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Genetics

Tn*10* transposition promotes RecA-dependent induction of a λ prophage

(SOS induction)

DENISE ROBERTS* AND NANCY KLECKNER†

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

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ABSTRACT **We present evidence that Tn*10* transposition, or a closely correlated event, induces expression of bacterial SOS functions. We have found that λ prophage induction is increased in *Escherichia coli* λ lysogens containing increased Tn*10* transposase function plus single or multiple copies of an appropriate pair of transposon ends. This increase occurs by the normal pathway for prophage induction, which involves RecA-mediated cleavage of the phage λ repressor protein. We also present evidence that Tn*10* promotes induction of expression of the *E. coli sfiA* gene. Tn*10* transposes by a nonreplicative mechanism. We propose that the signal for RecA protease activation and SOS induction is generated by degradation of the transposon donor molecule and suggest that SOS induction is biologically important in helping a cell undergoing transposition to repair and/or recover from damage to the transposon donor chromosome.**

Exposure of *Escherichia coli* cells to DNA-damaging agents coordinately induces the expression of a set of cellular functions called SOS functions, which help the cell to repair and recover from DNA damage (see refs. 1–4 for reviews). Among the functions induced are a protein involved in cell division inhibition (SfiA; ref. 5) and recombination or repair enzymes, including RecA (6), RecN (7), and Ruv (8).

SOS functions are coordinately controlled by a negative regulator, LexA (6, 9, 10). A cellular signal generated by DNA damage activates the protease activity of RecA protein, which then cleaves LexA, relieving repression of SOS genes. *In vitro*, RecA protease is activated by binding of single-stranded DNA (11–13). This has led to the suggestion that the direct signal for SOS induction *in vivo* could be single-stranded DNA generated by DNA damage or blockage of a DNA replication fork.

Treatments that lead to induction of SOS genes also lead to induction of a λ prophage because of cleavage of phage λ repressor by RecA protease (ref. 14; see ref. 15 for review). λ repressor is more resistant to cleavage by RecA protease than is LexA (16–18), so under conditions that generate enough activated RecA protein for λ repressor to be cleaved, it is likely that LexA repressor also will be cleaved. Thus, induction or partial induction of a λ prophage is one indication that all or most SOS genes have also been induced.

Tn*10* is a composite transposon that consists of tetracycline resistance (Tet^r) genes flanked by inverted repeats of insertion sequence IS*10* (19). Tn*10* and IS*10* are thought to transpose by a nonreplicative mechanism (39). We describe below evidence that Tn*10* transposition, or some closely correlated process, can lead to λ prophage induction. We also present evidence that Tn*10* transposition causes induction of one SOS gene, *sfiA*, and argue that SOS functions are generally induced. We suggest that the signal required for RecA protease activation is generated by excision of the transposon segment from a donor molecule by a pair of transposase-promoted double-strand breaks during transposition.

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MATERIALS AND METHODS

Bacterial Strains. *E. coli* NK5449 [$\Delta(lac\ pro)_{XIII}$ Nal^r Arg$^-$] was obtained from J. Miller. NK5830 is NK5449 *recA56*/F′ *lac pro lacI*^{Q} (19). NK6641 is $\Delta(lac\ pro)_{XIII}$ *recA56* Str^r λ^r (Str^r = streptomycin resistance). NK7368 is NK6641/pOX38::mini-Tn*10*-Lac (20). NK5898 is $\Delta(lac\ pro)_{XIII}$ Nal^r Rif^r Arg$^-$ Su2 *metB*. SG21083 (Δ*lon0510 rcsA*::Δ*kan* Δ$lacZ_{U169}$) was obtained from S. Gottesman.

Kan^r (kanamycin resistance) and Tet^r chromosomal mini-Tn*10* insertions or *nadA*::IS*10-kan809* was introduced into NK5449 by phage P1 transduction. F′ *lac pro lacI*^{Q} was introduced into NK5449, with and without the chromosomal mini-Tn*10* insertions, from NK5830 by passage of the F′ plasmid first to NK6641. Strains containing the F′ plasmid were lysogenized with λ112 (wild type) or λ609 (*cI ind*$^-$). Lysogens and nonlysogens were transformed with the plasmids described in the legend to Table 1.

Media and Chemicals. Bacteriological media have been described by Foster *et al.* (19) and Miller (24). Supplements were added at the following concentrations: amino acids, 50 μg/ml; ampicillin (Amp), 100 μg/ml; Kan, 50 μg/ml; and Str (sulfate), 150 μg/ml. Amino acids, drugs, and mitomycin C (MitC) were purchased from Sigma. Isopropyl β-D-thiogalactopyranoside (IPTG) was from Calbiochem and 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) was from BRL.

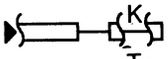
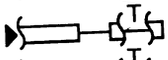
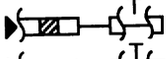
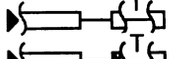
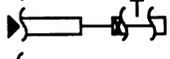
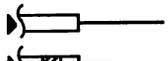
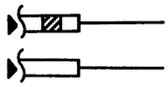
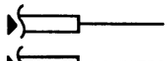
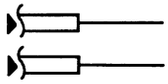
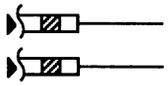
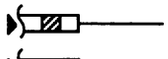
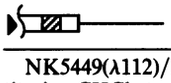
Measurement of Transposition and Phage Release. Isogenic lysogenic and nonlysogenic strains were assayed in parallel for phage release and transposition, respectively. Nonlysogenic strains were used for transposition assays to avoid possible complications because of the killing of cells in which phages had been induced. Strains were transformed with appropriate plasmids and plated on minimal plates with Amp and without proline (to retain the F′ *lac pro lacI*^{Q}). IPTG or MitC were not included in the transformation plates but were added when transformants were grown in liquid media.

For measurement of phage release, a single colony was picked into 1 ml of LB medium containing Amp, 1 mM $MgSO_4$, 0.2% glucose (to repress *lamB* and reduce readsorption of phage particles), and IPTG or MitC when needed. After growth for 6–7 hr at 37°C, cultures were titered for total viable cells. Several drops of chloroform were then added,

Abbreviations: MitC, mitomycin C; IS*10*, insertion sequence 10; Kan^r, kanamycin resistance; Tet^r, tetracycline resistance; IPTG, isopropyl β-D-thiogalactopyranoside; Str^r, streptomycin resistance; Amp, ampicillin; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

*Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
†To whom reprint requests should be addressed.

Table 1. Tn*10* requirements for increased phage release

Transposase and mini-Tn*10* present on plasmid	Plasmid	Chromosomal insertion	Phage yield, no. × 10^{-6} per ml		Transposition frequency × 10^{3}*	
			− IPTG	+ IPTG	− IPTG	+ IPTG
	pNK862	None	4.0	75	16	180
	pNK861	None	3.2	81	3.2	39
	pNK929	None	4.0	3.5	<0.1	<0.1
	pNK865	None	4.4	47	1.2	34
	pNK866	None	3.4	6.3	<0.1	0.6
	pNK474	None	3.8	4.0	—	—
	pNK537	None	3.0	2.4	—	—
	pNK474	Mini-Tn*10*-1	3.6	9.4	0.065	2.8
	pNK474	Mini-Tn*10*-2	2.4	26	0.049	1.8
	pNK474	Mini-Tn*10*-3	4.7	12	0.087	3.9
	pNK537	Mini-Tn*10*-1	2.7	3.0	—	—
	pNK537	Mini-Tn*10*-2	3.3	2.6	—	—
	pNK537	Mini-Tn*10*-3	4.4	4.0	—	—

NK5449(λ112)/F′ *lac pro lacI*^{Q} derivatives, transformed with the indicated plasmids, were tested for spontaneously released λ phages by titering $CHCl_3$-treated saturated cultures. Cultures of identical strains *lacking* the λ prophage were tested for the frequency of transposition of mini-Tn*10* elements by a "mating-out" assay. Phage release and transposition were measured with mini-Tn*10* elements inserted in plasmids and in the bacterial chromosome. pNK861, pNK862, pNK865, pNK866, and pNK464 carry the intact transposase coding region (base pair 70 to base pair 1329 of IS*10*) expressed from the strong, IPTG-inducible hybrid *trp-lac* promoter (P_{tac}; ref. 30) (▶). Transposase expression was induced by addition of 1 mM IPTG. pNK929 and pNK537 are transposase-defective derivatives of pNK861 and pNK474 and have a 300-base-pair deletion (▨) internal to the transposase reading frame (from base pair 606 to base pair 897 of IS*10*-Right). A mini-Tn*10* element is present on pNK861, pNK862, pNK865, pNK866, and pNK929. Mini-Tn*10* elements consist of inverted repeats of the outer 70 base pairs of Tn*10* flanking the genes for Kan^{r} or Tet^{r} as indicated (K or T). The mini-Tn*10* of pNK866 is transposition-defective because of a point mutation in one of the 70-base-pair Tn*10* terminal repeats (mutant 980 indicated by X, ref. 20). pNK474 and pNK537 lack the mini-Tn*10* element. Details of the structure and construction of pNK861, pNK862, and derivatives have been described (21, 22). Mini-Tn*10* elements inserted into the *E. coli* chromosome are insertions of the Kan^{r} mini-Tn*10* element of pNK862. Mini-Tn*10*-1 is linked to *hip* at 20 min, mini-Tn*10*-2 is linked to *himA* at 37 min, and mini-Tn*10*-3 is linked to *tna* at 83 min (23). Cell titers in prophage induction assays and frequencies of proline-independent exconjugants in transposition assays were constant in all experiments (0.8–1.6 × 10^{9} per ml and 0.5–1.4 × 10^{8} per ml, respectively).

*Number of drug-resistant exconjugants divided by the total number of exconjugants in 1 ml of mating mixture.

phenotype of the released phages was tested by superinfecting phage from single plaques into a λ lysogen and plating cells on plates containing Kan.

Transposition was measured by the "mating-out" assay (19). Single colonies were picked from transformation plates into 1 ml of LB medium with Amp and with or without IPTG as indicated. After growth to saturation (8 hr at 37°C), cultures were diluted 1:20 into 1 ml of LB medium without Amp and with IPTG as in the previous culture. Cells were grown with vigorous aeration for 1.5 hr at 37°C and then 0.5 hr with gentle aeration. A recipient culture (2.5 ml) was then added, and the mix was incubated with gentle aeration for 1 hr. Mating mixtures were then diluted and plated for total proline-independent (Pro^{+}) exconjugants, and for Kan^{r} (or Tet^{r}) exconjugants. Transposition frequencies are calculated as the number of drug-resistant exconjugants divided by total exconjugants.

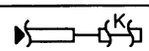
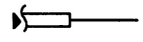
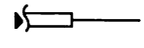
Transposition rates per cell per generation (R) were calculated from frequencies (f) as described by Morisato *et al.* (25) by using the formula: $R = 4.3f/(\log N - \log N_o)$, where N_o is the initial number of cells and N is the number of cells in the population at the time of sampling. In these transposition experiments, $N_o = 1$ and $N = 10^{10}$. The rate of phage induction per cell per generation (R') was calculated by using the equation $T = (\Sigma[2^{n} \times R' \times B])/2^{n}$, where T is the measured titer of phage per cell, n is the number of generations, and B is the number ofphage produced per induction event (burst size). In these phage induction experiments, $n = 30$.

For MitC induction donor cultures were grown overnight in the indicated MitC concentrations. For concentrations below 5 pg/ml, donors were diluted for mating into the same MitC concentration. At concentrations greater than 5 pg/ml, recovery of the RecA^{-} recipient decreased to <10%; thus, for the higher MitC treatments, donor cultures were diluted into 5 pg/ml MitC for the 2-hr growth period prior to recipient addition. The mating mix was plated onto LB plates containing Kan and Str and containing Str and X-Gal. Transposition frequencies were calculated as the number of Kan^{r} exconjugants divided by the number of blue (pOX38::Tn*10*-Lac-containing) recipient colonies. MitC at the concentrations used here did not significantly decrease viability or mating ability of the RecA^{+} donor strains; the ratio of exconjugants (blue colonies) to total recipients (blue plus white colonies) was constant (0.3 to 0.5).

RESULTS

Transposition, or a Closely Correlated Event, Induces a λ Prophage. In cultures of wild-type *E. coli* K-12 strains lysogenic for phage λ, a basal level of spontaneous prophage induction results in production of mature phage particles. The titer of free phage particles present in a saturated culture increases 20-fold when the lysogen carries a multicopy Tn*10*-derived plasmid that gives high levels of Tn*10* trans-

Table 2. Detailed comparison of transposition and phage release for mini-Tn*10* elements inserted in the plasmid and chromosome

Exp.	Transposase and mini-Tn*10* on plasmid	Chromosomal insertion	IPTG, μm	Phage yield, no. × 10^{-6} per ml	Transposition frequency × 10^3*
1		—	—	5.5	13
			10	8.3	39
			25	14	75
			50	40	160
			100	88	170
			1000	95	170
2		Mini-Tn*10*-1	—	4.4	0.14
			10	4.8	0.32
			25	5.9	0.95
			50	7.7	1.9
			100	11	3.3
			1000	12	3.3
3		Mini-Tn*10*-2	—	4.9	0.12
			10	5.2	0.23
			25	7.2	0.58
			50	15	1.2
			100	23	1.8
			1000	27	2.0

Selected strains from Table 1 were examined for transposition and phage release as a function of induction of P_{tac} by IPTG. Strains 1, 2, and 3 are equivalent to the first strain in the upper portion of Table 1 and the first two strains in the lower portion of Table 1. Phage release and transposition were tested as described in the legend to Table 1. As a control, strains containing the chromosomal mini-Tn*10* insertions were transformed with pNK862; phage titers and transposition frequencies were identical to those obtained with strains transformed with pNK862 but lacking the chromosomal insertions (data not shown).
*As in Table 1.

position (Table 1). Two such plasmids, pNK861 and pNK862, contain a pair of suitably oriented Tn*10* termini flanking a Tet^r or Kan^r segment and an IS*10* transposase gene fused to a strong IPTG-inducible promoter (P_{tac}).

Both an intact transposase gene and intact Tn*10* termini are required for enhanced prophage induction. Phage induction is stimulated by IPTG and is eliminated by a deletion mutation in the transposase coding region (pNK929). It is also eliminated by a single base change that inactivates one of the two termini (pNK866) or by deletion of the entire transposon segment (pNK474).

Increased prophage induction is also observed when the transposon segment exists as a single-copy insertion in the chromosome and with transposase provided in trans by a multicopy plasmid (Table 1). In fact, when the transposon segment is in the chromosome, prophage release is much higher relative to the frequency of transposition than when the transposon segment is present on the multicopy plasmid. For example, the phage produced per transposition event is 8-fold higher for the chromosomal mini-Tn*10*-1 complemented by pNK474 than for pNK862 (Table 1).

For a given transposon insertion, the amount of prophage induction is roughly proportional to the transposition frequency. When transposase expression from P_{tac} is varied over a 20-fold range by varying the concentration of IPTG, prophage induction increases as transposition frequencies increase (Table 2). This correlation is observed with the transposon segment either on the multicopy plasmid or inserted in the chromosome.

Additional evidence for Tn*10*-promoted cleavage of phage λ repressor is provided by the observation that the Tn*10* plasmids in Table 1 cause induction of genes fused to λ promoters in defective prophages. In strains carrying either a *lacZ* gene fused to λ P_L (BR293; ref. 28) or a *gal* gene fused to λ P_L (ES1; ref. 29), expression of the fusion genes is induced by the same plasmids that cause prophage induction (data not shown).

Prophage Induction by Tn*10* Plasmids Requires Wild-Type RecA Protein and Wild-Type λ Repressor. Tn*10* plasmids appear to enhance prophage induction through the normal pathway of RecA protease activation. λ prophage induction by UV light or other DNA-damaging agents requires cleavage of λ repressor by activated RecA protease (14). As with UV induction of prophages, induction by Tn*10* plasmids is blocked by mutations in λ repressor (λ *cI ind*$^-$) or in *recA* itself (*recA56*; Table 3). The total number of phage produced is greatly reduced (by 3–4 orders of magnitude) in the presence of these mutations. Among the residual phages recovered, an IPTG-inducible increase in phage production is still observed, but this increase is due to transposon insertions into the *cI* gene. The phages recovered in *recA56* strains with a wild-type λ prophage or in *recA*$^+$ with an *ind*$^-$ prophage form clear plaques and have acquired a Kan^r phenotype (Table 3). In *recA*$^+$ with a wild-type prophage, the majority of plaques produced both by spontaneous induction and by Tn*10*-enhanced induction are turbid and kanamycin-sensitive.

Comparison of the Rates of Transposition and Phage Induction. The number of cells in which transposition has occurred and in which the λ prophage has been induced can be estimated from the data presented above. For example, in the strain containing the chromosomal mini-Tn*10*-2 insertion and pNK474 and in the presence of 100 μM IPTG, 1.8×10^{-3}

Table 3. *E. coli* RecA and phage λ *cI* requirements for increased prophage release

Transposase and mini-Tn*10* present on plasmid	Genotype		Phage, no. per ml		Plaque type*	
	Host	*cI*	− IPTG	+ IPTG	% turbid	% clear
	recA$^+$	WT	4.8×10^6	99×10^6	>99.9 (0/36)	<0.1 (15/17)
	recA$^+$	*ind*$^-$	8.9×10^3	250×10^3	0.1	99.9 (36/36)
	recA56	WT	9.2×10^2	49×10^2	0.4	99.6 (36/36)
	recA$^+$	WT	3.0×10^6	3.2×10^6		
	recA$^+$	*ind*$^-$	0.6×10^3	0.8×10^3		
	recA56	WT	2.5×10^2	2.8×10^2		

Isogenic *recA*$^+$ and *recA56* strains (NK5449/F' *lac pro lacI*^{Q} and NK5830) were lysogenized with wild-type phage λ (λ112) or with λ carrying the *cI ind*$^-$ mutation (λ609) and tested for phage release in the presence of Tn*10* plasmids as described in Table 1. pNK862 and pNK537 are described in the legend to Table 1. With fully induced P_{tac} (1 mM IPTG), transposition frequencies of mini-Tn*10* elements in the RecA$^+$ and RecA$^-$ strains were 0.15 and 0.09 Kan^r exconjugant per total exconjugant, respectively. WT, wild type.
*Numbers in parentheses indicate Kan^r plaques per total plaques tested.

Table 4. MitC differentially affects transposition and prophage induction

MitC, pg/ml	Phage yield, no. × 10^{-6} per ml	Transposition frequency × 10^{5}*
—	4.0	1.9
0.1	4.0	1.9
1.0	5.0	1.7
5.0	15	1.7
10	18	2.0
50	200	1.9
100	330	1.5
250	760	2.7

The effect of MitC on prophage induction was measured in NK5449(λ112) containing pOX38::mini-Tn*10-lac* (26) and an IS*10* element marked with Kan^r genes (IS*10-kan*, ref. 26) inserted in *nadA*. IS*10-kan* transposition was tested in an isogenic strain lacking the λ prophage. Strains were grown in the indicated MitC concentrations for 8 hr. Lysogens were directly titered for cells and phage, and nonlysogens were diluted and tested for transposition by the mating-out assay. Except when MitC was at 250 pg/ml, cell titers in phage induction assays and frequencies of Lac$^+$ exconjugants were constant for all MitC concentrations (1.1–1.7 × 10^6 per ml and 1.1–1.7 × 10^8 per ml, respectively). At 250 pg/ml, cell titers and total exconjugants were 0.4 × 10^6 per ml and 0.4 × 10^8 per ml, respectively.
*As in Table 1.

Kan^r exconjugates per total exconjugant were obtained in a mating-out transposition assay (Table 2, experiment 3). This corresponds to a total rate of transposition to all sites in the genome of about 8 × 10^{-4} events per cell per generation (ref. 25; see *Materials and Methods*). The titer of mature phage particles was 23 × 10^6, or about 0.02 phages per viable cell. This corresponds to a rate of prophage induction events of 1 × 10^{-4} per cell per generation if we assume that each cell undergoing prophage induction yields a burst of 100 phages and that released phages are never readsorbed. This rate is likely to be an underestimate since phage λ burst sizes are frequently less than 100 under suboptimal conditions and since at least some readsorption of phage particles is likely.

Tn*10*-Promoted Induction of *sfiA* Expression. Induction of SOS functions in Lon$^-$ mutants of *E. coli* is a lethal event because these mutants are unable to degrade an SOS-induced inhibitor of cell division, SfiA protein (31). A Lon$^-$ strain carrying a chromosomal mini-Tn*10* insertion (SG21083) cannot be transformed efficiently by plasmids carrying the P_{tac}-transposase gene fusion either with or without the transposon ends (pNK862 or pNK474; data not shown). These strains can be transformed efficiently by the isogenic plasmids containing an internal deletion of transposase (pNK929 or pNK537).

SOS Induction Is Not Required for Tn*10* Transposition. Two observations suggest that there is no synergistic relationship between SOS induction and transposition. First, induction of the SOS system does not lead to an increase in transposition frequency. MitC is a potent inducer of SOS functions (32, 33). Exposure of a phage λ lysogen to sublethal concentrations of MitC results in an increase in the titer of free phage particles but does not stimulate IS*10* transposition (Table 4) or Tn*10*-promoted deletion formation (data not shown). Second, inability to induce SOS genes have no adverse effect on transposition. Transposition frequencies are not altered by the RecA-defective (*recA56*) mutation or by the RecA-uninducible (*recA430*) mutation (legend to Table 3 and data not shown), demonstrating that induction of SOS functions is not required for efficient transposition. These experiments do not rule out the possibility that some component of the SOS system is required for transposition if the basal level of that component is sufficient for full transposition activity.

DISCUSSION

The experiments presented above strongly suggest that Tn*10* transposition, or a closely correlated event, results in cellular DNA damage sufficient for activation of the RecA protease and resultant cleavage of λ repressor. The release of mature phage particles from a λ lysogen is increased in cells containing a multicopy transposase plasmid plus an active pair of transposon ends located either on the same plasmid or in the bacterial chromosome. Further evidence for Tn*10*-promoted induction of λ gene expression is provided by induction of λ P_L-*lac* and P_R-*gal* fusions.

Tn*10* transposition, or a closely correlated event, also induces cellular SOS functions. The observed increase in prophage induction provides indirect evidence to this effect, since induction of a λ prophage by DNA damage is known to require a stronger inducing treatment than induction of other SOS genes (16, 17).

Additional direct evidence for induction of SOS functions is provided by the observation that Tn*10* plasmids are lethal to a Lon$^-$ strain. This lethality is attributable to induction of expression of one of the SOS genes, *sfiA*. We sought additional evidence for induction of *sfiA* expression by examining a wild-type strain carrying a *sfiA-lacZ* fusion; we found that Tn*10* plasmids do not detectably increase *lacZ* expression in this strain (O. Huisman, D.R., and N.K., unpublished data). However, this result is not totally unexpected. The SOS-inducing signal generated by Tn*10* is probably transient and present in only a fraction of the cells at any given time. In addition, expression of SOS functions can be rapidly turned on and off (34–36). Thus, the effects of the Tn*10*-induced signal on gene expression may be difficult to detect above background. The prophage induction and Lon$^-$ lethality assays should be more sensitive because the initial signal leads to an irreversible response. Furthermore, in the prophage induction case, the response is amplified by replication of the induced phages.

Additional evidence that Tn*10* promotes induction of cellular SOS functions is provided by independent observations that active Tn*10* plasmids stimulate homologous plasmid recombination. Plasmids such as pNK862 stimulate in *trans* the recombinational reduction from dimers to monomers of a compatible non-Tn*10*-containing multicopy plasmid present in the same cell (D. Morisato and N.K., unpublished data). This result suggests that Tn*10* transposition promotes a general increase in cellular recombination capacity. Plasmid and/or cellular recombination is known to be stimulated by agents that induce SOS function (27, 37) and by host mutations such as *lexA*::Tn5, *tif*, and *spr*/*lexA*(Def) that result in increased expression of SOS genes in the absence of DNA damage (38).

Nature of the Inducing Event. Both an intact transposase gene and intact Tn*10* termini are required for elevated prophage induction, and the phage yield is proportional to the transposition frequency. This strongly suggests that the Tn*10*-promoted event that causes prophage and SOS induction is transposition itself, although we have not ruled out possibilities such as nonspecific DNA nicking or degradation of abortive transposition products.

Genetic evidence suggests that Tn*10* transposes by a nonreplicative mechanism in which the element is excised from the donor site and inserted into the target site without concomitant transposon replication (39–41). Physical and biochemical experiments have shown that Tn*10* can be excised by transposase-dependent double-strand cleavage at Tn*10* termini (21, 42). The fate of the donor molecule is not precisely known, but it is probably not resealed as an intrinsic part of the transposition process itself (ref. 43; J. Kuo, J. Bender, and N.K., unpublished data).

Several steps in transposition could conceivably lead to an SOS-inducing signal. However, the most straightforward possibility is that a signal is provided by degradation of donor DNA after transposase-directed double-strand cleavage at the Tn*10* termini. The overall rate of transposition by fully induced pNK861 and pNK862 is sufficiently high, 0.1 or more events per cell per generation, that many cells in the plasmid-containing culture will have experienced a transposition event and, thus, may have seen a double-strand break and/or degraded backbone (23).

Chromosomally inserted elements cause higher levels of phage release than plasmid-borne insertions even though the transposition frequency is lower. Perhaps more DNA is degraded per transposition event from a chromosomal donor than from a much smaller plasmid donor. Alternatively, by analogy to observations that different UV-damaged replicons cause "indirect induction" of SOS genes to different extents (16, 18, 37), the cell may respond more readily to damage or replication interference of the chromosome than of a multicopy plasmid.

Biological Significance of Transposition-Induced SOS Induction. Induction of SOS functions by transposition could play an important biological role in helping the cell to recover from the potentially lethal double-strand break generated by transposition. An SOS-induced delay of cell division and/or induction of SOS repair functions might help the cell to repair the break, perhaps by recombinational repair using another DNA molecule in the cell as template ("double-strand gap repair"). This idea fits particularly well with evidence suggesting that IS*10* activity is regulated in such a way that transposition occurs preferentially after passage of a replication fork (26). This type of regulation would ensure that a second intact copy of the donor chromosomal region is usually present in the cell at the time of transposition; furthermore, the presence of this second copy on a sister chromosome might be particularly favorable for recombinational repair.

For the above model to be biologically meaningful, a single Tn*10* or IS*10* transposition event must be sufficient to induce SOS functions. SOS genes probably can be induced by a single DNA lesion; in a Uvr^- Lon^- strain, UV doses that produce on the average one thymine dimer per cell are sufficient to cause transient nonlethal filamentation in at least 50% of the cells (E. Witkin, personal communication). One double-strand break might also be expected to provoke a response. From the above results with the chromosomal mini-Tn*10*, for which repair of double-strand breaks is likely to be more important than for the plasmid insertions, we estimate that transposition results in λ prophage induction more than 10% of the time. Since SOS functions can be fully induced with levels of inducer one-fifth that required for full λ induction (16), these results are compatible with the possibility that a single transposition event is sufficient to induce SOS functions.

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