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SHORT PAPER

P element excision in *Drosophila* is stimulated by gamma-irradiation in transient embryonic assays

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Summary

The influence of gamma-irradiation on *P* element excision and excision-site repair mechanisms was directly tested by embryonic somatic excision assays. Preblastoderm *P*[*ry*$^{+}$, Δ*2–3*](*99B*) embryos, having a stable source of somatically active *P* transposase, were irradiated previous to injection with *P* excision indicator plasmids. Frequencies of precise or nearly precise *P* excision increased with gamma-ray doses ranging from 0·5 to 3·5 Gy. Higher doses resulted in frequencies close to that in unirradiated embryos, though considerable embryonic lethality was also evident at these doses. A direct positive interaction between gamma-irradiation and *P* element activity is concluded.

1. Introduction

Positive or synergistic interactions between gamma-irradiation and transposon activity have been demonstrated for a variety of transposable element systems (see Arnault & Dufournel, 1994). In *Drosophila melanogaster*, the induction of recessive lethal mutations by the *MR* factor was enhanced by x-irradiation (Sobels & Eeken, 1981) and, more recently, reactivity in the *I–R* system was shown to be positively correlated with gamma-ray sensitivity (Laurencon & Bregliano, 1995; Bregliano *et al.*, 1995). For the *P* element system X-linked lethality, chromosome loss and hybrid sterility caused by P–M hybrid dysgenesis has, similarly, been shown to be enhanced by, or have a synergistic interaction with, x-irradiation (Margulies *et al.*, 1986; 1989). As noted by Engels (1989), however, for some of these studies the statistical significance of premeiotic mutational events resulting in clusters of mutant progeny is difficult to evaluate. Moreover, where hybrid dysgenesis and mutagenic agents result in similar yet quantitatively variable genetic aberrations, accurate correlations are difficult to determine. These tests, in addition, were not able to distinguish between effects on transposase activity, or direct effects on transposase target sites.

As a means of observing more directly the influence of gamma-irradiation on *P* transposase activity, in the context of a single functional *P* element per haploid genome, we assayed *P* element excision from plasmids injected into irradiated preblastoderm embryos. We previously demonstrated that precise or nearly precise excision occurs from indicator plasmids injected into *P*[*ry*$^{+}$, Δ*2–3*](*99B*) strain embryos (Handler *et al.*, 1993), which contain a single chromosomal source of highly active *P* transposase (Robertson *et al.*, 1988). The *P* Δ*2–3* gene, having its third intron deleted, functions as a somatically active helper which catalyses *P* excision from injected plasmids after cellularization. We reasoned that the influence of gamma-irradiation on *P* movement could be assessed directly in embryos irradiated shortly before introduction of the indicator plasmid. This would exclude any direct effects on the *P* element being mobilized. In this study we observed a significant increase in *P* excision and subsequent repair of the excision site correlated with increasing doses of gamma-rays up to a level where embryonic lethality obscured or interfered with this effect.

2. Materials and methods

(i) *Strains and rearing*

The *ry*^{506} *P*[*ry*$^{+}$, Δ*2–3*](*99B*) *D. melanogaster* transformant strain (referred to as Δ*2–3*) was originally created by Laski *et al.* (1986) and isolated as a subline containing a single and stable *P*[*ry*$^{+}$, Δ*2–3*] insertion by Robertson *et al.* (1988). The *Adh*^{fn23} *cn;ry*^{506} (*Adh*^{fn23}) strain has an M cytotype and is routinely used as a *P* excision assay host strain requiring exogenous *P* transposase. Strains were maintained

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on standard cornmeal–agar–molasses medium at 22–24 °C. Embryos for injection were collected on agar–grape juice egg-laying plates.

(ii) *Egg irradiation and handling*

Newly oviposited eggs were collected for 30 min after a 30 min prelaying period. Eggs were manually dechorionated, placed on damp filter paper and irradiated with a caesium-137 source in a Gammator M (Radiation Machinery) with a rotating turntable for specified time periods. The relatively short exposure periods required for low dosages (0·23 Gy/s) increases the variability of actual dosage at these levels. We assume an approximate 10% variability. Irradiated eggs were immediately prepared for injection.

(iii) Excision assays

The embryonic *P* excision assay used in this study is a modification of mobility assays described previously (O'Brochta & Handler, 1988; O'Brochta *et al*., 1991; Handler *et al*., 1993). Briefly, the assay involves the transient expression of an indicator plasmid, pISP-2 (Rio *et al*., 1986), in insect embryos which allows excision of a *P* element from a *lacZ* gene to be monitored. The pISP-2 plasmid was injected (1·0 mg/ml) into either the *P*[*ry*$^+$ Δ*2–3*](*99B*) or the *Adh*^{fn23} M host strain. Plasmids were injected into preblastoderm embryos, harvested after 18–24 h at 24 °C, and transformed into DH5α *E. coli*. Bacteria were plated on LB plates containing ampicillin (75 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; 40 μg/ml), allowing precise or nearly precise *P* element excision to be detected as LacZ$^+$ (blue) colonies. Excisions were verified by plasmid size determination on agarose gels, and frequencies were computed by dividing the number of excisions by the total number of pISP-2 plasmids screened (white and blue colonies). Final excision frequencies were derived from combined data of replicate experiments. Probit analysis was performed using the Statistical Analysis System (SAS Institute, Cary, NC).

3. Results and Discussion

To determine the influence of gamma-irradiation on *P* activity, we observed the effects of irradiation directly on *P* excision from plasmids injected into *D. melanogaster* embryos shortly after irradiation. To control for the possibility that irradiation has a direct influence on *P* excision independent of transposase, possibly due to stimulation of mechanisms facilitating deletion or recombination, excision was assayed in *Adh*^{fn23} M strain embryos being devoid of *P*. These M strain embryos support *P* excision at frequencies of approximately $1{\cdot}5–2{\cdot}0 \times 10^{-3}$/pISP-2 plasmids assayed, but require co-injection of a *P* encoding helper plasmid (O'Brochta & Handler, 1988; O'Brochta *et al*., 1991). These embryos were either unirradiated, or irradiated with 2·0 Gy and then injected only with pISP-2 (Table 1). Excision was not detected in either unirradiated or irradiated embryos, indicating that any positive influence of gamma-irradiation is dependent upon *P* transposase activity.

The influence of gamma-irradiation on *P* activity was determined for the *P*[*ry*$^+$, Δ*2–3*](*99B*) strain. These embryos have a somatically active source of *P* transposase resulting from the transgenic insertion of a functional, though immobile, *P*[*ry*$^+$, Δ*2–3*] vector. Although excision frequencies are lower in this strain than in *Adh*^{fn23} injected with plasmid-encoded heat-shock-regulated *P* transposase, the genomic source of transposase in Δ*2–3* is more consistent, thereby limiting variability among experimental samples. Due to nuclear divisions occurring during embryogenesis, and in contrast to limited cell division in subsequent developmental stages, embryos are highly sensitive to the somatic lethal effects of ionizing irradiation (Würgler & Ulrich, 1976). To assess the influence of radiation on viability, samples of sibling Δ*2–3* embryos were irradiated and not injected, with viability determined as larval hatching (Table 2). These viability rates are approximate since the embryos were not highly synchronized, and lethal radiosensitivity varies with nuclear cycle during the preblastoderm stage (Würgler & Ulrich, 1976).

The frequency of excision in control Δ*2–3* embryos injected with pISP-2 and not irradiated was $0{\cdot}20 \times 10^{-3}$/pISP-2 with more than 400 000 plasmids assayed. Sibling embryos were irradiated with doses ranging from 0·5 to 4·0 Gy. Significant increases in excision frequency were observed at 0·5 Gy, with further dose-related increases occurring at doses up to 3·5 Gy, yielding an excision frequency of $1{\cdot}04 \times 10^{-3}$/pISP-2 assayed. This represents nearly a fivefold increase in excision relative to unirradiated embryos, indicating a positive influence of gamma-irradiation on transient *P* element excision. The significance of the correlation between dosage and excision frequency is shown by probit analysis of the proportion of excision events over the total number of plasmids screened and that of gamma-irradiation up to 3·5 Gy which yielded the equation: Probit $= -3{\cdot}5 + 0{\cdot}001$ (dosage), with the standard error of the slope being 0·0002. The slope is positively significant from zero ($P = 0{\cdot}0001$) in that the proportion of excisions increased with an increase in dosage.

At 4·0 Gy excision decreased precipitously, below unirradiated levels, but this dose also resulted in one-third viability of unirradiated embryos. It is reasonable to assume that the ability of these embryos to support *P* excision is related to the fitness of their cellular state. Therefore it is likely that *P* activity at the higher doses is diminished due to disruption of cellular functions, and the frequencies observed may actually

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Table 1. *Excision frequencies of gamma-irradiated preblastoderm embryos*

Strain	Dose (Gy)	Replicate injection	Excisions/ plasmids screened	Frequency[a] ($\times 10^{-3}$)
Adh^{fn23} *cn*; *ry*^{506}				
	0	2	0/67600	0
	2·0	2	0/79800	0
P[*ry*$^{+}$, Δ*2–3*](*99B*)				
	0	9	90/442900	0·20
	0·5	3	12/30500	0·39
	1.0	6	63/134800	0·47
	2·0	3	24/42200	0·57
	3·0	7	70/104700	0·67
	3·5	3	28/27000	1·04
	4·0	6	18/97400	0·18

[a] See Section 2 for probit statistical analysis.

Table 2. *Larval hatching of P*[*ry*$^{+}$, Δ*2–3*](*99B*) *embryos after gamma-irradiation*

Dose (Gy)	Embryos (*n*)	Hatched (*n*)	Survival (%)
0	682	629	92·2
1·0	180	131	72·7
2·0	260	126	48·5
4·0	272	84	30·9
6·0	208	15	7·2
8·0	295	16	5·4
10·0	291	5	1·7

underestimate the positive interactions between *P* and radiation. It should also be noted that specific dose–excision frequency correlations are approximate due to possible variations in sensitivity with nuclear cycles and variations in actual dosage due to relatively short exposure periods. Therefore, the critical dosage of 3·5–4·0 Gy observed here is not absolute, but probably reflects a general dosage level at which the positive influence of gamma-irradiation on *P* excision intersects with the negative biological effects of the radiation.

Synergistic interactions between transposon movement and irradiation, as well as other forms of cellular stress, have been demonstrated or inferred for a variety of systems (Arnault & Dufournel, 1994). Given the general abundance of the types and numbers of mobile elements in most organisms, and the considerable influence of their activity on genome organization, external factors that influence their movement are of considerable importance to organismal evolution and the horizontal transmission of genetic information. Positive influences on transposon movement may also be useful to the stimulation of transposon-based vectors for use in gene-transfer and marking. In this study we have demonstrated a direct positive interaction between gamma-ray dose and *P* excision activity. Notably, the influence of the radiation was limited to a direct effect on transposase and/or DNA repair mechanisms, or factors affecting their activity.

Caveats to this conclusion are that *P* excision was artificially catalysed in somatic tissue, and that movement occurred from plasmids and not from normal chromosomal insertion sites (but having proximal chromosomal insertion site DNA from *w*^{hd80k17} in the plasmid; Rio *et al*., 1986). Excision plasmids, lacking homologue templates which exist in chromosomes, can only be recovered after ligation of the double-stranded breaks, without restoration of the *P* insertion.

The advantages of this type of study are that a known type of *P* element activity (precise or nearly precise excision) was assayed directly and not statistically derived from a frequency of premeiotic mutation events involved in hybrid dysgenesis. Importantly, a defined genetic background was used in terms of functional *P* genes, in contrast to less well characterized P strains which cause varying levels of dysgenic traits.

Despite the importance of mutagen–transposon interactions and the attention it has been given, the actual mechanisms occurring have only been speculated upon. The direct or indirect effect of DNA repair systems has long been implicated. Given that the recognition of excised plasmids (after bacterial transformation) requires repair of double-stranded breaks, it is likely that elevated *P* excision frequencies resulted, at least in part, from stimulation of DNA repair mechanisms. These systems were found to influence the synergistic effects of ionizing radiation and *P* activity in the Harwich P strain (Margulies & Griffith, 1991), and inducible DNA repair mechanisms, such as the SOS system in bacteria have been implicated as well, though no direct evidence has been reported. Bregliano *et al*. (1996) gave strength to this argument in terms of *I–R* reactivity level being promoted by

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DNA synthesis inhibitors, as well as gamma-rays, both of which induce the SOS response. The ability to observe a similar interaction between gamma-irradiation and *P* excision in a controlled and quantifiable manner using transient assays, may provide a system for further elucidating these mechanisms.

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