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

JAN C. J. EEKEN1,2*, RON J. ROMEYN1,2, ANJA W. M. DE JONG1,2, GEORGE YANNOPOULOS3 AND ALBERT PASTINK1

1 Department of Radiation Genetics and Chemical Mutagenesis, Sylvius Laboratories, State University of Leiden, Wassenaarseweg 72, Leiden, The Netherlands
2 J. A. Cohen Interuniversity Research Institute for Radiopathology and Radiation Protection, Leiden, The Netherlands
3 Department of Genetics, University of Patras, Patras, Greece

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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 F1 (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.
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-nl, MR-GB39/Cy, MR-T007/Cy and MRF-31.1/CyL4. 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 and Pelecanos, 1977); MRF-31.1 is kept in stock balanced over a CyL4 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 F1, 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 (Würinger et al. 1977).

Sex-chromosome-linked visible mutations were scored in the offspring of F1, MR-carrying, males from the same parental cross as described above, after crossing them to C(1)DX, yf 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 F1, MR-carrying, males, progeny of a cross between females homzygous for a particular MR-induced visible mutation and males from the MR strains. The reversions were detected after crossing these F1 males to C(1)DX, yf 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 Hind III–Sal I fragment of pnr25.1, 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. 1. Physical map of pnr25.1 according to OHare & Rubin (1983). Indicated are pHS used to recognize full length P elements. Some restriction sites are indicated: EcoR I (E), Sal I (S), Hind III (H), Sst I (Sst) and Hae III (Ha).
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-hl2/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 PdJll, a derivative of L47.1 (A amBam; P. de Jong, unpublished), cleaved with BamHI. EMBL4- and PdJll-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-hl2/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) SI protection experiments
To determine small deletions or duplications in the cloned P elements, SI-nuclease protection experiments were performed. As protection fragments, two fragments were subloned from p1wc (O’Hare & Rubin, 1983) into M13mp10. The first fragment contains the left end of P from restriction site SstI (1150) to a BamHI site in the flanking sequence of p1wc, M13mp10-Bam/Sst. The second fragment, a HaeIII fragment from p1wc, includes the right end of P element we from restriction site HaeIII (724) to the SalI site at 2882 and some pBR322 sequences. The ends of the HaeIII fragment were end-filled and cloned into the SmaI 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 mM- NaCl was added to the final concentration of 0.2 M

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**Fig. 2.** Physical map of the cloned P elements from the MR strains MR-hl2/Cy and MR-T007/Cy. Some restriction sites are shown: EcoRI (E), SalI (S), HindIII (H) and SstI (Sst). Underneath each element the subcloned fragments are indicated that were used for DNA sequencing.
Table 1. The induction of SLRL mutations by MR strains

| MR strain | Sex-linked recessive lethal mutations |
|-----------|--------------------------------------|
|           | Chromosomes (n) | Lethal (n) | % lethal |
| T-007     | 1889             | 52        | 2.7 ± 0.4 |
| MRF-31.1  | 3182             | 51        | 1.6 ± 0.2 |
| CyL⁺      | 3113             | 38        | 1.2 ± 0.2 |
| n1        | 2529             | 38        | 1.5 ± 0.2 |
| GB39      | 2461             | 26        | 1.1 ± 0.2 |
| h-12      | 6072             | 69        | 1.1 ± 0.1 |
| Cy(h-12)  | 6342             | 9         | 0.1 ± 0.1 |

* See Sobels & Eeken (1981).
* 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 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'CCTCTCAA-CAAGCAAACG-3'(5'[0]-3'[73]) and PR (right) 5'-TACGACCTCAGAGATCT-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⁺, 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⁺, 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 F₁ 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 & Hirai, 1973), 1.4 and 3.0% for v⁶ (Q strain) and π₂ (Simmons et al. 1980, 1984), 2.5% for both π₂ 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...
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,

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

|   | T007 | 31.1 | CyL4 | n1 | GB39 | h12 |
|---|------|------|------|----|------|-----|
| n1 | sn 46 | 6/40 (15) | 2/10 (20) | 90/411 (20) | 122/1501 (81) | 76/1652 (46) | 60/3394 (18) |
| y 25.2 | 43/174 (25) | 35/170 (20) | 44/603 (7) | 113/3628 (31) | 127/3422 (37) | 10/2061 (05) |
| sn 30 | 35/427 (74) | 2/35 (6) | 27/361 (75) | 110/3789 (29) | 64/2414 (27) | 17/2933 (06) |
| h12 | sn 17A | 134/1147 (11) | 44/326 (13) | 69/829 (83) | 113/2807 (4) | 158/4973 (32) | 16/1944 (08) |
| sn 39B1 | 56/670 (84) | 21/188 (11) | 44/514 (85) | 157/3386 (46) | 162/4148 (39) | 179/12664 (14) |
| sn 25A | 61/1768 (3-5) | 41/568 (7) | 41/1167 (3-5) | 111/3099 (3-6) | 44/3737 (12) | 14/3332 (04) |
| T007 | sn 42 GY | 35/643 (5) | 105/3763 (2-8) | 21/995 (2-1) | n.t. | n.t. |
| y 18 GY | 7/790 (0-4) | 9/686 (1-3) | 11/1059 (10) | 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 (02) |
| GB39 | ras 44.2 | 1/50 (2) | 3/24 (12) | 27/483 (5-4) | 48/2790 (1-7) | 23/3175 (07) | 1/3461 (02) |
| ras 44s2 | 1/154 (0-7) | 2/16 (12) | 37/1288 (2-9) | 59/3036 (19) | 23/3073 (07) | 6/3749 (02) |
| ras 44s1 | 6/83 (0) | 2/27 (7-5) | 16/677 (2-4) | 50/4169 (1-2) | 33/3530 (10) | 5/5379 (01) |
| F-31.1 | sn 22A | 27/1145 (2-4) | 21/598 (3-2) | 14/1937 (2) | 10/8938 (1) | 8/4712 (02) | 1/4655 (02) |
| y 42B | 17/4396 (0-4) | 8/1271 (0-6) | 22/5380 (0-4) | 0/4973 (0) | 4/5721 (007) | 0/1342 (0) |
| sn 4B | 4/6875 (0-1) | 1/6675 (001) | 0/4822 (0) | 0/9496 (0) | 0/9856 (0) | 1/7555 (02) |
| CyL4 | ras 44A | 4/2088 (0-2) | 0/187 (0) | 8/1329 (0-6) | 0/3815 (0) | 28/6555 (0-4) | 44/4711 (0-9) |
| sn 9A | sterile | 1/26 (4) | n.t. | 2/4597 (0-04) | 2/3910 (005) | 4/5051 (0-1) |
| y 46A | 0/103 (0) | 0/677 (0) | 0/2730 (0) | 0/6069 (0) | 0/5693 (0) | 1/2916 (0-03) |

* n.t., not tested.

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

|   | T007 | F31.1 | CyL4 | 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. | n.t. | n.t. | n.t. | n.t. |
| sn 42A | n.t. | n.t. | n.t. | n.t. | n.t. |
| sn 18 GY | 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 |
| CyL4 | ras 44A | 12 | 17 | 16 | 4 | 4 | 28 |
| sn 9A | 100 | 78 | n.t. | 4 | 14 | 11 |
| y 46A | 89 | 73 | 48 | 6 | 1 | 30 |

* n.t., not tested.
Table 5. Male sterility induced by MRF31.1 and CyL4 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 | CyL4 | MRF-31.1 | CyL4 | MRF-31.1 | CyL4 | MRF-31.1 | CyL4 |
| n1    |           |       |           |       |           |       |           |       |
| sn 46 | 95       | 60   | 81       | 34   | 20       | 20   | 46       | 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 |
| CyL4  |           |       |           |       |           |       |           |       |
| 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/CyL4 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 F1 males carrying the MR chromosome were selected. For MR-n1, kept as a homozygous stock, this implies that all F1 males could be used. In case of MRF-31.1/CyL4, the MRF-31.1 as well as the CyL4 chromosomes were tested separately. On average 80 F1 males were subsequently crossed individually to 2–4 C(1)DX females and the F2 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
induced by MR-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 MR-F31.1 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 (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 pH5 with the MR strains is predominantly with Acc I fragments of 2-4 kb. Rehybridization with pH5 (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, MR-F31.1/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
The reversion of unstable mutations by the Cy homologue of MR strains at 25 °C

Table 6. (total reversions/number of F₂ male progeny scored)

|          | T007 | Cy-T007 | GB-39 | Cy-GB39 | h12 | Cy-h12 |
|----------|------|---------|-------|---------|-----|--------|
| h12 ras 5B² | n.t. | n.t.    | n.t.  | n.t.    | 8/243 | 0/14471 |
| sn 39B1²  | n.t. | n.t.    | n.t.  | n.t.    | 204/12664 | 4/1357 |
| h12 sn 17A* | 134/1147 | 158/4973 |       |       |      |        |
| sn 25A*   | 61/1768 |        | 44/3737 |        |      |        |
| h12 sn 17A (trans) | 174/1805 | 0/4217 | 136/2832 | 3/4119 |      |        |
| sn 25A (trans) | 59/1551 | 0/5289 | 45/2495 | 1/3636 |      |        |
| sn 39B1 (trans) | n.t. | n.t.    | n.t.  | n.t.    |      |        |
| 134/1147  | 61/1768 | 174/1805 | 59/1551 | 134/1147 | 61/1768 | 174/1805 | 59/1551 |

* Data taken from Eeken (1982).
See Table 4b.

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/CyL² 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 CyL² chromosome, since, upon outcrossing, the CyL² 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.
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 CATGATGAATAACATATAGGGTCCCGTCG . . . . CGACGGGGACCACCTTATGTTATTTCATCATG ATGGAGGT GTT

HA51  TGA GCCGAAAC CATGATGAATAACATATAGGGTCCCGTCG . . . . CGACGGGGACCACCTTATGTTATTTCATCATG GCCGAAAC TA

HB    TT GATTATA CATCATG( )GTGGTCCCGTCG . . . . CGACGGGGACCACCTTATGTTATTTCATCATG ATGATGCC ATT
       (HB carries a deletion: GAATGGACCCGGAT[1054...1577GAT]GAATGATTGGT

MR-T007/Cy

TA11  GGT GTAAGAGG CATGATGAATAACATATAGGGTCCCGTCG . . . . CGACGGGGACCACCTTATGTTATTTCATCATG GTACAGAG GT

TB7   AGC GTTGATGG CATGATGAATAACATATAGGGTCCCGTCG . . . . CGACGGGGACCACCTTATGTTATTTCATCATG GTTGATGG AG

TC1   TGT GATTATA CATGATGAATAACATATAGGGTCCCGTCG . . . . CGACGGGGACCACCTTATGTTATTTCATCATG GTTTATAA TTT

TB8   CCCGAATGAAAGCGCCAATGACGACGCACTAGATGACGACGCACTAGATGACGACGCACTAGATGACGACGCACTAGATGACGACGCACTAGATGACGACGCACTAGATGACGACGCACTAGATGACGACGACGTG
       (TB8 shows an extra 8 bp direct duplication near the P-element)

TA2   T ATGGTCATA CATGATGAATAACATATAGGGTCCCGTCG . . . . CGACGGGGACCACCTTATGTTATTTCATCATG GGGTTCATA 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, 1983a,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 F1 offspring). F1 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 F1 Cy(-T007) males and females, lanes 4 and 5 F1, MR-T007/Cy males and females, lanes 7 and 8 F1, CY(-h12) males and females and lanes 9 and 10 F1, 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).

SI 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 SI 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 F₂ male/F₂ female progeny scored) | Reversion/number F₂ offspring (%) |
|---------------------------------------------------------------|----------------------------------|
| (1) Muller-5, sn⁺; HA41 males                                 | 4/524 (0.8)                      |
| (2) Muller-5, sn⁺; Cy males                                   | 0/398 (0.0)                      |
| (3) Muller-5, sn⁺/HA51; HA41 females                         | 26/2494 (1.0)                    |
| (4) Muller-5, sn⁺/HA51; Cy females                            | 2/1825 (0.1)                     |

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. F₁ 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⁺ and B). Muller-5, sn⁺ females were crossed to MR-h12/Cy males. Four types of F₁ dysgenic progeny can be recovered: (1) Muller-5, sn⁺; HA41 males, (2) Muller-5, sn⁺; Cy males, (3) Muller-5, sn⁺/HA51; HA41 females and (4) Muller-5, sn⁺/HA51; Cy females. The F₂ males were crossed to C(1)DX females and the F₂ 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 F₁ Cy males). The F₁ females were crossed to FM7, sn⁺/FM7 males. Reversions of the sn⁺ can be scored in the F₂ Muller-5, sn⁺ males as well as in the F₂ Muller-5, sn⁺/FM7, sn⁺ females. This experiment determines the ability of the HA41 (together with the HA51) to revert the sn⁺ mutation in females, as well as the ability of the HA51 alone (the F₂ Cy females) to revert this sn⁺ 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 pn25. 1 P element could be found (data not shown).

4. Discussion

MR chromosomes were originally characterized by their ability to induce recombination in F₁ 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
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 24 kb internal Acc I fragment, same level of hybridization signal after hybridization with an internal fragment of P, pH5) could be positively correlated with the ability to revert P insertion mutations. However, a more detailed analysis of MR-hl2/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-hl2/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-hl2/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-hl2/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 p725.1 sequence (O'Hare & Rubin, 1983). In fact the complete P element on the X chromosome of MR-hl2/Cy, that has no genetic effect, has been sequenced and no alteration compared to p725.1 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)KMC G (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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