Mol Gen Genet (1983) 189:263–268

MGG

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Recombination-Induced Suppression of Cell Division Following P1-Mediated Generalized Transduction in *Klebsiella aerogenes*

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Summary. *Klebsiella aerogenes* recombinants resulting from bacteriophage P1-mediated generalized transduction failed to increase in number for approximately six generations after transduction. Nevertheless these recombinants continued to grow and became sensitive to penicillin after a transient resistance, suggesting that the cells were growing as long, non-dividing filaments. When filamentous cells were isolated from transduced cultures by gradient centrifugation, recombinants were 1000-fold more frequent among the filaments than among the normal-sized cells. The suppression of cell-division lasted for six generations whether markers near the origin (*gln, ilv*) or terminus (*his, trp*) of chromosome replication were used, despite a 50-fold difference in transduction frequencies for these markers. The suppression of cell division was a host response to recombination rather than to P1 invasion since cells lysogenized by P1 in these same experiments showed only a short (two generation) suppression of cell division. We speculate that the suppression of cell-division is an SOS response triggered by the degraded DNA not incorporated in the final recombinant. We demonstrate that both the filamentation and the transient penicillin resistance of recombinant cells can be exploited to enrich greatly for recombinants, raising transduction frequencies to as high as 10^{-3}.

Introduction

Bacteriophage P1 mediated generalized transduction is one of the most widely used and trusted techniques in studying the genetics of *Escherichia coli* (Bachman and Low 1980). The utility of P1 was further increased when a simple method for isolating P1-sensitive derivatives of many enteric bacteria was described (Goldberg et al. 1974). During studies of the *glnA* region of the *Klebsiella aerogenes* chromosome, (Streicher et al. 1975) several anomalous results arising from P1 mediated transduction led us to investigate the process of recombination further. These investigations indicate that recombination following P1 mediated transduction, caused a prolonged suppression of cell division in enteric bacteria.

The mechanism of P1-mediated generalized transduction has been studied in many laboratories since its first characterization by Lennox (1955). The process can be divided into three stages: the generation of transducing particles (Ikeda and Tomizawa 1965; Harriman 1972) the recombination of some portion of a transducing particle with the homologous region of a bacterial chromosome (Sandri and Berger 1980; Newman and Masters 1980) and the biological effects of the recombination event on the recombinant cell. This last stage is the focus for this work.

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Materials and Methods

Bacterial and Phage Strains. Bacterial strains in this study are derived from *K. aerogenes* strain W70 (MacPhee et al. 1969) and were made sensitive to phage P1 by either of two methods (Goldberg et al. 1974; Goldberg and Magasanik 1975). Strains MK9000 (prototroph), MK9011 (*glnA6, ilvA1*), and MK9282 (*glnA20, rha-2*) have been described previously (Streicher et al. 1975). Strain MK9541 (*glnA6, ilvA1, his-3, trp-3*) was derived from MK9011 following mutagenesis with ethylmethane sulfonate. Bacteriophage P1 is P1 *clr100* Km described previously (Goldberg et al. 1974). Growth of bacteria was at 30° C in LB gln medium (Bender et al. 1975). Phage growth has been described (Goldberg et al. 1974). *K. aerogenes* was used for these experiments because the yield of transductants per plaque-forming-unit is about ten-fold higher with *K. aerogenes* lysates than with *E. coli,* allowing recovery of sufficient transductants even when the multiplicity of infection must be kept low.

Transductions. Bacteria were grown to late exponential phase (about 5×10^8/ml) in broth (LB gln) and concentrated 10-fold by centrifugation and resuspension in broth supplemented with 5 m*M* $CaCl_2$. Phage were added to a final ratio of about 0.5 or 5 plaque forming units per cell (strains MK9282 or MK9541 respectively). After allowing adsorption at 30° C for 20 min, the mixture was either plated directly on selective medium to assay for transductants or else it was diluted to about 1.5×10^7/ml in prewarmed broth and allowed to grow with vigorous aeration at 30° C. Adsorption was not monitored in the experiments shown in Fig. 1–5, but in similar experiments adsorption was about 10% and 1% for strains MK9282 and MK9541 respectively; thus the actual multiplicity of infection was well below 1. Selective medium for recombinants was W salts (Bender et al. 1975) supplemented with 0.4% glucose (w/v) and 0.2% (w/v) ammonium sulfate, and where appropriate, glutamine, isoleucine, tryptophan, and histidine. Specialized transductants were selected on broth plates to which kanamycin, at 50 µg/ml, was added.

Sucrose Gradient Centrifugation. Sucrose gradients containing 10–35% (w/v) sterile sucrose over a cushion of 60% (w/v) sucrose were prepared in nitrocellulose tubes. About 10^{10} cells were layered on top in a volume of 0.5 ml and the tubes were placed in a Sorval GLC-2B or Beckman TJ-6 centrifuge and subjected to centrifugation at 1000xg for 10 min at room temperature. The bottom of the tube was pierced with a sterile needle and fractions of 0.2–1.0 ml were collected and assayed for total viable cells and for transductants. The concentration of sucrose in each fraction was determined by the refractive index.

Results

Cell Division is Suppressed After Transduction

Phage grown on the prototrophic strain MK9000 were mixed with cells of the glutamine auxotroph, strain MK9282 (*glnA20*), and this transduction mixture was diluted into broth and allowed to grow in exponential phase for 10 h. Samples were taken at intervals and the growth of the Gln^+ transductants was compared with the growth of the untransduced cells. The data in Fig. 1 show that the Gln^+ transductants do not increase in number for 3.5 h and then their growth exactly parallels that of the culture as a whole. The 30–35-min doubling time observed for the culture as a whole from time zero, and the Gln^+ transductants after 3.5 h, is characteristic of *K. aerogenes* growing in broth at 30° C and so demonstrates that the manipulation of the culture during the adsorption of phage did not affect the bulk of the cells, but rather led to a specific effect on the transductants.

Phage Adsorption and DNA Injection do Not Cause Suppression of Cell Division

The transducing phage used in these experiments is P1 *clr100*Km (Goldberg et al. 1975) which confers resistance to the antiobiotic kanamycin on cells lysogenic for the phage. The P1 prophage is maintained as a plasmid, therefore the formation of lysogens requires phage adsorption and DNA injection, as does generalized transduction, but lysogen formation does not involve a recombinational event with the chromosome, the key event in generalized transduction (Ikeda and Tomizawa 1965). The data in Fig. 1 show that the lysogenized (kanamycin-resistant) cells display only a brief lag of about 1–1.5 h before they begin increasing in number at the log-phase rate of the rest of the culture. Thus the physical events involved in getting transducing DNA into cells (adsorption and injection) cannot explain the long lag in cell division for the Gln^+ transductants.

Transductants Grow But do Not Divide

If the Gln^+ transductants were growing but not dividing they would be sensitive to penicillin, which lyses actively growing cells. Therefore strain MK9282 (*glnA20*) was transduced with P1 grown on MK9000 and diluted into prewarmed broth containing penicillin. As can be seen in Fig. 2, the culture as a whole is immediately sensitive to penicillin, confirming that growth begins immediately upon dilution. The Gln^+ transductants are transiently resistant to penicillin (about 30 min) but then become as sensitive to penicillin as the bulk of the culture.

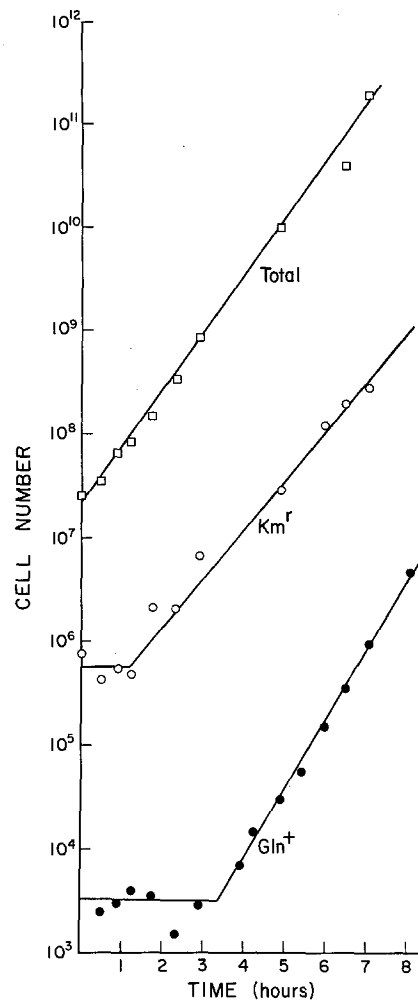

Fig. 1. The kinetics of growth following transduction. Phage P1 grown on the prototrophic strain MK9000 was adsorbed to cells of strain MK9282 (Gln^-) as described in Materials and Methods. The entire mixture was diluted to 10^7 cells/ml and allowed to grow at 30° C with vigorous aeration and periodic dilution to maintain log phase growth. Samples were withdrawn periodically and titred for transductants (on minimal medium lacking glutamine), for lysogens (on broth plates supplemented with kanamycin), and for total viable cells (on broth plates). The first time point was plated immediately before the dilution and all titres are expressed relative to the initial dilution. Gln^+ (●), lysogens (○), total viable cells (□). The slightly faster growth of the Gln^+ transductants is characteristic of this strain where even 0.2% glutamine is insufficient to allow maximum growth rate of the glutamine auxotroph (data not shown)

The data in Fig. 1 show that transductants did not increase in number before 3.5 h while the data in Fig. 2 show that the transductants grew at an apparently normal rate after a brief lag. Taken together, these two observations suggest that the transductants were filamenting. To test directly for filamentation, we analyzed the transduced cultures by sucrose gradient centrifugation. Strain MK9282 (*glnA20*) was transduced with P1 grown on MK9000, diluted into broth, and allowed to grow at densities less than 10^9/ml for 3 h. The cells were then sedimented through a sucrose gradient and the fractions were assayed for viable cells and Gln^+. Figure 3a shows that a fast sedimenting fraction containing a very small portion of the population contains a substantial portion of the transductants. When

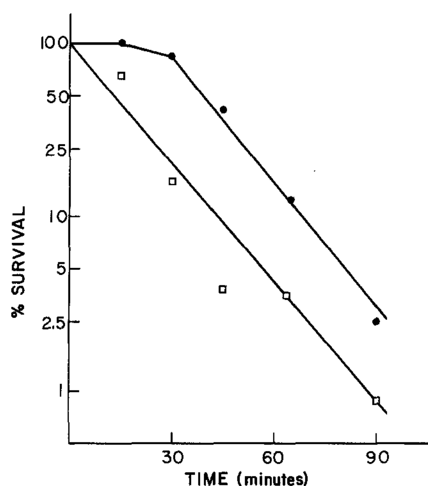

Fig. 2. Sensitivity of transductants to penicillin. Phage P1 grown on the prototrophic strain MK9000 was adsorbed to strain MK9282 (*glnA20*) as described in Materials and Methods. The adsorption mixture was diluted to 1×10^7 cells/ml into prewarmed broth supplemented with 5000 units/ml of penicillin G. Samples were withdrawn periodically and titred for Gln^+ transductants (●) and for total viable cells (□). Values are expressed relative to initial values

the transductant frequency (Gln^+/total cells) is calculated for the various fractions (Fig. 3b) the frequency of transductants among the fast-sedimenting cells was found to be at least 10^{-3}. This value is to be compared with the value of 6×10^{-6} for the fractions containing the majority (65%) of the viable cells. The peak of Gln^+ transductants clearly sediments faster than the bulk of the cells (corresponding to a shoulder on the whole cell count) and the fastest-sedimenting cells are greatly enriched for Gln^+ transductants. When this fastest-sedimenting peak was viewed with the phase contrast microscope, it consisted almost exclusively of long filamentous cells (not shown).

In an analogous experiment the transduction mixture was applied to the sucrose gradient immediately after adsorption with no time allowed for growth except that which may have occurred in the adsorption mixture. The Gln^+ transductants again sedimented in two peaks. In the fast moving shoulder the transduction frequency was as high as 5×10^{-3}, suggesting that virtually every cell in the viable count shoulder was transduced for some gene or other.

One further observation can be made by comparing the experiments shown in Figs. 3a and 4a. At t=0, the Km^R (lysogenic) cells cosedimented with the Gln^+ transductants while at t=3 hours the Km^R (lysogenic) cells cosedimented with the bulk (Gln^-) of the cells (data not shown). This suggests that the Km^R cells lag only briefly, and are dividing at the normal rate well before t=3 hours (Fig. 1).

Length of Cell-Division Lag is Marker Independent

Fast-growing enteric bacteria have several replication forks traversing their chromosome at any one time, resulting in a large number of copies of genes near the origin of replication and a much reduced number of copies of genes near the terminus of replication (Cooper and Helmstetter 1968). Since *glnA* lies near the origin of replication (Streicher et al. 1975; Zyskind et al. 1981) the time before Gln^+ cells begin to increase in number might have reflected the time required

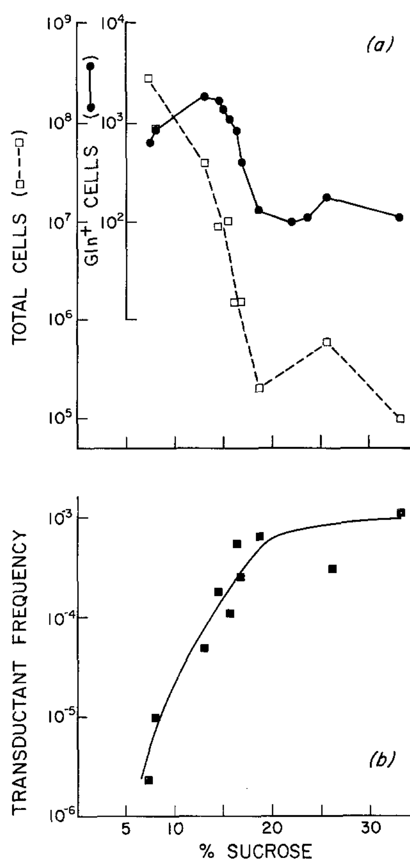

Fig. 3a, b. Separation of recombinant and non-recombinant cells by sucrose velocity gradient centrifugation. Phage grown on the prototrophic strain MK9000 were used to transduce strain MK9282 to Gln^+ as described in Materials and Methods. After adsorption, the cells were diluted into fresh broth and allowed to grow at 30° C with vigorous aeration with log phase growth maintained by periodic dilutions. After 3 h of growth, the cells were concentrated by centrifugation and 10^{10} cells were applied to a 10–30% sucrose gradient. After centrifugation and collection as described in Materials and Methods, the fractions were titred for Gln^+ recombinants, for kanamycin-resistant lysogens, and for total viable cells. The refractive index of each fraction was also measured to determine the sucrose concentration. **a** Gln^+ transductants (●); total viable cells (□). **b** Transduction frequency, calculated as the ratio of Gln^+ to total viable cells

for segregation of the multiple copies of the *glnA20* allele. If segregation were the explanation for the lag seen in Fig. 1, then there should be a strict marker dependence with origin-proximal markers like *glnA* and *ilvA* showing longer lags and terminus-proximal markers like *trp* and *his* showing little or no lag. When phage grown on the prototropic strain MK9000 were used to transduce the multiply auxotrophic strain MK9541 (*glnA, ilvA, trp, his*), transductants again failed to increase in number for about 3.5 h. Moreover, the length of the lag was the same for all four markers. The data for the Gln^+ and Trp^+ transductants are shown in Fig. 5. With this strain, MK9541, as with strain MK9282, the culture as a whole grew without a lag upon dilution and the lysogens began dividing after a brief lag of 1–1.5 h (compare Fig. 1 and Fig. 5).

To show that the length of the lag was the same for all four loci, we compared the *relative* transduction frequencies at t=0 (before dilution) and late in the experiment

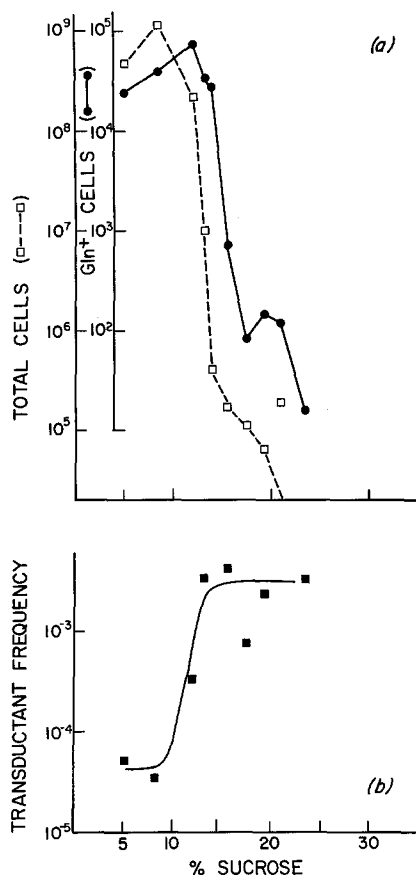

Fig. 4a, b. The same experiment as Fig. 3 except that the cells were subjected to sucrose gradient centrifugation immediately after adsorption at t=0. **a** Gln^+ transductants (●); total viable cells (□). **b** Transduction frequency

(where statistically significant numbers of transductants could be obtained). If the length of the delay in cell divisions were longer for some markers, then their relative transduction frequencies would be decreased more than the transduction frequencies of markers with shorter lags. However, if all markers displayed an equivalently long lag, the relative transduction frequencies would remain the same as they were at time zero. As can be seen from the data in Table 1, the relative transduction frequencies of the four markers did not change appreciably, confirming that all lagged for about the same length of time, about 3.5 h.

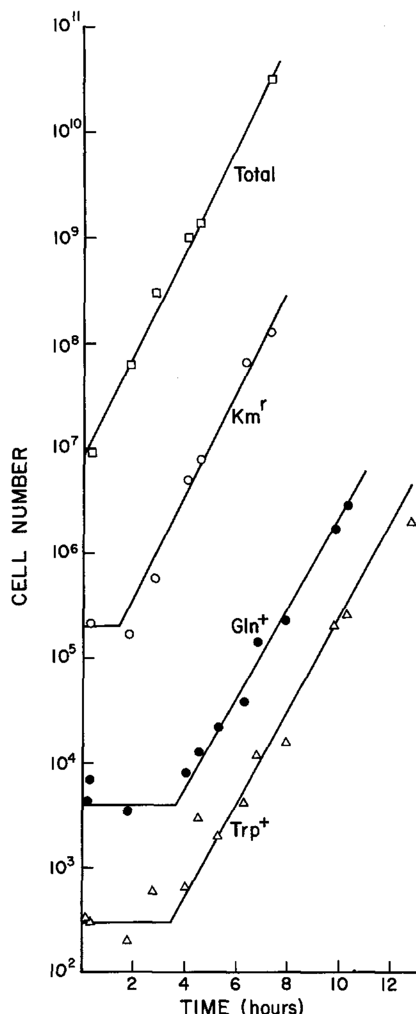

Fig. 5. Marker independence of cell-division lag. Phage P1 grown on the prototrophic strain MK9000 was adsorbed to cells of strain MK9541 (Gln^-, Ilv^-, Trp^-, His^-) as described in Materials and Methods. The entire mixture was diluted to 1×10^7 cells/ml and allowed to grow at 30° C with vigorous aeration and periodic dilution to maintain log phase growth. Samples were withdrawn periodically and titred for transductants (on minimal medium lacking glutamine, isoleucine, tryptophan, or histidine), for lysogens (on broth plates supplemented with kanamycin), and for total viable cells (on broth plates). All titres are expressed relative to the initial dilution. Trp^+ (△); Gln^+ (●); total viable cells (□); kanamycin-resistant lysogens (○)

Discussion

The data presented here show clearly that recombinants arising after P1-mediated transduction suppress cell division for 6 or more generation times while other cells in the culture – even those lysogenized by P1 – grow with little or no lag. Three issues warrant further discussion: the generality of this phenomenon, the direct applicability of the observation in the field of bacterial genetics, and the SOS response in enteric bacteria.

Our data are limited to recombinants arising from P1 mediated generalized transduction in *K. aerogenes*. Other data (Sandri and Berger 1980; Helling 1970), while collected for different purposes, strongly suggest that the effect is identical for P1 transduction in *E. coli*. Furthermore Ebel-Tsipis et al. (1972) showed a similar though shorter delay in cell division following P22-mediated transduction in *S. typhimurium*. Nor is this cell-division lag following recombination unique to transductionally derived recombinants. In his pioneering studies on *E. coli* fertility, Hayes (1957) showed that Hfr generated recombinants of *E. coli* fail to divide for 5 generation times when resuspended in broth. Therefore, we suggest that the cell-division lag we see following P1 mediated generalized transduction may be characteristic of all *recA* mediated recombination in the enteric bacteria. Tomizawa (1960) interpreted similar suppressions of cell division following Hfr crosses as the result of segregation of multiple copies of the genome. In particular, he did not report observing filaments at the frequencies we would predict. We argue that segregation is unlikely for our results because origin proximal markers (*glnA* and *ilv*) show the same delay in replication as terminus proximal

Table 1. Invariance of relative transduction frequencies

Genetic marker	Relative transduction frequency[a]		
	0 h	7 h	11 h
gln$^+$	(1.0)	(1.0)	(1.0)
ilv$^+$	0.35	0.30	0.34
trp$^+$	0.08	0.08	0.09
his$^+$	0.02	0.03	0.05

Transduction of strain MK9541 with P1 grown on strain MK9000 was performed as described in Materials and Methods. After adsorption, cells were diluted to 1.5×10^7/ml in broth and allowed to grow with periodic dilutions to keep the cell density between 1×10^7/ml and 1×10^9/ml.

[a] Transduction frequencies were normalized to the *gln*$^+$ frequency (set at 1.0) which was 3×10^{-5} Gln$^+$/viable cell at t=0, 1.2×10^{-6} Gln$^+$/viable cell at t=7 h, and 8×10^{-7} Gln$^+$/viable cell at t=10 h

markers (*his* and *trp*). Since fast growing cells have significantly more copies of the origin region of the chromosome than of the terminus region (Cooper and Helmstetter 1968), a segregation model requires that origin proximal markers take longer to segregate than terminus markers, in contradiction to our data. Furthermore, the integrated DNA in Hfr recombinants has replicated at least twice by the time any increase in the number of cells displaying the recombinant phenotype is seen (cited in Wollman et al. 1956).

The fact that recombinants can be separated from nonrecombinants by physical methods as simple as sucrose gradients means that it is now feasible to use P1 mediated transduction to isolate transductants whose phenotype is scoreable but not selectable. One limiting feature of P1 mediated transduction is the inability to isolate stable high-frequency-transducing variants of P1 (Wall and Harriman 1974). The ability to attain transduction frequencies in excess of 10^{-3} obviates the requirement for such a variant. In one pilot experiment, we plated about 1000 cells from the fast sedimenting peak of a 3 hour gradient like that shown in Fig. 3. In this experiment the recipient was wild type and the P1 lysate was grown on a *rha*$^-$ strain. Among the 1000 cells that grew on rhamnose indicator agar (MacConkey), one *rha*$^-$ colony was found. We assume such high frequencies will not be found when genes in less transducible regions of the chromosome (like the *his-trp* region) are used, but even so, the 100-fold increase in transduction frequency among cells from this region of the gradient may allow selection for recombinants in crosses where high reversion frequencies would otherwise obscure the results.

Finally, our observations regarding the effects of recombination on cell division need to be considered in light of our current models of the SOS response and the role of *recA* in this response to DNA damage. It is generally held that the basal level of *recA* protein expression is sufficient to mediate homologous recombination since prior induction of *recA* synthesis by U.V. irradiation does not increase recombination frequencies of average markers appreciably (Newman and Masters 1980). Our data suggest that it is inherent in the recombination event itself that the cells destined to become recombinants filament – a phenotype characteristic of the SOS response caused by U.V. irradiation or other DNA-damaging agents. If the filamentation is in fact part of the SOS response then each recombinant would appear in a cell with induced levels of *recA* product (as part of the SOS response). It is thought that small pieces of DNA resulting from degradation are the actual inducers of the SOS response. Such degradation must accompany P1 transductionally-mediated recombination since only about 10% of the DNA from the transducing particle actually becomes integrated into the chromosome (Sandri and Berger 1980). This model of recombination-induced SOS response would also allow a qualitative explanation of the lengths of the supression of cell division in three cases. The 90% of the average transducing DNA that fails to become part of the recipient chromosome is presumably degraded. This represents about 55×10^6d of DNA in P1-mediated transduction. In P22-mediated transduction, about 20×10^6d of DNA is thought to be degraded in transductants. P1 lysogeny results from an intramolecular recombination resulting in the loss (and presumed degradation) of about 7×10^6d of DNA. The suppression of cell division in these three cases are about six, three, and two generations respectively, suggesting that the duration of the response may reflect the amount of degraded DNA. The possible causal linkages between recombination and induction of SOS functions can be tested using *E. coli* mutants that fail to filament: *lexA* in which SOS is uninducible and *sulB* in which SOS is normal except that cell division is not suppressed. Neither *lexA* nor *sulB* displays a Rec$^-$ phenotype, so induction of SOS is not essential for recombination. These mutants should settle the question of whether the suppression of cell division following recombination is mediated via the SOS system or by an independent mechanism.

In summary, our data show that a widely used technique, P1-mediated generalized transduction, has an unexpected consequence, suppression of cell division. This recombination-induced suppression of cell division at least superficially resembles that seen in other recombination systems, especially Hfr matings. This filamentation is potentially useful when enriching for transductants in a population.

Acknowledgements. We thank S.L. Streicher for helpful discussions and S. Allen and J. Adams for their careful reading of the manuscript. This work was supported by grants from the NIH-PHS (GM27111 and A115822) to R.A.B. who was recipient of an American Cancer Society Junior Faculty Research Award (JFRA-3).

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Communicated by G. O'Donovan

Received November 13, 1982