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Mobility of P elements in drosophilids and nondrosophilids

(transposons/excision assay/Drosophilidae/Tephritidae)

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ABSTRACT  The mobility properties of the Drosophila melanogaster P element in drosophilid and nondrosophilid species has been determined using a P-element mobility assay that is conducted transiently in insect embryos. P elements are mobilizable in all drosophilids tested, including species outside the genus Drosophila but not in the related Tephritidae (order: Diptera), although the P-element gene necessary for mobility, transposase, is transcribed. These results show that without modifications P elements will not serve as general insect gene vectors and suggest that nonconserved host-encoded factors participate in the transposition of P elements. Our methods will be generally useful for analyzing the cis- and trans-acting factors required for P-element mobility in vivo and could be used to analyze the mobility properties of other transposable elements in insects.

P elements are highly mobile transposable elements originally isolated from Drosophila melanogaster, but also found in other species in the genus Drosophila (1-4). The mobility properties of this transposon allowed its development into an efficient gene vector, which significantly enhanced our ability to study all aspects of Drosophila biology (5, 6). The ability of P elements to be mobilized in the sibling species Drosophila simulans (7) and the more distantly related species Drosophila hawaiiensis (8) suggested that P-element movement is phylogenetically unrestricted, allowing its use as a general gene vector in a variety of insect and noninsect systems. This possibility was enhanced by the development of P-element vectors carrying the dominant selectable marker neomycin phosphotransferase, which potentially allowed testing of P-element transformation in heterologous systems (9). Nevertheless, the most straightforward transformation experiments would not measure P-element mobility directly and would rely on a selection system that is untested in most insects. Thus, negative results would not necessarily reflect a failure of P elements to be mobilized and would reveal few insights into the function of the P-element transposon system in nondrosophilids. Although a single gene-transformation event was reported for one nondrosophilid species, Anophetes gambiea (10), this event did not result from P-element transposition.

An initial step in assessing the utility of the P element as a general gene vector in insects is to directly determine the phylogenetic limits of P-element mobility. If limits do exist, understanding their basis will undoubtedly reveal information about the mechanisms and regulation of P-element transposition as well as an understanding of the origin and distribution of P elements within the genus. Determining the phylogenetic limits of P-element mobility will depend upon monitoring either P-element transposition and insertion or P-element excision. P-element insertions often cause partial or complete gene inactivation, and reversion of these insertional mutations usually results from the precise, or nearly precise, excision of the transposon (11, 12). In D. melanogaster, P-element excision is related to P transposition in that it requires the P-encoded polypeptide transposase and an M cytotype (6, 13). The mechanistic relationship between P-element excision and transposition is unknown, but because P-element excision is biochemically related to transposition, it provides a way of directly monitoring the function of the P-element transposon system.

Rio et al. showed that P elements can excise not only from resident sites in the genome, but also from plasmids introduced into insect (14) and mammalian cell (15) lines producing P transposase. Here we describe a modification of the in vitro P-element excision assay of Rio et al. (14) to directly assess P-element mobility in insect embryos. This assay can evaluate function of the P-element system in nondrosophilids and facilitates an analysis of mechanisms that regulate P-element mobility in Drosophila. We find that P elements can be efficiently mobilized in a variety of drosophilids, including representatives outside the genus Drosophila, but not in the related Tephritidae. These results are significant because they indicate that P-element mobility in vivo is phylogenetically restricted, limiting the utility of P elements as a general insect gene vector. Furthermore, these results suggest that nonconserved, host-encoded functions other than the P-element-encoded transposase are necessary for P-element mobility. This method should be generally applicable to the analysis of P-element function in other insects and to the identification and analysis of other insect transposable elements.

MATERIALS AND METHODS

Plasmids. The plasmids pISP and pISP-2 are P-element excision indicator plasmids constructed and used by Rio et al. (14) to assess P transposase activity in cell lines (Fig. 1). These plasmids consist of a 47-base pair (bp) fragment from the D. melanogaster white gene containing a P-element target sequence inserted into the polylinker region of pUC8 so as to retain the lacZa-encoded peptide-complementing function of that plasmid. Into the P-element target sequence was inserted a 600-bp nonautonomous P element resulting in an 8-bp duplication of the white sequences adjacent to P and a loss of LacZa complementation (14). The plasmids pISP and pISP-2 were used interchangeably and are referred to as pISP. pUCHsπ2Δ3-3 has been described and consists of the 4.6-kilobase (kb) BamHI fragment from pCNΔ2-3 containing the P-element transposase gene (open reading frames 0–3) lacking the third intron and under the promoter control of the D. melanogaster 70-kDa heat shock protein gene inserted into the BamHI site of pUC8 (16). Insertion of the BamHI fragment eliminates the LacZa-complementing function of this plasmid. The plasmid pACYCCHhsπ2Δ3 was constructed by inserting the 4.6-kb BamHI fragment containing hsπ2Δ3 from pUCHsπ2Δ3 into the BamHI site of pACYC184 (17), which does not contain the LacZa peptide-coding region or confer ampicillin resistance. Although both pUCHsπ2Δ3-3 and pACYCCHhsπ2Δ3 encode transposase, the P-element se-
RESULTS

Using an in vivo P-element excision assay we measured the mobility of P elements in embryos of seven drosophilid and two tephritid species, including the Caribbean fruit fly Anastrepha suspensa and the papaya fruit fly Toxotropane curvicauda. Table 1 shows that P-element excision from the pISP indicator plasmid occurred at a frequency ranging from $10^{-4}$ to $10^{-7}$ per pISP plasmid recovered from embryos of all drosophilids tested including the D. melanogaster P strain Harwich, Chymomyza pronemesis, and Zaprinus tuberculatus. Restriction endonuclease digestion of LacZ+ pISP plasmids with HindIII and EcoRI confirmed the loss of P sequences (Fig. 2). The frequency of excision in the distantly related drosophilids C. pronemesis and Z. tuberculatus was less by a factor of 10 than in the D. melanogaster M strain. Screening more than $10^8$ plasmids from the tephritids A. suspensa and T. curvicauda failed to reveal evidence of P-element excision events (Table 1).

In control experiments in which only pISP or pUCHsΔ2-3 was injected, LacZ+ plasmids were occasionally recovered from some species. These LacZ+ plasmids were only recovered when plasmid DNA was maintained in insect embryos and were not recovered when purified plasmid DNA was used to transform DH5α cells (data not shown). Therefore, the LacZ+ plasmids were not rare contaminants of the original plasmid preparation but were the result of recombination events. Although the frequency with which LacZ+ plasmids were recovered after injection of only pUCHsΔ2-3 was one per $10^5$ plasmids, we find that this plasmid perhaps due to its larger size is not recovered as efficiently as pISP. In coinjection experiments using pISP and pUCHsΔ2-3, 10% of recovered plasmids were pUCHsΔ2-3. Therefore, during coinjection experiments LacZ+ pUCHsΔ2-3 plasmids were a small fraction of the total LacZ+ plasmids recovered. Because these putative spontaneous rearrangements were rare and inconsistently detected,
Table 1. Frequency of P-element excision from plasmids transiently maintained in insect embryos

<table>
<thead>
<tr>
<th>Host insect</th>
<th>pUChsΔ2-3 (helper 1) Frequency (× 10⁻²)</th>
<th>n (× 10⁵)</th>
<th>pACYChsΔ2-3 (helper 2) Frequency (× 10⁻²)</th>
<th>n (× 10⁵)</th>
<th>No helper Frequency (× 10⁻²)</th>
<th>n (× 10⁵)</th>
<th>pUChsΔ2-3 alone Frequency (× 10⁻³)</th>
<th>n (× 10⁵)</th>
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<tr>
<td>Drosophilidae</td>
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<td>Subgenus Sophophora</td>
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<tr>
<td>D. melanogaster M (Adh623 en; ryr3006)</td>
<td>1.7</td>
<td>82</td>
<td>1.6</td>
<td>11</td>
<td>0</td>
<td>69</td>
<td>0.15</td>
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<td>D. melanogaster P Harwich</td>
<td>0.5</td>
<td>82</td>
<td>0.6</td>
<td>15</td>
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<td>22</td>
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<td>D. simulans</td>
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<td>47</td>
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<td>C. pronemis</td>
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<td>Toxotrypana curvicauda</td>
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</table>

*Frequency = LacZ⁺ per pISP recovered; n = pISP recovered; when pUChsΔ2-3 was used as a helper, we estimated n to be 0.9 × ampicillin-resistant colonies (data not shown).

† Frequency = LacZ⁺ per pUChsΔ2-3 recovered; n = pUChsΔ2-3 recovered.

their occurrence did not significantly affect the observed excision frequencies from pISP derived from coinjection experiments. To avoid any possible ambiguity from recovery of LacZ⁺ helper plasmids, we constructed a plasmid containing hs870-2 on the P15A replicon pACYChs184, which does not contain a LacZa peptide-coding region or a β-lactamase gene (17). This permitted screening of only pISP plasmids after coinjection of pISP and pACYChsΔ2-3. Table 1 shows that use of this new helper plasmid resulted in excision frequencies similar to those obtained using the helper pUChsΔ2-3.

Although heat shock of D. melanogaster embryos was not required to detect P-element excision (data not shown), the transcriptional activity of the hsp70 promoter in tephritids was undetermined. Therefore, RNA blot analysis was conducted on total RNA isolated from A. suspensa and D. melanogaster (M strain) embryos injected with the helper plasmid pUChsΔ2-3. The results demonstrated the presence of P-element-homologous RNA species 3.0, 1.5, and 1.0 kb in length in both A. suspensa and D. melanogaster (Fig. 3). These transcription products were not detected in uninjected embryos. In both species the 3.0-kb transcript was seen in all experiments; however, the occurrence of the 1.5- and 1.0-kb transcripts was variable. We also occasionally detected a 3.5-kb transcript in both D. melanogaster and A. suspensa embryos injected with helper plasmids. In comparison, the most prominent P-element transcripts in the P strain r2 are 2.5, 1.4, and 0.9 kb, whereas strains harboring a single copy of the P-element Pcryr, from which hsp870-2 was derived, contain P-element transcripts between 2.5 and 3.0 kb (25). The P-element transcription products seen in tephritids are therefore consistent with transcript sizes in Drosophila seen here and in previous studies. These results demonstrate that the hsp70 promoter can function in tephritid species.

Fig. 2. Restriction endonuclease digestion of LacZ⁺ pISP plasmids recovered during the excision assay. All plasmids were digested with EcoRI and HindIII. Digestion of pISP (lane a) and pISP2 (lane b) results in a 600-bp fragment containing the P element (arrow). pISW⁺ (lane c), a pUC8 plasmid containing only a 47-bp fragment of the Drosophila white gene, and all Lac-Z⁺ pISP plasmids (lanes d–g) did not have the 600-bp fragment containing P sequences. DNA size standards are in kb.

Fig. 3. Detection of P-element transcripts in D. melanogaster and A. suspensa embryos injected with pUChsΔ2-3. Lanes: a, total RNA from 100 Adh623 cnyr3006 embryos; b, total RNA from 100 Adh623 cnyr3006 embryos injected with pUChsΔ2-3; c, total RNA from 100 A. suspensa embryos; d, total RNA from 100 A. suspensa embryos injected with pUChsΔ2-3. Transcript sizes are in kb. Arrows refer to plasmid DNA that is occasionally recovered during RNA preparation from injected embryos. The probe was a uniformly labeled 700-nucleotide single-stranded antisense RNA complementary to transposase open reading frame 0 and a portion of open reading frame 1.
DISCUSSION

We report data that indicate *D. melanogaster* P-element mobility in vivo to be phylogenetically restricted. Using a modification of the P-element excision assay of Rio et al. (14), we directly assessed P-element mobility in insect embryos. P-element excision was detected in all drosophilids tested, including species outside the genus *Drosophila*. With this assay P-element excision was found to be transposase dependent in all drosophilids tested except *D. melanica* and *Z. tuberculatus*, where spontaneous excision from pISP was observed with a frequency lower by a factor of 10 (5 × 10^{-5}) than when transposase was present. Because these species do not contain P elements (ref. 4 and unpublished observations) these excisions might be catalyzed by a functionally homologous protein encoded by another host-encoded gene. Spontaneous excisions were not observed in *A. suspensa* and *T. curvicauda* or in the other drosophilids tested. Thus the in vivo P-element excision assay represents a sensitive method to monitor P-element mobility.

The frequency of P-element excision in drosophilids depended on the relatedness of the species to *D. melanogaster*—with distantly related species being less capable of supporting P-element mobility. Embryos of species outside the family Drosophilidae, *A. suspensa* and *T. curvicauda* (family: Tephritidae), were incapable of supporting P-element excision. Although drosophilids and tephritids diverged ~120 million years ago (26) a number of other families are more closely related to Drosophilidae than are tephritids. Analyzing the mobility of P elements in representatives of these families should allow us to determine more precisely the limits of this phylogenetic restriction. Preliminary results of the excision assay conducted in species from families more and less related to Drosophilidae than are tephritids support our conclusion that P mobility is phylogenetically restricted. Significantly, these results suggest that without modification P elements will not be useful as gene vectors in Diptera other than drosophilids.

Although interest exists in the use of P elements as gene vectors in insects of economic and medical importance (27, 28), attempts to transform the germ line of the mosquito *A. aegypti* (10), and the Mediterranean fruit fly, *Ceratitis capitata* (family: Tephritidae; ref. 29), using P-element vectors carrying a dominant selectable marker (neomycin phosphotransferase) have failed to yield a P-element-mediated transformant. It is not known whether the failure of these experiments resulted from the absence of P-element sequences required for vector mobility in nondrosophilids, the lack of P-element insertion sites, the inability to confer whole-animal resistance to neomycin (in the case of *C. capitata*), or the absence of additional host-encoded trans-chemical factors required for transposition. Determining the mobility properties of P elements in heterologous systems has therefore remained an important unachieved goal.

Use of the in vivo P-element-excision assay to indicate P-element mobility in heterologous systems is subject to neither the limitations associated with using P-element vectors carrying a dominant selectable marker nor the reliance on germ-line transformation. (i) P elements carrying exogenous DNA will result in P-element excision, and the mobility is inversely proportional to the amount of DNA contained within the P-element vector (30). Such structural constraints may affect the mobility of P elements in heterologous systems to a greater degree than they do in *D. melanogaster*. For example, Brennan et al. (8), while trying to recover germ-line transformants of *D. hawaiiensis* using a P-element vector containing the *D. melanogaster* alcohol dehydrogenase gene, only recovered transformants containing autonomous, unmodified P elements. This result suggests that either structural features essential for P-element mobility in *D. hawaiiensis* were eliminated during vector construction or the presence of exogenous DNA more severely limits the mobility of P-element vectors in this system. (ii) The ability to confer whole-insect resistance to neomycin has only been demonstrated in *D. melanogaster* (9) and *A. aegypti* (10). The utility of this marker in other insects is not known, and its use in tephritids, which have high concentrations of symbiotic bacteria in their gut (31), is particularly problematic. Therefore, failure to attain germ-line transformation using a P-element vector carrying a dominant selectable marker has not allowed conclusions regarding the function of the P-element transposon system. The in vivo excision assay allows direct assessment of P-element mobilization without relying on genetic selection or germ-line transformation.

Most DNA injected into preblastoderm insect embryos is incorporated into somatic tissue (32), and as a consequence, P-element excision has been monitored in the somatic. Nevertheless, our results show that functional transposase in the embryonic soma can support a level of P-element mobility comparable to that observed in germ-line tissue: the frequency of P-element excision from the pISP indicator plasmid (1.7 per 10^6 pISP plasmids recovered) is comparable to the reversion frequency (i.e., P-element excision) of the P-element insertion mutation w^tdhok17^ (4 per 10^3 X chromosome somes) occurring in the germ line of mutant *D. melanogaster* (1). The mutation w^tdhok17^ consists of a 600-bp nonautonomous P element inserted into the coding region of the white gene of *D. melanogaster*. The nonautonomous P element and 47 bp of flanking white sequence from w^tdhok17^ were used in constructing pISP (14). We believe the quantitative and qualitative similarities in P-element mobility in germ-line and somatic cells permit use of the somatic in vivo excision assay as a reliable indicator of P-element function in the germ line.

Recently Rio et al. (15), using the P-element excision assay, reported P mobility in mammalian cell lines producing transposase. These results are significant because they show that under certain conditions P elements can be mobilized in heterologous cells. However, copia-like elements, which are relatively immobile in vivo, have increased mobility in vitro (32), suggesting that in cultured cell lines control of transposable element mobilization may be generally altered relative to cells in vivo. Developing a method for the difference in vivo and in vitro should enhance our understanding of regulation of P-element function and our ability to fully exploit P-element vectors.

Understanding the basis of P-element dysfunction in nondrosophilids should reveal important aspects of the mechanism and regulation of P-element transposition and excision. Lack of P-element excision in tephritids is not attributable to failure of P transposase gene transcription. Helper plasmid pUCHsΔΔ2-3 is transcribed in both *A. suspensa* and *T. curvicauda* embryos, and although some variability was seen in P-element transcript lengths in these embryos, transcripts of identical size were found in both species—with one transcript corresponding in length to a correctly processed transposase transcript. The transposase gene contains three introns, and although pUCHsΔΔ2-3 lacks intron III, introns I and II are present and require splicing (16). Furthermore, intron II contains an alternative splice site by which a unique splicing event is preserved (16). The alternative splicing event is present in the tephritid embryos, suggesting that the alternative splicing event should occur normally in tephritid embryos, thus explaining the lack of P-element excision. Alternatively, the pres-
ence of negative regulatory factors or the absence of cellular components required as cofactors for P-element mobility may also result in immobilization of P elements. Species that do not support P-element excision will offer useful systems for testing putative host-encoded factors on P-element mobility.

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