

Germline transformation of *Drosophila melanogaster* by purified P element transposase

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P transposable elements are used as vectors for germline transformation of *Drosophila melanogaster* (1–3). P element transposition requires an 87 kD P element-encoded transposase protein (4). The conventional transformation protocol involves microinjection of embryos with a genetically marked, non-autonomous P element along with a second DNA encoding the transposase gene. Transposase synthesized from the second DNA acts in *trans* to stimulate transposition of the nonautonomous P element into germline chromosomes (1–3). We have purified P element transposase protein (5), and wished to determine if it retained the ability to stimulate P element transposition *in vivo* upon microinjection into *D. melanogaster* embryos. The transposase used was the TdT 0.3M KCl chromatographic fraction prepared essentially as described (5). Transposase was diluted to approximately 1.5 μg/ml in buffer (20 mM Hepes-KOH, pH 7.6, 20% glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.05% Nonidet P-40), supplemented with 25 μg/ml bovine serum albumin, 70 μg/ml PMSF, 0.2 mM sodium metabisulfite, and 0.5 μg/ml antipain, leupeptin, pepstatin, chymostatin and aprotinin. This transposase concentration corresponds to approximately 0.05 pmol per 3 μl aliquot. The transposase/DNA complexes were formed by adding 1 μl of 1 mg/ml pDm30 (6) DNA in standard injection buffer (3) to a 3 μl transposase aliquot on ice; these were either frozen in liquid nitrogen and stored at −80°C or used directly to load a microcapillary for embryo injection. $rosy^{506}$ embryos were injected as per published protocols (1–3).

Three different molar ratios of transposase protein to $rosy^{+}$ marked P element vector DNA (0.3:1, 1:1 and 3:1) were used to microinject embryos. All three ratios gave rise to stable germline transformants (Table I). Our frequency of approximately 50% transformation of fertile G_0 flies was similar to that typically observed with the DNA coinjection method. Previous studies using the transposase-encoding helper plasmid pΠP25.7wc have shown that $rosy^{+}$ marked transposons of similar size were stably transformed at overall frequencies of 30–60% of fertile G_0 flies (3).

This experiment demonstrates the biological activity of purified transposase; experiments are underway to determine whether it will stimulate P element transformation of other organisms. Previous attempts to transform distant heterologous organisms using the transposase gene (4, 7, 8) have failed, but in these cases it was not known if active transposase protein was ever synthesized. The observation of transposase-dependent P element excision in transfected mammalian cells (9) suggests that P element transposition may not be limited by host factors involved in the transposition reaction itself. Direct injection of active transposase protein may therefore facilitate transposition in diverse organisms.

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Table I. Results of microinjection of P element transposase and pDm30 DNA

Molar Ratio Transposase: DNA	Embryos injected (no.)	hatched (no.)	Eclosed (no.)	Fertile adults (no.)	Transformed (no.)	(%)
1	1809	187	81	49	20	41%
0.3	486	88	46	24	12	50%
3	316	74	44	15	8	53%

The number of larvae hatched, adults eclosed, fertile adults, and transformed G_1 offspring were scored for three molar ratios of transposase to DNA. Progeny were scored as transformed by the presence of wild-type red eye color (1–3).

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