-h12*
h12	*ras 5B2*[a]	n.t.	n.t.	n.t.	n.t.	8/2433	0/14471
	sn 39B1[a]	n.t.	n.t.	n.t.	n.t.	204/12664	4/1357
h12	*sn 17A*[b]	134/1147		158/4973			
	sn 25A[b]	61/1768		44/3737			
h12	*sn 17A*	174/1805	0/4217	136/2832	3/4119		
	sn 25A	59/1551	0/5289	45/2495	1/3636		

[a] Data taken from Eeken (1982).
[b] See Table 4b.

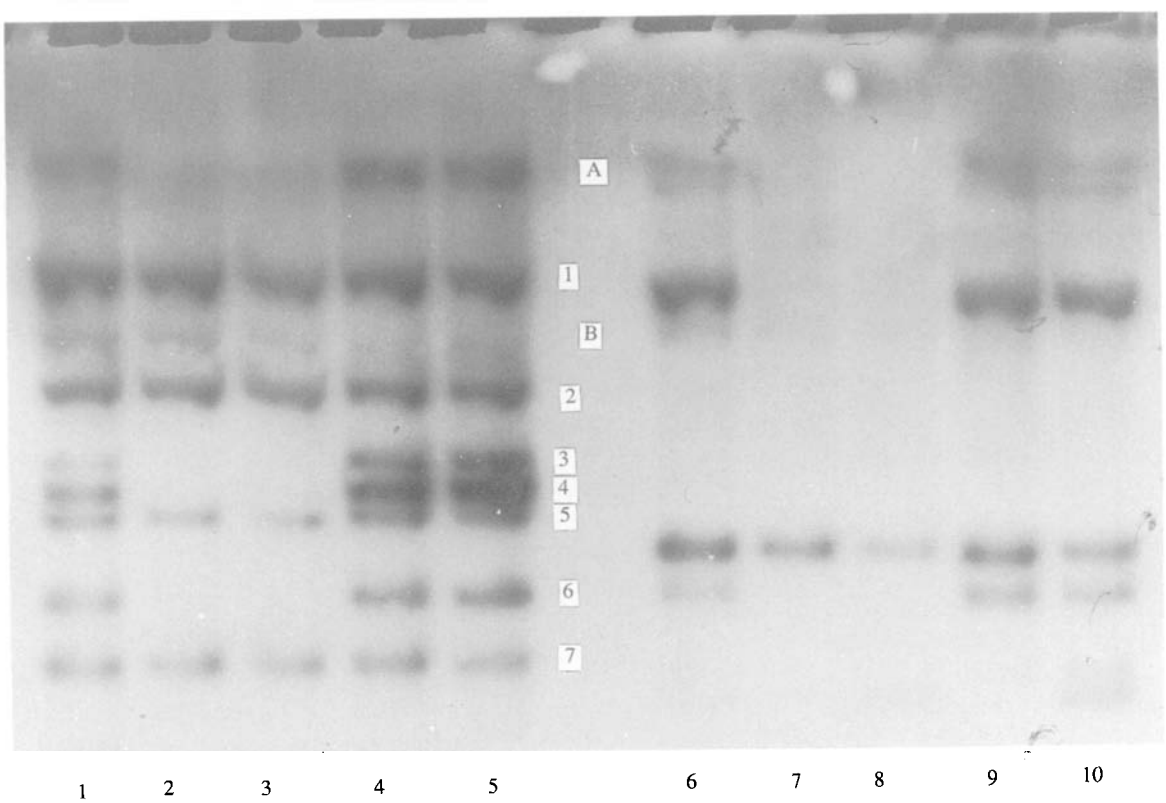

Fig. 5. *Sal* I digest of total genomic DNA of the *MR* strains: *MR-T007/Cy* and *MR-h12/Cy* after outcrossing to P-free *Berlin K* males. Lane 1, the original *MR-T007/Cy* strain; lanes 2 and 3, F_1 *Cy(T007)* males and females respectively; lanes 4 and 5, F_1 *MR-T007* males and females respectively; Lane 6, the original *MR-h12/Cy* strain; lanes 7 and 8, F_1 *Cy(h-12)* males and females respectively; lanes 9 and 10, F_1 *MR-h12* males and females respectively. Probe pHS.

the *MR-T007/Cy, MR-GB39/Cy* and *MR-h12/Cy* strains, the *Cy* (non-*MR*) male progeny have no or hardly any *MR* activity (Table 6), most, if not all of the 2·4 kb *Acc* I copies detected in these three strains should be localized on the *MR* chromosomes. Since *MR-n1* is kept as a homozygous stock, it is assumed that the number of 2·4 kb *Acc* I copies responsible for the reversions, is one-half that determined directly in the *MR-n1* strain, e.g. 5 or 6 copies. *MRF-31.1/CyL*^{4} contains over 16 copies of the 2·4 kb *Acc* I fragment; presumably these copies are distributed equally over the *MRF-31.1* and the *CyL*^{4} chromosome, since, upon outcrossing, the *CyL*^{4} chromosome shows approximately the same genetic activity as does the *MRF-31.1* chromosome itself. The reversion frequency of unstable mutations as described by these two chromosomes should be due to about eight or more complete P elements. The number of complete P elements as inferred from the *Acc* I digestion experiments, is confirmed by the determination of the integration sites after digestion with the enzyme *Sal* I (Fig. 4). If we compare the pattern of bands obtained after *Sal* I digestion, the strains *MR-n1* and *MR-GB39* appear clearly related. This resemblance is not surprising in view of the fact that both strains originate from two, not too distant, sites in California. The

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Sequence of inverted repeats and integration site of the cloned P-elements
the strains MR-h12/Cy and MR-T007/Cy

MR-h12/Cy

HA41 AAG ATGGAGGT CATGATGAAATAACATAAGGTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG ATGGAGGT GTT

HA51 TGA GCCGAAAC CATGATGAAATAACATAAGGTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG GCCGAAAC TA

HB TT GTATTATA CATCATG( )GTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG GTATAGCC ATT
(HB carries a deletion: GAATGGACCCGGAT[1054...1577GAT]GAATGATTGGT

MR-T007/Cy

TA11 GGT GTACAGAG CATGATGAAATAACATAAGGTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG GTACAGAG GT

TB7 AGC GTTGATGG CATGATGAAATAACATAAGGTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG GTTGATGG AG

TC1 TGT GTTTATAA CATGATGAAATAACATAAGGTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG GTTTATAA TTT

TB8 CCCGAATGAAACGACGCGAAATGTGACGGT GACTGAGC CATGATGAAATAACATAAGGTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG GACTGAGC ACCCTCGAAAACGACGCCTCGCTG
(TB8 shows an extra 8 bp direct duplication near the P-element)

TA2 T ATGGTCTA CATGATGAAATACACATAAGGTGGTCC CATGATGAAATAACATAAGGTGGTCCCGTCG.... ....CGACGGGACCACCTTATGTTATTTCATCATG GGGTGCTA CCT
(TA2 carries a partial duplication of the left 31 bp inverted repeat)

Fig. 6. The sequence of the inverted repeats, the duplication at the integration site and some flanking sequences of the P elements cloned from the *MR-h12/Cy* strain (HA41, HA51, HB) and the *MR-T007/Cy* strain (TA11, TB7, TC1, TB8, TA2).

three bands detected after *Sal* I digestion in the *MR-h12/Cy* strain are more than expected from the *Acc* I digestion experiments. In addition, the reisolated *MR-h12* chromosome, the *MR-13-1*, has retained only one band identical to the *MR-h12/Cy* strain, whereas one new band has appeared. It seems reasonable to assume that if a P element on the *MR* chromosome is causally related to the genetic events observed then the band present in both strains represents this P element. The results obtained so far suggest a positive correlation between the number of complete P elements and the ability to revert P-insertion mutations.

In order to prove the above hypothesis, the *MR-h12/Cy* and *MR-T007/Cy* strains were examined in more detail. These two strains were chosen for several reasons: (1) the ability to revert P-insertion mutations is lowest in *MR-h12* and highest in *MR-T007*, (2) genetic evidence is provided that in both strains the *Cy* homologue has no genetic effect (Table 6). (3) both strains have been used more extensively in several other genetic tests, especially *MR-h12/Cy* (Eeken & Sobels, 1981, 1983*a*, *b*, 1986) and (4) the genetic localization of the activity is determined for both strains (Slatko & Green, 1980). The genetic experiments (Table 6) indicate that the functional P element in the two *MR/Cy* strains should be localized exclusively on the *MR* chromosomes. *MR-h12/Cy* and *MR-T007/Cy* females were crossed to P-free *Berlin K* males (the reciprocal cross in terms of P–M hybrid dysgenesis, to avoid transposition events in the F_1 offspring). F_1 males and females carrying either the *MR* or the *Cy* chromosome were collected separately, DNA isolated and digested with *Sal* I, fragments separated electrophoretically, blotted and hybridized with the pHS probe. The result is shown in Fig. 5. In lanes 1 and 6 the pattern of the original *MR-T007/Cy* and *MR-h12/Cy* strains are shown. Lanes 2 and 3 show the pattern of F_1 *Cy*(-T007) males and females, lanes 4 and 5 F_1 *MR-T007* males and females, lanes 7 and 8 F_1 *Cy*(-h12) males and females and lanes 9 and 10 F_1 *MR-h12* males and females. Surprisingly, the *Cy* flies also show numerous bands, especially in the case of *MR-T007/Cy*. Comparing Fig. 5 with Fig. 4, bands numbered A and B (Fig. 5) are most likely partial digestion artefacts. In lanes 1–5 (-T007), bands numbered 1, 2, 5 and 7 are clearly present in all five lanes, indicating that they should be present either on the III, IV autosome or the X chromosome. Only band numbers 3, 4 and 6 appear to be localized on the *MR-T007* chromosome. In case of the *MR-h12/Cy* strain, the middle heavy band appears to originate from a P element not localized on the *MR* chromosome. This is in agreement with the result shown in Fig. 4, where in the reisolated *MR-h12* chromosome (*MR-13-1*) this band is eliminated. From Fig. 4 it also is clear that the lower band from the *MR-h12/Cy* strain has disappeared, suggesting that in fact the top band in the *Sal* I digest is the only candidate for the functional P element in the *MR-h12/Cy* strain, located on the *MR-h12* chromosome. The results can be interpreted in more than one way. First, the detected bands in the *Sal* I digest, using the internal sequences of the P element (pHS) as probe, detects a number of non-functional P elements with small internal deletions. Therefore, it is incorrect to simply assume that the remaining detectable bands that are located uniquely on the *MR* chromosomes are in fact all complete and functional P elements. The assumption that pHS detects non-functional P elements with small internal deletions is, however, contradicted by the results obtained with the *Acc* I digestion experiments. These showed that nearly all the detected P elements have in fact an internal *Acc* I fragment of exactly 2·4 kb. Alternatively, all detected P elements are in fact complete P sequences, but they are not all active. Also in this case it is not possible to compare genetic activity with the number of complete P elements located on the *MR* chromosome, since possibly not all these elements contribute equally to the observed genetic effects. To resolve some of the contradictions the complete/nearly complete, active/non-active, P elements from the *MR-T007/Cy* and *MR-h12/Cy* strains were cloned. From the *MR-T007/Cy* strain five different elements were isolated (TA2, TA11, TB7, TB8 and TC1). From the *MR-h12/Cy* strain, three different elements were cloned, HA41, HA51 and HB. According to Southern analysis of several restriction sites, all these different P elements appear complete with the exception of P-element HB that contains a large deletion (see Fig. 2).

S1 nuclease protection experiments were performed to determine whether the cloned P elements contained deletions or duplications smaller than 50 bp, the detection limit of blot hybridization. All the P elements, with the exception of the HB element from *MR-h12*, showed protected fragments of the expected size, indicating that no deletions/insertions larger than 4–5 bp (the detection limit of the S1 protection technique) are present. This result shows that in the *MR-T007/Cy* and the *MR-h12/Cy* strain complete P elements are almost certainly present that are not located on the *MR* chromosome and therefore, complete P elements may be present in the genome (1) that are not activated in a dysgenic cross or (2) that carry small changes inactivating the element. The DNA sequence of the ends of all cloned P elements was determined. Part of the results of the sequence analysis is presented in Fig. 6. The inverted repeats of P elements HA41, HA51, TA11, TB7, TB8 and TC1 are exact copies of those reported by O'Hare & Rubin (1983). One element from *MR-T007/Cy*, TA2, shows an imperfect partial duplication of the left inverted repeat. All the complete P elements show a perfect 8 bp duplication at the integration site. From the cloned elements (with the exception of HA41 and the right part of HA51) we also compared the sequence of the internal P-transposase binding sites (by 48–66 and

Table 7. *The reversion of a P-insertion mutation by HA41 and HA51 at 25 °C*

(total reversions/number of F_2 male/F_2 female progeny scored)

	Reversion/number F_2 offspring (%)
(1) Muller-5, sn^P; HA41 males	4/524 (0·8)
(2) Muller-5, sn^P; Cy males	0/398 (0·0)
(3) Muller-5, sn^P/HA51; HA41 females	26/2494 (1·0)
(4) Muller-5, sn^P/HA51; Cy females	2/1825 (0·1)

2855–2871; Kaufman *et al.* 1989); no changes were observed in the sequence of these binding sites in any of the elements analysed.

An *in situ* hybridization experiment was performed to determine the integration sites of various P elements. *MR-T007/Cy* and *MR-h12/Cy* females were crossed to P-free *Berlin K* males. F_1 non-*Cy* female larvae were used for chromosome spreads. The sites of hybridization with pHS are: 1A, 3E and 6E on the X chromosome and 25C, 25F, 34F, 47A and 47D on the II-chromosome for *MR-T007* and 2B (X chromosome), 38A and 55A (II chromosome) for *MR-h12* (data not shown). Using isolated flanking sequences from the clones HA41 and HA51, it could be shown that: (1) HA41 corresponds to the top band in the *Sal* I digest of *MR-h12/Cy* and is localized at site 38A of the *MR* chromosome and (2) HA51 corresponds to the heavy middle band of the *Sal* I pattern and is localized at 2B on the X chromosome of the original *MR-h12/Cy* strain.

Knowing the distribution of complete P elements in the *MR-h12/Cy* strain, it is obvious that the measured genetic effects of this strain are due exclusively to the presence of one complete P element, the HA41, on the *MR-h12* chromosome. A number of genetic tests were devised to examine the activity of the second complete P element in this strain, HA51, localized at the X chromosome. For this purpose we tested the ability of HA41 and HA51 to revert a *singed* (P-insertion) mutation induced by *MR-h12* in a Muller-5 balancer chromosome (marked with w^a and *B*). Muller-5, sn^P females were crossed to *MR-h12/Cy* males. Four types of F_1 dysgenic progeny can be recovered: (1) Muller-5, sn^P; HA41 males, (2) Muller-5, sn^P; *Cy* males, (3) Muller-5, sn^P/HA51; HA41 females and (4) Muller-5, sn^P/HA51; *Cy* females. The F_1 males were crossed to *C(1)DX* females and the F_2 males scored for reversions of *sn*. This experiment determines the ability of the HA41 to revert this particular P-insertion *sn* mutation, as well as the control frequency (the F_1 *Cy* males). The F_1 females were crossed to FM7, sn^{X2} males. Reversions of the sn^P can be scored in the F_2 Muller-5, sn^P males as well as in the F_2 Muller-5, sn^P/FM7, sn^{X2} females. This experiment determines the ability of the HA41 (together with the HA51) to revert the sn^P mutation in females, as well as the ability of the HA51 alone (the F_1 *Cy* females) to revert this sn^P mutation. The data of this experiment are given in Table 7. Although the numbers are small it is obvious that the *Cy* homologue has no activity by itself. HA41, on the *MR* chromosome has nearly 10 times the activity of HA51, on the X chromosome. To ensure that the HA51 is a functional P element, the complete sequence was determined. Not 1 bp difference between the HA51 and the published (O'Hare & Rubin, 1983) sequence of the active pπ25.1 P element could be found (data not shown).

4. Discussion

MR chromosomes were originally characterized by their ability to induce recombination in F_1 males when inherited from their fathers and by the fact that in these males they induced high frequencies of locus-specific unstable mutations (Green, 1977, 1978), due to the insertion of P elements (Eeken *et al.* 1985). P-insertion mutations revert with high frequency in the presence of *MR* chromosomes (Eeken, 1982), strongly suggesting that complete P elements are located on these chromosomes. In this investigation we analysed several *MR* strains with respect to their ability to (1) induce P-insertion mutations and (2) revert P-insertion mutations and the number and localization of complete P elements.

Several different *MR* chromosomes were characterized genetically. The ability of all tested *MR* strains to induce forward mutations is similar, but differences in their ability to revert unstable (P-insertion) mutations could be demonstrated. The *MR-h12* chromosome is in this respect the weakest, the *MR-T007* chromosome one of the strongest. Although there is no direct relationship between the ability to revert P-insertion mutations and the ability to induce male sterility in the tested *MR* strains, it could be shown for two of the *MR* chromosomes that reducing the temperature at which the dysgenic males are raised not only decreases the male sterility but also the ability to revert P-insertion mutations.

To correlate the ability to revert P-insertion

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mutations with the number of complete P elements, genomic DNA of the *MR/Cy* strains was isolated and digested with *Acc* I and with *Sal* I. The results indicated that the number of apparent complete P elements (containing a 2·4 kb internal *Acc* I fragment, same level of hybridization signal after hybridization with an internal fragment of P, pHS) could be positively correlated with the ability to revert P insertion mutations. However, a more detailed analysis of the *MR-h12/Cy* (weak genetic effect) and *MR-T007/Cy* (strong genetic effect) strains showed that the complete P elements are not only localized on the *MR* chromosomes but also on other chromosomes (mainly the X chromosome, as shown by *in situ* hybridization experiments). In fact the *in situ* hybridizations indicate that in the *MR-h12/Cy* strain one complete element is localized on the X chromosome and one on the *MR-h12* chromosome, in the *MR-T007/Cy* strain, 3 hybridization signals can be detected on the X chromosome and 5 on the *MR-T007* chromosome. The *in situ* hybridization data of the *MR-T007/Cy* strain are not completely in agreement with similar data obtained for this strain using Southern-blot hybridization. Genetic tests using the *MR-h12/Cy* strain clearly show that the complete P element on the X chromosome in this strain has no genetic activity. In order to determine whether the detected apparent complete P elements are actually complete elements, we isolated 5 of these elements from the *MR-T007/Cy* and 2 from the *MR-h12/Cy* strain. S1 protection experiments show that all these 7 P elements do not have deletions/insertions larger than 4–5 bp (the detection limit of the S1 protection technique) compared to the pπ25.1 sequence (O'Hare & Rubin, 1983). In fact the complete P element on the X chromosome of *MR-h12/Cy*, that has no genetic effect, has been sequenced and no alteration compared to pπ25.1 sequence could be detected. Furthermore it was shown that in all the seven cloned complete P elements, the 31 bp inverted repeats as well as the two internal P-transposase binding sites (46–66 and 2855–2871 bp; Kaufman *et al.* 1989) are intact.

The results clearly show that the induction of P-insertion mutations is similar in all tested strains, irrespective of the number of apparent complete P elements present. Furthermore, although the ability to revert P-insertion mutations is localized on the *MR* chromosomes, this ability cannot be correlated directly with the number of the complete P elements present on these chromosomes. Complete P elements present on other chromosomes in the *MR* strains are not active with respect to mutation induction and reversion.

It is tempting to attribute the recombination in the dysgenic males carrying *MR* chromosomes to transposition of P elements, as is customary for sterility in dysgenic females. The genetic factor involved in male recombination is mapped between *Tft* and *pr*; Slatko & Green, 1980). In the *MR-h12* chromosome a complete P element is present in the same region (38A) as the genetic localization of the *male recombination* activity. However, this is not the case for the *MR-T007* chromosome. Where the main genetic activity of this chromosome was also located between *Tft* and *pr*, no P elements are present at this particular region. A similar situation appears in a III chromosome with *MR* activity, +(*3*)*KMCG* (Green, 1986); also in this chromosome, the genetic localization of the activity does not coincide with P elements present on that chromosome (Green, pers. comm.). Although it is clear that *MR* strains behave as P strains with the active P elements located on the second chromosome, the relation between P transposition and male recombination remains unsolved.

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