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THE INFIDELITY OF CONJUGAL DNA TRANSFER IN *ESCHERICHIA COLI*

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ABSTRACT

The accuracy of replication and transfer of a *lacI* gene on an F′ plasmid was measured. Following conjugal transfer of the F′, a small but reproducible increase (1.8-fold) in the frequency of *lacI*⁻ mutations was detected. Among these, however, the frequency of nonsense mutations was 15-fold higher than in the absence of transfer. This corresponds to a 300-fold increase in the rate of base substitutions per round of replication compared with normal vegetative DNA replication. The amber mutational spectra revealed that, following conjugal transfer, mutation frequencies were increased markedly at all sites detected. In addition, an increase in G:C → A:T transitions was noted and was due almost entirely to an enhanced proportion of mutants recovered at the spontaneous hotspots (amber sites 6, 15 and 34). *recA*-dependent processes were not responsible for the increase in mutation, since similar results were observed with various *recA*⁻ donor and recipient combinations. These results demonstrate that the fidelity of conjugal DNA replication is considerably lower than that of vegetative DNA replication.

CONJUGAL transmission of the F episome in *Escherichia coli* has been studied extensively over the past two decades. Much has been learned about the genetics of conjugation, physical interactions between conjugating cells and the regulation of plasmid transfer [for recent reviews see CLARK and WARREN (1979) and WILLETS and SKURRAY (1980)]. With respect to DNA transfer, biochemical and genetic evidence indicates that a single DNA strand of the F plasmid, the strand with a 5′ terminus at the origin of transfer, is passed from donor (F^+) to recipient (F^-) during conjugation (GROSS and CARO 1966; COHEN et al. 1968; HOWARD-FLANDERS *et al.* 1968; OHKI and TOMIZAWA 1968; RUPP and IHLER 1968; VIELMETTER, BONHOEFFER and SCHUTTE 1968; VAPNEK and RUPP 1970, 1971; KINGSMAN and WILLETS 1978). DNA synthesis produces complementary strands using the strand transferred to the recipient and the strand retained in the donor as templates (OHKI and TOMIZAWA 1968; VAPNEK and RUPP 1970, 1971). This conjugal DNA synthesis is not prevented in donor and recipient cells that have been heavily irradiated to abolish vegetative DNA replication (GROSS and CARO 1966; GREENBERG, GREEN and BAR-NUN 1970; GREEN, BRIDGES and RIAZUDDIN 1971), suggesting that conjugal DNA synthesis

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circumvents DNA damage that causes a block in vegetative DNA replication. Mechanisms that endow the cell with the ability to bypass template damage during DNA synthesis might be expected to alter the fidelity of DNA replication.

To test the fidelity of conjugal DNA synthesis, we have employed the *E. coli lacI* system which permits the detection of amber and ochre mutations at 65 independent sites within the *lacI* gene (COULONDRE and MILLER 1977a,b; MILLER *et al.* 1977; SCHMEISSNER, GANEM and MILLER 1977). Since the *lacI* DNA sequence and the location of the nonsense mutations have been established, each mutation can be attributed to a specific base change (COULONDRE and MILLER 1977a; FARABAUGH 1978; MILLER, COULONDRE and FARABAUGH 1978). As an integral part of this system, the *lacI* gene is situated on the F′ carried by donor cells, and the homologous chromosomal gene is deleted in both donor and recipient strains. Thus, for the purpose of our study, *lacI*⁻ mutations could be selected subsequent to the conjugal transfer of the F′ and conjugal fidelity compared with the fidelity of vegetative DNA replication.

We have found that the frequency of *lacI* nonsense mutations detected after conjugation is 15-fold higher than the spontaneous frequency for untransferred F′ DNA. This corresponds to a 300-fold increase in the rate of base substitution per round of conjugal DNA replication compared with the error rate per round of vegetative DNA replication. An examination of mutational spectra revealed that the enhanced frequency of nonsense mutations associated with conjugation is due to increases in both transition and transversion events. Quite similar spectra for amber mutations are obtained with or without F′ transfer, the exception being an increase in the proportion of mutants isolated at the mutational hotspot sites at ambers 6, 15 and 34. Experiments with *recA*⁻ donors and/or recipients demonstrated that the elevated frequencies of nonsense mutations observed subsequent to conjugation are not the consequence of *recA*-dependent processes. These findings are consistent with the idea that conjugal DNA synthesis may be inherently less accurate than vegetative DNA replication. Other possible mechanisms that might account for conjugation-associated mutation are discussed.

MATERIALS AND METHODS

Strains and media: Unless otherwise stated, suppressor and deletion strains and media and techniques for the *lacI* system were the same as those described by COULONDRE and MILLER (1977a,b). The wild-type strain KMBL3835 [F′ *pro-lac/ara*⁻, Δ(*pro-lac*), *thi*⁻, *trpE9777*] has been described previously (GLICKMAN 1979). Strain KMBL3883 [F′ *pro-lac/ara*⁻, Δ(*pro-lac*), *thi*⁻, *recA56*] was constructed by conjugation between KA273 (Hfr KL16, *recA56*) and KMBL3831 [F′ *pro-lac/ara*⁻, Δ(*proc-lac*), *thi*⁻, *thy*⁻] through phenotypic suppression of KMBL3831. Following conjugation, Thy⁺ recipients were selected and screened for UV sensitivity. Strain S90Ac [F⁻ Δ(*pro-lac*), *ara*⁻, *thi*⁻, *tet*^{R}, *strA*, *recA56*] was constructed by P1-*vir*-mediated transduction using JC10240 (*argE3, his-4, leu-6, proA2, thr-1, rpsL31, galK2, lacY1, tsx-33, ara-14, xyl-5, mtl-1, supE44, srl-300*:Tn*10*, *recA56*) as the donor and S90c [F⁻ Δ(*proc-lac*), *ara*⁻, *thi*⁻, *strA*] as the recipient. Tetracycline-resistant transductants were selected and screened for UV sensitivity. Spontaneous nalidixic acid-resistant derivatives of S90c and S90Ac (S90cN and S90AcN, respectively) were selected on nutrient medium containing 40 μg/ml of nalidixic acid.

Conjugation and mutant selection: To select spontaneous *lacI*⁻ mutants, exponential phase cells of

strain KMBL3835 were inoculated into wells of 96-well microtiter plates containing 0.2 ml of nutrient broth (approximately 10 cells per well) and incubated at 37° overnight. Aliquots of 10 μl each were withdrawn from each well and plated on minimal medium supplemented with phenyl-β-D-galactoside as the sole carbon source (pgal medium) and incubated at 37° for 48 hr. Sufficient growth occurs on these plates to ensure full expression of all mutants (TODD and GLICKMAN 1982). To ensure the independent origin of mutants chosen for further analysis, only the first amber and first ochre mutation detected from each culture were selected. To select mutants after conjugation, donor and recipient strains were grown at 37° to 3–4 × 10^8 cells/ml in nutrient broth. Aliquots of 1 ml each were withdrawn from donor cultures, the cells were washed twice in VB solution [0.2 g/liter of $MgSO_4 \cdot 7H_2O$, 2 g/liter of citrate, 10 g/liter of K_2HPO_4, 3.5 g/liter of $Na(NH_4)HPO_4 \cdot 4H_2O$, pH 7.0], diluted, spread on pgal medium and incubated at 37° for 48 hr to determine the spontaneous frequencies. Donor and recipient cultures were concentrated by centrifugation, mixed in nutrient broth (donors:recipients = 1:10) and incubated at 37° for 1 hr. Nalidixic acid was then added (final concentration for all steps: 40 μg/ml) to prevent further initiation of F′ transfer (BARBOUR 1967), and the donor-recipient mixture was washed thrice with, and resuspended in, VB solution containing nalidixic acid. *lacI*⁻ exconjugants were selected by plating 0.2 ml of the donor-recipient mixture in 3 ml of soft agar (7.5 g/liter of agar in VB solution containing nalidixic acid) on pgal medium containing nalidixic acid and streptomycin (0.2 g/liter) to select against donor cells. Growth of recipient cells (*pro*⁻) lacking the F′ was prevented by omitting proline from the medium. Transfer of the F′ was detected by plating 0.1 ml of diluted donor-recipient mixture in 3 ml of soft agar (containing nalidixic acid) on minimal proline omission medium containing streptomycin and nalidixic acid.

The possibility that nalidixic acid in the selection plates behaved as a mutagen was guarded against in two ways. Control experiments showed no mutagenic effect associated with the presence of nalidixic acid. In addition, identical spectra were obtained in the absence of nalidixic acid (data not shown). However, in the later case crossing on the plate makes frequency arguments impossible. We, therefore, have used nalidixic acid to prevent crossing from occurring on the plate.

Calculation of mutation rates: The rates of nonsense mutation per round of DNA synthesis were calculated according to the method of DRAKE (1970) using equation (5-9): $m = (f - f_o)/lnN - lnN_o$ for conjugal DNA replication and equation (5-10): $m = (0.4343 f)/(\log N - \log N_o)$ for vegetative DNA replication, where m = the probability of mutation per replication, N = the total population size at a given time, f = the mutant frequency at that time and N_o and f_o = the total population size and the mutant frequency, respectively, at the beginning of the experiment. Use of these formulas is based on the assumptions that the number of mutants is much smaller than N, that the number of mutants is not altered significantly by reversion and that mutants and nonmutants grow at the same rate (DRAKE 1970). In addition, it was assumed that due to the selective conditions employed, most *lacI* nonsense mutants recovered after F′ transfer arose as a consequence of mutations that occurred during the single round of conjugal DNA synthesis in recipient cells. Spontaneous mutants were selected in the donor strain after at least ten rounds of DNA replication preceding the initial formation of *lacI*⁻ mutants.

RESULTS

Experimental rationale behind the lacI *conjugation system*: The production of a mutational spectrum requires the isolation and characterization of thousands of mutants. To facilitate these procedures, the *lacI*⁻ mutations are studied on the transferable F′ *proc-lac* episome in a host strain having a deletion of the homologous chromosomal region. *lacI*⁻ mutants are selected on medium containing phenyl-β-D-galactoside, a noninducer of the *lac* operon, as the sole carbon source. By appropriate modifications of this procedure, *lacI*⁻ mutants can be isolated either prior or subsequent to conjugal transfer of the F′. Since the only copy of *lacI* resides on the F′, no contribution of spontaneous lesions from homologous chromosomal DNA is possible. Thus, any spontaneous point

mutations arising within the *lacI* gene must be the result of DNA synthesis or repair processes acting on F′ DNA.

To identify nonsense mutations, the F′ *lacI*⁻ mutations are transferred by replica mating into a series of suppressor strains which are, in turn, replicated onto indicator medium containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside. On this medium, nonsuppressed *lacI*⁻ mutants form blue colonies, whereas suppression of the mutations leads to the emergence of white colonies. Deletion mapping and suppression pattern analysis of the nonsense mutants, carried out by replica-mating transfer of the F′ into deletion strains and suppressor strains, is used to identify particular sites within the *lacI* gene. Finally, the *lacI* DNA sequence (FARABAUGH 1978) can be employed to identify the specific base substitutions that give rise to the nonsense mutations at these sites.

Analysis of lacI *mutants*: The *lacI* mutational responses are summarized in Table 1. When mutants were selected after conjugation, a small (1.8-fold) but reproducible increase in the *lacI*⁻ frequency was found. Fewer than 2% of the mutations that arose spontaneously in untransferred F′ DNA were nonsense mutations. However, analysis of the *lacI*⁻ mutants isolated subsequently to conjugation revealed that amber and ochre mutations comprised approximately 14% of the total examined. Thus, the frequency of nonsense mutation following F′ transfer was approximately 15-fold higher than the corresponding frequency for untransferred F′ DNA. To fully appreciate the significance of these increases, it must be realized that the spontaneous mutants were selected in the donor strain after growth for at least ten generations, following the initial appearance of *lacI*⁻ mutants. In contrast, there is but one round of conjugal DNA replication in which mutations can occur in order for *lacI*⁻ colonies to form under the selective conditions employed. On this basis, the rate of *lacI* nonsense mutation (base substitution) per round of conjugal DNA replication can be calculated to be about 300-fold greater than the corresponding rate for vegetative DNA replication (Table 2). Thus, there is a marked reduction of fidelity associated with conjugal DNA transfer.

Mutagenesis in response to DNA damage in *E. coli* often occurs concomitantly with the induction of a *recA*-dependent DNA repair mechanism (RADMAN 1974; WITKIN 1976; KIMBALL 1978; HANAWALT *et al.* 1979; HALL and MOUNT 1981; LITTLE and MOUNT 1982). Transfer of UV-damaged F′ DNA elicits a number of such *recA*-dependent processes in the recipient, including induction of λ prophage, UV-reactivation of UV-damaged λ phage, cleavage of λ repressor and synthesis of *recA* protein (BOREK and RYAN 1958, 1960; GEORGE and DEVORET 1971; GEORGE, DEVORET and RADMAN 1974; MOREAU, PELICO and DEVORET 1982). Moreover, a proposed mechanism of misrepair involves reduced fidelity of DNA replication leading to bypass of template lesions (VILLANI, BOITEUX and RADMAN 1978). Thus, it seemed conceivable that *recA* functions might play a role in the reduction of fidelity during conjugal DNA synthesis. This possibility was tested in experiments using various combinations of *recA*⁻ donors and recipients. There was a slight decrease in the percentage of nonsense mutations recovered among the *lacI*⁻ mutants subsequent to F′ plasmid transfers involving *recA*⁻ strains, but mutation rates were mostly un-

TABLE 1

Influence of conjugal transfer on mutation

Genotype[a]			Amber mutants		Ochre mutants		Total nonsense mutants		
Donor F′	Recipient F^-	*lacI*$^-$ frequency	%	Frequency	%	Frequency	%	Frequency	Total *lacI*$^-$ mutants screened
recA$^+$ (no transfer)		3.2	0.96	0.03	0.61	0.02	1.6	0.05	67,568
recA$^+$	*recA*$^+$	5.6	10.0	0.56	3.7	0.21	13.7	0.77	1,931
recA$^+$	*recA*$^-$	10.7	6.6	0.71	1.3	0.14	7.9	0.85	6,428
recA$^-$	*recA*$^+$	5.3	3.5	0.19	1.4	0.07	4.9	0.26	6,583
recA$^-$	*recA*$^-$	8.8	5.9	0.52	0.5	0.04	6.4	0.56	4,589

Frequencies are per 10^6 viable cells (row 1: the spontaneous control, no transfer of F′) or per 10^6 F′ transferred (rows 2–5).

[a] Genotypes refer only to the presence or absence of a wild-type *recA* allele in the donor (F′) or recipient (F^-) strains.

TABLE 2

lacI *mutation rates*

Genotype[a]					
Donor F′	Recipient F^-	*lacI*$^-$ mutation rate ($\times 10^6$)	Amber mutation rate ($\times 10^6$)	Ochre mutation rate ($\times 10^6$)	Total nonsense mutation rate ($\times 10^6$)
recA$^+$ (no transfer)		0.14 (1)	0.0013 (1)	0.0009 (1)	0.0022 (1)
recA$^+$	*recA*$^+$	2.4 (17)	0.53 (410)	0.19 (220)	0.72 (340)
recA$^+$	*recA*$^-$	7.5 (54)	0.68 (520)	0.12 (140)	0.80 (370)
recA$^-$	*recA*$^+$	1.8 (13)	0.16 (120)	0.05 (60)	0.21 (100)
recA$^-$	*recA*$^-$	5.3 (38)	0.49 (380)	0.02 (23)	0.51 (230)

Mutation rates are per pound of replication and were calculated as described in MATERIALS AND METHODS. Numbers in parentheses are the relative rates.

[a] Terminology as for Table 1.

affected (Tables 1 and 2). Clearly, the reduced fidelity of conjugal transfer does not depend on a functional *recA* gene in either recipient or donor.

Mutational spectra: The amber mutations recovered during the screening of the *lacI*$^-$ mutants were analyzed to identify the base substitutions responsible. Because of their prevalence, amber rather than ochre mutants were selected for analysis. Also seven suppressors are available for characterization of amber mutations (only three suppressors act on ochre mutations). Furthermore, in excess of 1300 amber mutations were to be examined and so the greater facility of amber mutant analysis became an important consideration. Finally, characterization of amber mutations permits the detection of all five base substitution events that can give rise to nonsense mutations in the *lacI* gene.

The mutational spectra for *lacI* amber mutations selected in the wild-type donor strain, or recovered subsequently to conjugation between the wild-type donor and wild-type or *recA*$^-$ recipients, are given in Figure 1. Although the mutation frequencies of individual amber sites are as much as 140-fold greater in the conjugational spectra, the three spectra present similar profiles, all hav-

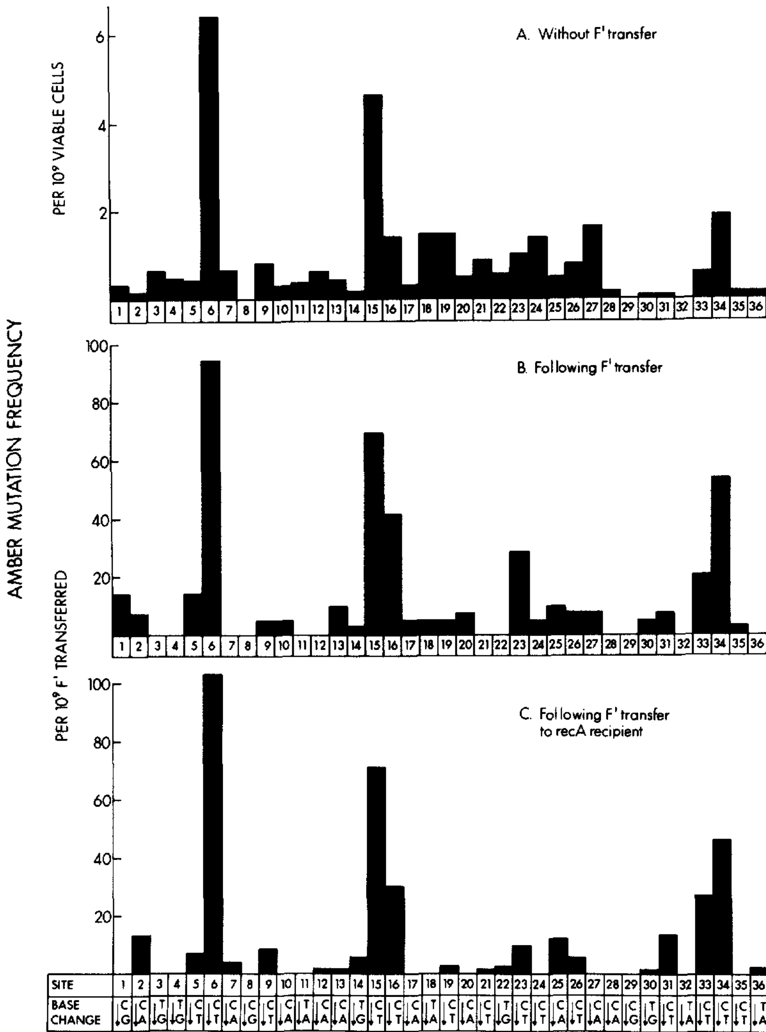

FIGURE 1.—Spectra of *lacI* amber mutations. There are 36 characterized amber sites within the *lacI* gene. The single base substitutions indicated for each site are those that convert the wild-type codon to the nonsense codon. *A*, The top spectrum represents spontaneous mutations obtained in the donor strain KMBL3835 without transfer of the F′ (464 amber mutants were analyzed). *B*, The middle spectrum represents mutations obtained following transfer of the F′ from donor strain KMBL3835 to recipient strain S90cN (186 mutations were analyzed). *C*, The bottom spectrum represents mutations obtained following transfer of the F′ from donor strain KMBL3835 to the *recA*$^-$ recipient strain S90AcN (272 mutations were analyzed). Note that there is a 15-fold increase in the frequency scale for mutations selected after F′ transfer.

ing hotspots at amber sites 6, 15 and 34. Each of these sites contains a 5-methylcytosine, and the amber mutations arise as the consequence of G:C → A:T transitions (COULONDRE and MILLER 1977b; FARABAUGH 1978; MILLER, COULONDRE and FARABAUGH 1978). It has been proposed that the hotspots are the result of spontaneous deamination of 5-methylcytosine to thymine

(COULONDRE *et al.* 1978). Other G:C → A:T transition sites (*e.g.*, ambers 16 and 33) also are prominent in the conjugational spectra. With few exceptions, sites detected for spontaneous mutation in the untransferred F′ also are found when mutants are selected after conjugation. The mutational spectra for crosses involving *recA*⁻ donors and wild-type or *recA*⁻ recipients were very similar to the spectra in Figure 1 (data not shown).

Table 3 summarizes the mutational specificities of transfer of the F′ *vs.* nontransfer in terms of the base pair substitutions required to generate the amber mutants. [An important feature of the *lacI* system is that because A:T → G:C transitions cannot generate nonsense codons in the *lacI* gene, only the G:C → A:T pathway for transition mutagenesis can be scored (MILLER, COULONDRE and FARABAUGH 1978).] Whether or not the F′ is transferred, G:C → A:T transitions predominate over the various transversions. However, this transition does occur more frequently among those mutants selected following conjugation. Analysis of the spectra reveals that this increase in G:C → A:T transitions occurs almost entirely at the amber 6, 15 and 34 sites. In fact, the transfer of the F′ seems to have little effect on the proportion of G:C → A:T transitions at the remaining transition sites. Although the proportion of transversions is reduced among the amber mutants selected subsequently to conjugation, too few isolates were recovered to determine whether their distribution among available sites is affected (Table 3).

DISCUSSION

For the purposes of this study, an essential feature of the *E. coli lacI* system is the location of the *lacI* gene on an F′ and its absence from the chromosome of both donor and recipient. Ordinarily, *lacI*⁻ mutations are selected in the donor strain and then characterized by transfer to specific recipients. In this study, we selected the *lacI*⁻ mutants following transfer of the F′ so that the influence of conjugation on spontaneous mutation frequencies could be determined. We found that transfer of the F′ leads to a 15-fold increase in the frequency of nonsense mutations (Table 1). This corresponds to a 300-fold elevation in the rate of base substitution per round of replication (Table 2). The inclusion of a defective *recA* allele in the donor and/or recipient strain did not prevent the reduction in fidelity during conjugal transfer (Tables 1 and 2). We conclude that conjugal DNA synthesis is inherently much less accurate than vegetative DNA replication.

Following conjugal transfer, a small (<twofold) increase in the frequency of *lacI*⁻ mutations was detected, however, there was a tenfold enhancement in the fraction of nonsense mutations (Table 1). This preferential recovery of nonsense mutants among the *lacI*⁻ population indicates that the decrease in fidelity is largely due to base substitution events. Spectra for amber mutations occurring with or without transfer were similar although a greater proportion of transitions was seen following F′ transfer (Figure 1, Table 3) This enhanced level of G:C → A:T transitions among mutations selected after conjugation is due almost solely to events at amber sites 5, 16 and 34. This observation is intriguing because these sites also are hotspots for spontaneous mutation that

TABLE 3

Distribution of base substitutions leading to lacI *amber mutations*

Base substitution	Sites available	Sites found				
		F′*recA*$^{+a}$ (no transfer)	F′*recA*$^+$ F$^-$*recA*$^+$	F′*recA*$^+$ F$^-$*recA*$^-$	F′*recA*$^-$ F$^-$*recA*$^+$	F′*recA*$^-$ F$^-$*recA*$^-$
G:C → A:T	14	14 (71.8)	13 (82.8)	12 (87.9)	13 (84.6)	13 (84.6)
G:C → T:A	10	9 (16.4)	7 (11.3)	5 (8.8)	6 (6.0)	7 (9.0)
A:T → T:A	4	3 (6.2)	1 (1.1)	1 (0.7)	2 (4.4)	
A:T → C:G	5	5 (4.7)	2 (1.6)	3 (2.6)	3 (3.3)	3 (5.0)
G:C → C:G	3	1 (0.9)	1 (3.2)		2 (2.2)	2 (1.5)
Total ambers mapped		464	186	272	182	201

Numbers in parentheses are the percents of total occurrences.
[a]Terminology as for Table 1.

is thought to be the result of spontaneous deamination of 5-methylcytosine residues (COULONDRE *et al.* 1978; DUNCAN and MILLER 1980). The basis for this proposal is that, whereas uracil, the deamination product of cytosine, is efficiently removed from DNA by uracil-*N*-glycosylase, thymine, the deamination product of 5-methylcytosine, is not. To account for our observation of the enhanced recovery of amber 6, 15 and 34 mutations after F′ transfer, an impressive increase in the rate of cytosine deamination during conjugation would be required. Although cytosine deamination does occur more readily in single-stranded DNA (at elevated temperatures) (LINDAHL and NYBERG 1974), it seems unlikely that the brief period of F′ transfer during which the DNA is single-stranded is sufficient to allow any significant degree of deamination under physiological conditions. Moreover, transition and transversion events at other amber sites also are increased following transfer. Thus, we do not favor the view that the increased mutagenesis found at amber sites 6, 15 and 34 subsequent to F′ transfer is related to the spontaneous deamination of 5-methylcytosine residues. Instead, we prefer the hypothesis that 5-methylcytosine templates the misincorporation of adenine more often than does cytosine, and reduced replicational fidelity during conjugal transfer enhances this effect. Such an alteration in the base-pairing properties of 5-methylcytosine would be consistent with the observed 2-aminopurine hotspots at the same sites (B. W. GLICKMAN and C. JANION, unpublished result). Alternatively, some yet to be characterized repair system which does not act during F′ transfer may recognize A:C mispairings and suppress the G:C → A:T transitional event.

Although *E. coli* DNA polymerase III, the enzyme responsible for vegetative DNA replication, is also thought to catalyze conjugal DNA synthesis (WILKINS and HOLLOM 1974; KINGSMAN and WILLETS 1978), there are some differences. For example, unlike vegetative DNA replication, conjugal DNA synthesis is not blocked by *dnaB* mutations (MARINUS and ADELBERG 1970; VAPNEK and RUPP 1971; BRESLER, LANZOV and LIKHACHEV 1973) or adenine starvation (GROSS and CARO 1966). Furthermore, conjugal DNA synthesis occurs when vegetative DNA replication in both donor and recipient strains has been

blocked by UV or ionizing radiation (GROSS and CARO 1966; GREENBERG, GREEN and BAR-NUN 1970; GREEN, BRIDGES and RIAZUDDIN 1971). This raises the possibility that conjugal DNA synthesis can bypass template damage. Although both vegetative and conjugal DNA replication appear to be carried out by the same DNA polymerase, clearly these processes differ substantially in their accuracy. If, in fact, conjugal DNA synthesis is inherently less accurate, this could be due to differences in the composition of the polymerase complex or could reflect specific alterations by a *recA*-independent mechanism. Alternatively, F factors code for a number of products involved in their metabolism (CLARK and WARREN 1979; WILLETS and SKURRAY 1980), and some of these, such as single strand-binding protein (KOLODIN *et al.* 1983) may play a role in the maintenance of replicational fidelity. The absence of these products in the recipient during and immediately following F′ transfer may be the cause of the reduction in fidelity.

Other possibilities exist. Rather than being a direct consequence of the fidelity of DNA replication *per se*, the reduction in transfer fidelity might mirror the inaction of methylation-instructed mismatch repair on transferred DNA (GLICKMAN, VAN DEN ELSEN and RADMAN 1978; GLICKMAN and RADMAN 1980; GLICKMAN 1982). In this case, replication errors normally repaired by mismatch correction would lead to enhanced mutation rates. If so, the spontaneous mutational spectra for mismatch repair-deficient strains should closely resemble the mutational spectrum for F′ transfer. This is not the case. The spontaneous spectra obtained in *dam*⁻ strains (GLICKMAN 1979) and *mutH*⁻, *mutL*⁻, *mutS*⁻ and *uvrE*⁻ strains (B. W. GLICKMAN, R. L. DUNN and W. MOESSEN, unpublished results) show increased G:C → A:T transition rates but fail to show the specific enhancement of mutation at amber sites 6, 15 and 34 as found after transfer. Thus, reduced fidelity of conjugal DNA synthesis through inefficiency or absence of methylation-instructed error-avoidance repair seems unlikely. Another possible explanation for a decrease in the fidelity of conjugal DNA replication is that transferred F′ DNA may bear an inordinate amount of DNA damage. Such damage might accumulate during vegetative replication of the F′ DNA in the donor or might be caused by the transfer process itself. It is conceivable that mutations detected following conjugation might arise from the fixation of lesions present in the F′ DNA and which go unrepaired upon transfer.

Although the molecular mechanism remains undefined, the altered fidelity of DNA synthesis associated with conjugal transfer demonstrates how exquisitely the accuracy of vegetative DNA replication is controlled. Our observation provides one more example of how vegetative and conjugal DNA synthesis differ.

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