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Analysis and Possible Role of Hyperrecombination in the Termination Region of the *Escherichia coli* Chromosome

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The frequency of excisive homologous recombination has been measured at various positions along the *Escherichia coli* chromosome. The reporter system makes use of a λ *c*I857 prophage integrated by homologous recombination within Tn*5* or Tn*10* transposons already installed at known positions in the *E. coli* chromosome. The excision frequency per cell and per generation was determined by monitoring the evolution of the relative number of temperature-resistant (cured) bacteria as a function of the age of the cultures. Excisions, due to RecA-dependent homologous exchanges, appeared to occur more frequently in the preferential termination zone for chromosome replication. The highest frequency of excision observed is compatible with a recombination event at each replication cycle in this region. On the basis of these data, we propose a model involving homologous recombination in the final steps of bacterial chromosome replication and separation.

Analysis of the termination step of chromosome replication in *Escherichia coli* has led to the discovery of site-specific pausing of replication forks (9, 17), which is now well documented. The number, position, and orientation of polar pause sites have been determined (12, 16, 20). An associated protein required for the inhibition of fork movement, Tus, has been recently characterized (18, 19), and its mode of action as a site-specific inhibitor of DnaB helicase has been defined (22, 25). Site-specific pausing, however, is dispensable, since deletions of the *tus* locus and of pause sites are not severely detrimental (15, 18). Site-specific pausing delimits the region where termination occurs, but is probably accessory among events involved in completion of replication, which remain to be described.

The results reported here open the possibility that homologous recombination may be a major actor in termination of chromosome replication and final separation of chromosomes. Our evidence stems from an analysis of the variations of homologous recombination capacity along the chromosome. We have monitored recombination capacity by measuring the frequency of a defined excisive recombination event at many different chromosomal locations. Our main finding is that the region of the *E. coli* chromosome where replication terminates is more prone to excisive RecA-directed homologous recombination than is the rest of the chromosome. The position where excision frequency was maximal is located between the most frequently used pause site and *dif*, a locus of very active site-specific recombination (6, 24). A model is presented implicating homologous recombination in the termination process.

MATERIALS AND METHODS

Plasmids, bacteriophages, and bacterial strains. Excision frequency measurements were performed on a set of isogenic strains all derived from CB0129 (W1485 F$^-$ *leu thyA deoB* or *C supE*), carrying different Tn*5* or Tn*10* insertions, introduced by transduction with a bacteriophage P1 *vir*. Tn*10* insertions have been previously described (2, 29). The *pyrF*::Tn*5* and *zdd-263*::Tn*5* insertions were obtained from P. Kuempel. The *lacZ*::Tn*5* insertion was isolated in this laboratory. Hfr KL16-99 *srl*::Tn*10* and strain LN967 (AB2494 *malE52*::Tn*10*) were used as donors of the *recA1* and *lexA1* alleles, respectively. Bacteriophage λTSK has been previously described (12, 13). Its repressor is insensitive to RecA protease, the *c*I gene carrying the *ind* mutation in addition to the 857 allele. After lysogenization by λTSK, all strains were rendered λ resistant by crossing in the *lamB* allele from Hfr LN2463. Hfr LN2463 is CB0129 *metE*::pVF9 *lamB* (11). Plasmid pGY5353, a mini-F carrying the *recA*$^+$ gene and an ampicillin resistance determinant, was a gift of R. Devoret.

Culture conditions. Population evolution was monitored on cultures grown in rich LB medium (32). Overnight cultures were diluted in LB medium at an initial optical density at 540 nm (OD_{540}) of 0.1 and grown with aeration at 30°C. The frequency of cured temperature-resistant bacteria was determined at an OD of 0.5 (initial value in Table 1), by plating on LA plates (32) incubated at 42°C. After reaching an OD close to 5, the culture was diluted to an OD of 0.05 and grown again at 30°C up to an OD close to 5. The same culture cycle was repeated several times to obtain a cumulated cell number increase ranging between 10^8 and 10^{11}. The frequency of cured bacteria was again determined (final value in Table 1).

Genetic procedures. All genetical experiments, either in vivo or in vitro, involved already published or standard procedures (29, 32).

RESULTS

Experimental strategy for recombination frequency measurements. We used a bacteriophage λ derivative, λTSK, which carries fragments from Tn*5* and Tn*10* transposons in place of the *att int xis* region (12, 29). A stable lysogenic state can be established after prophage integration by homologous recombination between a copy of Tn*5* or Tn*10* already installed in the chromosome and the corresponding homologous sequence carried by the prophage (about 3 kb in each

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TABLE 1. Frequencies of prophage excision at various chromosomal positions and dependency on RecA activity

Strain	Relevant genotype	Insertion site[a]	Frequency of Tr bacteria[b]			Excision frequency
			Initial	No. of doublings	Terminal	
LN2076	*malK*::Tn*10*	91.5	8.9×10^{-5}	26	2.4×10^{-4}	0.6×10^{-5}
LN2442[c]	LN2076 *recA1*	91.5	8.5×10^{-7}			
LN2464	*lac*::Tn*10*	8.0	2.5×10^{-4}	49	9.4×10^{-4}	1.4×10^{-5}
LN2423	*lac*::Tn*5*	8.0	1.0×10^{-4}	27	3.4×10^{-4}	0.9×10^{-5}
LN2446[c]	LN2423 *recA1*	8.0	1.1×10^{-5}			
LN2419	*mdoA*::Tn*10*	23.4	6.2×10^{-5}	26	2.3×10^{-4}	0.6×10^{-5}
LN2442[c]	LN2419 *recA1*	23.4	1.1×10^{-6}			
LN2430	*trpB*::Tn*10*	27.9	1.3×10^{-3}	29	2.4×10^{-3}	3.8×10^{-5}
LN2426	*pyrF*::Tn*10*	28.4	1.4×10^{-3}	38	5.6×10^{-3}	1.1×10^{-4}
LN2428	*pyrF*::Tn*5*	28.4	1.8×10^{-3}	38	6.6×10^{-3}	1.3×10^{-4}
LN2421	*zci-233*::Tn*10*	29.0	8.3×10^{-4}	26	5.7×10^{-3}	1.9×10^{-4}
LN2446[c]	LN2421 *recA1*	29.0	2.1×10^{-5}			
LN2409	*zdd-263*::Tn*5*	33.8	3.3×10^{-1}	36	8.7×10^{-1}	4.0×10^{-2}
LN2447[c]	LN2409 *recA1*	33.8	3.0×10^{-5}			
LN2536[d]	LN2409 *lexA1*	33.8	2.3×10^{-1}			
LN2513	*zde-395*::Tn*10*	34.7	5.0×10^{-3}	49	5.1×10^{-2}	0.9×10^{-3}
LN2427	*zdf-237*::Tn*10*	35.8	4.5×10^{-3}	38	8.1×10^{-3}	0.9×10^{-4}
LN2420	*zdg-232*::Tn*10*	36.3	1.2×10^{-3}	26	5.6×10^{-3}	1.7×10^{-4}
LN2519	*zdh-57*::Tn*10*	37.0	8.5×10^{-4}	49	4.0×10^{-3}	6.4×10^{-5}
LN2429	*his*::Tn*10*	44.0	3.4×10^{-4}	38	7.0×10^{-4}	0.9×10^{-5}
LN2466	*purC*::Tn*10*	53.2	4.2×10^{-4}	49	7.8×10^{-4}	0.7×10^{-5}
LN2465	*metC*::Tn*10*	65.0	2.7×10^{-4}	49	4.9×10^{-4}	0.4×10^{-5}
LN2425	*tnaA*::Tn*10*	83.7	3.2×10^{-4}	31	7.6×10^{-4}	1.4×10^{-5}
LN2434[e]	*tnaA*::Tn*10 zdd-263*::Tn*5*	83.7	5.2×10^{-4}			

[a] Map locations are from Bachmann (1), except for the 28- to 36-min interval, for which positions in minutes were calculated according to marker positions in kilobases on the physical map of Bouché (3), aligned on the genetic map by placing the *trp* locus at 28 min and *uidR* at 35.9 min and considering that 1 min = 48.6 kb in this interval.

[b] Tr, temperature resistant.

[c] These strains are derived from the preceding ones in the list by introduction of the *recA1* allele linked to a *srl*::Tn*10* insertion (mating with Hfr KL16-99 *srl*::Tn*10*, selection of Tc^r transconjugants, then screening for UV sensitivity).

[d] This strain derives from LN2409 (*zdd-263*::Tn*5*::λTSK) by cotransduction of the *lexA1* allele with *malE*::Tn*10* from LN967 (selection of Tc^r transductants then screening for UV sensitivity).

[e] Strain LN2434 is CB0129 *tnaA*::Tn*10 zdd-263*::Tn*5* lysogenized with λTSK, then screened for a Tc^s temperature-sensitive phenotype.

case). Integrated in this manner, the prophage DNA (45 kb) is flanked by directly repeated sequences (Fig. 1). The prophage can be excised by homologous recombination between these direct repeats and is subsequently lost. Since the repressor encoded by the prophage is temperature sensitive, bacteria cured of the prophage become temperature resistant and are therefore easily selectable among the temperature-sensitive lysogens. For a given transposon, whatever its chromosomal location, excisive recombination always involves the same sequences. Its rate per cell per generation is therefore directly related to the local capacity of recombination.

The rationale for curing frequency determinations is as follows. Evolution of a population obtained from a single lysogen will depend on the rate of prophage loss per cell and per generation, f, and on the respective generation times of the lysogens, T_l, and of the cured derivatives, T_c. When the number of individuals of the rarest type is sufficiently large, the evolution between time t and time $(t + T_l)$ of the numbers of lysogens, U, and of nonlysogens, V, should follow the equations

$$U_{t+T_l} = 2U_t(1-f)$$
$$V_{t+T_l} = 2fU_t + V_t 2^{T_l/T_c} \quad (1)$$

When $T_l = T_c$ and f is small [i.e., when $(1 - f)$ is close to 1], the frequency of cured bacteria increases linearly with generations, according to a simplified version of the previous equations, which expresses in a classical way the variation of ratio V/U between times t_1 and t_2 (corresponding to n doublings):

$$(V/U)_{t_2} = (V/U)_{t_1} + (n \times f) \quad (2)$$

Our estimations of f necessitated careful determinations of the V/U ratio at different ages of the culture. This method minimizes the risk of error due to random drift, since the number of individuals of the rarest phenotype may be kept large, but requires precise estimates of relative generation times. Since one or two divisions must separate a prophage excision event from the subsequent birth of a cured bacterium, f is an underestimate of the prophage excision frequency by a factor of 2 to 4, the higher value of this factor being expected when the prophage maps near *oriC*. In the following, we neglect this correction and assimilate prophage loss frequency and prophage excision frequency.

Variation of excision frequencies as a function of map position. The map positions of the 17 insertions (14 Tn*10* and 3 Tn*5*) used as targets are given in Table 1 and Fig. 2. Correct λTSK integration was confirmed as described in the legend to Fig. 1. To avoid killing of cured bacteria by free phages liberated by spontaneous induction, a mutation conferring λ resistance was introduced into the various lysogens (Materials and Methods). In no case was the number of free phages per bacterium found to be above 10^{-4}. Our treatment depends critically on a knowledge of the growth rates of lysogens and nonlysogens. Reconstruction experiments have been performed by using one-to-one mixtures of some

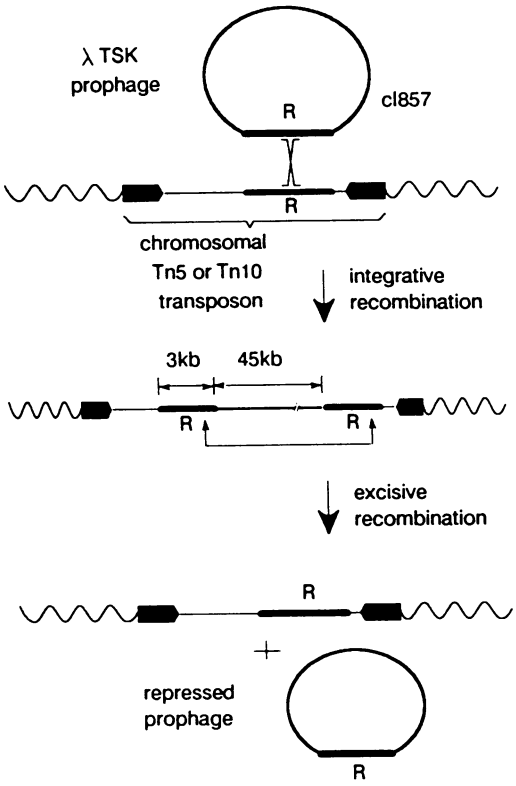

FIG. 1. Excision test. Phage λTSK carries in place of the *att int xis* region a 2.9-kb fragment carrying the *tet* region of Tn*10*, with the *tetA* (the Tc^r determinant) gene disrupted by insertion, at two positions 0.9 kb apart, of a 2.0-kb fragment carrying an Sm^r gene and of a Tn*5*-derived 3.3-kb fragment carrying a Km^r locus. Stable temperature-sensitive lysogens may arise from homologous recombination with resident Tn*10* or Tn*5* transposons. In the first case, 30% of the integration events result in the disruption of the two *tetA* loci, hence a Tc^s Km^r Sm^r phenotype (30), which was systematically sought since it indicates integration at the desired location. In the second case, integration was checked by Southern analysis of Tn*5*-containing fragments or by the loss of the λTSK prophage when the wild-type allele of the Tn*5*-induced mutation is introduced by P1 transduction. Excision of the repressed prophage eventually leads to temperature-resistant derivatives, whose frequency was scored. That excision was due to homologous recombination between the repeated flanking sequences could be easily checked when integration occurred within a Tn*10*: an active *tetA*$^+$ locus was reconstituted in an average of 30% of the excision events (30). When a Tn*5* was the integration site, Southern analysis of cured clones was required for the same purpose, with far fewer clones tested.

lysogens with one of their cured derivatives. In all cases, the ratio of temperature-resistant to temperature-sensitive bacteria monitored for approximately 40 successive doublings remained essentially invariant (data not shown). This indicates that the presence of a λTSK prophage has little or no influence on bacterial growth under permissive conditions, and therefore equation 2 is, in general, sufficient to account for population evolution.

In populations derived from a single lysogenic bacterium, the frequency of cured bacteria generally increased by a factor of 2 to 4 between the initial and final determinations (Table 1). This fits equation 2, since the number of doublings at the first determination was similar to the number of doublings which subsequently occurred prior to the second determination (between 30 and 50) and confirms again that the presence of a prophage had no effect under the growth conditions used. It also appeared that at a given locus (*lac*, *pyrF*), excision frequencies were about the same irrespective of the transposon used for integration, as expected from the equivalence in size of the homology regions between λTSK and Tn*5* or Tn*10*.

The excision frequency is low and relatively constant (around 10^{-5}) along most of the chromosome (for insertion sites lying between *his* [44 min] and *mdoA* [23 min] on the *oriC* side). It increases significantly in the region of pause sites delimiting the termination region (Fig. 2). The increase was moderate (10-fold) around the site inhibiting leftward-moving forks, *pslA* or *terA* (28.4 min [12, 16, 20]), but the effect was dramatic near *psrA* or *terC* (34.0 min [12, 16]), reaching a maximal increase of nearly 10^4 at insertion *zdd-263*::Tn*5* (strain LN2409). In this latter strain, a colony derived from a single lysogenic cell usually contains 30 to 50% cured bacteria. Use of the Sm^r phenotype associated with λTSK is required to determine the fraction of lysogenic cells.

It was essential, in view of the high segregation levels, to determine whether the presence of a prophage at position *zdd-263*::Tn*5* had any effect on growth. The following specific protocol was required. The relative generation times were estimated in a reconstruction experiment involving an initial ratio of 1 lysogen per 10 nonlysogens, to minimize the contribution of new cured bacteria appearing during the reconstruction experiment (the lysogens were scored as Sm^r bacteria). The nonlysogen-to-lysogen ratio remained invariant for 10 successive doublings. Thus, no prophage-induced variation in generation time was detectable. Equations 1 were used for calculation of the excision frequency since f could no longer be considered small in this strain. Assuming no difference in generation times, the excision frequency was estimated to reach the very high value, reported in Table 1, of 4×10^{-2}. Assuming that lysogens grow 5% slower than cured bacteria (an overestimate of the possible difference), the estimate for excision frequency was still above 2×10^{-2}.

We have previously shown that at a position located 30 kb to the left of *psrA* (as drawn in Fig. 2), *oriC*-initiated forks are still moving in the rightward direction. This observation was made on a derivative of strain CB0129 (9). It is thus probable that, in our strain family, the rightward-moving forks are inhibited at *psrA* in most replication cycles and that termination occurs at, or immediately to the left of, *psrA*. This is consistent with the fact that the distance from *oriC* to *psrA*, calculated from the physical map of Kohara et al. (23), appears slightly shorter in the rightward direction (Fig. 2). The highest excision frequency point, *zdd-263*::Tn*5*, at less than 10 kb to the left of *psrA*, is therefore located in the preferential zone for termination.

Analysis of excisive recombination at the *zdd-263*::Tn*5* position. To determine whether excisive recombination at the *zdd-263*::Tn*5* locus requires a proficient homologous recombination machinery, we introduced a *recA1* mutation into the lysogenic strain. The frequency of cured bacteria was reduced 10,000-fold (Table 1). This is a much more dramatic effect than at any other locus tested, where the presence of *recA1* results in a 10- to 100-fold decrease in the frequency of temperature-resistant bacteria (Table 1). Recovery of a RecA$^+$ phenotype by introduction of plasmid pGY5353, a low-copy-number plasmid carrying the *recA*$^+$ allele (10), restored the original very high excision frequency. Moreover, since spontaneous induction is necessarily low owing to the *c*Iind allele, phage-controlled functions are most probably not participating in the excision process.

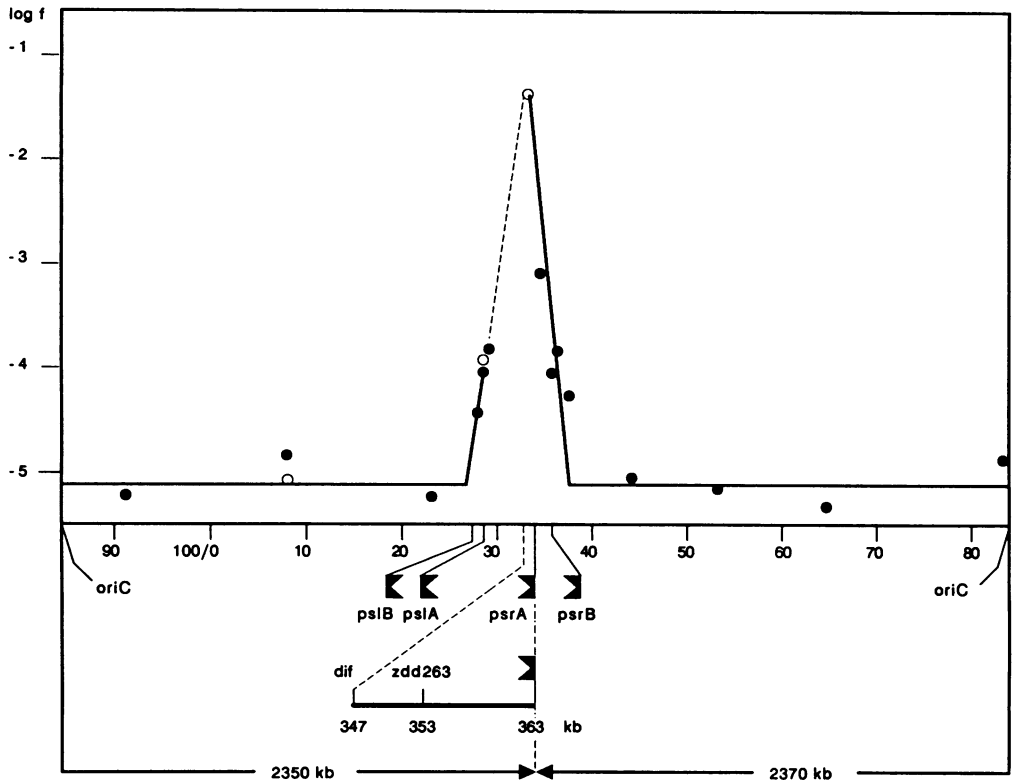

FIG. 2. Variations of excision frequency as a function of map position. Excision frequencies, *f*, are plotted on a log scale versus the position of transposons used as integration sites along the chromosome, open at *oriC*. Pause sites *pslA* and *pslB* inhibit leftward-moving forks, and *psrA* and *psrB* inhibit rightward-moving forks. Map positions of pause sites, recalibrated as in Table 1 from reference 11, are as follows: *pslB*, 27.3 min; *pslA*, 28.4 min; *psrA*, 34.0 min; *psrB*, 35.6 min. The region around *zdd-263*::Tn*5* is further enlarged, to show the respective positions of *dif* (23a), *zdd-263*::Tn*5*, and *psrA*; the ordinates (in kilobases) are those of the Bouché map (3). The distances in both directions from *oriC* to *psrA* were calculated on the basis of the map of Kohara et al., with correction for the *rrnD-rrnE* inversion (25). Symbols: ●, Tn*10* integration sites; ○, Tn*5* integration sites.

In addition, introduction into strain LN2409 of a *lexA1* allele, which prevents SOS induction (37), has no effect on high-frequency curing (Table 1), which therefore does not depend on the SOS system.

The fact that homologous recombination is responsible for the extremely high frequency of excision at *zdd-263*::Tn*5* was confirmed by a direct analysis of the recombination products. This insertion maps at position 353 kb on the Bouché map (3; data not shown). The predicted sizes of the *Eco*RI fragments responding to a Tn*5*-specific probe are different in a single lysogen, in a "free" prophage (or a double lysogen), and in a cured bacterium (Fig. 3B). The corresponding Southern analyses are presented in Fig. 3A. In nonlysogenic parental bacteria, the only detectable *Eco*RI material is an 18-kb fragment which harbors the Tn*5* insertion alone. Note also that nonlysogenic parental and cured bacteria display the same set of *Bam*HI-*Eco*RI fragments responding to the probe. This invariance is consistent with prophage integration and excision by homologous recombination. In the *recA1* lysogen, two fragments of 7.8 and 14.5 kb represent the prophage-chromosome junctions. In the Rec^+ lysogen, three fragments give a strong response to the probe: the 7.8- and 14.5-kb junction fragments and a 18-kb fragment which comes from the large fraction of cured bacteria (about 70%) in the clones analyzed. Several bands were hardly detectable on the original autoradiogram, in particular at 4.7 kb. This band could be due to double lysogens or excised prophages, which could be present at very low levels in the culture. The other faint bands remain unexplained. None of them were detectable in the $RecA^-$ derivative.

A direct effect of the *zdd-263*::Tn*5* insertion on functions required in *trans* for prophage excision has been excluded. The insertion was first transduced into a *tna*::Tn*10* strain. The resulting strain was then lysogenized with λTSK, and Sm^r Tc^s temperature-sensitive derivatives were isolated (the Tc^s phenotype ascertained that prophage integration occurred within the *tna*::Tn*10* locus). The frequencies of cured bacteria remained unaffected, whether the *zdd-263*::Tn*5* insertion was present or not (Table 1, see the two last lines).

A Tn*5* insertion introduces a set of inverted repeated sequences (the two IS*50*s bordering the transposon) in the chromosome. Since homologous recombination appears to be extremely frequent at *zdd-263*::Tn*5*, a high rate of exchanges between the IS*50*s is possible. The resulting Tn*5* inversions could be as frequent as λTSK deletions at this site. Two new Tn*5*-containing *Bam*HI-*Eco*RI fragments should result from these inversions in nonlysogenic bacteria (Fig. 3B). These new fragments have never been detected either in the original strain or in cured derivatives (Fig. 3A). Exchanges in the *zdd-263* region are thus strongly directed toward deletions rather than toward other figures of homologous recombination.

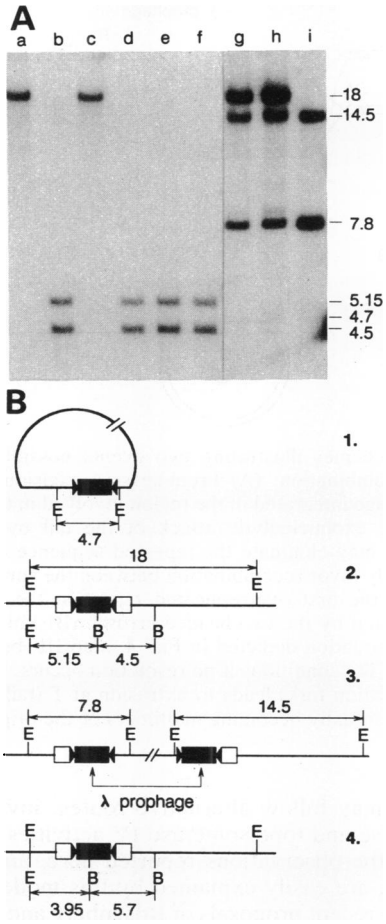

FIG. 3. Integration and excision of a λTSK prophage at position *zdd-263*::Tn*5*. (A) Southern analysis of Tn*5*-containing fragments. Total cellular DNAs were digested with *Eco*RI (lanes a, c, g, h, and i) or *Eco*RI plus *Bam*HI (lanes b, d, e, and f), electrophoresed in a 1% agarose gel for 18 h at 1.5 V/cm, denatured in situ, and transferred onto a nylon membrane. The membrane was subsequently incubated under DNA-DNA hybridization conditions with a ^{32}P-labeled Tn*5*-specific probe (a pBR322::Tn*5*) and autoradiographed. Lanes: a,b and c,d, two subclones of the *zdd-263*::Tn*5* parental strain; e and f, two cured (temperature-resistant) clones derived from strain LN2409; g and h, two subclones of strain LN2409 (*zdd-263*::Tn*5*::λTSK); i, strain LN2447 (LN2409 *recA1*). (B) Known and predicted Tn*5*-responding fragments. Line 1: prophage λTSK organization. The 3.3-kb Km^r *Hin*dIII fragment from Tn*5* (solid box plus arrowheads) is included within a 4.7-kb *Eco*RI fragment (12; unpublished data). Prophage duplication (tandem dilysogen) is also characterized by the same 4.7-kb *Eco*RI fragment. Line 2: chromosome map in the vicinity of *zdd-263*::Tn*5*. Transposon Tn*5* (5.5 kb) is installed at position 353 kb on the Bouché map in a 11.6-kb *Eco*RI fragment 1.7 kb to the left of a *Bam*HI site (the orientation is the same as in Fig. 2). This map was deduced from Southern analysis data partly presented in panel A. Line 3: predicted fragments in a lysogen resulting from integrative homologous recombination of λTSK within *zdd-263*::Tn*5*. Line 4: predicted *Eco*RI-*Bam*HI fragments at the *zdd-263*::Tn*5* position assuming that the central part of the transposon is inverted by recombination between the two inverted IS*50*s bordering the transposon. Abbreviations: E, *Eco*RI; B, *Bam*HI.

DISCUSSION

The present data show unambiguously that the same repeated sequences separated by a constant piece of DNA recombine with each other at a frequency which depends on their chromosomal location. To our knowledge, this is the first systematic analysis of this type made in *E. coli*. Vagner and Ehrlich (36) have found that the frequency of excisive recombination between identical direct repeats installed at various positions along the *Bacillus subtilis* chromosome can also vary considerably from place to place. They observed a 30-fold difference between the least and most active locations in recombination, but found no correlation with the chromosomal replication pattern. In *E. coli*, excision frequencies are rather constant along most of the chromosome, but increase considerably in the vicinity of replication pause sites.

Why is the terminus region hyperactive in excision by homologous recombination? We suspect that the major RecBCD recombination pathway, which is generally not used for recombination between direct repeats on the chromosome to generate deletions (26), might, in contrast, operate actively in the termination region. The terminus might differ from the rest of the chromosome, where free-ended DNA, the preferred RecBCD substrate (33), is probably scarce, in that it frequently harbors points of entry for the RecBCD complex. Two candidate sites may be considered (Fig. 2): the pause site *psrA*, at position 363 kb on the Bouché map (12, 16), and the *dif* site, at position 347 kb, located on the opposite side of the *zdd-263* position (353 kb). The *dif* site is a *cis*-acting locus, whose deletion induces a RecA-dependent filamentation (24). Very recently, it has been shown by Clerget that the same region (between 345 and 350 kb on the Bouché map) harbors a 50-bp sequence homologous to, and displaying similar properties as, a locus of plasmid R1drd19 which supports very active RecA-independent site-specific recombination (5, 6). In addition, Clerget (6) has found that the recombinase acting at *dif* is most probably the XerC enzyme described by Colloms et al. (8).

The tentative model presented below proposes that a recombination event is initiated at *psrA* and terminated at *dif* and may occur at the end of most replication cycles, facilitating the physical separation of daughter chromosomes. Our model for terminal recombination is presented in Fig. 4 and can be described as follows. The rightward-moving replication fork stops at *psrA* (Fig. 4, step I). We postulate that the progression of the leftward-moving fork induces a torsional strain on the stalled fork. This strain forces the parental strands to re-pair and the fork to move backward (branch migration). RecA protein can then invade the freshly replicated strands and favor their displacement and subsequent reciprocal pairing, to form a double-stranded tail. The stalled fork is now converted into the equivalent of a Holliday junction (Fig. 4, step II). The leftward-moving fork continues its progression beyond *psrA* and enters into a region already replicated by the rightward fork (which keeps moving backwards, with consequent tail extrusion). The region is now triplicated (Fig. 4, step III). Two distinct recombination events can then occur. The first is circularization, in which the tail, which is a typical target for the RecBCD enzyme (33), recombines with one branch of the leftward fork. Such recombinations might be oriented by choice of one of the two branches: the strand harboring Okazaki fragments might be the more accessible since it may carry single-stranded stretches (Fig. 4, step IV). The second event is separation, in which the Holliday junction is resolved in a directional fashion: the tail, which is potentially free of topological constraints, is cut in a way which favors the physical separation of sister chromosomes (Fig. 4, step V). We propose that resolution is *dif* specific only because

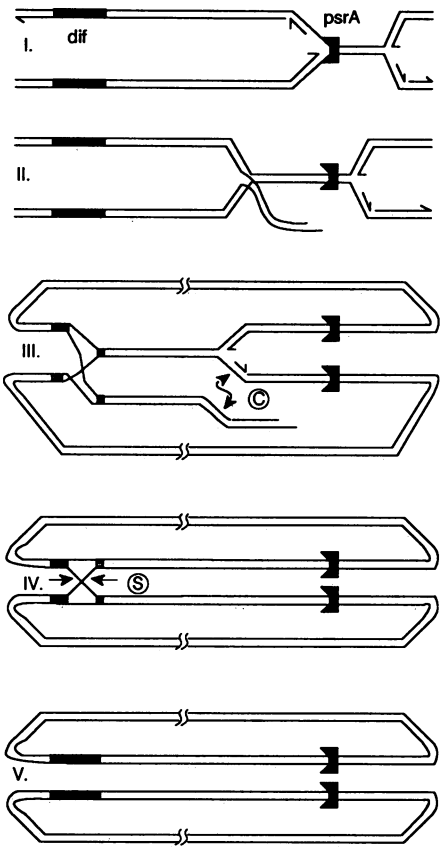

FIG. 4. Terminal-recombination model. Step I: the rightward-moving fork stops at *psrA*. The opposite fork is still progressing and introduces a positive torsional strain in the unreplicated material. Step II: the strain on the stalled fork leads to reciprocal pairing of the parental strands. The two freshly replicated strands are thus displaced and subsequently pair with each other, forming the tail. Step III: the leftward-moving fork continues its progression beyond *psrA* and overreplicates the region already replicated by the first fork, which is now converted into a Holliday junction, itself moving leftward. Step IV: circularization (C). Recombination becomes possible between the tail and one branch of the leftward fork, with recircularization and formation of a figure-eight shape. Step V: separation (S). Resolution by site-specific recombination occurs at the *dif* locus.

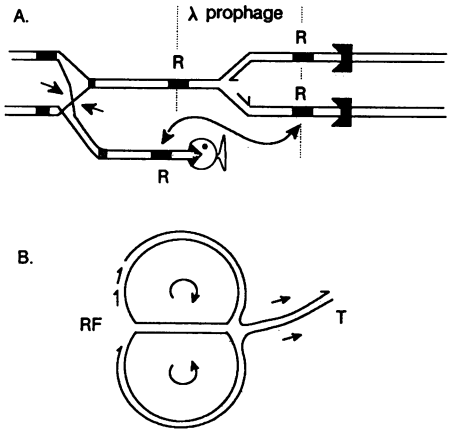

FIG. 5. Schemes illustrating two events possibly mediated by terminal recombination. (A) Proneness to excision. In case of a λTSK prophage integrated in the region involved in terminal recombination, the exonucleolytic attack of the tail by RecBCD (the piranha fish) may eliminate the repeated sequence closest to *psrA* and eventually favor recombination between the survivor repeat on the tail and the first overreplicated repeat at the rightward fork (event indicated by the two-headed arrow). (B) Rolling-eight replication. The situation depicted in Fig. 4, step III, becomes endless under RecBCD$^-$ conditions if no resolution occurs. DNA synthesis at RF (replication fork) leads to extrusion at T (tail) of long linear products, eventually becoming multimers of the original molecule.

directionality of exchanges is a usual feature of site-specific recombination.

How frequent might be terminal recombination events? If one assumes that the very high frequency of exchanges between the 3-kb repeats flanking the λ prophage at position *zdd-263*::Tn*5* (4×10^{-2} per cell per generation) is valid for the *dif-psrA* segment (69 kb in strain LN2409), the region might undergo recombination at most if not all termination events. Thus, terminal recombination could be the usual mechanism for separation of sister chromosomes, acting as a "comfort device" reducing the number of topoisomerase interventions for decatenation of daughter chromosomes. At least this model provides a new way of considering the long-suspected possibility that homologous recombination plays a role in the cell cycle (4). However, it is worth recalling that pause sites (15) and the associated gene *tus* (18), the *dif* locus (15, 24), and of course the homologous recombination machinery are dispensable. Chromosome separation may follow alternative routes, involving for instance gyrase and topoisomerase IV activities (21, 35).

Most of the observations reported here, and some additional ones, are easily explained by this model, which also fits with the recent proposals of Rosenberg and Hastings (30) for RecBCD activity. These explanations are as follows. (i) For prophage excision, the exonucleolytic attack of the tail from its free end may result in a preferential loss of the repeated copy proximal to *psrA*. This may favor a recombination between the distal copy on the tail and a proximal one on fork branch, as indicated in Fig. 5A. The final result, deletion without concomitant duplication or free-prophage formation, is consistent with our physical analysis data of Fig. 3 and also with the strong bias in favor of deletions over inversions. Our excision model implicates no double-strand break in the prophage separating the direct repeats and therefore excludes the uncomfortable hypothesis that lambda sequences could be more sensitive to double-strand breaks when they are installed in the terminus region. (ii) For the phenotype of *dif*-deleted bacteria, if resolution of terminal recombination occurred at *dif*, this would restrict this event to the *psrA-dif* interval. This region is large enough to capture all the RecA protein normally present in the cell at the terminal recombination step (about 1,200 monomers each covering 3 bp of double-stranded DNA [31, 34]). The filamentation phenotype observed in *dif*-deleted cells (24) could be a consequence of a longer half-life of terminal recombination intermediates as a result of a defect in resolution, which may result in a higher probability that the proteolytic function of RecA protein would be activated, and hence that induction of the SOS pathway and filamentation of the *dif* mutant would occur. (iii) For aspects of RecBCD$^-$ phenotype, terminal recombination may explain the observation that in *recBC* mutants only 20% of the bacteria present in a culture are able to form a colony (4): the first, RecA-dependent step of terminal recombination occurs, but

the RecBCD-dependent circularization step is inhibited, leading to a circular (viable) plus a linear (inviable) chromosome if resolution occurs. Note also that abortive terminal recombination under RecBCD$^-$ conditions is assimilable to a unidirectional replication yielding a linear product, in a "rolling-eight" mechanism (Fig. 5B). The formation of linear multimers of plasmids pBR322 and mini-F in RecBC$^-$ or RecD$^-$ hosts (7, 28) could be explained by this mechanism.

Though attractive, the terminal-recombination model is only one possible explanation for the high excision frequency at the *zdd-263* locus. At present, the possibility has not been excluded that the *zdd-263* region harbors a site of entry for recombination enzymes which functions independently from the termination process. Indeed, we have recently obtained preliminary indications that presence of a chromosomal *dif*-carrying fragment (4.4 kb) cloned into a replication temperature-sensitive plasmid vector allows high persistence of plasmid-associated characteristics at 42°C (presumably due to plasmid integration) not only in RecA$^-$ bacteria, as expected for XerC-dependent *dif*-specific recombination, but also in XerC$^-$ bacteria. It was only when the strain carried both *recA* and *xerC* mutations that integration of this plasmid dropped to low levels. This suggests that two recombination processes, one RecA dependent and the other XerC dependent, are elicited in the *dif* region. More work is clearly needed to determine sites and functions involved in the phenomenon of hyperrecombination in the *zdd-263* region. Nevertheless, if homologous recombination is normally involved at each division cycle and is the usual mechanism for spatial separation of sister chromosomes, this is its prominent role, a concept that may contribute to explaining the past evolution of recombination enzymes.

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