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A *P* element of *Scaptomyza pallida* is active in *Drosophila melanogaster*

(transposable elements/evolution/Drosophilidae/hybrid dysgenesis)

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ABSTRACT **Several results suggest that *P* elements have recently invaded natural populations of *Drosophila melanogaster* after a horizontal transfer from another species. The donor species is thought to come from the *willistoni* group, which contains *P* elements very homologous to those of *D. melanogaster*. However, more divergent *P* elements are present in many other Drosophilidae species. We have analyzed such elements from *Scaptomyza pallida*, a species phylogenetically distant to *D. melanogaster*. We report here the isolation of two coding *P* elements from *S. pallida* (PS2 and PS18) that are 4% divergent from one another. At least one of these elements (PS18) is active since it is able to transpose in *D. melanogaster* and to mobilize a *D. melanogaster* defective *P* element, even though its nucleotide sequence is 24% divergent from the canonical *P* element of *D. melanogaster*. To our knowledge, a *P* element that is active and strongly divergent from the *D. melanogaster P* element has not been reported previously. Sequence comparison between the complete *P* elements of *D. melanogaster* and *S. pallida* reveals that the structural characteristics are maintained: PS2 and PS18 contain terminal inverted repeats and internal repeats very similar to those of the *D. melanogaster P* element. In addition, the noncoding regions cis necessary for the transposition are more conserved than the coding sequences. Two domains found in the *D. melanogaster P* transposase (helix–turn–helix and leucine zipper) are well conserved in the putative proteins encoded by PS2 and PS18. This study provides insights into which parts of *P* elements are functionally important and correlates with functional studies of the *P* element in *D. melanogaster*.**

Since the first descriptions of transposable elements, the question of their origin and evolution has been of great interest. A simple way to study this problem is to determine the distribution of transposable elements within a set of species whose phylogenetic relationships are well known. The Drosophilidae family is an excellent model for this analysis, since it has been subject to extensive phylogenetic studies (1, 2) and since >30 different families of transposable elements have been described in *Drosophila melanogaster* (3). The survey of the distributions of various transposable elements in the Drosophilidae family has led to the conclusion that transposable elements are unstable components of the genomes. In most cases, their distribution within the species shows phylogenetic discontinuities, which suggests two properties of transposable elements: (*i*) they can diverge rapidly and may be lost in some species by divergence or genetic drift (4–6); (*ii*) their transmission does not seem to be strictly vertical and some data might be explained by horizontal transfer between species (6–8).

The evolutionary history of *P* elements is particularly interesting since *P* elements are present only in a subset of strains within the *D. melanogaster* species. These elements are widely distributed in natural populations but are completely lacking in older laboratory strains (9). The particular distribution of *P* elements in *D. melanogaster* is responsible for PM hybrid dysgenesis. Indeed, *P* elements have been shown to be the causative agents of this phenomenon (a set of genetic abnormalities), which appears in the progeny of certain crosses between strains containing many *P* elements and strains devoid of them (see ref. 10 for review). Two types of *P* elements occur in *D. melanogaster*: the autonomous or complete *P* elements, which are 2.9 kilobases (kb) long and encode a transposase, and the nonautonomous or defective ones, which contain internal deletions of varying length. Both kinds of elements are terminated by 31-base-pair (bp) inverted repeats and, except for the internal deletions, they are very homogenous in sequence. Defective elements are able to transpose if transposase is provided in trans; the transposition of all *P* elements produces an 8-bp direct duplication at the insertion site.

The lack of *P* elements in older strains of *D. melanogaster*, as well as in the species most closely related to *D. melanogaster* (11), suggests that *P* entered the *D. melanogaster* genome recently, probably by horizontal transfer from another species. Extensive surveys of the distribution of *P* homologs in the Drosophilidae have shown that they are present in many of the species throughout the four species groups (*melanogaster, obscura, willistoni*, and *saltans*), which form the subgenus *Sophophora* (6, 7, 12–15). *P* elements from the *willistoni* group appear to be the most similar to those of *D. melanogaster* (6, 14), and recently a functional *P* element has been isolated from *D. willistoni* (6). Its sequence is identical to the canonical *P* element of *D. melanogaster*, except for one base substitution. This strongly suggests a horizontal transfer of *P* transposon between *D. willistoni* and *D. melanogaster*. More divergent *P* elements occur in other species from the *Sophophora* subgenus and from some closely related subgenera (reviewed in ref. 6), but they are also found in species from the *Scaptomyza* genus, more distant to the *Sophophora* subgenus (6, 13). We have studied these divergent *P* elements from *Scaptomyza pallida* to determine if they could be functional and to compare their behavior with that of the *D. melanogaster P* elements.†

MATERIALS AND METHODS

***Drosophila* Strains.** The *S. pallida* stock originated from flies caught in France in 1985 by Georges Périquet and maintained in our laboratory.

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Abbreviation: ORF, open reading frame.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M63341 for PS2 and M63342 for PS18).

The *D. melanogaster* strain $z^1w^{1118}P[w^{dl}9.3]$ (19DE) carries the $P[w^{dl}]$ transgene, which contains 0.8 kb of *P* extremity sequences and the whole white gene (16).

Construction of the *S. pallida* Library and Cloning of *P* Elements from This Species. A random library of the *S. pallida* stock (France) was constructed in phage λEMBL4 as described (8). This library was screened by plaque hybridization using a mixture of two internal *P* fragments from pπ25.1 (17) (1.5-kb *Sal* I–*Hin*dIII and 0.8-kb *Hin*dIII–*Hin*dIII) labeled with ^{32}P by nick-translation. These two fragments cover 2.3 of the 2.9 kb of the *D. melanogaster* autonomous *P* element. Hybridization conditions were as follows: 5× Denhardt's solution and 6× standard saline citrate (SSC)/0.5% SDS at 65°C; wash at 65°C in 2× SSC/0.1% SDS. Nine defective *P* elements and two potentially full-length elements (PS10 and PS2) were isolated in this screen. In an effort to isolate other full-length *P* elements from *S. pallida*, the library was rescreened using, in succession, four probes corresponding to four different internal fragments of PS10. The four fragments cover the whole sequence of PS10 and only phages hybridizing to all four probes were selected. Another two elements, one defective and one full-length (PS18), were isolated in this second screen. The location of the *P* elements in the recombinant phage was determined by restriction mapping and Southern blotting using different parts of pπ25.1 as probes. Hybridization conditions were as described above. DNA fragments containing PS10, PS2, and PS18 were subcloned into pUC8 and pUC13.

DNA Sequencing. Appropriate restriction fragments were cloned into M13 vectors. Nested deletions were generated using exonuclease III (18). Sequences were determined by dideoxy chain-termination sequencing (19) using Sequenase (United States Biochemical).

Transformation. A 6-kb *Sal* I–*Hin*dIII fragment containing PS18 was cloned into pUC8. The resulting plasmid, pPS18HS, was purified on a CsCl density gradient and microinjected (800 μg/ml) into embryos from the $z^1w^{1118}P[w^{dl}9.3]$ (19DE) strain of *D. melanogaster* according to the standard procedure (20).

Other Procedures. All other procedures were carried out as described (8).

RESULTS

***S. pallida* Contains Full-Length *P* Elements.** From preliminary Southern analyses, it appeared that *P* elements of *S. pallida* were divergent from the *P* elements of *D. melanogaster* based on the signal strength and the absence of internal restriction fragments of the *D. melanogaster* autonomous *P* element in the *S. pallida* genome. To clone these divergent *P* elements, a random genomic library of the *S. pallida* stock (France) was made in the bacteriophage λEMBL4. This library was screened first with the *D. melanogaster* *P* element DNA [pπ25.1 (17)] and then with DNA from a full-length *P* element of *S. pallida* (see *Materials and Methods*). Thirteen independent *P* elements from the *S. pallida* genome were isolated and analyzed by restriction mapping.

Each of these elements isolated possesses a specific restriction map, differing from one another and also differing from the autonomous *P* element of *D. melanogaster* restriction map (M.S., unpublished data). Most of the *S. pallida* *P* elements are defective, but three (PS10, PS2, and PS18) appear to be full-length. The restriction maps and the sequence of the ends of these three elements are shown in Fig. 1. Like the autonomous *P* element of *D. melanogaster*, all three are about 3 kb long and they are terminated by 31-bp inverted repeats, similar in sequence to those of *D. melanogaster* *P* elements. PS2 and PS18 have perfect terminal repeats, which differ from the terminal repeats of the *D. melanogaster* *P* elements by only 1 bp for PS2 and 2 bp for PS18. Thus, for PS2 and PS18, the inverted repeats are

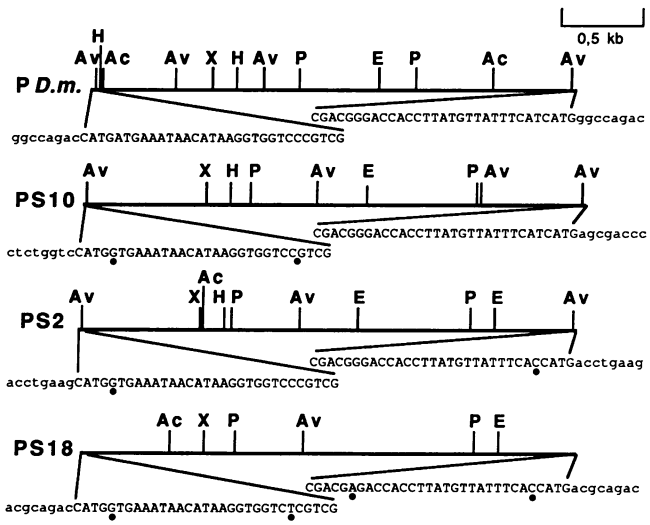

FIG. 1. Restriction maps of the full-length *P* elements of *S. pallida* and *D. melanogaster* (*D.m.*) and sequences of their extremities. The 31-bp inverted repeats are marked in uppercase letters. Dots indicate mismatches in these repeats between *S. pallida* and *D. melanogaster* *P* elements. The 8-bp target sequences are indicated in lowercase letters. For the *D. melanogaster* *P* element, the target sequence given is the published consensus (17). Ac, *Acc* I; Av, *Ava* II; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; X, *Xho* I.

identical within an element, but they are different between elements. This suggests that these repeats may have been homogenized during or after the transposition event. Moreover, like the *D. melanogaster* *P* elements, PS2 and PS18 are flanked by the direct duplication of an 8-bp target sequence. As expected, the target site is different between PS2 and PS18, but for each element the 8-bp duplication is perfect. These results indicate that no mutational event has occurred at or near the ends of these two elements since their last transposition. In contrast, the terminal repeats of PS10 are 2 bp divergent from one another, and this element is not flanked by an 8-bp direct repeat. PS10 could have resulted from a rearrangement between two different *P* elements. Such *P* elements with recombinant ends have been described in *D. melanogaster* and are found at the breakpoints of chromosomal rearrangements (21).

The restriction maps of these three elements of *S. pallida* are related, but they are quite different from that of the *D. melanogaster* autonomous *P* element (Fig. 1). This suggests that the internal regions are not as well conserved as the terminal repeats.

Sequence Analysis of Two Potentially Active *P* Elements of *S. pallida*. We have determined the complete sequence of PS2 and PS18 (Fig. 2). These two *P* elements of *S. pallida* are not identical; the overall divergence between them is 4% and suggests that they have been evolving independently for several million years. Nevertheless, both of them contain four ORFs that match those of the *D. melanogaster* autonomous *P* element (17) (see Fig. 2).

PS2 and PS18 are 77% and 76%, respectively, homologous to the autonomous *P* element of *D. melanogaster*. This homology is widespread throughout both coding and noncoding sequences. The level of homology is lower over the last ORF (ORF3, see Fig. 2) (73%), but it is high (83–85%) over the noncoding regions that flank the terminal repeats (≈100 bp at the 5′ end and 170 bp at the 3′ end). This suggests that these noncoding sequences play an important role. The ends of *P* elements including the terminal inverted repeats and internal sequences beyond them have been shown to be cis necessary for transposition in *D. melanogaster* (24, 25). Moreover, *in vitro* DNA-binding experiments with the *D.*

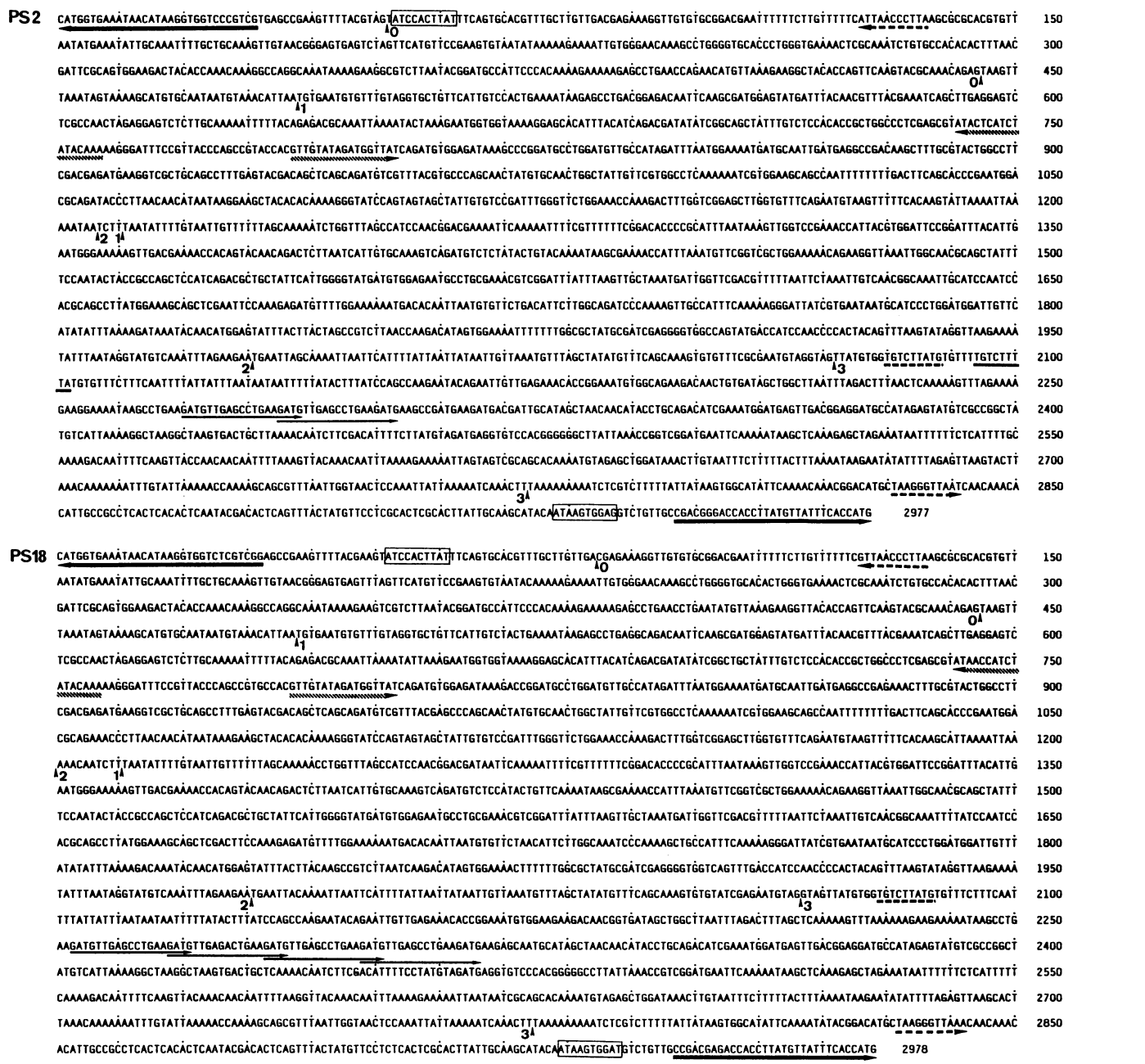

FIG. 2. Nucleotide sequences of PS2 and PS18. The inverted terminal repeats are underlined with solid arrows. For PS18 these repeats even extend to 32 bp, as for the functional *P* element of *D. willistoni* (6). Internal repeats, either inverted or direct, that have been described in *D. melanogaster* (10) are underlined with broken, shaded, or thin arrows. The 10-bp transposase binding sites that have been defined at both ends of the *D. melanogaster P* element (22) are boxed. The 9-bp motif that matches the Tn*3* resolvase binding site (23) is indicated with a thin line and the 9-bp sequence that resembles this motif (see text) is underlined with a broken line. Limits of the four open reading frames (ORFs) (0, 1, 2, and 3) are indicated by vertical arrowheads.

melanogaster transposase have allowed the definition of a 10-bp transposase binding site near each end of the *D. melanogaster P* element (22). The corresponding sequences for PS2 and PS18 are shown in Fig. 2. They differ from the *D. melanogaster* transposase binding sites by only one or two base substitutions and, in the case of PS18, both sites form a perfect inverted repeat.

In addition to the terminal repeats, internal repeats, either inverted or direct, occur in the *D. melanogaster P* element (10). Their functional significance is not known, except for the 11-bp subterminal inverted repeat whose presence, although not strictly necessary, appears to aid in transposition (25). These internal repeats exist in PS2 and PS18 and are depicted in Fig. 2. They are, in general, not perfect within these elements; however, they are more conserved than the rest of the entire *P* sequence when compared with the *D. melanogaster P* element. The direct repeat located in ORF3 of PS18 is unusual in that it contains four copies of the same sequence rather than two (see Fig. 2). Such rearrangements of a duplicated sequence within an engineered *P* element have been described for *D. melanogaster* and are transposase-dependent (D. Coen and D.A., unpublished data).

A 9-bp motif has been identified in intron 2-3 of the *D. melanogaster P* element, which matches the resolvase binding site of the bacterial transposon Tn*3* (23). An identical sequence is found in a similar region of PS2 (see Fig. 2). In PS18, the region containing this motif is deleted; however, another 9-bp sequence that is still well conserved with

respect to the Tn*3* resolvase binding site consensus [TGTCYNNTA (26)] occurs 5 bp upstream of the deletion site (see Fig. 2). This 9-bp sequence in PS18 contains one mismatch with respect to the consensus. A similar sequence is also found upstream of the Tn*3* resolvase binding motif of PS2 and the *D. melanogaster P* element. It is not known whether this 9-bp sequence has a function.

When the putative proteins encoded by PS2 and PS18 are compared with the *D. melanogaster P* transposase, the overall homology is similar to that obtained at the DNA level. This suggests that most nucleotide substitutions are not silent. The *D. melanogaster P* transposase contains a helix–turn–helix motif near the ORF1–ORF2 boundary, which corresponds to a potential DNA-binding domain (27) and a leucine zipper, located in ORF1, which could be involved in transposase dimerization (28). The conservation of both motifs in PS2 and PS18 is shown in Fig. 3 and reinforces the hypothesis of their functional importance.

Recently, two other regions in the *D. melanogaster* transposase have been described as leucine zipper-like motifs (amino acids 283–311 in ORF1 and 497–525 in ORF2) (28). However, their accordance with the leucine zipper motif is weaker. Interestingly, in PS2 and PS18, both of these regions should not correspond to functional leucine zippers, since the first one contains a proline, which would prevent the helical structure, and in the second one a hydrophilic amino acid is present where there should be a leucine or another hydrophobic amino acid.

PS18 Is Active in *D. melanogaster*. To test the ability of PS18 to mobilize a defective *D. melanogaster P* element, PS18 DNA was microinjected into embryos from the *D. melanogaster* strain $z^1w^{1118}P[w^{dl}9.3]$ (19DE) (see *Materials and Methods*). This strain contains only one *D. melanogaster* defective *P* element ($P[w^{dl}9.3]$), which is labeled with the white gene and gives a colored eye phenotype (16). Excision of this defective *P* element would result in the loss of the white gene and a white eye phenotype. We recovered white-eyed progeny in 1 of the 34 lines established after microinjection. This suggests that PS18 encodes a protein that is capable of recognizing a defective *P* element of *D. melanogaster* and of inducing its excision. To determine if PS18 could transpose itself in the *D. melanogaster* genome, we performed squash blot experiments [hybridization of flies squashed onto nylon filters (29)] on the 34 injected lines using a probe specific to PS18 (1.4-kb *Pst* I–*Pst* I internal fragment of PS18; see Fig. 1). A strong hybridization with the probe was detected in flies from one of the lines (data not shown) and probably resulted from the integration of PS18 in the genome of this line. This was confirmed by DNA blotting and *in situ* hybridization experiments. In Fig. 4*a*, DNAs from the transformed line and from a plasmid including PS18 were digested with *Pst* I and hybridized with a clone containing the 1.4-kb *Pst* I–*Pst* I internal fragment of PS18. This 1.4-kb fragment is specific to *S. pallida* full-length *P* elements and is not present in the *D. melanogaster* autonomous *P* element (see Fig. 1). The presence of this fragment in the transformed line's DNA indicates that PS18 has been integrated into the genome of this line. The location of integration in 13A, on the X chromosome, was determined by *in situ* hybridization experiments (Fig. 4*b*).

These results suggest that PS18 is able to catalyze its own insertion into the *D. melanogaster* genome. It is thus an autonomous *P* element: it possesses the coding capacity for transposase and the structure necessary for the transposition.

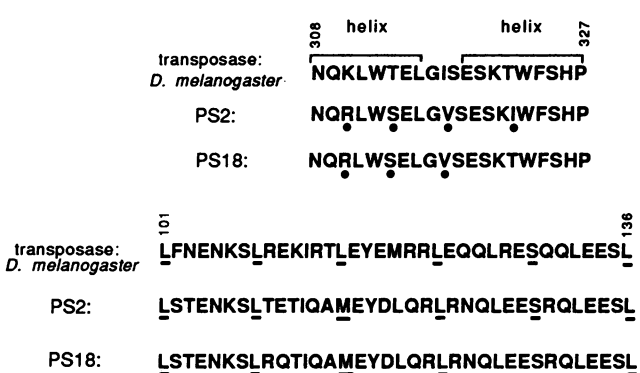

FIG. 3. Comparison of the helix–turn–helix and leucine zipper domains of the *D. melanogaster* transposase and of the putative proteins encoded by PS2 and PS18. (*Upper*) Helix–turn–helix domain. The dots indicate amino acid changes between the *S. pallida* and *D. melanogaster* proteins. This region is highly conserved between *S. pallida* and *D. melanogaster* (82% homology for PS2 and 88% for PS18) and the amino acid changes, in PS2 and PS18, do not prevent the bihelical motif. (*Lower*) Leucine zipper domain. The leucines found every seven amino acids are underlined. In the *D. melanogaster* transposase, what would be the fifth leucine is a serine, and there is an additional sixth leucine. In proteins potentially encoded by PS2 and PS18, the positions of the leucines are strongly conserved, although the whole region presents only 60% homology between *S. pallida* and *D. melanogaster*.

DISCUSSION

Our results show that functional *P* elements can be divergent from the *D. melanogaster* canonical *P* element. Two other species, *Drosophila nebulosa* and *D. willistoni*, have been extensively examined for the presence of active *P* elements. Although no such element was found in *D. nebulosa* (30), one functional element was isolated from *D. willistoni*. However, the sequence of this element is identical to the *D. melanogaster* autonomous *P* element, except for 1 bp (6). Sequence comparison between the *D. melanogaster* autonomous *P* element and *S. pallida* full-length elements PS2 and PS18 reveals that although they are divergent (23% for PS2 and 24% for PS18), their general structures are similar. Like the

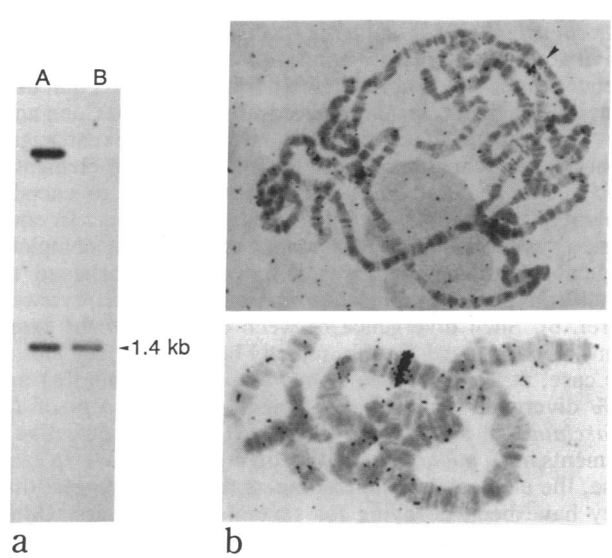

FIG. 4. Detection (*a*) and localization (*b*) of PS18 in the *D. melanogaster* transformed line. (*a*) *Pst* I digests of the PS18-containing plasmid (lane A) and of the transformed line genomic DNA (lane B) probed with a plasmid containing the 1.4-kb *Pst* I–*Pst* I internal fragment of PS18 (see Fig. 1). (*b*) *In situ* hybridizations to polytene chromosomes of the transformed line. ^{3}H-labeled PS10 DNA was hybridized to chromosome squashes. Only one *P* site per genome was detected (upper); it is located in 13A on the X chromosome (lower). The transposon $P[w^{dl}9.3]$ in 19DE, which contains 0.8 kb of *D. melanogaster P* sequence, is not detected with this heterologous probe.

autonomous *P* element of *D. melanogaster*, PS2 and PS18 are flanked by an 8-bp direct duplication; they contain 31-bp terminal inverted repeats and internal repeats that are particularly well conserved with respect to those of the *D. melanogaster P* element. For PS2 and PS18, the inverted terminal repeats are perfect within an element. Moreover, the noncoding sequences directly adjacent to these terminal repeats are more conserved than the coding regions. This is in good agreement with functional studies of the *P* element in *D. melanogaster*, which have shown that the terminal inverted repeats, as well as some sequences adjacent to them, are cis required for transposition (24, 25). These regions include the 10-bp transposase binding sites found at both ends (22). These data suggest that a strong selection pressure maintains the structural organization of *P* elements. The sequencing of a defective *P* element from another species, *Drosophila bifasciata*, has given results that correlate with ours, since the terminal repeats and the subterminal noncoding sequences are also well conserved in this species (31).

The selection pressure appears to be weaker at the coding sequence level. The putative proteins deduced from the nucleotide sequences of PS2 and PS18 contain the helix–turn–helix and leucine zipper motifs found in the *D. melanogaster P* transposase (Fig. 3). When compared with the *D. melanogaster* transposase, the C-terminal part of PS2 and PS18 proteins, encoded by the end of ORF3, presents a weaker homology (70% for PS2 and 73% for PS18) than the rest of the protein (76% for PS2 and 77% for PS18). In *D. melanogaster*, the amino acids encoded by ORF3 are necessary for transposase function (27) and shorter proteins whose translation stops in intron 2-3 or within ORF3 correspond to repressors of transposition (32, 33).

We have shown that although the region that is supposed to be essential for transposase activity is less well conserved, the protein encoded by PS18 has functions similar to those of the *D. melanogaster* transposase. This protein is able to promote PS18 integration into the *D. melanogaster* genome and to excise a *D. melanogaster* defective *P* element. The strong structural similarities between *P* elements from *D. melanogaster* and *S. pallida* may account for the interspecies activity of the *S. pallida* transposase. Moreover, the sequence amplification noted in PS18 (Fig. 2) suggests that this protein, like the *D. melanogaster* transposase (D. Coen and D.A., unpublished data), might be able to produce rearrangements of duplicated sequences located within a *P* element.

Although PS2 and PS18 are potentially able to encode proteins, they are 4% divergent from one another. In contrast, *D. melanogaster P* elements show almost complete sequence homogeneity. This homogeneity is believed to result from the recent presence of *P* in this species (reviewed in ref. 6). Such divergence between elements of the same family, within a species, has already been described. This is the case, at least, for two *D. nebulosa P* elements that are 10% divergent (30), for two defective *P* elements of *D. bifasciata* that are 8.5% divergent (31), and for defective *I* elements in *D. melanogaster* that diverge by 7% (34). In each case, the elements are noncoding, and the data suggest that they have been decaying for several million years. Other results concerning the structure and the genomic location of defective *P* elements in *S. pallida* (M.S., unpublished data) indicate that *P* elements are old components of the genome of this species. Thus, sequence divergence between *P* elements from *S. pallida* is to be expected. However, the question still remains of why two different coding *P* elements have been maintained in this *S. pallida* population. One hypothesis is that the function of PS2 may differ from that of PS18. It should be noted that the divergence in the C-terminal part of the *D. melanogaster* and *S. pallida* proteins is higher for PS2 than for PS18 (see above). The study of PS2 function as well as a study of its functional relationships with PS18 are possible in *D. melanogaster* and may provide a better understanding of the evolution and the regulation of *P* elements. Alternatively, the divergence could be explained by the entry of either PS2 or PS18 in the *S. pallida* genome by horizontal transfer from a closely related species. *P* homologs have, indeed, been found in other *Scaptomyza* species (6).

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