**P-Element Repression in Drosophila melanogaster by a Naturally Occurring Defective Telomeric P Copy**

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**ABSTRACT**

In Drosophila melanogaster, hybrid dysgenesis occurs in progeny from crosses between females lacking P elements and males carrying P elements scattered throughout the genome. We have genetically isolated a naturally occurring P insertion at cytological location 1A, from a Tunisian population. The Nasr’Allah P(1A) element [NA-P(1A)] has a deletion of the first 871 bp including the P promoter. It is flanked at the 3’ end by telomeric associated sequences and at the 5’ end by a Hef-A element sequence. The NA-P(1A) element strongly represses dysgenic sterility and P transposition. However, when testing P-promoter repression, NA-P(1A) was unable to repress a germlinally expressed P-lacZ construct bearing no 5’-homology with it. Conversely, a second P-lacZ construct, in which the fusion with lacZ takes place in exon 3 of P, was successfully repressed by NA-P(1A). This suggests that NA-P(1A) repression involves a homology-dependent component.

The P-transposable element is a recent invader of natural populations of Drosophila melanogaster. It is thought to have entered the genome of this species within the last 50 years (Kidwell 1983). Strains that possess P elements are called P strains; strains that do not are called M strains. When P males are crossed to M females, the resulting progeny exhibit a syndrome of germline abnormalities (Kidwell et al. 1977) due to P-element activity. This syndrome, called hybrid dysgenesis, is repressed by various mechanisms (Engels 1989). In some populations, repression is maternally inherited—a condition called P cytophate (Engels 1979). In others, it is biparentally transmitted (Kidwell 1985; Black et al. 1987; Simmons et al. 1990). Of the 30–60 P copies present in a P-strain genome, one-third are complete P elements (Bingham et al. 1982; O’Hare and Rubin 1983; O’Hare et al. 1992). These elements can produce the transposase required for their mobility (Karass and Rubin 1984; Rio et al. 1986) and are therefore autonomous. The other two-thirds of the P elements in a P-strain genome are nonautonomous because they are structurally incomplete; however, many of these defective elements can be mobilized in trans by complete P elements (Engels 1984, 1989) because they possess the sequences recognized by the P transposase.

P cytophate represses transcription from the P promoter (Lemaitre and Coen 1991; Lemaitre et al. 1993; Coen et al. 1994). Although in the short term the cytophate is maternally determined, in the long term, it is chromosomally determined by the P elements themselves (Engels 1979, 1989). Transformation of an M genome with P elements can induce the development of P repression over generations (Presston and Engels 1989). Conversely, the removal by segregation of the P elements of a P strain leads to the loss of repression capacities in the progeny (Sved 1987). The repression ability of a P element depends on its structure and its insertion site (Robertson and Engels 1989; Misra and Rio 1990; Higuet et al. 1992; Gloor et al. 1993; Misra et al. 1993; Rasmusson et al. 1993). We have previously sought to identify regulatory P elements in the chromosomes of natural populations (Ronsseray et al. 1989; Biémont et al. 1990) because selection has probably favored their retention. We isolated, in a genomic context devoid of other P elements, a pair of autonomous P elements inserted near the telomere of an X chromosome (cytological site 1A on the polytene chromosomes) from a Russian natural population (Ronsseray et al. 1991). The resulting line, called Lk-P(1A), can completely repress P-induced hybrid dysgenesis, P transposition, and transcription from the P-element promoter in germline cells (Ronsseray et al. 1993; 1996; Lemaitre et al. 1993). Furthermore, this repression is maternally transmitted but is lost if the P elements at 1A are removed by segregation (Ronsseray et al. 1993). The Lk-P(1A) P elements are inserted in telomeric asso-

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ciated sequences (TAS; Karpen and Spradling 1992; Ronsseray et al. 1996), which have some properties of heterochromatin, including the ability to silence transgenes inserted within them (Waller and Elgin 1995; Cyderman et al. 1999). In addition, the repression ability of the P(1A) elements is sensitive to the dosage of HP1, a nonhistone heterochromatin protein (James and Elgin 1986; Wustmann et al. 1989), which binds mostly to centromeres and telomeres (James and Elgin 1986; James et al. 1989; Fant et al. 1998); heterozygosity for a null allele of Su(var)205, the gene that encodes this protein, strongly impairs the repression ability of the Lk-P(1A) P elements (Ronsseray et al. 1996, 1997). Genetic and molecular studies show that the P(1A) elements are expressed (Ronsseray et al. 1991, 1996; Roche et al. 1995). This expression is thought to be important for the repression ability of the Lk-P(1A) stock because genetic analysis has revealed the existence of a maternally transmitted component of repression termed the “pre-P cytotype” (Ronsseray et al. 1993). Indeed, females heterozygous for the Lk-P(1A) P elements produce oocytes that do not carry the Lk-P(1A) elements; however, they transmit in their oocytes a condition that stimulates P repression in their progeny.

It is possible that the regulatory properties of the P(1A) elements do not depend solely on the expression of these elements. P-lacZ insertions at cytological site 1A and a P-w-ry insertion at an autosomal telomere (100F) have been shown to prevent the germline expression of an euchromatic P-lacZ insertion, a phenomenon termed trans-silencing (Roche and Rio 1998), and to stimulate the regulatory properties of other P elements in a P-strain genome (Ronsseray et al. 1998). These phenomena are strongly associated and have been interpreted to result from chromatin-chromatin interactions involving the telomeric P reporter and the euchromatic P insertions. However, the telomeric P transgene insertions do not by themselves repress P-induced hybrid dysgenesis (Ronsseray et al. 1998), probably because of their inability to encode a P polypeptide. The regulatory properties of the P elements at cytological site 1A are therefore thought to involve both P-encoded product(s) and chromatin-chromatin interactions.

In this article, we report the genetic and molecular characterization of a naturally occurring P insertion at the 1A site that is derived from a Tunisian population (Nasr'Allah). Unlike previous naturally occurring P insertions at 1A, this element [NA-P(1A)] has a large deletion at its 5' end, encompassing both the 31-bp terminal repeat and the P promoter. The properties of this new P(1A) element are investigated.

MATERIALS AND METHODS

Drosophila stocks: Canton is a typical M line (Kidwell et al. 1977) containing no P elements and marked with a spontaneously occurring allele of yellow.

Muller-5 is an M line carrying the Muller-5 (Basc) balancer X chromosome marked with Bar and w (Lindsley and Zimm 1992).

Harwich-2 is a P line. The subline used here shows >80 P labels by in situ hybridization on polytene chromosomes. It has an unidentified autosomal recessive marker (sepiacolored eye), which appeared spontaneously in the stock.

M S/sn; π2 is a P line with the genetic background of the P strain π2. It carries numerous autonomous P elements (Engel's 1984) and has both the Muller-5 balancer chromosome and an X chromosome marked with the hypermutable allele sn, which causes a slight malformation of the bristles (Engel's 1984). This allele is the result of two defective P elements inserted at the singed locus. In the M cytotype (absence of P regulation), sn is unstable in the presence of transposase, mutating to sn' (with a more extreme phenotype) or to sn− (wild type). In each case, the change of phenotype is due to the excision of one of the two P elements. The excision rate of these P elements can be quantified by measuring the instability of sn in appropriate crosses.

w cv sn′ (Umea-30200) is a stock containing an extreme sn allele. When made heterozygous with the various derivatives of sn, the sn′ allele enhances their expression and makes scoring easier. The dominance relations of the singed alleles are sn' > sn > sn = sn′ (Simmons 1987). ry506 Sb P[rty Δ2-3] (998) TM 6 Ubx (abbreviated Sb Δ2.3) is a line with the Δ2-3(998) P element linked to a dominant bristle marker (Stubble, abbreviated Sb).

Birmingham 2/Cyo (abbreviated Birm2/Cyo) is a strain with numerous defective P elements on the Birm2 second chromosome. It is devoid of repression abilities. w1118; Su(var)251/Cv Roi is a stock with a Su(var)205 allele that encodes a truncated nonfunctional HP1 protein (Eisenberg et al. 1992). This allele strongly impairs the repression ability of Lk-P(1A) (Ronsseray et al. 1996, 1997).

Nasr'Allah ("N31") is an inbred line that was collected at Nasr'Allah, Tunisia in 1985 (Izzaabel et al. 1987). The N31 line had ~30–40 P elements, including a defective copy at the cytological site 1A (Izzaabel et al. 1988), and it possessed a maternally inherited P regulatory ability that appeared to be correlated with the P element at 1A (Izzaabel et al. 1988).

P[ry177 = IAR]A171.1F1; py506 (synonym WG-1152) is a line, from Walter Gehring, which carries a P-lacZ-ry-adh construct at the cytological site 1A. It is inserted in TAS (Roche and Rio 1998). No expression is detected in the female germ-line, even after an overnight staining.

Gonadal dysgenesis assay: The ability of lines to repress the occurrence of gonadal dysgenic (GD) sterility was measured by the "A" assay" (Kidwell et al. 1977). Females of the tested line were crossed with strong P males (Harwich-2). For each test cross, 3–10 pairs were mated en masse and immediately placed at 29°. Parents were discarded after 3 days of egg laying. Approximatively 2 days after the onset of eclosion, G0 progeny were collected and allowed to mature for 2 days at room temperature. Twenty-five to 50 G1 females were then taken at random for dissection. Dissected ovaries were scored as unilaterally dysgenic (51 type) or bilaterally dysgenic (50 type; Schaeffer et al. 1979). The frequency of gonadal dysgenesis was calculated as %GD = %50 + %51 and is referred to as percentage of GD A* (%GD A*). The M cytotype, which allows P elements to be active, results in a high percentage of GD A*, whereas the P cytotype, which represses P-element activity, results in a low percentage of GD A* (~5%). An intermediate percentage indicates incomplete repression.

P-excision assay: sn− hypermutability was used to assay the capacity of lines to regulate P-element excision in the germ-line. In the P cytotype, sn− is almost completely stable even in the presence of transposase. Tested females (10–15) were crossed en masse with 15 sn−; π2 males at 20°. Fifteen G1 virgin
females were crossed en masse with 15 w or sn females at 25°C and allowed to lay eggs in successive bottles for 10 days. Among the G1 females, sn and sn+ individuals were scored. The mutation rate is equal to u = (sn/ (sn+ + sn+)) x 100. The absence of regulation results in a high mutation rate, whereas strong regulation results in a low or null mutation rate.

**Assay for repression of pupal lethality in the soma:** P-element transposition is naturally restricted to the germline due to an inhibition of the splicing of the last intron of the transposase gene in the somatic tissues (Laski et al. 1986; Siebel and Rio 1990). The Δ2-3 P element is an in vitro-modified P element from which the last intron has been removed. Consequently, this element produces transposase in both the somatic and germline tissues (Robertson et al. 1988). Δ2-3 has been inserted at cytological location 998 on chromosome 3, where it is immobile. In the absence of P regulation, Δ2-3 combined with numerous defective P elements results in pupal lethality, most likely because of somatic chromosome breakage (Engel et al. 1987). In the presence of P regulation, this combination does not produce pupal lethality. Twenty females from the line under test were crossed at 18°C with 20 Sb Δ2-3 males. Twenty G1 males carrying the Δ2-3 element were crossed with 20 Birm 2/Cy0 females. The G2 progeny were allowed to develop at 25°C and the Sb+ and Sb phenotypes were scored among the G2 females that were not Cy. The Sb individuals, which carry Δ2-3, normally die at the pupal stage in the absence of P repression, whereas in the presence of P repression, they survive. The Sb+ individuals, which lack Δ2-3, survive in the presence or absence of P repression and serve as a viability control. Thus among the non-Cy progeny, a percentage of Sb ~50% indicates complete repression of pupal lethality.

**Repression of P-lacZ expression in ovaries:** G1 females derived from a cross between test line females and males from a line bearing a transgenic P-lacZ element were examined for their capacity to repress the P promoter in ovaries by staining or by a quantitative assay for enzyme activity. Two 5'P-lacZ constructs were used. The structures of these constructs are diagrammed along with the results (see Figure 4). In the first one, P[lac, ry'1A], lacZ is fused in frame with the first 587 bp of P, which include exon 0 and part of exon 1. The BQ16 insertion of this construct was isolated in a screen for female sterile mutations. It is located on the second chromosome and expresses the P-lacZ transgene in the germine tissues of the ovaries and testes (J. L. Coudérec and F. A. Laski, personal communication). However, the genes adjacent to this insertion are still unidentified. In the second construct, PLH, lacZ is fused in frame with the first 2410 bp of P, which span from exon 0 to part of exon 3 (Kobayashi et al. 1993). This construct is driven by the hsp70 promoter. Without heat shock, no staining is detected in the female germline even after an overnight staining. When heat-shock induced, the PLH3 insertion of this construct is expressed in the nurse cells and in the mature oocyte. Staining of ovaries and enzyme assays to measure lacZ expression was performed as described in Lemaître et al. (1993). The results of the enzyme assays are given in nanomoles per minute per milligram of protein. High activity indicates an absence of P repression and low activity indicates strong P repression (Lemaître et al. 1993).

**Sensitivity of repression to Sn(var)205:** NA-P(1A) females were crossed at 20°C with heterozygous males, which had a wild-type allele of Suv(1A)205 on a balancer chromosome (Cy) and a mutant allele (Suv(1A)205) on a Cy+ chromosome. The regulatory properties of the two kinds of G1 females, which had paternally inherited a mutant or a wild-type Suv(1A)205 allele, were tested by crossin 5-10 females with P-strain (Harwich-2) males at 29°C. For each replicate cross, the GD sterility percentage was determined by the dissection of 25-50 daughters.

**Pre-P cytotype assay:** The ability of the NA-P(1A) line to elicit the pre-P cytotype, a maternally transmitted component of P repression (Rönnser ay et al. 1993), was also tested. The mating scheme is presented along with the results.

**Sequences around the P elements at 1A:** Primes: For the primes deriving from the P sequence (denoted by the letter P), the number indicates the position in the P element (O'Hare and Rubin 1983). Orientations are shown in Figure 1.

P1118: 5'-TTGTTTTCGGTACCTAAATCG-3'
P1282: 5'-GCCTGGGTGTCGCCAAAGCC-3'
P1950: 5'-ACGCCATTCTTTAATTTGCTAC-3'
P2751: 5'-CCACGACATGCTAAAGGTTAA-3'
M13 forward: 5'-GTAAACACGACGCCAGT-3'
M13 reverse: 5'-TACGACTCATTAGGGCC-3'.

**Inverse PCR:** The DNA adjacent to the 3' end of the P element was amplified by inverse PCR performed on a PstI digest of genomic DNA from NA-P(1A), using primers P1950 and P2751 and annealing temperature of 51°C.

**PCR with an adapter:** The DNA adjacent to the 5' end of the P element was obtained using the rapid amplification of genomic DNA end method developed by Mizobuchi and Fröhman (1993), in which the Bluescript SK plasmid was used as an adapter: NA-P(1A) genomic DNA and Bluescript SK plasmid DNA (5 μg each) were digested overnight with SspI plus EcoRI and with HindII plus BamHI, respectively, to direct the ligation. PCR was performed on the ligation products using the M13 forward and P1282 primers and an annealing temperature of 53°C. A nested PCR was then performed using internal primers M13 and P1118 at an annealing temperature of 56°C.

**RNA blot hybridization:** Total RNA was isolated from sets of 360 pairs of ovaries using the RNAzol reagent (Bioprobe Systems, Montreuil Sous Bois, France). Poly(A)+ RNA was purified by chromatography through an oligo(dT) column, separated by electrophoresis in a 1.3% agarose/formaldehyde gel, and transferred onto a nitrocellulose membrane under conditions recommended by the supplier (Schleicher and Schuell, Keene, NH).

**Statistical analysis:** The repression capacities as measured by GD sterility percentages were compared using the nonparametric Mann-Whitney test performed on *A* assay replicates.

**RESULTS**

**Synthesis of a line with a single P-hybridization site at 1A:** A line carrying the tip of the X chromosome from the inbred N31 line (Izabel 1988) in an M-type chromosomal background was synthesized as described previously for the synthesis of the Lk-P(1A) line (Ronsser ay et al. 1991), except that an M line with autosomal balancers was used to substitute the autosomal complement. The resulting Nas'Allah-P(1A) line [NA-P(1A)] was labeled only at 1A by in situ hybridization with a P-element probe (data not shown). This line is genetically marked with a w+ allele.

**Molecular analysis of the structure of the P element of NA-P(1A) and characterization of the adjacent sequences:** Figure 1 shows the structure of the canonical P element, the probes and primers used in the analysis, and the map of the NA-P(1A) P element derived from Southern blot analysis and DNA sequencing. Southern blotting has shown that the NA-P(1A) P element is deleted at its 5' end. The deletion breakpoint maps be-
Figure 1.—Structure of the P element of NA-P(1A). (Top) The restriction map is shown with the probes (S1, S2, S3) used for Southern blot analysis and the primers (solid arrows) used for PCR. All sites from SspI (160) to XhoI (729) were found to be absent in the NA-P(1A) element but the sites from Avall (1045) to Avall (2883) were found to be present. (Bottom) The deleted part of NA-P(1A) is shown (dashed box) on a canonical P-element map (Rio et al. 1986). Positions of exons are given below the map with the position of the first nucleotide present in the NA-P(1A) element in parentheses. The arrowheads indicate the 31-bp inverted terminal repeats of the P element.

Figure 2.—Sequences flanking the NA-P(1A) P element. Boldface italic, HeT-A homologous sequence; small caps, P-element homologous sequence; boldface, TAS-homologous sequence. The first nucleotide at the 5’ end of the P element corresponds to nucleotide 872 in the canonical P-element sequence (O’Hare and Rubin 1983).
shows that, as expected, the M strain Canton
genetic analysis has shown that the
NA-P
the
Lk-P
from the ovaries of the
another transcript that was longer (
Karess
et al.
1984). Canton
y
(at 29° from the cross of M females (Can-
ton)) with P males (Harwich-2) are sterile due to atrophy of the gonads (100% gonadal dysgenesis). Conversely, P females ([Harwich-2 or Lk-P(1A)] crossed with P males produce G1 females with trivial percentages of GD steril-
y (0.3 and 1.1%, respectively) due to a repressive com-
ponent transmitted by the P females. With NA-P(1A) females, there is also nearly complete repression of GD sterility (1.9%). The level of repression of this line is as strong as that of Lk-P(1A).

Repression of excision of the defective P elements in the unstable singed-weake allele (Table 1, column 2): Both the sn* allele and autonomous P elements, which supply transposase, were paternally introduced in the G0.

TABLE 1
Repression of P-element activity

<table>
<thead>
<tr>
<th>Tested lines</th>
<th>GD A* excision</th>
<th>Pupal lethality</th>
<th>P-lacZ activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton M</td>
<td>100</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(1519)</td>
<td>(588)</td>
</tr>
<tr>
<td>Harmonich-2</td>
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<td>0.0</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(332)</td>
<td>(1320)</td>
</tr>
<tr>
<td>Lk-P(1A)</td>
<td>1.1</td>
<td>0.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(35)</td>
<td>(820)</td>
<td>(728)</td>
</tr>
<tr>
<td>NA-P(1A)</td>
<td>1.9</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(0450)</td>
<td>(732)</td>
</tr>
</tbody>
</table>

GD A*, the mean percentage of GD sterility (first line), the standard deviation over replicates (second line), and the number of replicates performed (in parentheses, third line). For each replicate cross, 50–100 ovaries were examined in the progeny. GD A* results obtained for Harwich-2 and Lk-P(1A) are from Ronsseray et al. (1998). sn* excision, repression of P excision at the singed locus. The percentage of sn* females among sn* and sn* G1 females (first line) and the total number of flies scored (in parentheses, second line) are given. Pupal lethality, the percentage of adults carrying the Δ2-3 element (first line) and the total number of adults (in parentheses, second line). P-lacZ activity, results are given in nanomoles per milligram of protein. The mean of replicates is given (first line), with the standard deviation (second line), and the number of replicates (in parentheses, third line).
pected to live. There are no surviving Sb adult flies in the progeny from the M strain Canton\^r (Table 1, column 3), whereas half of the adult progeny are Sb (50.3\%) in the progeny of the P strain Harwich-2. Lk-P(1A) and NA-P(1A) fail to rescue pupal lethality since 1.2 and 0.4\% of Sb flies are produced, respectively. These data are consistent with previous results showing that P(1A) lines are almost devoid of somatic repression capacities (Ronsseray et al. 1996).

Repression of a P-lacZ element in the germline (Figures 4–6; Table 1, column 4): The BQ16 P-lacZ insertion was used initially in this assay. The structure of this P reporter is shown in Figure 4; 587 bp of P are present upstream of the lacZ fusion. In M strains, this insertion is strongly expressed in the nurse cells and in the mature oocyte (Figure 5A). By contrast, G\(_1\) females from the cross of Lk-P(1A) females with BQ16 males strongly repress lacZ activity in the germline (Figure 5B). P-1152 is an insertion of a P-lacZ construct at 1A in TAS (see materials and methods) with the first 587 bp of P present upstream of the lacZ fusion. Females carrying the P-1152 transgene also strongly repress BQ16 expression (Figure 5C), thereby demonstrating that the trans-silencing effect previously described with a P-vasa-lacZ target (Ronsseray et al. 1998) also works on a P-lacZ target. Surprisingly, G\(_1\) females from the cross between NA-P(1A) females and BQ16 males show strong lacZ staining (Figure 5D). Assays of lacZ activity were also performed on the ovaries of G\(_1\) progeny from test crosses (tested line females × BQ16 males). According to these assays, NA-P(1A) females strongly repress the lacZ activity of BQ16 (Table 1, column 4).

The failure of NA-P(1A) to repress P-lacZ expression was also tested by staining assays with two other euchromatic insertions of the same P-lacZ construct as in BQ16, called AB00 and BA37 (J. L. Couderc and F. Laski, personal communication). Both are located on the second chromosome, at different genomic locations from BQ16. AB00 is expressed in nurse cells and mature oocytes. Here again, Lk-P(1A) repressed the AB00 P-lacZ insertion in the germline, whereas NA-P(1A) did not (data not shown). This shows that the inability of NA-P(1A) to repress is not restricted to the BQ16 genomic insertion site. BA37 is expressed in the follicle cells. It is repressed by Harwich but not by Lk-P(1A), P-1152, or NA-P(1A) (data not shown). This result is consistent with the fact that the repression ability of P(1A) lines is restricted to the germline (Ronsseray et al. 1996).

Because of these results, the repression capacities of NA-P(1A) were tested with another P-lacZ structure in which the lacZ fusion is in exon 3 of the P element (PLH3, see Figure 4). By contrast to BQ16, which shares no 5’ homology with NA-P(1A), the PLH3 construct has >1.5 kb of homology with NA-P(1A) upstream of the lacZ fusion. Figure 6, A–C, shows that, after a heat shock, the PLH3 transgene is strongly expressed in the germ-line and is repressed by both Lk-P(1A) and P-1152. Furthermore, it is also strongly repressed by NA-P(1A) (Figure 6D). This indicates that although NA-P(1A) is unable to repress the P promoter of the P-lacZ construct in BQ16, it can repress the P-lacZ construct in PLH3, suggesting that the homology between the 5’ sequence of the telomeric P element and the P-lacZ transgene is important.

From the above tests, it appears that NA-P(1A) parallels the Lk-P(1A) line except for the ability to repress P-lacZ expression in the germline of different transgenic strains. The repression capacities as inferred from the different tests are therefore not strictly correlated.

**Maternal inheritance assay of repression of GD sterility:** Depending on the strain, the regulatory properties in the P-M system can be maternally inherited (“P cytotype”; Engels 1979) or biparentally transmitted (“P susceptibility”; Kidwell 1985). The repression ability of Lk-P(1A) has been shown to be maternally inherited (Ronsseray et al. 1991). Consequently, we tested the inheritance of the NA-P(1A) repression ability. M females (Canton\^r) were crossed to NA-P(1A) males (cross termed “M-MI” for M maternal inheritance) and reciprocally, NA-P(1A) females were crossed to Canton\^r males (cross termed “P-MI” for P maternal inheritance) at 20\°. In both cases, G\(_1\) females were tested for their regulatory properties by the A\(^*\) assay and the percentage of GD sterility was determined for G\(_2\) females (Table 2, columns 1 and 2). Data from a similar experiment performed with Lk-P(1A) individuals instead of NA-P(1A) are also presented. With Lk-P(1A), P-MI G\(_1\) females show strong repression ability because they have inherited the P cytotype from their Lk-P(1A) mothers; conversely, M-MI G\(_1\) females show almost no repression ability because of the M cytotype inherited from their Canton\^r mothers. With NA-P(1A), the two types of females also differed in their repression abilities (Table 2, columns 1 and 2; \(P < 0.01\) by the Mann-Whitney test) but the level of repression found with the P-MI G\(_1\) females from NA-P(1A) was weaker than that found with the corresponding females from Lk-P(1A) (74.4 vs. 2.2\%). Nonetheless, these experimental results indicate that NA-P(1A) has a maternally transmitted repression ability similar to the P cytotype but much weaker than that of Lk-P(1A).

**Sensitivity of NA-P(1A) repression ability to a mutation in Su(var)205:** The repression ability of Lk-P(1A) is strongly sensitive to mutations in Su(var)205, a gene that encodes HP1 (Ronsseray et al. 1996). The presence of a null allele of Su(var)205 abolishes the ability of the Lk-P(1A) P elements to repress GD sterility. We tested the sensitivity of the repression capacities of NA-P(1A) to Su(var)205 by comparing females carrying two wild-type alleles of Su(var)205 with their sisters carrying a wild-type and a mutant allele (Table 2, columns 3 and 4). We detected a strong effect. Females that inherit the NA-P(1A) P element maternally and that carry two wild-type alleles of Su(var)205 repress GD sterility at a
Figure 4.—P-lacZ structures used in repression assays. The NA-P(1A) element is also shown to allow easier comparisons with the P-lacZ structures. BQ16 and PLH3 are P[\text{lac, ry}']A and PLH constructs, respectively. Numbers below the constructs correspond to nucleotide positions in the 2097-bp P-element sequence. tr.m., transformation markers. The fragment with the hsp70 promoter in the construct in PLH3 is 344 bp long. For a detailed structure of PLH3, see Kobayashi et al. (1993).
level (70.0%) close to that of the P-MI group in the maternal inheritance assay (74.4%, column 1). However, their sisters, which differ only by the presence of a mutated allele of Su(var)205, showed little or no repression capacity (95.2% sterility). This difference is highly significant (P < 0.01) by the Mann-Whitney test. NA-P(1A) repression ability is therefore sensitive to Su(var)205.

Thermosensitivity of NA-P(1A) repression ability: The determination of P-repression capacities in G1 females from crosses between P and M individuals is highly thermosensitive (Ronsseray et al. 1984; Ronsseray 1986). During imaginal life, an 18°C treatment results in a decrease of repression capacities whereas a treatment at temperatures >25°C results in an enhancement of repression capacities. These modifications can be at least partially reversible. Lk-P(1A) repression is also thermosensitive (Ronsseray et al. 1991; data reproduced here in Table 2, columns 5–7). The thermosensitivity of repression ability in G1 females resulting from crosses between NA-P(1A) females and Canton males was investigated by aging sets of virgin G1 females at different temperatures. Table 2 (columns 5–7) shows that the G1 females, which have partial repression ability (58.3% sterility) at emergence, evolve toward a lack of repression capacities (81.0%) after 15 days at 18°C and toward...
nearly complete repression ability (3.1%) after 10 days at 28.5°C. Thus, G1 females from crosses between M and NA-P(1A) individuals show strong thermosensitivity in the determination of their repression ability.

Ability of NA-P(1A) to transmit a pre-P cytotype: Genetic experiments have previously shown that the Lk-P(1A) line is able to transmit a maternally inherited component, not linked to the presence of any P element, called pre-P cytotype (Ronsseray et al. 1993). We tested the ability of NA-P(1A) to produce such a component (Figure 7). The two reciprocal crosses between individuals from NA-P(1A) and an M line [Muller-5 (M 5)] were performed at 20°C. The M 5 line has a balancer of the X chromosome marked with a semidominant mutation (Bar). The G2 cross with NA-P(1A) females is referred to as the “P-grandmother” (P-GM) cross and the reciprocal cross is referred to as the “M-grandmother” (M-GM) cross. G1 females from the P-GM cross are expected to have significant repression ability due to maternal inheritance (see Table 2, column 1) but G1 females from the M-GM cross are expected to have weak repression ability. This was confirmed by the A* assay performed on G1 females (data not shown).

We have tested whether the M 5 gametes produced by P-GM G1 females can transmit a “memory” of this repression ability although this gamete does not carry any P element. G1 females were crossed to Lk-P(1A) males and the repression ability of G2 females that inherited the M 5 chromosome was tested with the A* assay. Such a memory is expected to stimulate the repression ability of the paternally introduced Lk-P(1A) chromosome. As a control, the M 5-bearing gamete from M-GM G1 females was similarly analyzed. Figure 7 shows that G2 females that inherit the M 5 chromosome from P-GM G1 females have significantly stronger repression ability than corresponding females produced by M-GM G1 females. The difference is highly significant as determined by the Mann-Whitney test (P < 0.01). The two M 5-bearing oocytes (although devoid of P elements in both cases) do not have the same capacity to stimulate P repression when regulatory P elements are introduced by the spermatozoid. This illustrates the capacity of the P-GM G1 females to transmit the pre-P cytotype. The same experiment with Lk-P(1A) in G0 instead of NA-P(1A) produces a weaker percentage of GD sterility (P-GM = 4.2%, s = 3.3, n = 13; M-GM = 40.4%, s = 19.1, n = 15; Ronsseray et al. 1993). These results therefore show that NA-P(1A) is able to elicit the strictly maternally transmitted component of repression termed the pre-P cytotype.

The foregoing genetic analysis shows that the repression ability of NA-P(1A) resembles that of Lk-P(1A) but is weaker. Furthermore, the NA-P(1A) and Lk-P(1A) strains differ in their effects on P-lacZ repression in the germine.

DISCUSSION

NA-P(1A), a new P-element structure at the site 1A, exerts unusual P-repression capacities: The naturally occurring P element NA-P(1A) located at 1A, isolated from a Tunisian population, is a nonautonomous 5’-truncated P-element variant. Like the autonomous regulatory P elements at 1A previously isolated from French and Russian populations, NA-P(1A) (i) strongly represses dysgenic sterility, (ii) is capable of eliciting the pre-P cytotype, and (iii) has a repression ability apparently restricted to the germline. Furthermore, NA-P(1A) strongly represses P-element excision and its ability to repress dysgenic sterility is maternally transmitted.

<table>
<thead>
<tr>
<th>Tested lines</th>
<th>Maternal inheritance</th>
<th>Sensitivity to Su(var)205</th>
<th>Thermosensitivity aging at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-MI</td>
<td>M-MI</td>
<td>(Cy/+</td>
</tr>
<tr>
<td>Lk-P(1A)</td>
<td>2.2</td>
<td>94.3</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>4.3</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(10)</td>
</tr>
<tr>
<td>NA-P(1A)</td>
<td>74.4</td>
<td>99.8</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>10.4</td>
<td>0.4</td>
<td>21.3</td>
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<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
<td>(14)</td>
</tr>
</tbody>
</table>

All results are GD sterility percentages. The mean percentage of GD sterility (first line), the standard deviation over replicates (second line), and the number of replicates performed (in parentheses, third line) are given. For each replicate test cross, 50-100 ovaries were examined. For the maternal inheritance assay, the A* test cross was performed on sets of 5–10 G1 females from the G0 cross (tested females × Canton1 males, denoted P-MI, or Canton1 females × tested males, denoted M-MI) performed at 20°C. For the Su(var)205 sensitivity assay, the G0 cross was tested females × Su(var)205/Cy males at 20°C. The regulatory properties of the two kinds of G1 females (Cy/+ and Su(var)/+, respectively) were tested with the A* assay. For the thermosensitivity assay, the G0 cross was tested females × Canton1 males at room temperature (~24°C). The A* cross was performed on sets of 5–10 G1 females subjected to different aging treatments before mating. nt, no treatment (flies tested at emergence); 18°C, 15 days of aging at 18°C; 29°C, 10 days of aging at 29°C. Results obtained with Lk-P(1A) are from Ronsseray et al. (1991, 1996).
However, repression by NA-P(1A) is impaired by a mutant allele of Su(var)205. Unlike the autonomous regulatory P elements studied previously, the ability of NA-P(1A) to repress a P-lacZ transgene depends on the structure of the transgene. NA-P(1A) acts as a strong repressor of lacZ reporter expression in the PLH3 strain. As shown in Figure 4, this construct consists of a large P sequence upstream of the lacZ fusion. Conversely, NA-P(1A) does not repress a typical P-lacZ construct in line BQ16, composed only of a 5' section of the canonical P element absent from the NA-P(1A) element. Apparently, the NA-P(1A) repression capacity appears to depend on homology between itself and the euchromatic P-lacZ target.

**Is the P-element repression ability observed in the NA-P(1A) line caused by the expression of a putative HeT-A-P fusion protein?** The P element of NA-P(1A) has not been completely sequenced but restriction enzyme mapping allowed us to localize sites at the positions corresponding to the canonical P element for all enzymatic sites tested downstream of the AvaI site at nt 1045 (Figure 1). This suggests that the remaining P sequence in NA-P(1A) is similar to the corresponding sequence in the canonical P element. In spite of the fact that the telomeric NA-P(1A) P element is devoid of sequences homologous to the canonical P promoter, this element might be transcriptionally active. NA-P(1A) expression could be driven from an external flanking promoter in the adjacent HeT-A element (Figure 2). Using promoter mapping studies, Danilevskaya et al. (1997) have shown that the 3' section of a HeT-A sequence acts as a promoter when fused to a lacZ reporter gene. In addition, primer extension analysis has indicated that the transcripts extended to a position 62 nt upstream of the 3' end of the HeT-A element. A homologous sequence is found in the HeT-A segment located just upstream of the NA-P(1A) P element. A potential ATG start codon for translational initiation is also found between this position (62 nt upstream) and the P sequence, which can give rise to an in-frame fusion open reading frame (ORF). This ORF has 18 amino acids (aa) belonging to the HeT-A sequence fused with half of P-element exon 1. It is followed by exon 2 of P (and exon 3 if the last P intron is removed). NA-P(1A) could have the coding capacity for a 43-kD (or a 64-kD) protein, similar to an N-terminal truncated P polypeptide devoid of the first 221 aa encoded by exon 0 and part of exon 1. Thus, the deduced P protein of NA-P(1A) lacks the DNA-binding domain of the P polypeptides that resides in the 88 N-terminal amino acids (Lee et al. 1996; Lee and Rio 1998). This amino acid segment includes a CCHC putative metal-binding motif that is required for site-specific DNA binding (Miller et al. 1995; Lee and Rio 1998). The NA-P(1A) deduced protein also lacks the coiled-coil domain within the first leucine zipper (Andrews and Gloor 1995; Miller et al. 1995; Belenkaya et al. 1998). Hence it seems reasonable that the putative polypeptide of the NA-P(1A) element would not be able to recognize and block a canonical P promoter. However, the putative NA-P(1A) protein still retains two additional leucine zippers located in exons 1 and 2 of the canonical P (Rio 1990). In addition, the fusion of HeT-A with a P results in the formation of a presumptive new leucine zipper (data not shown). Following this line of argument, the deduced NA-P(1A) protein might still be capable of repressing P-element mobility via protein-protein interaction, e.g., by heterodimer formation with the P transposase (Rio 1990).

The ability of NA-P(1A) to elicit the pre-P cytotype suggests that a diffusible product plays a role in its repression capacity. It was first postulated that the pre-P cytotype in Lk-P(1A) corresponds to a deposit of a polypeptide repressor in the mature oocyte (Ronsseray et al. 1993). The P elements of Lk-P(1A) are expressed since transposase activity is genetically detectable using the sn’ excision assay (Ronsseray et al. 1991, 1996). However, the Lk-P(1A) expression is undetectable by Western analysis using an antibody against P proteins (S. E. Roche, S. Misra and D. C. Rio, personal communication). This suggests that such a low level of protein cannot be detected easily by immunological analyses.
and thus we cannot exclude that NA-P(1A) females produce and deposit a P-encoded polypeptide in oocytes.

_Does a trans-silencing effect play a role in the P-repression ability of NA-P(1A)?_ A case of homology-dependent transgene silencing, induced by telomeric transgenes, has been previously reported in tobacco (Vaucheret et al. 1993, 1994). In this study, a plant numberer "271" carried a telomeric silencer locus that has multiple copies of a chimeric transgene: an antisense nitritereductase cDNA (RN) under the control of the 35S promoter of the cauliflower mosaic virus. This telomeric silencing locus is highly methylated and is able to silence any euchromatic gene under the control of the same promoter (Vaucheret et al. 1993, 1994). The authors hypothesized that the silencing capacity of the telomeric 271 locus is due to the location of this locus. The telomeric position could allow a rapid scanning of the genome; the silencer locus could then recognize a homologous target and inactivate it (Matzke et al. 1994). This transcriptional silencing is correlated to the methylation of the target transgene (Vaucheret 1993). As the genome of _D. melanogaster_ was not found to be methylated at a detectable level, the structural modifications of chromatin involved in such a model for a trans-silencing effect in Drosophila could be mediated by trans-heterochromatinization (Roche and Rio 1998).

Alternatively, transgene silencing in tobacco can also involve a post-transcriptional repression component (Park et al. 1996; Vaucheret et al. 1996). It is proposed that the telomeric transgene produces RNAs that lead to the degradation of the homologous RNAs. In Drosophila, it is also possible that the pre-P cytotype is caused by a similar RNA-mediated mechanism. P-RNAs produced by the telomeric P insertion could be aberrant RNA or double-stranded RNA (dsRNA) molecules, which could induce cosuppression. In fact, post-transcriptional RNA-mediated cosuppression has been recently shown to regulate transposable element activity in Drosophila. As shown by Jensen et al. (1999a,b), non-coding RNAs of the D. melanogaster retrotransposon I can efficiently repress I-element activity in vivo. This effect is inducible by introducing modified I elements, which produce either noncoding or antisense RNAs. In addition, Kennerdel and Carthew (1998) have shown that dsRNA in Drosophila is capable of causing a null phenotype of the frizzled gene. Regarding P repression, Simmons et al. (1996) have shown that expression of antisense RNAs can lead to partial P repression. Reverse transcriptase (RT)-PCRs in Lk-P(1A) adults allowed detection of P transcripts (Roche et al. 1995). In our survey, we were unable to detect P-element transcription via Northern analysis for both Lk-P(1A) and NA-P(1A). Hence, we cannot exclude the possibility that the P element of NA-P(1A) is transcribed at a very low level in some tissues or developmental stages and the sensitivity of the method used in our study is insufficient for detecting such a weak expression level.

Under these two nonmutually exclusive models, i.e., transcriptional and post-transcriptional silencing, target sequence homology seems to play a crucial role. The NA-P(1A) transgene repression capacities, which depend on homology with the target transgene, evoke such trans-silencing phenomena. Further experiments will be necessary to determine the nature of the molecular support implied here.

The molecular genesis of the telomeric NA-P(1A) P-element insertion: At its 3’ end, the NA-P(1A) element is flanked by a TAS element, a structural feature resembling the insertion sites of the P elements of Lk-P(1A) and of Ch-P(1A) (Ronsseray et al. 1996). In general, P elements are known to insert preferentially inside TAS elements, thus providing a hotspot for P insertions (Karpen and Spradling 1992). The molecular position of NA-P(1A) within a TAS repeat differs from those of Lk-P(1A) and Ch-P(1A) (Ronsseray et al. 1996). This shows that the target elements of P(1A) insertions sampled in natural populations are not identical by descent but come from independent transpositional events. In the NA-P(1A) line, the P element lacks the first 871 bp. The He-A-P fusion observed here can therefore be explained by the following scenario: a full-sized P element is first inserted inside TAS, then a terminal deletion occurs, removing the upstream TAS section and the 5’ end of the P element (5’ inverted repeat, exon 0 and half of exon 1); an incomplete replication of the chromosome over generations may have helped to achieve this. Finally, a He-A copy transposes at the telomere, thus healing the broken X chromosome. In situ hybridization on polytene chromosomes with a TAS probe showed signals at 1A in all three P(1A) lines tested [i.e., Lk-P(1A), Ch-P(1A), and NA-P(1A)], but the signal intensity obtained on the tip of the X chromosome for NA-P(1A) was significantly weaker compared to those of Lk-P(1A) and Ch-P(1A) (data not shown), suggesting that the 1A TAS cluster of NA-P(1A) is shorter than those of the other two P(1A) lines. This observation is consistent with our terminal deletion model. Furthermore, the genomic organization of the telomeric NA-P(1A) element, derived from a natural sample, resembles that of some telomeric P-reporter insertions that originated in the laboratory. The P-w½ line harbors a P-w+ transgene in one of the telomeres of the second chromosome (2L). This transgene was found to be truncated at its 5’ end. Like NA-P(1A), it is flanked in 3’ by a TAS and in 5’ by a He-A sequence (H. Biessmann and J. Mason, personal communication). Furthermore, in the P-833 line harboring a P-w+ transgene in the telomere of the third chromosome (3R), Sheen and Levis (1994) showed that spontaneous terminal deletions and incomplete replication removed a part of the transgene and the flanking distal DNA; the telomere was then rescued by a terminal TART insertion.

From an evolutionary point of view, the NA-P(1A) insertion is an immobile regulatory P element since it
lacks the 5' terminal repeat: such a copy could confer a selective advantage by suppressing the deleterious effects caused by P-induced hybrid dysgenesis. Furthermore, a molecular structure like the one described in the present study could thus serve as a starting point for an evolutionary process termed “molecular domestication” (Miller et al. 1992): independent molecular transitions of formerly active P elements into chromosomal neogenes have indeed been described earlier in other Drosophila species (Paricio et al. 1991; Miller et al. 1992, 1995; Nouaud and Anxolabéhère 1997; Nouaud et al. 1999; for recent review, see Miller et al. 2000).

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