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Redundant Homosexual F Transfer Facilitates Selection-Induced Reversion of Plasmid Mutations

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F plasmids use surface exclusion to prevent the redundant entry of additional F plasmids during active growth of the host cells. This mechanism is relaxed during stationary phase and nonlethal selections, allowing homosexual redundant plasmid transfer. Homosexual redundant transfer occurs in stationary-phase liquid cultures, within nongrowing populations on solid media, and on media lacking a carbon source. We examined the relationship between homosexual redundant transfer, which occurs between F^+ hosts, and reversion of a plasmid-encoded *lac* mutant allele, *lacI33ΩlacZ*. Sodium dodecyl sulfate (SDS) and mutations that prevent normal transfer to F^- cells reduced redundant transfer and selection-induced reversion of the *lacI33ΩlacZ* allele. A *recA* null mutation reduced redundant transfer and selection-induced reversion of the *lacI33ΩlacZ* mutation. Conversely, a *recD* null mutation increased redundant transfer and selection-induced reversion of the *lacI33ΩlacZ* allele. These results suggest an explanation for why SDS and these mutations affect reversion of the plasmid *lacI33ΩlacZ* allele. However, a direct causal relationship between transfer and reversion remains to be established. These results suggest that Rec proteins play an active role in redundant transfer and/or that redundant transfer is regulated with the activation of recombination. Redundant homosexual plasmid transfer during a period of stress may represent a genetic response that facilitates evolution of plasmid-encoded functions through mutation, recombination, reassortment, and dissemination of genetic elements present in the populations.

The molecular biology of the fertility (F) plasmid of *Escherichia coli* has been studied extensively (see reviews in references 12, 16, 17, and 32). Most studies with F and related plasmids use a heterologous system in which an F^+ male donor is mated to an F^- female recipient for a short time in exponentially growing liquid cultures. Under these conditions, plasmid transfer is generally assumed to occur once (reviewed in references 12, 16, 17, 18, and 32); and transfer between F^+ males is inhibited by a process, termed surface exclusion (19), that requires the products of two genes, *traS* and *traT* (1). In laboratory matings, filters and solid media are often used to increase the probability of successful plasmid transfer. It is mechanistically unclear how these treatments and growth conditions affect mating efficiency and whether surface exclusion is an effective barrier under all conditions. In stationary-phase cultures, surface exclusion is relaxed (19, 25), resulting in the accumulation of more than one F plasmid in a single host (3). This increase in ploidy is temporary because copy number returns to one plasmid per cell upon exponential growth (3). The accumulation of more than one F plasmid within a host allows exchange of genetic information between F genomes; this process combined with the segregation of recombinant genomes into daughter cells can be viewed as a sexual cycle for the plasmid.

Homosexual F plasmid transfer occurs between F^+ cells during nonlethal selection in a process termed redundant transfer (25). We are interested in the significance of homosexual redundant transfer and how it might influence the evolution of prokaryotic genomes under different environmental conditions. To better understand redundant transfer, we constructed strains that allowed us to monitor transfer and reversion of the plasmid-encoded *lacI33ΩlacZ* allele. We found that redundant transfer occurred during various selective and nonselective conditions, both in liquid media and on solid media, and was reduced by sodium dodecyl sulfate (SDS) and by a mutation that blocks expression of the F-encoded *tra* genes. During nongrowth conditions, the amount of redundant transfer was sensitive to the presence and absence of the RecA and RecD proteins. Treatments and mutations that affected redundant transfer, in a parallel fashion, affected the amount of selection-induced reversion of the plasmid-encoded *lacI33ΩlacZ* allele, suggesting there may be a linkage between homosexual redundant transfer and reversion of the plasmid-encoded *lacI33ΩlacZ* allele during selection.

MATERIALS AND METHODS

Media and growth conditions. Media, M63 buffer, and antibiotic concentrations were as described by Silhavy et al. (30). Rifampin was used at 100 μg/ml. Liquid cultures were grown in LB media at 37°C on a roller drum. SDS was added at a concentration that does not interfere with cell growth, 0.01% (wt/vol) (2, 27).

Bacterial strains. The strains used are described in Table 1. Strain IB29 was constructed in two steps. First a *malB*::Tn*5* (kanamycin resistance) mutation was introduced into FC29 via P1 transduction, yielding a strain unable to grow on minimal maltose media (Mal^-). The strain was transduced to Mal^+ by using a P1 lysate prepared on MCR106. The transductants were screened by cross-streaking over phage λ to identify those that acquired the Δ*lamB106* allele. P1 transductions were done as described by Silhavy et al. (30). The Tn*5*, Tn*10*, and *tra-701* mutations were introduced into the various F′ *lac* plasmids by P1 transductions. In brief, P1 lysates were grown on strains carrying F plasmids or derivatives carrying the desired insertion marker (Table 1) and used to move the desired marker onto the F′ *proAB* Δ(*lacI-lacZ*) or F′ *proAB lacI33ΩlacZ* plasmids by selecting for resistance to the appropriate antibiotic.

Plasmid transfer assays. Transfer in liquid media was determined as follows. Five-milliliter cultures of the donor and F^+ recipient were grown overnight in LB media, diluted 1:50 in 10 ml of LB media (with and without SDS), and then grown to stationary phase (~8 h). One milliliter was removed from the F^+ recipient cultures and replaced with 1 ml of the donor culture. The mixed cultures were incubated for 0, 0.5, 2, and 4 h on a roller drum before assays of plasmid transfer. To assay transfer, the cells were washed twice with 10 ml of M63 buffer, and transfer from the donor into the F^+ recipient population was determined by using their antibiotic resistance determinants as described previously (25). Transfer on solid medium was assayed as follows. Cultures were

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TABLE 1. Bacterial strains and F′ plasmids used

Name	Genotype	Reference or construction
Strains		
MCR106	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR* Δ*lamB106*	8
FC29	*ara* Δ(*lac-proB*)$_{XIII}$ *thi* [F′ *proAB* Δ(*lacI-lacZ*)]	7
FC40	*ara* Δ(*lac-proB*)$_{XIII}$ *thi* (Rif^r) (F′ *proAB lacI33*Ω*lacZ*)	7
SBM410	MCR106 (F′ *proAB lacI33*Ω*lacZ*)	25
SBM420	FC40 Δ*lamB106* (F′ *proAB lacI33*Ω*lacZ*)	25
IB29	FC29 Δ*lamB106* [F′ *proAB* Δ(*lacI-lacZ*)]	Materials and Methods
IB39	IB29 *rpsL150* [F′ *proAB* Δ(*lacI-lacZ*)]	P1 (MCR106) × IB29
JP30	IB29 [F′ *proAB* Δ(*lacI-lacZ*) Tn*5*]	P1 (JP184) × IB29
JP36	JP30 *rpsL150* [F′ *proAB* Δ(*lacI-lacZ*) Tn*5*]	P1 (MCR106) × JP30
JP181	SBM420 (F′ *proAB lacI33*Ω*lacZ* Tn*5*)	F′ (JP184) × SBM420
JP182	SBM410 (F′ *proAB lacI33*Ω*lacZ* Tn*10*)	25
JP183	SBM420 (F′ *proAB lacI33*Ω*lacZ* Tn*10*)	F′ (JP182) × SBM420
JP184	SBM410 (F′ *proAB lacI33*Ω*lacZ* Tn*5*)	P1 (RY01) × SBM410
DPB271	λ$^-$ *recD1903*::mini-Tet	6
GP01	MC4100 Δ*recA*::Cmam^r	Greg Philips
RY01	MC4100 F′ Tn*5*	Ryland Young
Plasmids		
pOX38	45.4 *Hin*dIII fragment, circularized (*tra*$^+$)	20
pOX38-*tra-701*	Kan^r in a P_{T7} linker, replaces P_Y	20

grown overnight in LB media and washed with M63 buffer, and 10^8 CFU of the donor plus 10^9 CFU of the F$^+$ recipient were mixed and spread on solid agar medium. At various times, samples of the lawn were removed as an agar plug with a sterile test tube (18 by 150 mm), and the cells were washed free by vortexing in M63 buffer. Transfer frequencies were assayed as described previously (25). Each strain set was independently assayed at least three times; the exception was the liquid transfer assay, which was done twice. Transfer frequencies in Fig. 1 to 4 show data from a single representative transfer assay; in every case, replicate experiments gave analogous results.

Nonlethal selection conditions. Cells were grown overnight in LB media and washed twice with M63 buffer. Mutants able to grow with lactose as the sole carbon source (Lac$^+$) were selected on minimal M63 lactose medium, using either whole plates or minilawns. When whole plates were used, 10^8 donors plus 10^9 F$^+$ recipient cells were mixed and plated (0.2 ml). For minilawns, 10^7 donor cells plus 10^8 F$^+$ recipient cells were applied to the plate as 50-μl spots. Minilawns behaved identically to whole plates, i.e., the Lac$^-$ cell population did not increase (data not shown). Use of minilawns allowed us to test >20 samples from each culture set, improving statistical analysis. Mutants able to grow with maltodextrin as their sole carbon source (Dex$^+$) were selected by plating approximately 10^8 cells as 50-μl spots as minilawns on minimal M63 maltodextrin medium; cell populations plated under these conditions do not increase in size during the selection (data not shown).

Determination of mutant background. To determine whether the Lac$^+$ colony originated from the rifampin-resistant donor or the streptomycin-resistant F$^+$ recipient population, 15 to 30 Lac$^+$ revertants that appeared on day 2 or 6 were purified on minimal lactose medium from each independent culture set. An isolated Lac$^+$ colony was picked from the purification plate grown on lactose minimal medium and replica plated to LB-streptomycin and LB-rifampin media. For the *rec*$^+$ tests, five independent culture sets were tested (three, JP183 plus JP36; two, JP181 plus IB39). For the *recA* (JP183 Δ*recA*::Cam plus JP36Δ*recA*::Cam) and *recD* (JP181 *recD*::mini-Tet plus IB39 *recD*::mini-Tet) strains, three independent culture sets were tested for each allele.

RESULTS

We quantified the transfer that occurred between F$^+$ populations during conditions commonly employed in the laboratory, using strains in which the chromosomes and F plasmids had been tagged with unique antibiotic resistance determinants (Table 1). When describing plasmid transfer, one usually refers to an F$^+$ strain as the donor and an F$^-$ strain as the recipient. However, these terms are ambiguous when both populations are F$^+$ because each donor is a potential recipient and each recipient is a potential donor. We therefore use the terms donor and F$^+$ recipient for the two F$^+$ hosts to designate the direction of plasmid transfer being monitored. Because we were also interested in exploring the relationship of plasmid transfer and the reversion of a plasmid-encoded allele, the host carrying the revertible F′ *lacI33*Ω*lacZ* allele was always monitored as the donor, and the F$^+$ recipient carried the nonrevertible F′ Δ(*lacI-lacZ*) plasmid (Table 1).

F plasmid transfer during various nongrowth conditions. It was previously shown that surface exclusion is relaxed in cells once they reach stationary phase (19, 25), suggesting the possibility of redundant transfer among F$^+$ cells during stationary phase. Two nearly isogenic populations were used to learn the extent of redundant F plasmid transfer within an F$^+$ population during stationary phase. We used strain JP183 as the donor and strain JP36 as the F$^+$ recipient. We found that nearly 20% of the donor cells transferred plasmids to F$^+$ recipient cells (Fig. 1). When transfer was assayed in the presence of 0.01% SDS, an agent that reduces transfer in actively growing cells, transfer decreased more than 100-fold (Fig. 1).

We also determined the frequency of redundant plasmid transfer for donor and F$^+$ recipients on minimal lactose agar medium (Fig. 2). This medium selects for cells that have reverted the *lacI33*Ω*lacZ* allele and can form colonies in the nongrowing bacterial lawn (7). Redundant transfer occurred at

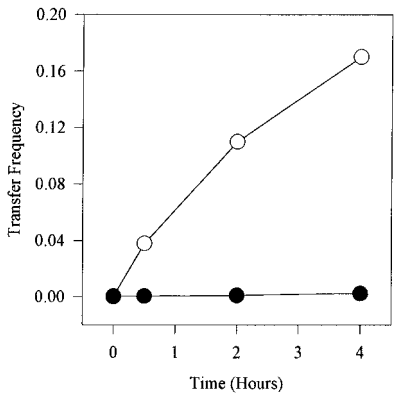

FIG. 1. Frequency of redundant F plasmid transfer during overnight culture and effect of SDS. Plasmid transfer from the donor population (JP183) to an F$^+$ recipient population (JP36) was monitored during the stationary phase of an LB culture (open circles) and an LB culture with 0.01% SDS (filled circles) as described in Materials and Methods.

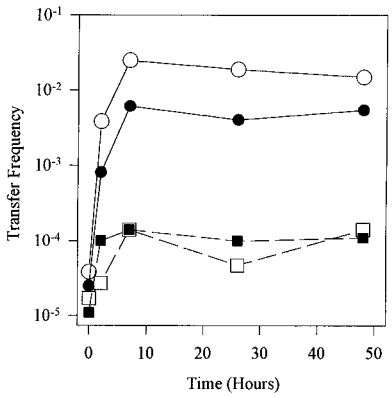

FIG. 2. Frequency of redundant F plasmid transfer on minimal lactose media and effect of SDS. Plasmid transfer was monitored from the donor population (JP183) to an F^+ recipient population (JP36) on minimal lactose medium without (circles) and with (squares) 0.01% SDS for populations pregrown in LB (open symbols) or in LB with 0.01% SDS (filled symbols) as described in Materials and Methods.

a frequency approximating that previously found on minimal maltodextrin medium a different selective medium on which these strains cannot readily grow (5, 25). As in previous assays using minimal maltodextrin medium (25), we could not detect additional transfer events after 10 to 12 h. It is unknown if the observed plateau in transfer frequency results from cessation of transfer or the inability of the cells to form new mating pairs in the lawn with nonclonal partners. The assay system detects only the first transfer to an F^+ recipient; if additional transfers to the same F^+ recipient or between clones occur, they are not detected in this system.

The presence of SDS in the minimal lactose agar medium reduced transfer, as it did in rich liquid medium (Fig. 2). This reduction in transfer was independent of whether the cells were pregrown in SDS. Transfer on solid media (in the absence of SDS) was affected by pregrowth in SDS; i.e., pregrowth in SDS reduced transfer severalfold (Fig. 2). Although the reduction in transfer was only three- to fivefold, it was reproducible and not due to reduced initial cell numbers (data not shown). This finding suggests that prior growth conditions can affect transfer frequencies attained after imposition of the selection, even when an agent is no longer present. This may help to explain why transfer frequencies obtained at different times with the same type of medium show some variation. However, the relative transfer frequencies, SDS versus no SDS and mutant versus wild type, were always consistent within experiments. We observed that transfer on M63 medium lacking a carbon source occurred at a similar frequency and plateau, i.e., $\sim 5 \times 10^{-3}$ (data not shown). This finding suggests that washed stationary-phase cells have sufficient energy reserves to allow redundant transfer to continue for at least 10 to 12 h or that a carbon source is not needed to allow redundant transfer to occur.

Redundant transfer in strains with the *tra-701* promoter deletion. The *tra-701* mutation removes P_Y, the major promoter of the *tra* operon, and reduces transfer to F^- cells more than 10^6-fold, to an undetectable level, in actively growing cultures (20). This mutation did not have as dramatic an effect on redundant transfer on solid minimal lactose medium at later time points, when it reduced redundant transfer by approximately 100-fold (Fig. 3). During the first 6 h, transfer was not detected; i.e., the rate was $<10^{-6}$. Three possible explanations for the different effects of the *tra-701* mutation on normal F^+-to-F^- transfer and redundant transfer are as follows: (i) redundant transfer during nongrowth conditions occurs by a different mechanism than F^+-to-F^- transfer; (ii) redundant transfer is reduced to the same extent as in actively growing cells, but the actual amount of transfer on solid media is masked because most of the transfer events are to the same F^+ recipient (recall that we can detect only the first transfer to an F^+ recipient); and (iii) the extended period available for mating, e.g., >40 h in our assay versus 0.5 h in the F^+-to-F^- transfer (20), is sufficient to partially overcome the deficiencies resulting from the *tra-701* mutation. When the *tra-701* mutation is present only in the donor strain, the results are analogous to those observed when both strains contained the mutation (data not shown). Normal levels of transfer were observed when only the F^+ recipient carried the *tra-701* mutation (data not shown), suggesting that the *tra-701* mutation did not make the strain a better recipient than the tra^+ strain.

Effects of *rec* mutations on redundant transfer. Other forms of gene transfer, e.g., transformation, may be regulated with recombinational activation in *Bacillus* and *Haemophilus* species (21, 24). While it has been suggested that recombination functions are not required for F plasmid transfer (32), a recent review suggests an involvement of RecA during conjugation (16). To test if recombination plays a role in redundant transfer, we determined the effects of *recA* and *recD* null alleles. When the *recA* mutation was present in both the donor and the F^+ recipient, transfer decreased more than 100-fold (Fig. 4). When *recA* was present in only one of the two backgrounds, we observed intermediate levels of transfer (data not shown). When the *recD* mutation was present in both the donor and the F^+ recipient (Fig. 4) or in the donor alone (data not shown), redundant transfer increased more than 50-fold. Transfer frequencies did not change when the *recD* mutation was in only the F^+ recipient strain (data not shown). These findings suggest that redundant transfer during nongrowth conditions involves the RecA and RecD proteins.

Relationship between transfer and reversion of plasmid-encoded alleles. It has been shown that the *lacI33ΩlacZ* allele reverts at a higher frequency when present on an F plasmid than when on the chromosome (27). Several groups have suggested that this difference can be attributed to processes encoded by the *tra* operon of the F plasmid (10, 13, 25, 27). An argument has been made that conjugation itself may be re-

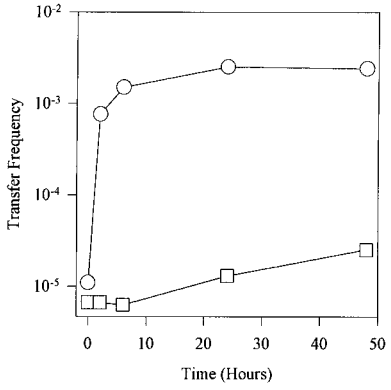

FIG. 3. Frequency of redundant F plasmid transfer on minimal lactose media and effect of the *tra-701* mutation. Plasmid transfer was monitored from the donor population (JP183) to an F^+ recipient population (IB39) on minimal lactose medium as described in Materials and Methods. No transfers ($<10^{-6}$) between the *tra*-minus strains were detected during the first 6 h of incubation. Symbols indicate results found with the following culture sets: JP183 F′ tra^+ plus IB39 F′ tra^+ (circles) and JP183 F′ *tra-701* plus IB39 F′ *tra-701* (squares).

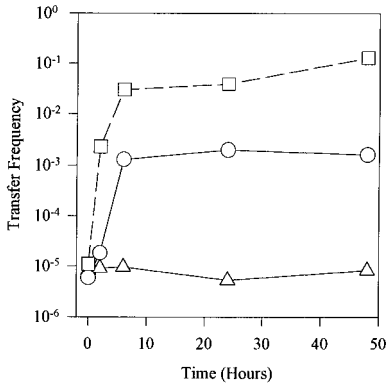

FIG. 4. Frequency of redundant F plasmid transfer on minimal lactose media and effects of *recA* and *recD* null alleles. Plasmid transfer was monitored as described in Materials and Methods. Symbols indicate the results found with the following culture sets: JP181 *rec*$^+$ plus IB39 *rec*$^+$ (circles), JP181 *recD*::mini-Tet plus IB39 *recD*::mini-Tet (squares), and JP183 *recA*::Cam plus JP36 *recA*::Cam (triangles).

sponsible for the increased reversion of the plasmid-encoded allele (13, 27), although which step in the transfer-related processes is responsible for reversion of the *lacI33ΩlacZ* allele is debated (10). Since we can monitor transfer and reversion of the plasmid-encoded *lacI33ΩlacZ* allele, we looked at the relationship between the two phenomena.

Radicella et al. (27) showed that addition of SDS to the lactose selection medium reduced reversion of the plasmid-encoded *lacI33ΩlacZ* allele, but its effects on transfer during selection were not monitored. Having shown that SDS affects transfer within the F$^+$ population in the overnight culture and on minimal lactose medium (Fig. 1 and 2), we monitored the appearance of Lac$^+$ revertants under these same conditions. We obtained results analogous to those reported by Radicella et al. (27); i.e., plating on minimal lactose medium containing SDS greatly reduced the number of Lac$^+$ revertants (Fig. 5). Our results extend those of Radicella et al. (27) by showing that pregrowth of donor cells in SDS reduced the number of late-appearing revertants on lactose medium with or without SDS (Fig. 5). This result suggests that transfer-competent aggregates form in the donor population before plating and that these aggregates yield Lac$^+$ revertants many days after plating. Alternatively, SDS may prevent the accumulation of multiple plasmids in the revertible F′ *lacI33ΩlacZ* cells prior to selection. As with the observed transfer frequencies, prior growth conditions affect the signal that is assayed. This may help to explain why it is commonly found that reversion frequencies vary between experiments, since preselection conditions, as well as the selection environment, can affect the number of Lac$^+$ colonies obtained.

Mutations affecting reversion and plasmid transfer. Mutations in *recA* and *recD* are reported to affect reversion of the *lacI33ΩlacZ* plasmid-encoded allele (9, 15). Given the effects of the *recA* and *recD* null alleles on plasmid transfer, we tested the effects of these mutations on reversion of the *lacI33ΩlacZ* allele carried on the F plasmid. Our findings match those previously reported (9, 15): the *recD* mutation increased the number of Lac$^+$ revertants, and the *recA* mutation reduced the number of Lac$^+$ revertants (Fig. 6). Thus, there is a striking correlation between the effects of the *rec* mutations on plasmid transfer and reversion to Lac$^+$ (Fig. 4 and 6). When we tested the effect of the *tra-701* allele on Lac$^+$ reversion, a similar correlation was observed; i.e., the reduction in transfer correlated with a reduction in late appearing Lac$^+$ revertants (Fig. 3 and 7). Other *tra* alleles are reported to have similar effects (10).

Lac$^+$ revertants in the nonrevertible F$^+$ recipient population. At the start of our selections, the lawn contains two cell populations: donor cells with the F′ *lacI33ΩlacZ* allele and F$^+$ recipient cells with the nonrevertible F′ Δ(*lacI-lacZ*) allele. Immediately following plating, redundant homosexual transfer would presumably generate a new F$^+$ recipient cell population containing both the F′ *lacI33ΩlacZ* plasmid and the F′ Δ(*lacI-lacZ*) plasmid. By comparing the frequency of transfer of the plasmid-encoded *lacI33ΩlacZ* allele into the nonrevertible F$^+$ recipient population with the frequency of *lacI33ΩlacZ* reversion in the F$^+$ recipient population, we can explore the relationship between redundant transfer and reversion.

It has been shown that once a Lac$^+$ reversion occurs, a visible Lac$^+$ colony develops within 2 days (7). Therefore, we examined Lac$^+$ colonies that first became visible on day 2 and those that first appeared on day 6. Lac$^+$ colonies that appeared 2 days after plating presumably represent reversion events that occurred before or immediately after imposition of the selection. Lac$^+$ colonies that appeared on day 6 presumably represent mutational events that occurred on day 4. On day 2, only 3% of the Lac$^+$ revertant plasmids were recovered in the F$^+$ recipient background (Table 2). In contrast, on day 6, 44% of the Lac$^+$ revertant colonies were formed by cells in which the Lac$^+$ allele is carried by an F$^+$ recipient cell. This percentage suggests a linkage between transfer and reversion. One possible explanation for the effects of *recA* and *recD* on reversion of the plasmid-encoded *lacI33ΩlacZ* mutation is that *recA* and *recD* affect the frequency of plasmid transfer (Fig. 6). If this is true, then the *recA* and *recD* mutations should also change the frequency of Lac$^+$ reversions recovered in the F$^+$ recipient background. When the *recA* mutation was present in both the donor and F$^+$ recipient populations, recovery of Lac$^+$ revertants in the F$^+$ recipient population at day 6 was reduced to 16% (Table 2). The *recD* mutation had the opposite effect: it increased the percentage of Lac$^+$ mutations in the F$^+$ recipient population to 58% on day 6 (Table 2).

***recA* and *recD* null mutations do not affect the frequency of late-arising chromosomal Dex$^+$ mutations.** Chromosomal genes are reported to experience mutations during selection in the absence of population growth (5, 14, 26). If plasmid transfer drives reversion of the F′ *lacI33ΩlacZ* allele, and if the *recA*

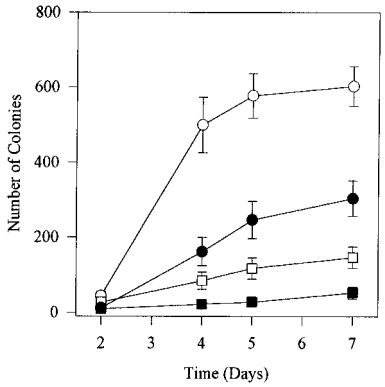

FIG. 5. Effect of 0.01% SDS, during pregrowth in LB and during selection, on Lac$^+$ colony formation. The experiment was carried out with whole plates as described in Materials and Methods, using the mixed population of JP183 plus JP36. Symbols show the results found on minimal lactose media without (circles) and with (squares) 0.01% SDS for populations pregrown in LB (open symbols) or in LB with 0.01% SDS (filled symbols). Error bars show standard errors of the means. Results are from four independent culture sets, each with four replicates.

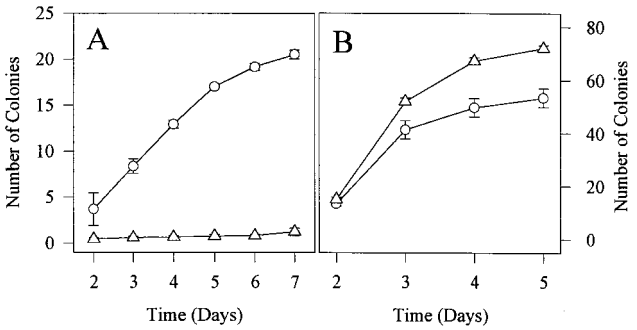

FIG. 6. Effects of *recA* and *recD* null alleles on Lac$^+$ colony formation. The experiment was carried out with minilawns as described in Materials and Methods. (A) Effect of *recA* on accumulated colony number for JP181 *rec*$^+$ plus IB39 *rec*$^+$ (circles) and JP181 *recA* plus IB39 *recA* (triangles). (B) Effect of *recD* on accumulated colony number for JP181 *rec*$^+$ plus IB39 *rec*$^+$ (circles) and JP181 *recD*::mini-Tet plus IB39 *recD*::mini-Tet (triangles). Results for the *recA* and *recD* alleles are from three and four independent culture sets, respectively, tested >20 times. Errors bars show standard errors of the means.

and *recD* mutations affect the reversion frequency by influencing transfer, then colony formation that stems from chromosomal mutations during selection might not be affected by alterations in these functions. To test this predication, we used a selection for growth on large maltodextrins as a chromosome-based system (5). The maltodextrin selection system uses strains that cannot grow on minimal maltodextrin medium (Dex$^-$) because they lack the LamB maltoporin that is required to transport large maltodextrins across the outer membrane (31). The Dex$^-$ phenotype can be overcome by mutations at four chromosomal genes that allow maltodextrin import (4, 22, 23, 29). As with the lactose system, Dex$^+$ colonies continue to appear during extended selection in the absence of population growth or an increase in unselected mutations (5). The *recA* and *recD* mutations do not significantly affect the formation of Dex$^+$ colonies (Fig. 8). Therefore, a Rec-independent mechanism must be responsible for the mutations that yield late-appearing Dex$^+$ colonies arising from chromosomal mutations. This has also been found for the *lacI33ΩlacZ* allele when it is present on the chromosome (10).

DISCUSSION

This work suggests a link between two of the functions implicated in the selection-induced reversion of the *lacI33ΩlacZ* mutation, F transfer and Rec dependence. We show that *recA*, *recD*, and *tra-701* mutations as well as treatment with SDS have parallel effects on transfer- and selection-induced reversion of the *lacI33ΩlacZ* allele. We show that a high level of redundant plasmid transfer occurs in liquid stationary-phase cultures but does not to lead to a detectable increase in reversion of the *lacI33ΩlacZ* allele. Previously Kunz and Glickman (18) showed a 1.8-fold stimulation in the formation of episomal *lacI* mutations during conjugal transfer to F$^-$ recipients. Our assay system is not sensitive enough to detect changes of this size. In contrast, homosexual redundant transfer on solid media during selective conditions increased the reversion of the plasmid-encoded allele 5- to 10-fold (Fig. 5 to 7) and resulted in recovery of Lac$^+$ revertants in the F$^+$ recipient population (Table 2).

Redundant transfer may occur continuously during selection. The observed transfer frequency reaches a stable plateau after 10 to 12 h when assayed on solid medium (Fig. 2 to 4) (25). Previously we have suggested that this plateau does not represent cessation of transfer (25). The transfer assay can detect only the first transfer into an F$^+$ recipient cell; i.e., an F$^+$ recipient that receives multiple plasmids scores the same as one with a single donated plasmid. When nongrowing cells could form new mating pairs, e.g., in a liquid environment on the roller drum, much higher levels of mating were detected (Fig. 1). Therefore, the observed plateau in transfer frequency presumably occurs because new genetically detectable donor-recipient mating pairs cannot form in the static and highly structured lawn environment. The transfer frequencies in Fig. 1 through 4 do not portray the full extent of transfer occurring in the population; they represent only the initial transfer of the marked F′ from the donor into the F$^+$ recipient population, and similar levels of transfer are occurring in the opposite direction (25) and presumably among siblings within the donor and F$^+$ recipient populations. With ongoing redundant plasmid transfer, plasmid copy number may rise, existing plasmids may recycle to provide precursors for the replication of an incoming plasmid, or the incoming plasmid may remain single stranded.

Transfer and reversion of plasmid alleles during selection. It is suggested that plasmid transfer functions stimulate reversion of the F′ *lacI33ΩlacZ* allele (10, 13, 25, 27). Our data support and extend this suggestion. We found that SDS, the *tra-701* mutation, and mutations in *recA* and *recD* have parallel effects on transfer and reversion of the plasmid-encoded allele. Other mutations in the F plasmid *tra* operon have also been found to affect reversion of the *lacI33ΩlacZ* allele (10), but the effects of these mutations on transfer have not been determined. Thus far, it has been impossible to uncouple transfer and reversion of the *lacI33ΩlacZ* allele so as to test the possibility of a causal relationship between transfer and reversion of the *lacI33ΩlacZ* allele. Support for interdependence of these two phenomena comes from the observation that the *recA* and *recD* mutations change the occurrence of Lac$^+$ revertants in the F$^+$ recipient cells in a manner consistent with their effects on plasmid transfer (Table 2). It is unknown whether the *rec* mutations play roles in reversion of the *lacI33ΩlacZ* allele beyond their effects on plasmid transfer. How these *rec* mutations alter redundant transfer frequencies is unknown.

Reversion of a plasmid allele is stimulated by transfer during selection. We found that the high degree of redundant transfer in stationary-phase liquid cultures is reduced by SDS

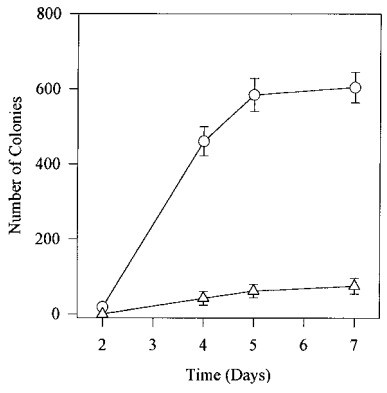

FIG. 7. Effect of *tra-701* on reversion of the plasmid-encoded *lacI33ΩlacZ* allele. The experiment was carried out as described in Materials and Methods, using whole plates. Accumulated colony number was monitored for JP183 F′ *tra*$^+$ plus IB39 F′ *tra*$^+$ (circles) and JP183 F′ *tra-701* plus IB39 F′ *tra-701* (triangles). Results are from four independent culture sets, each with four replicates plated. Error bars show standard errors of the means.

TABLE 2. Percentages of Lac^+ revertants in the donor and F^+ recipient strains[a]

Relevant genotype	% of Lac^+ revertants			
	2 days		6 days	
	Donor	F^+ recipient	Donor	F^+ recipient
rec$^+$	97 (96)	3 (3)	56 (134)	44 (105)
recA	99 (86)	1 (1)	84 (58)	16 (11)
recD	100 (90)	0 (0)	42 (56)	58 (78)

[a] Strain background was determined as described in Materials and Methods. The number of isolates of each type is given in parentheses.

(Fig. 1). Since we consistently observed the same quantity of Lac^+ colonies at day 2 irrespective of whether SDS or the *tra-701* mutation was present (Fig. 5 and 7), transfer before imposition of selection is not mutagenic per se. If all redundant homosexual transfer were mutagenic, then we would have observed fewer Lac^+ colonies on day 2 when transfer was reduced by SDS or the *tra* mutation; we did not. However, transfer of the plasmid-encoded *lacI33ΩlacZ* allele during selection on solid media stimulates reversion. When we compared the occurrences of Lac^+ colonies in the donor and the newly formed F^+ recipient populations carrying the *lacI33ΩlacZ* allele, on day 6, we found that this latter population yields 44% of the mutants (Table 2). Similar results were obtained with colonies that arose on day 8 (data not shown). This level is higher than those reported by Radicella et al. (20%) (27), Rosenberg et al. (8%) (28), and Foster and Trimarchi (4%) (11); the difference in these percentages compared with that in Table 2 may reflect differences in plating techniques, e.g., soft agar overlays versus cell lawns. It is important to note that the newly formed F^+ recipient population carrying the *lacI33ΩlacZ* allele is only 1 to 5% of the size of the donor population (Fig. 2). If transfer of the *lacI33ΩlacZ* allele into the F^+ recipient population stops after 10 to 12 h, the mutation rate in this small population would have to be 50- to 100-fold higher than that of the donor population to yield >40% of the late-occurring Lac^+ colonies. This seems unlikely. The simplest explanation is that once mating aggregates are established on solid medium, ongoing redundant transfer increases the probability of plasmid mutations. Although we favor the latter explanation, transfer-associated reversion could also stem from the single-stranded nature of newly transferred plasmid DNA or unknown causes.

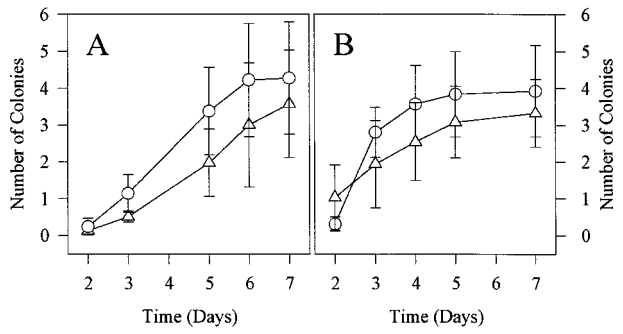

FIG. 8. Effects of *recA* and *recD* null alleles on Dex^+ colony formation. The experiment was carried out with minilawns as described in Materials and Methods. (A) Effect of *recA* on accumulated colony number for JP183 *rec*$^+$ (circles) and JP183 *recA*::Cam (triangles). (B) Effect of *recD* on accumulated colonies for JP181 *rec*$^+$ (circles) and JP181 *recD*::mini-Tet (triangles). Results for the *recA* and *recD* alleles are from three and four independent culture sets, respectively, tested >20 times. Error bars show standard errors of the means.

We do not find an exact correlation between the magnitude of the effects of SDS and the mutations on transfer and reversion of the plasmid-encoded *lacI33ΩlacZ* allele. However, in most cases, the magnitudes are similar. For example, the *tra-701* mutation reduces transfer approximately 100-fold (Fig. 3) and the number of Lac^+ revertants about 50-fold (Fig. 7). Differences in the sizes of the two effects are expected because of the different natures and limitations of the two tests. Recall that the plasmid transfer estimates provide only a lower limit for the transfer that actually occurs, and the maximum change in reversion frequency is limited to about 100-fold. Because the total number of revertants obtained is in the range of 400 to 600, and the basal level of Lac^+ revertants present in the overnight culture is in the range of 4 to 10. Additionally, moving the *lacI33ΩlacZ* allele to the chromosome does not abolish reversion to Lac^+ (references 10 and 27 and data not shown), and other non-F-associated changes such as frameshift suppressors can yield a Lac^+ phenotype (7). Together, these limitations suggest that one may not be able to measure the true size of the effects of SDS and mutations on transfer-stimulated reversion.

The cell's environment affects the recovery of mutations during selection. Environmental factors not directly related to the selection can affect the occurrence of late mutations. The presence of SDS in the pregrowth medium reduced reversion of the plasmid allele for many days (Fig. 5), even when cells were freed of SDS before plating. It is unclear if this reduction results from reduced plasmid accumulation in the donor population, decreased ability to form mating aggregates, a combination of these two effects, or an unknown nonspecific effect of the detergent. Other environmental factors such as the density of the F^+ recipient cells (27) and the presence of limiting carbon sources (11) affect the recovery of revertants in the lactose selection system, and changing the salt concentration in the media dramatically changes the profile of mutations isolated in the maltodextrin selection (5). In the maltodextrin system, it has been shown that this environmental effect is related to the expression of the genes that mutate allowing the cell to become Dex^+ (5).

Obviously the biochemical, cell-cell, and cell-environment interactions that lead to reversion of the *lacI33ΩlacZ* allele during extended selections are complex. Thus, it is not surprising that untangling the mechanism(s) that contributes to reversion has resulted in a variety of models and opinions (10, 13, 25, 27, 28). In this work, we show a positive correlation between transfer and reversion of the F′-encoded *lacI33ΩlacZ* allele, supporting our contention that in nature conjugal transfer can serve to stimulate genetic diversity.

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