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Conjugation Is Not Required for Adaptive Reversion of an Episomal Frameshift Mutation in *Escherichia coli*

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Adaptive reversion of a *lac* allele on an F′ episome in a strain of *Escherichia coli* is dependent on the RecA-BCD pathway for recombination and is enhanced by conjugal functions. However, conjugation, i.e., transfer of the episome, whether between distinct populations of cells or between newly divided siblings, does not contribute to the mutational process.

The mechanism by which adaptive mutations occur in a population of cells exposed to a nonlethal selection has been well studied in one strain of *Escherichia coli*, FC40. This strain has the *lac* operon deleted from its chromosome but carries a revertible *lac* allele, Φ(*lacI33-lacZ*), on an F′ episome. When lactose is the sole carbon source, Lac$^+$ revertants appear continuously at a high rate for several days (2). The occurrence of these revertants depends on the RecA-BCD pathway for recombination (2, 4, 8). In addition, RecA-dependent adaptive reversion (RADAR) requires that the *lac* allele reside on the episome (6, 7, 14) and is greatly enhanced by the expression of certain functions required for conjugation (6, 7). Because of these facts, it has been assumed that conjugation among the F′ (male) cells is an important component of RADAR (12, 15). Conjugation is the transfer of genetic material from donor to recipient, which for F$^+$ bacteria means transfer of the episome (9, 10). We previously showed that, during our experiments, the level of conjugation between genetically distinct male cells is low and that such conjugation does not raise the frequency of mutation in the population (6). Here we show that transfer of the episome between identical cells is not required for RA DAR and that conjugation, even between siblings (if it occurs), has no discernible effect on the frequency of mutation to Lac$^+$.

To test directly for episome transfer, we used strains of revertible cells that were differentially marked with drug resistances on their episomes and their chromosomes but were otherwise genetically identical. The chromosomal marker was rifampin resistance (Rif^r) (presumably a mutation in *rpoB*), and the episomal markers were *zzf-1831*::Tn*10*dTet, encoding tetracycline resistance (Tet^r) (obtained from J. Roth), and *zaj-3099*::Tn*10*dKan, encoding kanamycin resistance (Kan^r) (obtained from C. Gross). In one experiment we mixed FC453 (Rif^r/F′ Tet^r) with FC30 (no drug resistance); in a second experiment we mixed FC396 (Rif^r/F′ Kan^r) with FC509 (Rif^s/F′ Tet^r). In both experiments, 10^9 cells, consisting of equal aliquots from 20 independent cultures of each strain, were mixed and plated on M9–0.1% lactose plates (1). Each day from day 2 until the plates were crowded with Lac$^+$ colonies, two or more newly arisen Lac$^+$ colonies from each plate were purified by streaking on lactose plates. Three to five colonies from each Lac$^+$ isolate were then tested for their drug resistances on minimal lactose plates.

The results given in Table 1 show that 96% of the late-appearing Lac$^+$ mutations appeared in the parental genetic background. Therefore, even if episome transfer is inherently mutagenic (3, 11), transferred episomes make little contribution to RADAR. For some reason, Tet^r episomes gave rise to fewer mutants than the other episomes in both experiments. Part of this difference was because the Tet^r cells were in the minority (46% in the first experiment and 40% in the second experiment). But this difference is irrelevant to episome transfer because the proportion of Tet^r revertants was the same among late-appearing mutants as among those that appeared on day 2, which are due to mutations that occurred during nonselective growth before the populations were mixed.

While these experiments demonstrate that episome transfer

TABLE 1. Chromosomal and episomal phenotypes of Lac$^+$ revertants

Mutant class	No. of isolates on:	
	Day 2	Days 3 to 5
Expt 1		
Lac$^+$ (total)	40	120
Rif^s Tet^s (parental)	29	86
Rif^r Tet^r (parental)	11	31
Rif^s Tet^r (nonparental)	0	1
Rif^r Tet^s (nonparental)	0	2
Expt 2		
Lac$^+$ (total)	44	98
Rif^r Kan^r (parental)	32	70
Rif^s Tet^r (parental)	12	23
Rif^r Tet^r (nonparental)	0	0
Rif^s Kan^r (nonparental)	0	0
Other (nonparental)	0	5[a]
Both expts		
Lac$^+$ (total)	84	218
Parental	84	210
Nonparental	0	8

[a] Three isolates, two Rif^s and one Rif^r, carried both episomal drug markers, and two isolates carried no drug markers. The Rif^s Tet^r Kan^r isolates segregated the episomal markers, indicating that the original Lac$^+$ cell probably had two episomes. The others were stable, suggesting that they carried a recombinant episome.

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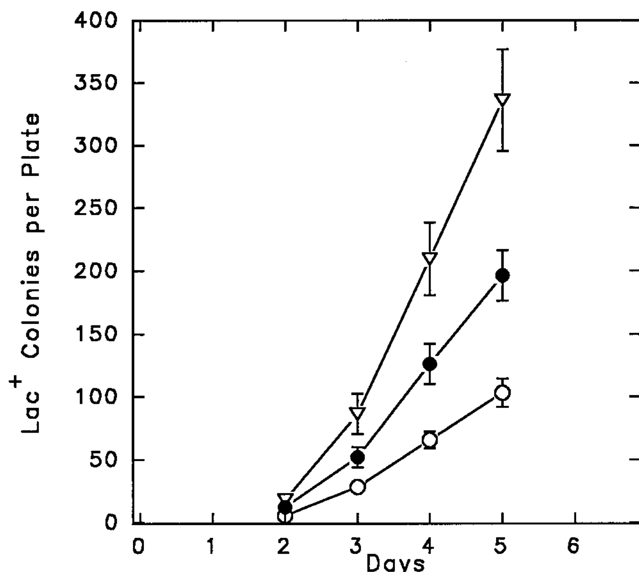

FIG. 1. Accumulation of Lac+ revertants of FC40 on lactose plates supplemented with glycerol. Five independent cultures of FC40 were used. Shown are the mean cumulative number of Lac+ colonies per plate ± the standard error of the mean. Open circles, no glycerol; closed circles, 1 μg of glycerol; triangles, 3 μg of glycerol.

among different populations of male cells is not required for RADAR, episome transfer could preferentially occur between siblings, and this transfer would be genetically invisible (1). If this hypothesis is true, mating must occur after the cells are on lactose plates, because when mating-pair formation is inhibited by adding a detergent before plating, mutation to Lac+ after plating is not inhibited (14).

No growth occurs on our lactose plates when 10^9 Lac− cells are plated (2, 5). Thus, to test whether mating between siblings contributes to Lac+ mutation, we ensured that every cell divided by adding a small amount of another carbon source, glycerol. A total of 10^8 revertible cells and 10^9 scavengers (FC29, which can neither revert nor recombine to give a Lac+ phenotype) were plated in top agar on plates without a carbon source and then overlaid with top agar containing no carbon source or 1 or 3 μg of glycerol. The plates were then overlaid with top agar containing 25 mg of lactose. The addition of 1 μg of glycerol allows all the cells to divide once, and an additional 2 μg allows many of these cells to divide again (data not shown). Thus, on the glycerol-supplemented plates, each cell was in close contact with several siblings and would have a much better chance of transferring its episome. If RADAR depends on intersibling transfer, then the rate of mutation should have been greatly enhanced. But, as shown in Fig. 1, glycerol increased the rate at which Lac+ mutants arose only two- and threefold, exactly as expected from the increase in revertible cells present.

These results indicate that transfer of the episome from one male cell to another, whether the cells are newly divided siblings or are derived from different populations, makes no significant contribution to the mutation rate to Lac+. The level of male-male conjugation reported to occur during nonlethal selections varies widely, apparently reflecting differing experimental conditions (6, 13, 14). Because the episome is replicated upon transfer, each act of conjugation produces two mutational targets where before there had been but one. Thus, conjugation, if it occurs at all, may increase the rate at which mutants appear among the population as a whole, but the mutation rate per mutational target may be unchanged. Our experiments demonstrate that conjugation is not required to produce RADAR. Therefore, conjugal functions must be required for reasons other than episome transfer. The most likely of these are nicking at the conjugal origin, *oriT*, and/or the initiation of DNA replication (6, 7).

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