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Conjugational Recombination in *Escherichia coli*: Genetic Analysis of Recombinant Formation in Hfr × F$^-$ Crosses

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ABSTRACT

The formation of recombinants during conjugation between Hfr and F$^-$ strains of *Escherichia coli* was investigated using unselected markers to monitor integration of Hfr DNA into the circular recipient chromosome. In crosses selecting a marker located ~500 kb from the Hfr origin, 60–70% of the recombinants appeared to inherit the Hfr DNA in a single segment, with the proximal exchange located >300 kb from the selected marker. The proportion of recombinants showing multiple exchanges increased in matings selecting more distal markers located 700–2200 kb from the origin, but they were always in the minority. This effect was associated with decreased linkage of unselected proximal markers. Mutation of *recB*, or *recD* plus *recJ*, in the recipient reduced the efficiency of recombination and shifted the location of the proximal exchange(s) closer to the selected marker. Mutation of *recF*, *recO* or *recQ* produced recombinants in which this exchange tended to be closer to the origin, though the effect observed was rather small. Up to 25% of recombinant colonies in *rec*$^+$ crosses showed segregation of both donor and recipient alleles at a proximal unselected locus. Their frequency varied with the distance between the selected and unselected markers and was also related directly to the efficiency of recombination. Mutation of *recD* increased their number by twofold in certain crosses to a value of 19%, a feature associated with an increase in the survival of linear DNA in the absence of RecBCD exonuclease. Mutation of *recN* reduced sectored recombinants in these crosses to ~1% in all the strains examined, including *recD*. A model for conjugational recombination is proposed in which recombinant chromosomes are formed initially by two exchanges that integrate a single piece of duplex Hfr DNA into the recipient chromosome. Additional pairs of exchanges involving the excised recipient DNA, RecBCD enzyme and RecN protein, can subsequently modify the initial product to generate the spectrum of recombinants normally observed.

THE conjugational system in *Escherichia coli* provides a simple model for the genetic control of recombination (CLARK 1971, 1973; CLARK and LOW 1988; SMITH 1991). More than 20 genes have been identified and the products of several have been used successfully to process model DNA substrates into recombinant products *in vitro* (CLARK 1991; DIXON and KOWALCZYKOWSKI 1991; DUNDERDALE *et al.* 1991; LLOYD and SHARPLES 1992; ROMAN *et al.* 1991; WEST 1992). Despite this knowledge, it is still not clear how recombinant chromosomes are produced *in vivo*. During conjugation, a single strand of Hfr DNA is transferred to the F$^-$ recipient where it provides a template for lagging-strand synthesis (WILLETTS and WILKINS 1984). While transfer is in progress, the leading 5′ end is probably attached to DNA helicase I at the site of DNA transfer replication so that in effect a growing loop of partially duplex DNA is presented to the recipient (MATSON *et al.* 1993) (Figure 1a). When mating terminates, the transferred DNA is released as a linear fragment with a 40 kb or so segment of F plasmid DNA at the leading end and a single-strand overhang of variable length at the distal 3′ end because of the failure to complete synthesis of the complementary strand. Recombinants arise from exchanges between this fragment and the circular recipient chromosome.

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For recombination to begin, we presume that one of these two DNA molecules must have a single-stranded region to bind RecA protein and allow polymerization of the helical RecA-DNA filament that initiates homologous pairing and strand exchange (WEST 1992). Two crossovers (or a higher even number) are needed to integrate the Hfr DNA and produce a viable (circular) recombinant chromosome. SMITH and co-workers have suggested that in ~80% of the matings, these crossovers are located near each end of the Hfr DNA fragment and are initiated by RecBCD enzyme, the product of *recB*, *recC* and *recD* (SMITH 1991; TAYLOR and SMITH 1992). This multifunctional protein complex binds to DNA molecules with flush or nearly flush duplex ends and has DNA helicase and nuclease activities that enable it to unwind and degrade both strands as it tracks along the molecule (SMITH 1991; TAYLOR and SMITH 1992; DIXON and KOWALCZYKOWSKI 1993; GANESAN and SMITH 1993). When it encounters the sequence 5′-

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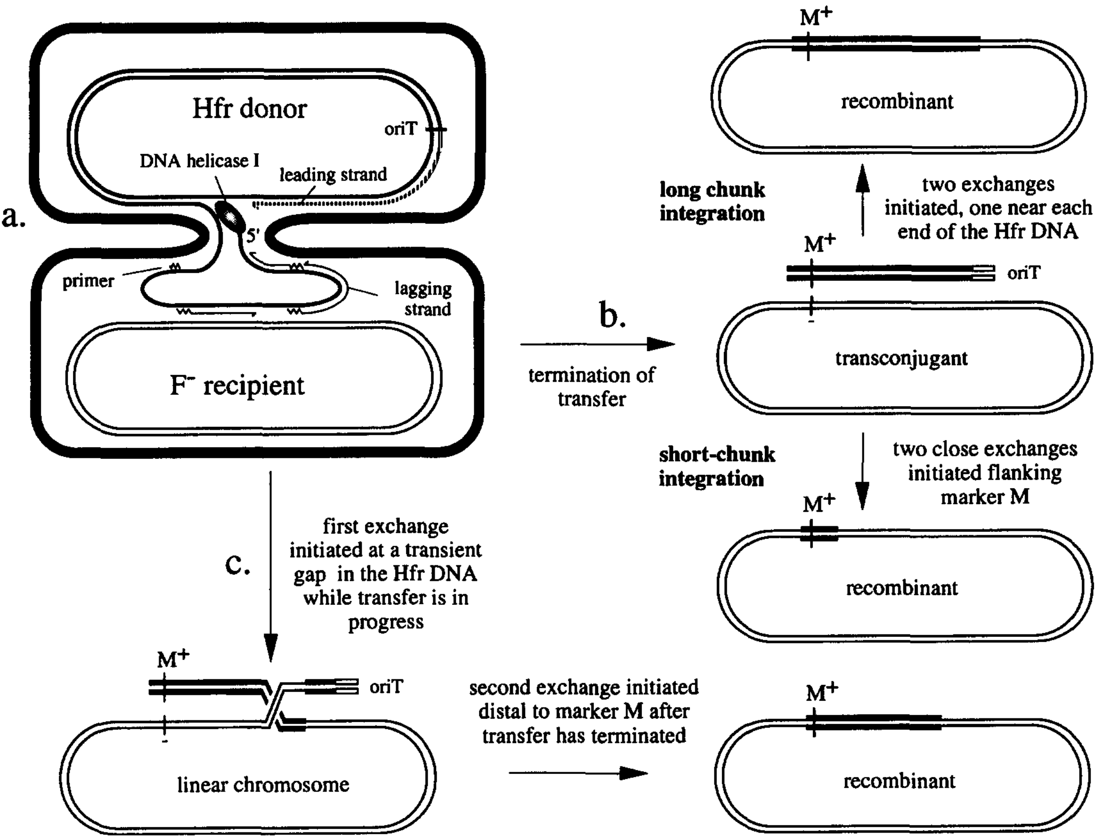

FIGURE 1.—Conjugation in *E. coli*. (a) DNA transfer between Hfr and F⁻ cells showing a loop of Hfr DNA being directed into the F⁻, with the leading 5′ end attached to DNA helicase I at the point of cell to cell contact and lagging strand synthesis of the complementary strand. *oriT* is the transfer origin located within the integrated F plasmid. (b) Integration of the linear Hfr DNA into the circular recipient chromosome by two exchanges initiated after transfer has terminated. (c) Formation of a recombinant chromosome with overlapping ends after initiation of recombination at a gap during transfer and of circular recombinant by a second exchange initiated after transfer has terminated. All exchanges are shown as splices (crossovers).

GCTGGTGG-3′ (Chi) from the 3′ end, the nuclease activity is modulated so that as the enzyme proceeds beyond Chi, the strand ending 3′ is no longer degraded and can be recruited by RecA to initiate exchange (SMITH *et al.* 1984; TAYLOR and SMITH 1992; DIXON and KOWALCZYKOWSKI 1993). Because Chi sequences occur every 5 kb on average (FAULDS *et al.* 1979; BURLAND *et al.* 1993) and exchanges initiated by Chi are resolved within a relatively short distance of the Chi site itself (CHENG and SMITH 1989), it is easy to see how RecBCD activity could lead to a crossover near each end of the Hfr DNA (SMITH 1991; TAYLOR and SMITH 1992). However, the single-strand overhang at the distal end would need to be removed by a single-strand exonuclease like Exonuclease I, the *sbcB* product (KUSHNER *et al.* 1971, 1972), or RecBCD itself (KARU *et al.* 1973). The segment of F plasmid DNA at the leading end would also have to be degraded to expose sequences homologous to the chromosome. The latter should present no problem for RecBCD as the enzyme is highly processive and F apparently has no Chi sequences to modulate nuclease activity (TAYLOR and SMITH 1980; SMITH 1991; ROMAN *et al.* 1992).

If RecBCD enzyme initiates precisely two exchanges, one near each end, the recombinants formed would be expected to inherit the Hfr DNA as a single fragment that excludes only those sequences between the transfer origin and the first Chi site to modulate RecBCD activity. Studies by PITTARD and WALKER (1967) and by WALKER and PITTARD (1970) revealed a high incidence of exchanges near the Hfr origin. By analyzing published data from crosses with multiply marked strains, SMITH (1991) came to the conclusion that some 80% of the recombinants are derived from events of this type, which he described as "long-chunk integration" (Figure 1b). The remainder seem to have arisen from "short-chunk integration" with both crossovers located near the selected marker (Figure 1b). Because recombination is reduced 100-fold or more in *recBC* mutants, short-chunk integration is assumed to involve at least one RecBCD-mediated exchange. Similar ideas were proposed in earlier models (BRESLER *et al.* 1981; LLOYD and THOMAS 1984). However, LLOYD and THOMAS (1984) added the possibility that recombination could also initiate during transfer by making use of a transient single-strand gap in the loop of Hfr DNA (Figure 1c). A

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viable recombinant would be produced when a second exchange is initiated after transfer has terminated and the distal end of the Hfr DNA fragment has become available to RecBC(D) enzyme. Earlier studies of conjugational recombination also reported that some recombinant colonies contain sectors of both donor and recipient genotype for an unselected marker (LOTAN *et al.* 1972; MAHAJAN and DATTA 1979; BRESLER *et al.* 1981). The exchanges that give rise to such recombinants have never been fully understood, though several mechanisms have been proposed (LOTAN *et al.* 1972; MAHAJAN and DATTA 1979; BRESLER *et al.* 1981).

Regardless of how and where the exchanges initiate, the final location of the crossovers that give rise to recombinants will depend on what happens to the joint molecules formed by RecA. Recombination in Hfr crosses depends on proteins that specifically target Holliday junctions (LLOYD 1991a). These are the symmetrical joints formed when DNA duplexes pair and exchange single strands. It follows that the exchanges initiated by the binding of RecA to single-stranded DNA must extend to adjacent duplex regions. The RuvAB and RecG proteins catalyze branch migration of the Holliday junction and will either extend the heteroduplex joint or abort the exchange, depending on which direction the junction is moved (IWASAKI *et al.* 1992; PARSONS *et al.* 1992; TSANEVA *et al.* 1992; LLOYD and SHARPLES 1993a,b; WHITBY *et al.* 1993). The aborting of recombination by reverse branch migration immediately raises the possibility that the first exchanges initiated by RecA need not be the ones that finally give rise to recombinants. The same conclusion can be drawn from the properties of RuvC. This protein resolves Holliday junctions by endonuclease cleavage across the point of strand exchange to give either "patch" (noncrossover) or "splice" (crossover) products (DUNDERDALE *et al.* 1991; IWASAKI *et al.* 1991). If these alternatives are equally likely, the probability of any pair of exchanges producing two splices to integrate a long chunk of Hfr DNA into a circular recombinant is 0.25. The most common outcome would be a combination of a splice and a patch, which would produce a linear chromosome with overlapping ends similar to that in Figure 1c. Further exchanges between the overlapping ends, presumably mediated by RecBCD, would be required until the appropriate splice was generated to complete integration. Because each engagement of a DNA end with RecBCD is expected to result in exonuclease digestion to at least the next appropriately oriented Chi site, the chances of integrating a donor marker will decrease with each successive attempt at an exchange, especially if the marker selected is near an end. This situation could be avoided by biasing resolution toward splices. An alternative possibility suggested by SMITH (1991) is that the 3′ single strands displaced by RecBCD activity at the ends of the Hfr DNA are aided by RecA to invade the recipient chromosome to form two D-loops that are then converted to replication forks. Completion of replication followed by resolution of the two Holliday junctions will produce either one recombinant chromosome plus one of recipient genotype or a circular dimer. Integration of the Hfr fragment would be limited therefore to one pair of exchanges regardless of how junctions are resolved.

In this paper we describe a genetic analysis of conjugational recombination in which we test several predictions of the models described. The data presented support the idea that the majority of recombinants arise from the integration of a single segment of duplex Hfr DNA into the recipient chromosome by a sequence of events that often involves more than one pair of exchanges. Studies with recombination-deficient *recD recJ* mutants lead us to suspect that some recombinants may arise from DNA splices made by annealing single-stranded DNA ends rather than by resolution of Holliday junctions.

MATERIALS AND METHODS

Bacterial strains: The *E. coli* K-12 strains used are listed in Table 1. Relevant genotypes were confirmed by P1*vir*-mediated backcrosses to appropriate strains and testing for the relevant phenotype among the transductants.

Media and general methods: Luria-Burrous (LB) broth and 56/2 salts media have been described (LLOYD *et al.* 1974). The salt concentration in LB media was 0.5 g/liter, except in broth used for matings when it was increased to 10 g/liter in standard crosses, or to 14.5 g/liter in crosses with *recA200* recipients. Previous studies showed that adding extra salt improves the efficiency of mating and is necessary for *recA200* zygotes to retain their ability to form recombinants at 35° during postmating incubation in broth before imposing selection on agar plates (LLOYD and JOHNSON 1979). LB media were supplemented with 100 μg/ml ampicillin, 40 μg/ml kanamycin or 20 μg/ml tetracycline, as required. 56/2 agar contained 40 μg of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) per ml and 22 μg of IPTG (isopropyl-β-D-thiogalactopyranoside) per ml for the detection of *lacZ*$^+$ cells. Minimal medium was 56/2 salts and was used in liquid form for cell dilutions or solidified with 1.5% (w/v) agar (Difco) for plates (LOW 1973a). Transductions with P1*vir* followed published protocols (MILLER 1972). The Tn*10* insertions in strain N3001, N3002, N3090 and N3153 were isolated after infection of W3110 with λNK55 as described (SILHAVY *et al.* 1984). Incubation was at 37° except with strains carrying *recA200*, which are temperature sensitive for recombination, when incubation was at 35° or 42° as indicated (LLOYD *et al.* 1974).

Matings: Hfr matings have been described (LLOYD and JOHNSON 1979; LLOYD *et al.* 1987). To control for day-to-day variation, the F$^-$ strains examined were always mated in parallel with the relevant control. Cultures were grown to an A_{650} of 0.4 (*cf.* 2×10^8 cells/ml as determined microscipically), and the donor-to-recipient ratio was 1:10. Unless stated otherwise, mating was allowed for 60 min before DNA transfer was terminated by chilling on ice and blending before spreading diluted samples of the exconjugant mixture on the surface of 56/2 agar plates supplemented as described by LOW (1973b) for the selection of recombinants. Hfr donors were counterselected by incorporating streptomycin into the plate agar at a final concentration of 100 μg/ml. Unless stated

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TABLE 1

***Escherichia coli* K-12 strains**

Strain	Relevant genotype[a]	Source or reference
AB1157	*rec*$^+$ *ruv*$^+$ *mut*$^+$	BACHMANN (1987)
AB2470	*recB21*	BACHMANN (1987)
AM561	Δ*ruvAC65 eda-51*::Tn*10*	MANDAL *et al.* (1993)
CS85	*ruvC53 eda-51*	SHURVINTON *et al.* (1984)
RDK1541	*recO1504*::Tn*5*	R. FISHEL via M. MARINUS
RF1075	*recJ284*::Tn*10*	R. FISHEL via M. MARINUS
RH213	*mutH34 thy*$^+$	HOESS and HERMAN (1975)
KD2216	*recQ1803*::Tn*3*	NAKAYAMA *et al.* (1985)
W3110	IN *(rrnD-rrnE)1*	BACHMANN (1987)
ES455	*mutS3 cys*$^+$	E.C. SIEGEL via K.B. LOW
KL425	*rec*$^+$ *ruv*$^+$ *mut*$^+$ *thyA54*	LLOYD *et al.* (1987)
MC109	*mutY zgd*::Tn*10*	K. B. LOW
V222	*recD1013 argA*::Tn*10*	A. CHAUDHURY
χ478	*purE42 proC32 lacZ36 leuB6 rpsL109 metE*	R. CURTISS via B.J. BACHMANN
SP256	*tyrA16*::Tn*10 recN262*	PICKSLEY *et al.* (1984)
GC2277	*pyrD*::Tn*5*	R. D'ARI
N1332	*recA200 proB48 rpsL*	LLOYD and JOHNSON (1979)
N2057	*ruvA60*::Tn*10*	SHURVINTON *et al.* (1984)
N2415	*rec*$^+$ *tna-300*::Tn*10*	LLOYD *et al.* (1987)
N2417	*recF143 tna*	LLOYD *et al.* (1987)
N2419	*rec*$^+$ *ruv*$^+$ *mut*$^+$	P1.AB2470 × KL425 to Thy$^+$
N2420	*recB21*	P1.AB2470 × KL425 to Thy$^+$
N2436	*recF tna?*	LLOYD *et al.* (1987)
N2453	*thyA54 tyrA*	P1.SP256 × KL425 to Tc^r
N2466	*recO*	LLOYD *et al.* (1987)
N2468	*recJ*	LLOYD *et al.* (1987)
N2477	*recN tyrA*	LLOYD *et al.* (1987)
N2485	*recF recN tna?*	LLOYD *et al.* (1987)
N2488	*ruvA*	P1.N2057 × KL425 to Tc^r and P1.AB2470 to Thy$^+$
N2498	*recN recF recO tna?*	LLOYD *et al.* (1987)
N2516	*recD1009 tna*	LLOYD *et al.* (1988)
N2517	*recD1009 recF*	LLOYD *et al.* (1988)
N2518	*recD1009 recO*	LLOYD *et al.* (1988)
N2519	*recD1009 recJ*	LLOYD *et al.* (1988)
N2520	*recD1009 recN tyrA*	LLOYD *et al.* (1988)
N2521	*recJ recF tna?*	LLOYD *et al.* (1987)
N2523	*recJ recO*	P1.RF1075 × N2466 to Tc^r
N2535	*recJ recF recO tna?*	P1.RF1075 × N2524 to Tc^r
N2539	*recJ recF recO tna? thyA*	Trimethoprim selection on N2535
N2588	*recJ recD1009 recO*	LLOYD *et al.* (1988)
N2589	*recJ recD1009 recF tna?*	LLOYD *et al.* (1988)
N2590	*recJ recD1009 recF recO tna?*	LLOYD *et al.* (1988)
N2594	*thyA54*	Tyr$^+$ selection on N2453
N2595	*recN thyA54*	LLOYD and BUCKMAN (1991a)
N2596	*thyA54 cysC*	P1.N3002 × N2594 to Tc^r
N2597	*recN thyA54 cysC*	P1.N3002 × N2595 to Tc^r
N2598	*recF recN tna? cysC*	P1.N3002 × N2485 to Tc^r
N2601	*cysC*	P1.RH213 × N2596 to Thy$^+$
N2602	*mutH34 cysC*	P1.RH213 × N2596 to Thy$^+$
N2603	*recN cysC*	P1.RH213 × N2597 to Thy$^+$
N2604	*recN mutH34 cysC*	P1.RH213 × N2597 to Thy$^+$
N2605	*mutS3*	P1.ES455 × N2601 to Cys$^+$
N2606	*recN mutS3*	P1.ES455 × N2603 to Cys$^+$
N2607	*recN recF mutS3 tna?*	P1.ES455 × N2598 to Cys$^+$
N2888	*recA200 proB48 recD1013 argA*	P1.V222 × N1332 to Tc^r
N2908	*recN recJ*	LLOYD and BUCKMAN (1991b)
N2914	*recN recJ recD1009*	LLOYD and BUCKMAN (1991b)
N2915	*recN*	LLOYD and BUCKMAN (1991b)

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TABLE 1

Continued

Strain	Relevant genotype[a]	Source or reference
N2936	*mutY zgd*	P1.MC109 × N2419 to Tc^r
N2940	*recN mutY zgd*	P1.MC109 × N2915 to Tc^r
N2965	*recD1013 recG258*	LLOYD and BUCKMAN (1991b)
N2967	*recG recN*	LLOYD and BUCKMAN (1991b)
N2971	*recG*	LLOYD and BUCKMAN (1991b)
N3001	*his-221*::Tn*10* IN(*rrnD-rrnE*)*1*	λNK55 × W3110 to Tc^r
N3002	*cysC95*::Tn*10* IN(*rrnD-rrnE*)*1*	λNK55 × W3110 to Tc^r
N3090	*pyrC24*::Tn*10* IN (*rrnD-rrnE*)*1*	λNK55 × W3110 to Tc^r
N3105	As AB1157 but *purE85*::Tn*10*	MANDAL *et al.* (1993)
N3153	*trp-77*::Tn*10* IN(*rrnD-rrnE*)*1*	λNK55 × W3110 to Tc^r
N3343	*recQ1803*::Tn*3 met*$^+$	LLOYD and BUCKMAN (1991a)
N3345	*recF recQ tna? met*$^+$	P1.KD2216 × N2436 to Met$^+$
N3346	*recO recQ met*$^+$	P1.KD2216 × N2466 to Met$^+$
N3347	*recN recQ met*$^+$	P1.KD2216 × N2915 to Met$^+$
N3348	*recG recQ met*$^+$	LLOYD and BUCKMAN (1991a)
N3755	*ruvC eda*	P1.CS85 × N2419 to Tc^r
N3756	Δ*ruvAC eda*	P1.AM561 × N2419 to Tc^r
N3842	*purE*	P1.N3105 × N2419 to Tc^r
N3854	*pyrD*	P1.GC2277 × N2419 to Km^r
N3855	*pyrC*	P1.N3090 × N2419 to Tc^r
N3856	*trp*	P1.N3153 × N2419 to Tc^r
N3857	*his*	P1.N3001 × N2419 to Tc^r
KL226	Hfr (Cavalli, PO2A) *relA1 tonA22*	K.B. LOW
AB259	Hfr (Hayes, PO1) *thi-1 relA1*	K.B. LOW
DF71	Hfr (Hayes, PO1) *thi-1 relA1 lacI22*	B.J. BACHMANN

[a] After the first full description, transposon insertions and *rec* alleles, except *recD*, are abbreviated to the gene symbol alone. KL425 and all the strains from N2415 though N3857 except N2888, N3001, N3002, N3090, N3105 and N3153 are also F$^-$ *thi-1 metE70 leuB6 proC32 lacI3 lacZ118 (ochre) ara-14 mtl-1 xyl-5 gyrA supD rpsL109 rpsE2015,* unless indicated otherwise. AB1157 is F$^-$ *thi-1 his-4* Δ(*gpt-proA*)*62 argE3 thr-1 leuB6 kdgK51 rfbD1*(?) *ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31.*

otherwise, broth supplementation of the selective agar to maximize the recovery of recombinants (RILEY and PARDEE 1962) was omitted to avoid background growth of nonrecombinant recipient cells. Conjugational DNA transfer to the strains examined has been determined independently by measuring transfer of F primes and zygotic induction of phage λ as described previously (LLOYD *et al.* 1988). With all strains, any deficiency detected was attributable to the reduced viability of the recipient. Cell viability was measured by counting the number of colony-forming units in the recipient cultures at an A_{650} of 0.4, using 56/2 agar selective for nonrecombinant cells. In crosses with *ruv* recipients, the minimal agar plates used were also supplemented with 0.005% isoleucine and 0.0025% valine. We have found that *ruv* mutants appear somewhat vulnerable to oxidative damage in that they grow better on minimal agar supplemented with branched-chain amino acids (R. G. LLOYD, unpublished data), as is the case with mutants disabled for the oxidative-stress response (CARLIOZ and TOUATI 1986). Colonies were scored after 2–3 days incubation. In temperature-shift experiments with *recA200* recipients, mating was for 40 min at 35° before blending to interrupt DNA transfer and diluting 100-fold into high salt (14.5 g/liter) LB broth prewarmed to 35° or 42°. Incubation was continued and samples were removed at intervals, mixed with 3 ml molten 0.75% water agar supplemented with 0.05 ml LB broth and overlaid on 56/2 agar selective for Pro$^+$ (*rpsL*) recombinants. Incubation was continued at 35° and colonies of recombinants were scored after 2 days.

Analysis of unselected markers: In the crosses described in full in the Appendix and summarized in Tables 7, 8 and 10, the Leu$^+$ (or Leu$^+$Ara$^+$) recombinant colonies selected were scored by their blue (*lacZ*$^+$), white (*lacZ*) or sectored (*lacZ*$^+$ and *lacZ*) phenotype on 56/2 agar supplemented with X-gal and IPTG. Between 4 and 10 plates (usually 300–2000 colonies in total) were scored in any one mating. Plates with crowded colonies or more than ~300 colonies in total were avoided. The Lac$^+$ and Lac$^-$ recombinants from blue/white sectored colonies analyzed for the unselected *pro* marker in Table 2 were first purified on MacConkey (Difco) lactose agar before testing. The crosses described in Table 11 also used the X-gal assay to monitor segregation of the unselected *lacZ* marker. To score unselected markers in other crosses, samples of the recombinant colonies were picked with a sterile needle directly from the selection plates, regrown overnight in regular arrays on selective agar of the same recipe and replica plated onto media diagnostic for the relevant markers: selective 56/2 minimal salts agar for auxotrophic markers and MacConkey agar supplemented with lactose or arabinose for the *lac* and *ara* markers, respectively. Recombinants containing cells of both donor and recipient genotype for one or more markers were scored as mixed. The frequency of mixed (sectored) colonies detected by this method is generally lower than revealed by monitoring segregation at *lacZ* on plates containing X-gal because the inoculum taken with a needle usually comes from a small area of the recombinant colony and in the case of mixed colonies may be restricted to a sector containing a single genotype. The chances of picking a single sector is likely to be affected by the size of the

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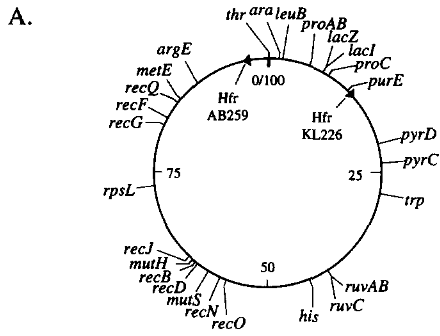
A.



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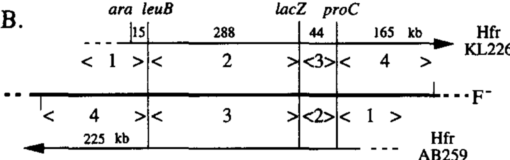

FIGURE 2.—(A) *E. coli* genetic map showing the location of genetic markers, recombination and mutator genes and Hfr origins of chromosomal DNA transfer (arrowheads). (B) Arrangement of markers and identification of genetic intervals for recombination in crosses with Hfr strains KL226 and AB259. The relevant section of the transferred Hfr DNA is indicated by the arrowed line. The F^- strains used are mutant for the markers shown (see Tables 2, 3, 7, 8, 10), whereas the Hfrs are wild type. Distances between markers, or between the first proximal marker and the Hfr origin, are indicated in kilobases.

colony. For these reasons, the values for sectored colonies given in Tables 3–6 and 12 must be regarded as minimal estimates. With the strains tested, no recombinants of mixed genotype were observed after purification of cells from sectored colonies. Estimates of distance between markers are based on published genetic and physical maps (KOHARA *et al.* 1987; BACHMANN 1990; YURA *et al.* 1992) and sequences in the Genbank database.

RESULTS

Analysis of recombinant genotypes in Hfr × F^- crosses: The models of conjugational recombination proposed by LLOYD and THOMAS (1984) and by SMITH (1991) make specific predictions about the spectrum of recombinants that would be expected from Hfr crosses with multiply marked F^- recipient strains. To test these predictions, we focused on well-defined genetic intervals extending clockwise from the *metE* locus at minute 86 to the IS*3c* insertion located at minute 12.5, the origin of transfer of the HfrC strain, KL226 (Figure 2A) (UMEDA and OHTSUBO 1989; BACHMANN 1990). We initially concentrated on the region extending some 500 kb from *leuB* to the Hfr KL226 origin. We used *lacZ* to divide this region into two intervals and at the same time to provide a convenient way of examining large numbers of recombinants for exchanges in these two intervals. In crosses between Hfr strain KL226 and an F^- *leuB lac rpsL* recipient, Leu^+ (*rpsL*) recombinants arising from a pair of exchanges near the ends of the Hfr DNA fragment (*i.e.*, long chunk integration) would be Lac^+, whereas those coming from a pair of exchanges immediately flanking *leuB* (*i.e.*, short chunk integration) would be Lac^- (Figure 2B). The frequencies of these two classes were determined by monitoring the segregation of blue and white colonies on plates containing X-gal.

We examined a total of 37,374 Leu^+ (*rpsL*) recombinants from 36 independent matings between Hfr KL226 and the rec^+ recipient, N2415, and found 65.2% to be Lac^+ and 25.7% Lac^-. A third category contained sectors of Lac^+ and Lac^- cells radiating from the center of the colony. These colonies accounted for the remaining 9.1%. The majority had one Lac^+ and one Lac^- sector, with each sector varying from 1/8 to 7/8 of the colony. Colonies with three or more sectors were also observed but very infrequently. Slightly ovoid colonies showing what appeared to be 50:50 sectoring were assumed to have arisen from the close juxtaposition of Lac^+ and Lac^- recombinant cells (see MATERIALS AND METHODS) and were scored as such. In a series of 12 additional crosses with the very closely related rec^+ strain, N2419, we observed an almost identical distribution of Lac^+, Lac^- and sectored $Lac^{+/-}$ colonies (data not shown).

Sectored recombinant colonies are a well-established feature of conjugational recombination (LOTAN *et al.* 1972; MAHAJAN and DATTA 1979). To investigate the nature of the exchanges that generate such recombinants, Lac^+ and Lac^- cells were purified from the sectored Leu^+ colonies selected on X-gal agar and tested in pairs for inheritance of the unselected *proC* marker located ~44 kb from *lacZ* (Figure 2B). Four categories of sectored colony could be distinguished (Table 2). Most were also sectored for *proC*, with the Lac^+ sector being Pro^+ and the Lac^- sector being Pro^-. Of the 651 sectored colonies analyzed in the crosses with strain N2415, 95.4% had a Lac^+ sector that was Pro^+, whereas 90.5% had a Lac^- sector that was Pro^-. The sectored colonies from one experiment were also tested for the distal *ara* marker located ~15 kb from *leuB*. Ninety-four percent (173/184) of both the Lac^+ and Lac^- sectors were Ara^+. Samples of the nonsectored colonies were also tested. Out of 800 Lac^+ colonies, 96% were Pro^+ and 91.4% were Ara^+, whereas of 400 Lac^- colonies, 92.8% were Pro^- and 85.3% were Ara^+. These values are very close to those obtained with the corresponding recombinants from sectored colonies, which suggests that the two categories (pure and sectored) arise from a similar spectrum of genetic exchanges.

The relatively small number of Lac^- Pro^+ (Leu^+) recombinants detected by these analyses suggest that multiple exchanges (four in this case) are rare. To test this directly, we analyzed samples of Leu^+ recombinants from several Hfr KL226 × N2415 crosses selected in the absence of X-gal (Table 3). The data show that recombinants arising from multiple exchanges (Leu^+ Lac^- Pro^+) represent only 1.6% of the total, or 2.3% of

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TABLE 2

Inheritance of *proC* in sectored Lac$^+$/Lac$^-$ colonies

Category of sectored colony[a]		% of total in each category	
		N2415 (*rec*$^+$) (n = 641)	N2477 (*recN*) (n = 181)
A	lacZ$^+$ proC$^+$ / lacZ$^-$ proC$^-$	85.9	56.3
B	lacZ$^+$ proC$^+$ / lacZ$^-$ proC$^+$	9.5	22.7
C	lacZ$^+$ proC$^-$ / lacZ$^-$ proC$^-$	4.3	19.3
D	lacZ$^+$ proC$^-$ / lacZ$^-$ proC$^+$	0.3	1.7

Lac$^+$ and Lac$^-$ colonies purified from sectored Leu$^+$ recombinants selected on X-gal plates were regrown in paired arrays and the *proC* marker carried was identified by replica plating. The data are from four (N2415) or nine (N2477) independent matings with Hfr KL226.

[a] The paired lines represent the Lac$^+$ and Lac$^-$ sectors of each colony.

the Leu$^+$ Pro$^+$ progeny. The frequency of mixed (sectored) colonies detected in these crosses (4.7%) is less than half that observed when the recombinant colonies were scored directly on the selection plates, using X-gal to monitor segregation at *lacZ*. This difference is probably due to the taking of inocula for the replica plate tests with a needle, a procedure that would be expected to often select a single recombinant genotype from a sectored colony (see MATERIALS AND METHODS).

From these data, it appears that the majority of the Leu$^+$ recombinants arise from the integration of a single segment of the Hfr DNA, with the proximal exchange located between *lacZ* and the origin of DNA transfer. Because the two intervals defined by *lacZ* are very long, we conducted further crosses and analyzed more markers to try to pin down the location of the exchanges in more detail. First, we mated strain N2415 with Hfr AB259, which transfers the chromosome in the opposite orientation to Hfr KL226 (Figure 2B). In this case, we selected ProC$^+$ recombinants and analyzed the segregation of both the *leuB* marker, which is ~225 kb from the Hfr origin, and *lacZ* to try to determine the position of the proximal exchange. The data obtained are summarized in Table 3. Again, a majority (66.5%) of the recombinants seemed to have inherited a single segment of Hfr DNA extending from *proC* to *leuB*. Of the remainder, >75% had a crossover in interval 3 to incorporate both the selected *pro*$^+$ allele and the unselected *lacZ*$^+$ allele. If these are defined as coming from short-chunk integrations, then it is clear that the mechanism involved must often incorporate ≥44 kb of Hfr DNA into the chromosome, this being the distance from *proC* to *lacZ*. Because the exchange distal to the selected marker is often located some distance away (note that ~85% of Leu$^+$ recombinants from the

TABLE 3

Analysis of unselected marker inheritance in crosses with Hfrs KL226 and AB259 and effect of *recF*, *recJ* and *recO* mutations

Crossover intervals or recombinant category	% total recombinants analyzed[a]							
	Hfr KL226 (Cavalli) donor				Hfr AB259 (Hayes) donor			
	N2415 (*rec*$^+$) (1993)	N2417 (*recF*) (500)	N2466 (*recO*) (500)	N2468 (*recJ*) (700)	N2415 (*rec*$^+$) (1200)	N2417 (*recF*) (999)	N2466 (*recO*) (1000)	N2468 (*recJ*) (1000)
Crossover interval[b]								
1 + 2	22.9	18.6	16.8	25.0	6.7	2.8	4.3	5.7
1 + 3	2.2	2.8	2.0	2.0	21.9	23.3	24.9	27.5
1 + 4	68.6	74.2	75.0	65.8	66.5	69.8	68.1	61.0
1 + 2 + 3 + 4	1.6	1.6	1.4	2.0	2.2	1.7	1.4	2.4
Mixed (sectored)	4.7	2.8	4.8	5.2	2.7	2.4	1.3	3.4
Linkage[c]								
Pro$^+$	73.6	78.0	80.3	71.5				
Lac$^+$	74.3	79.2	80.9	71.5	90.6	95.4	94.1	92.1
Leu$^+$					69.8	72.9	70.0	65.2

Mating was for 60 min before blending to interrupt transfer and plating appropriate dilutions for selection of Leu$^+$ (KL226 donor) or ProC$^+$ (AB259), *rpsL* recombinants.

[a] The number of colonies analyzed is shown below the strain genotype and were accumulated from two to four independent crosses in each case. Recombinants were analyzed by replica-plating colonies picked with a needle and regrown on selective agar.

[b] The arrangement of genetic markers and crossover intervals for the two Hfr crosses are shown in Figure 2B.

[c] Excluding recombinants in the mixed category.

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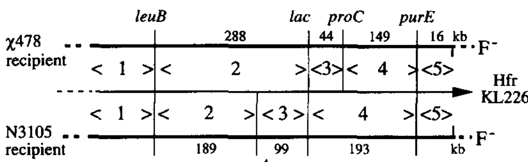

FIGURE 3.—Arrangement of markers and identification of genetic intervals for recombination in crosses between Hfr KL226 and F^- strains χ478 or N3105 (Table 4). The relevant section of the transferred Hfr DNA is indicated by the arrowed line. The F^- strains are mutant for the markers shown, whereas the Hfr is wild type. Distances between markers, or between the first proximal marker and the Hfr origin, are indicated in kilobases.

matings between N2415 and Hfr KL226 inherit the distal *ara*$^+$ marker, located ~15 kb from *leuB*), we suspect that the majority of these events integrate >44 kb of Hfr DNA. Only the 6.7% of recombinants inheriting the donor *leuB*$^+$ marker alone appear to have integrated <44 kb of Hfr DNA.

We next crossed Hfr KL226 with recipients carrying either a point mutation or a Tn*10* insertion in *purE*. The *purE* locus is only 16 kb from the IS*3c* defining the Hfr KL226 origin of chromosomal DNA transfer (KRÖGER *et al.* 1993). It provides therefore a convenient marker for the proximal end of the transferred DNA (Figure 3). In both cases, approximately half the Leu$^+$ recombinants appeared to have come from a single pair of exchanges, with the proximal exchange located in interval 4 toward the Hfr origin (Table 4). A smaller number had the proximal exchange in interval 5, which therefore must be within 16 kb of the Hfr origin. The actual number of exchanges in interval 5 varied between the two crosses. This variation may reflect differences in the genetic markers carried by the two recipient strains.

Although most of the Leu$^+$ recombinants analyzed seemed to have inherited a single segment of Hfr DNA, the number showing evidence of multiple (4) exchanges was significantly higher than in the crosses with strain N2415 (Table 3). Much of this increase was due to the ability to monitor segregation at *purE*. Presumably, fewer N2415 Leu$^+$ recombinants inherited Hfr DNA in a single long segment than was conveyed by the analysis simply because of the lack of markers to reveal multiple exchanges. We therefore crossed Hfr KL226 with N3842 and selected the more distal *metE*$^+$ marker rather than *leuB*$^+$. In this case, exchanges were analyzed in six proximal intervals (Figure 4, Table 5). Several significant differences emerged: (1) apart from *purE*$^+$, linkage of unselected donor markers was reduced; (2) the number of recombinants showing evidence of multiple exchanges was increased substantially and (3) recombinants with a single proximal exchange in the region between *proC* and the Hfr origin (intervals 6 and 7) to integrate Hfr DNA in one long segment were now only a quarter (26.1%) of the total. Some

TABLE 4

Analysis of unselected markers among Leu$^+$ recombinants from Hfr KL226 crosses with recipient strains χ478 and N3105

Crossover intervals or recombinant category	% total Leu$^+$ (*rpsL*) recombinants analyzed[a] Hfr KL226 × χ478	Hfr KL226 × N3105
Crossover interval[b]		
1 + 2	27.5	21.0
1 + 3	4.75	5.25
1 + 4	52.75	48.0
1 + 5	7.25	18.0
1 + 2 + 3 + 4	2.25	1.75
1 + 2 + 3 + 5	0.5	2.25
1 + 2 + 4 + 5	1.75	2.0
1 + 3 + 4 + 5	1.0	0.75
Mixed (sectored)	2.25	1.0
Total multiple exchanges	5.5	6.75
Linkage analysis[c]		
ProA$^+$		72.7
Lac$^+$	67.3	70.7
ProC$^+$	64.2	
PurE$^+$	10.7	23.0

Mating was for 60 min before blending to interrupt transfer and plating appropriate dilutions for selection of Leu$^+$ (*rpsL*) recombinants.

[a] Four-hundred recombinants analyzed in each cross. Recombinants were analyzed by replica-plating colonies picked with a needle and regrown on selective agar.

[b] The arrangement of genetic markers and crossover intervals for the two crosses are shown in Figure 3.

[c] Excluding recombinants in mixed category.

43.7% of the progeny did have an exchange within this 165-kb region, but a large fraction of these (40%) were associated with multiple crossovers.

The *purE* marker in strain N3842 is a Tn*10* insertion, which might have interfered with the distribution of exchanges. A control mating with the *pur*$^+$, but otherwise isogenic parent strain, N2419, was therefore conducted in parallel. The analysis is shown in Table 5. If the segregation at *purE* is excluded from the analysis for strain N3842 (column 3), it is clear that the range of recombinants obtained is almost identical for the two recipients and that the Tn*10* insertion is not affecting the results. From these data we can conclude that a successful exchange is initiated in the 165-kb interval between the Hfr KL226 origin and *proC* in less than half (~40%) of the matings that give Met$^+$ recombinants.

To confirm that the changes observed are due to the selection of a more distal marker, Hfr KL226 was crossed with the *argE* strain, N3105, which is from a different genetic background than N3842. Arg$^+$ recombinants were analyzed for exchanges in seven proximal intervals (Figure 5, Table 6). Compared with the cross in which we selected Leu$^+$ (Table 4, strain N3105 recipient), the data again revealed a reduction in the linkage

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TABLE 5

Analysis of unselected markers among Met$^+$ recombinants from Hfr KL226 crosses with recipient strains N2419 and N3842

Crossover intervals or recombinant category	% total Met$^+$ (*rpsL*) recombinants analyzed[a]		
	Hfr KL226 × N3482	Hfr KL226 × N3482[b]	Hfr KL226 × N2419
Crossover interval[c]			
1 + 2	36.4	41.7	42.9
1 + 3	1.5	1.5	0.25
1 + 4	8.0	8.5	11.0
1 + 5	0.75	1.0	2.5
1 + 6	19.1	26.1	25.3
1 + 7	7.0		
1 + 2 + 3 + 4	1.25	1.25	0.5
1 + 2 + 3 + 6	1.0	1.0	1.0
1 + 2 + 4 + 5	1.76	1.76	1.0
1 + 2 + 4 + 6	6.8	7.8	9.0
1 + 2 + 4 + 7	1.0		
1 + 2 + 5 + 6	1.0	1.5	1.0
1 + 2 + 5 + 7	0.5		
1 + 2 + 6 + 7	5.3		
1 + 3 + 4 + 6	0.5	0.5	0.75
1 + 4 + 5 + 6	0.75	0.75	0.25
1 + 4 + 6 + 7	0.5		
1 + 5 + 6 + 7	0.25		
Mixed (sectored)	6.5	6.5	4.5
Total multiple exchanges	20.6	14.6	13.5
Linkage analysis[d]			
Ara$^+$	41.1		42.0
Leu$^+$	41.4		42.5
Lac$^+$	40.9		41.5
Pro$^+$	40.3		39.1
Pur$^+$	15.6		

Mating was for 60 min before blending to interrupt transfer and plating appropriate dilutions for selection of Met$^+$ (*rpsL*) recombinants. Recombinant yields were 2.5×10^5 (N2419) and 2.1×10^5 (N3842) per ml of the mating mixture.

[a] The total of recombinants analyzed was 398 for N3842 and 399 for N2419. Recombinants were analyzed by replica-plating colonies picked with a needle and regrown on selective agar.

[b] Excluding the *purE* marker.

[c] The arrangement of genetic markers and crossover intervals for the two crosses are shown in Figure 4. No recombinants were detected for other combinations of exchange intervals not shown.

[d] Excluding recombinants in mixed category.

of proximal markers, a substantial increase in the number of recombinants showing multiple exchanges and relatively few progeny inheriting a single long segment

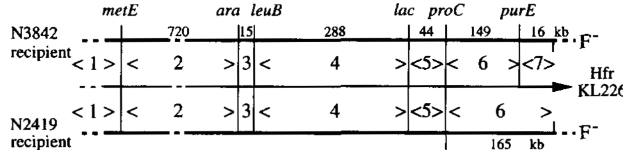

FIGURE 4.—Arrangement of markers and identification of genetic intervals for recombination in crosses between Hfr KL226 and F$^-$ strains N3842 or N2419 (Table 5). The relevant section of the transferred Hfr DNA is indicated by the arrowed line. The F$^-$ strains are mutant for the markers shown, whereas the Hfr is wild type. Distances between markers, or between the first proximal marker and the Hfr origin, are indicated in kilobases.

of Hfr DNA extending from the selected marker to near the Hfr origin. In this case, 38% of the progeny selected had an exchange in the ~209 kb between *lac* and the Hfr origin. Of these, 33% had multiple exchanges. The data for the *pur*$^+$ parent strain, AB1157, showed slightly different frequencies of exchanges in some of the intervals, but when *purE* was excluded, the results in general were the same as for N3105. Compared with previously published data for Leu$^+$ recombinants (Table 2, LLOYD 1991b), they also showed a substantial drift away from recombinants that inherit Hfr DNA in one long segment and in which the proximal exchange is near the origin.

The data in Tables 3–5 were also used to calculate a value for the percentage of the total exchanges falling within a given interval divided by the length of that interval in kilobases. The values obtained were 0.085,

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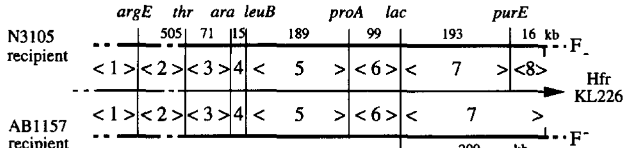

FIGURE 5.—Arrangement of markers and identification of genetic intervals for recombination in crosses between Hfr KL226 and F$^-$ strains N3105 or AB1157 (Table 6). The relevant section of the transferred Hfr DNA is indicated by the arrowed line. The F$^-$ strains are mutant for the markers shown, whereas the Hfr is wild type. Distances between markers, or between the first proximal marker and the Hfr origin, are indicated in kilobases.

0.086, 0.43, respectively, for intervals 2–4 in the N2415 × KL226 cross (Figure 2B, Table 3); 0.11, 0.19, 0.39, 0.66 for intervals 2–5 in the χ478 cross (Figure 3, Table 4); 0.14, 0.1, 0.27, 1.43 for intervals 2–5 in the N3105 cross (Figure 3, Table 4) and 0.08, 0.28, 0.07, 0.11, 0.23, 0.91 for intervals 2–7 in the N3842 cross (Figure 4, Table 5). In each case, the recombination is highest in the interval immediately adjacent to the Hfr origin. The disproportionately high frequency of exchanges in the most proximal interval in each case suggests that the leading end of the Hfr DNA fragment stimulates recombination, as was suggested by PITTARD and WALKER (1967).

Effect of mutations in *rec* and *ruv* genes on the distribution of crossovers: LLOYD and THOMAS (1984) suggested that the distribution of genetic exchanges in Hfr crosses might be affected by the products of *recF* and other genes needed for recombination in the *recBC sbcBC* genetic background. To investigate this possibility, recombination was examined in Hfr KL226 crosses with strains carrying various combinations of mutations in these genes. We used the X-gal plate assay to monitor segregation at *lacZ* among Leu$^+$ recombinants so that large numbers of progeny could be analyzed, thereby minimizing the effect of sampling error. We first looked at the effect of mutations that eliminate or alter the activity of RecBCD enzyme (Table 7A). Mutation of *recB* reduced recombination to <1% and shifted the balance of Leu$^+$ recombinants toward those that are Lac$^-$. This reduced inheritance of a proximal markers has been observed before (DE HAAN *et al.* 1972). Our data show that *recB* also reduces sectored colonies ninefold. These observations support the notion that the high level of exchanges normally detected near the proximal end are catalyzed by RecBCD enzyme. They also suggest that most sectored colonies arise from events catalyzed by RecBCD. In contrast, mutation of *recD* increased recombination to a slight but reproducible extent and doubled the number of sectored colonies at the expense of pure Lac$^+$ recombinants. Strains carrying *recD1013* or *recD1014* gave results that were almost identical to those for the *recD1009* strain (data not shown).

Mutation of *recF*, *recO* or *recQ* caused a modest shift from Lac$^-$ to Lac$^+$ recombinants. The effect was observed with single mutants and with *recF recO*, *recQ recF* and *recQ recO* strains (Table 7B and data not shown). It was also noticed when segregation of unselected markers was measured by replica plating samples of the recombinants (Table 3). The shift was reversed by the additional mutation of *recD* or partly by mutation of *recJ* (Table 7C). Mutation of *recJ* alone caused only minor changes (Tables 3 and 7C), but when *recJ* was combined with *recD* there was a marked shift in the other direction toward Lac$^-$ recombinants (Table 7D). As with the *recB* recipient, the shift was associated with a decrease in recombinant yield. However, the changes are not as great. The addition to *recJ recD* of mutations in *recF*, recO or both did not alter any further the yield or spectrum of recombinants produced.

The only substantial change in the spectrum of recombinants observed with mutations in *ruv* that inactivate either RuvAB or RuvC, or both, is a decrease in sectored colonies and a corresponding increase in pure Lac$^+$ recombinants (Table 7E). Mutation of *recG* also reduced the number of sectored colonies, but in this case there was an increase in both Lac$^+$ and Lac$^-$ recombinants (Table 7F). The addition to *recG* of a *recD* mutation reduced the yield of recombinants by selectively reducing those that inherit Lac$^+$, whereas adding a *recQ* mutation had the opposite effects.

Given the location of the *recF*, *recG* and *recQ* genes (Figure 2A), transfer of the Hfr *rec*$^+$ allele might be affecting the results in crosses with recipients mutant for one or more of these genes. The number of Met$^+$ recombinants in samples of the Leu$^+$ progeny analyzed was 12% (data not shown), which agrees with the fact that in 60-min matings with Hfr KL226, *rec*$^+$ recipients yielded nearly 20 times more Leu$^+$ recombinants than Met$^+$ recombinants (see footnotes to Table 5 and the APPENDIX). The low inheritance of *metE*$^+$ suggests that transfer of the more distal *recF,G,Q* wild-type alleles was not frequent enough in these crosses to significantly affect the results. Even so, a *recF143* derivative of Hfr KL226 was made and used as a donor in crosses with the *recF143* recipient, N2417. Hfr KL226 itself was used in parallel as a control. The results obtained with the two Hfrs were almost indistinguishable (data not shown) and very similar to those for the N2417 recipient in Table 7B.

Mutation of *recN* blocks the formation of sectored colonies with both *recD*$^+$ and *recD* recipients: Mutation of *recN* reduced the level of sectoring to ~1%, with a proportionate increase in both Lac$^+$ and Lac$^-$ recombinants (Table 8). A similar low level of sectoring was observed when we combined *recN* with mutations in other recombination genes, including *recD*. The latter effect is particularly striking because it is not matched

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TABLE 6

Analysis of unselected markers among Arg⁺ recombinants from Hfr KL226 crosses with recipient strains AB1157 and N3105

Crossover intervals or recombinant category	% total Arg^+ (*rpsL*) recombinants analysed[a]		
	Hfr KL226 × N3105	Hfr KL226 × N3105[b]	Hfr KL226 × AB1157
Crossover interval[c]			
1 + 2	46.3	47.9	39.8
1 + 3	3.4	3.6	3.5
1 + 4	0.4	0.4	
1 + 5	2.2	2.6	5.5
1 + 6	3.8	4.2	3.0
1 + 7	21.8	25.4	34.0
1 + 8	3.6		
1 + 2 + 3 + 4			0.5
1 + 2 + 3 + 5	0.2	0.2	0.25
1 + 2 + 3 + 7	0.6	0.8	1.25
1 + 2 + 3 + 8	0.2		
1 + 2 + 4 + 5	0.2	0.2	0.25
1 + 2 + 5 + 6	0.2	0.4	0.25
1 + 2 + 5 + 7	1.4	1.6	3.25
1 + 2 + 5 + 8	0.2		
1 + 2 + 6 + 7	1.0	1.6	2.25
1 + 2 + 6 + 8	0.6		
1 + 2 + 7 + 8	1.6		
1 + 3 + 4 + 5	0.2	0.2	
1 + 3 + 4 + 7			0.25
1 + 3 + 5 + 6			0.25
1 + 3 + 5 + 7			0.5
1 + 3 + 6 + 7	0.2	0.2	
1 + 3 + 7 + 8	0.2		
1 + 4 + 5 + 6	1.0	1.2	
1 + 4 + 5 + 7	0.6	0.6	0.25
1 + 4 + 6 + 7	0.2	0.2	
1 + 5 + 6 + 7	0.2	0.2	0.75
1 + 5 + 7 + 8	0.4		
1 + 6 + 7 + 8	0.4		
1 + 2 + 3 + 5 + 6 + 7	0.2	0.2	
1 + 2 + 4 + 5 + 6 + 7	0.4	1.2	
1 + 2 + 4 + 5 + 6 + 8	0.8		
1 + 2 + 5 + 6 + 7 + 8	0.2		
1 + 4 + 5 + 6 + 7 + 8	0.2		
Mixed (sectored)	6.9	6.9	4.0
Total multiple exchanges	11.4	8.8	10.0
Linkage analysis[d]			
Thr^+	41.9		50.1
Ara^+	38.8		47.5
Leu^+	37.7		47.3
Pro^+	36.9		44.9
Lac^+	34.5		44.4
Pur^+	9.1		

Mating was for 60 min before blending to interrupt transfer and plating appropriate dilutions for selection of Arg^+ (*rpsL*) recombinants. Recombinant yields were 2.84×10^5 (AB1157) and 2.57×10^5 (N3105) per milliliter of the mating mixture.

[a] The total of recombinants analyzed was 495 for N3105 and 399 for AB1157. Recombinants were analyzed by replica-plating colonies picked with a needle and regrown on selective agar.

[b] Excluding the *purE* marker.

[c] The arrangement of genetic markers and crossover intervals for the two crosses are shown in Figure 5. No recombinants were detected for other combinations of exchange intervals not shown.

[d] Excluding recombinants in mixed category.

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TABLE 7

Effect of *rec* and *ruv* mutations on the production and Lac phenotype of Leu$^+$ recombinant colonies in Hfr KL226 × F$^-$ crosses

Recipient strain	Genotype tested	Lac phenotype (% of total)			Relative yield of recombinants
		Lac$^-$	Lac$^+$	Mixed Lac$^{+/-}$	
A. Effect of *recB* and *recD*					
N2415	*rec*$^+$ *ruv*$^+$ *mut*$^+$	25.7	65.2	9.1	1.0
N2420	*recB*	64.2	34.8	1.0	0.006
N2516	*recD*	25.9	55.1	19.0	1.22
B. Effect of *recF*, *recO* and *recQ*					
N2417	*recF*	22.1	72.5	5.4	0.64
N2466	*recO*	18.8	76.0	5.2	0.58
N3343	*recQ*	16.6	76.6	6.8	0.89
N3345	*recF recQ*	17.7	75.8	6.5	0.69
N3346	*recO recQ*	16.5	76.4	7.1	0.64
N2517	*recF recD*	25.9	65.2	8.9	0.36
N2518	*recO recD*	25.9	65.4	8.7	0.64
C. Effect of *recJ*, with *recF*, *recO*, *recQ*					
N2468	*recJ*	27.8	66.3	5.9	0.93
N2521	*recJ recF*	26.4	66.5	7.2	0.58
N2523	*recJ recO*	22.0	72.4	5.6	0.59
N2535	*recJ recF recO*	22.7	72.3	5.0	0.53
N3349	*recJ recQ*	23.0	71.0	6.0	0.84
D. Effect of *recJ* with *recD* and *recF*, *recO*					
N2519	*recJ recD*	42.8	47.7	9.5	0.20
N2589	*recJ recD recF*	43.4	49.6	7.0	0.16
N2588	*recJ recD recO*	41.3	51.0	7.7	0.17
N2590	*recJ recD recF recO*	41.7	50.0	8.3	0.17
E. Effect of *ruv* mutations					
N2488	*ruvA*	24.8	72.0	3.2	0.19
N3755	*ruvC*	25.4	72.9	1.7	0.14
N3756	Δ*ruvAC*	24.4	73.5	2.1	0.25
F. Effect of *recG* alone, or with *recD*, *recQ*					
N2971	*recG*	28.8	68.5	2.7	0.11
N2965	*recG recD*	33.5	58.8	7.7	0.061
N3348	*recG recQ*	21.6	76.4	2.0	0.15

Mating was for 60 min before interruption. Leu$^+$ (*rpsL*) recombinants were recovered on selective minimal agar supplemented with X-gal to score the Lac phenotype. Details of the recombinant yields, colonies scored, cell viability and experimental variation are given in the APPENDIX. Yields of recombinants have been corrected for any deficiency in the viability of the recipient cells. The arrangement of genetic markers is shown in Figure 2B.

by mutations in other genes like *recG* and *ruv*, which when present in a *recD*$^+$ strain also reduce sectoring quite substantially (Table 7 and data not shown). It seems that RecN protein may have a role in recombination that specifically encourages sectoring. We made use of the *recN* background to examine further the effects of other *rec* genes on the location of crossovers relative to *lacZ*. The low numbers of sectored colonies made it easier to monitor the location of the exchanges. The results showed the same general trends as before with the proportion of Lac$^+$ recombinants increasing with mutation of *recF* and *recO* and decreasing with mutation of both *recJ* and *recD*. However, in this case mutation of *recQ* had no effect (Table 8).

All the constructs made showed a lower efficiency of recombination than is seen with a *recN* single mutant (Table 8). Yet, the proportion of sectored colonies remained fairly constant at around the 1% level. Perhaps some colonies scored as sectored were in fact plating artefacts caused by the close juxtaposition of two recombinant cells of different *lac* genotype. To test this possibility, mixtures of Lac$^+$ and Lac$^-$ cells (Leu$^+$ recombinants of the *recN* strain N2477 from a mating with Hfr KL226) were spread on X-gal plates at various densities,

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TABLE 8

Effect of a *recN* mutation, either alone or in combination with other *rec* mutations, on the production and phenotype of Leu$^+$ recombinant colonies in Hfr KL226 × F$^-$

Recipient strain	Genotype tested	Lac phenotype (% of total)			Relative yield of recombinants
		Lac$^-$	Lac$^+$	Mixed Lac$^{+/-}$	
N2415	*rec*$^+$	25.7	65.2	9.1	1.0
N2516	*recD*	25.9	55.1	19.0	1.22
N2477	*recN*	29.0	69.6	1.4	0.38
N2485	*recN recF*	22.5	76.4	1.1	0.11
N2498	*recN recF recO*	18.8	80.1	1.1	0.11
N3347	*recN recQ*	27.4	71.6	1.0	0.10
N2967	*recN recG*	37.6	61.3	1.1	0.10
N2520	*recN recD*	31.8	67.0	1.2	0.13
N2908	*recN recJ*	30.3	68.3	1.4	0.12
N2914	*recN recJ recD*	40.6	59.0	0.42	0.02

Mating was for 60 min before interruption. Leu$^+$ (*rpsL*) recombinants were recovered on selective minimal agar supplemented with X-gal to score the Lac phenotype. Details of the recombinant yields, colonies scored, cell viability and experimental variation are given in the APPENDIX. Yields of recombinants have been corrected for any deficiency in the viability of the recipient cells. The data for the control strain (N2415) and the *recD* mutant (N2516) are repeated from Table 7A for ease of comparison. The arrangement of genetic markers is shown in Figure 2B.

incubated for 2 days and scored. Colonies that would normally be scored unambiguously as sectored were clearly visible. A higher than usual proportion of these were half Lac$^+$, half Lac$^-$, as would be expected from equal growth of two cells plated next to each other. Also as expected, their frequency increased with increasing cell density and was higher when Lac$^+$ and Lac$^-$ cells were plated in equal number as opposed to a 2:1 ratio (Table 9). When the density of colonies was increased to 600–1000, the frequency of apparently sectored colonies increased even further, but their number could not be determined reliably because of clustering. Because the plates scored in matings typically carried between 50 and 300 recombinants, a significant fraction of the sectored colonies scored in crosses with the *recN* strain N2477 were probably the result of plating artefacts. Because these are less frequent at low densities of recombinants, six further matings were conducted with N2477 and Leu$^+$ recombinants were scored at a density of no more than 100 per plate. Of 7957 recombinants scored in total, 27.9% were Lac$^-$, 70.9% Lac$^+$ and 1.2% sectored. These values are almost identical to those for N2477 in Table 8. We suspect therefore that at least some of the recombinant colonies obtained with the *recN* recipient are genuinely sectored, though given the data obtained in the reconstruction experiment (Table 9) their frequency may be somewhat <1%.

Because mating occurs in aggregates (ACHTMAN 1975), the 1% sectored colonies observed with *recN* mutants might be derived from zygotes that receive DNA from two or more donors. To try to assess the incidence of biparental matings, the *rec*$^+$ recipient, N2415, was mated with a 50:50 mixture of Hfr Hayes strains AB259 (*lacI*$^+$) and DF71 (*lacI22*), and the number of sectored Pro$^+$ (*rpsL*) recombinants was monitored on X-gal plates lacking IPTG. The donor to recipient ratio was increased to 1:1 to provide more opportunities for detecting biparental mating. Pro$^+$ colonies with pale-blue (*lacZ*$^+$ *lacI*$^+$) and dark-blue (*lacZ*$^+$ *lacI22*) sectors were detected, but they were only 1.2% of the total (484 colonies analyzed). From these data, it seems that biparental matings could be responsible for some of the sectored colonies observed, but as the donor to recipient ratio was 1:10 in our standard crosses, events of this type would be expected to be <1% of the total.

Sectored colonies normally arise only occasionally from heteroduplex DNA: Donor and recipient markers that fall within the heteroduplex joints associated with crossovers would be expected to segregate after the first round of DNA replication unless they were first subject to correction. The DNA mismatch repair system controlled by *mutHLS* acts in conjugation (FEINSTEIN and LOW 1986; RAYSSIGUIER *et al.* 1989). To see whether this system affects the frequency of sectoring, we conducted crosses with recipients mutant for *mutH* or *mutS*. We also tested a *mutY* strain defective in very-short patch repair (NGHEIM *et al.* 1988). Because of the mutator phenotype of these strains, we imposed a double selection for Leu$^+$ Ara$^+$ recombinants so as to avoid revertants. We saw no increase in sectoring at *lacZ*, either in the *mut* single mutants or when *recN*, or *recN* and *recF* mutations were present to reduce the background levels (Table 10). Indeed, both *mutH* and *mutS* alone slightly reduced the proportion of sectored colonies. Because

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TABLE 9

Reconstruction experiment to measure effect of plating density on the frequency of sectored colonies

Ratio Lac^+:Lac^-					
2:1			1:1		
Colonies per plate	Total analyzed	% sectored	Colonies per plate	Total analyzed	% sectored
63–92	471	0.2	55–107	416	0.5
145–308	1468	0.6	178–314	857	0.9

Cultures of the Lac^+ and Lac^- strains were grown in LB broth to an A_{650} of 0.4 (~2×10^8 cells/ml) and mixed in the ratio indicated before diluting and plating on 56/2 glucose minimal agar supplemented with X-gal.

the *lacZ118* allele is an *ochre* mutation, it cannot give C-C mismatches, which would go undetected by the MutHLS system (SU *et al.* 1988). We conclude that the mismatch repair system rarely operates on the *lacZ* region in these crosses and that most sectored colonies probably do not arise from heteroduplex DNA. This would be expected if a single segment of duplex Hfr DNA is integrated into the chromosome with heteroduplex DNA restricted to the flanking exchanges. It also agrees with our general observation that by no means all of the sectored colonies were of the half Lac^+ and half Lac^- type expected from replication of a recombinant chromosome with a heteroduplex patch over *lacZ*.

However, the possibility remains that some sectored colonies could arise from $lacZ^+/lacZ118$ heteroduplexes associated with crossovers located nearby. Tables 3–5 reveal that a few exchanges do occur in the short interval between *lacZ* and *proC*. Heteroduplex DNA associated with some of these exchanges could extend across the *lacZ* region. Table 2 shows that 14.1% (categories B–D) of the 641 sectored colonies examined in rec^+ crosses show evidence of an exchange between *lacZ* and *proC*. Could these be derived from $lacZ^+/lacZ118$ heteroduplexes and could recombinants of this type be responsible for the residual sectored colonies detected with *recN* recipients? Compared with the rec^+ control, a much higher proportion of the sectored colonies obtained with a *recN* recipient are half Lac^+ and half Lac^- (data not shown), which would agree with the idea that they are derived from zygotes with heteroduplex DNA extending across *lacZ*. We examined the sectored recombinants obtained with a *recN* strain to see if the proportion with an exchange between *lacZ* and *proC* was increased. The data in Table 2 show that 43.7% of the sectored colonies fall into categories B–D, a threefold increase compared with the rec^+ cross. The real increase is probably higher because some of the sectored colonies obtained with the *recN* recipient were probably plating artefacts arising from the juxtaposition of Lac^+ and Lac^- cells, the vast majority of which would be expected to fall into category A. The increased frequency of exchanges between *lacZ* and *proC* detected in the sectored recombinants from *recN* crosses is consistent with the idea that these recombinants arise from $lacZ^+/lacZ^-$ heteroduplex DNA associated with these exchanges. In rec^+ crosses, the vast majority (85.9%) of sectored recombinants do not have an exchange in the *lacZ-proC* interval. We suspect therefore that these are derived from *recN*-dependent exchanges that do not involve heteroduplex DNA across *lacZ*, though we have not shown this directly.

Mutation of *recD* increases the half-life of Hfr DNA in transconjugant cells: The high frequency of sectoring observed with *recD* strains provides a clue to the mechanism responsible for the majority of sectored recombinants in rec^+ crosses. Several lines of evidence indicate that the half-life of linear DNA molecules is increased in *recD* cells because of the reduced exonuclease activity of RecBCD (AMUNDSEN *et al.* 1986; LOVETT *et al.* 1988; RUSSELL *et al.* 1989; DABERT *et al.* 1992; KUZMINOV *et al.* 1994). In *recD* transconjugants, any section of the recipient chromosome excised during integration of Hfr DNA would be expected to survive longer than usual. If this fragment provided a substrate for further recombination and some of the additional exchanges were to occur after a recombinant chromosome had replicated but before the cell divided, it is easy to imagine how a sectored colony might arise from a single transconjugant.

To see if this was a feasible explanation for sectoring, we used the *recA* temperature-sensitive allele, *recA200* (LLOYD *et al.* 1974), to monitor the fate of Hfr DNA in *recD* transconjugants. Previous studies showed that if newly formed *recA200* transconjugants are transferred to 42°, they rapidly lose the ability to form recombinants when incubated subsequently at the permissive temperature of 35°, because in the absence of RecA activity the Hfr DNA fragment is probably degraded through nuclease attack (LLOYD and JOHNSON 1979). Figure 6 shows the results of a similar experiment conducted with *recD recA200* transconjugants. It is clear that mutation of *recD* substantially increases the half-life of the Hfr $proB^+$ marker during the period when it cannot

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TABLE 10

Effect of *mut* gene mutations, alone or with *recN*, on the production and Lac phenotype of Leu$^+$ Ara$^+$ recombinant colonies in Hfr KL226 × F$^-$ crosses

Recipient strain	Genotype tested	Lac phenotype of recombinants (% total) Lac$^-$	Lac$^+$	Mixed Lac$^{+/-}$	Relative yield of recombinants
N2419	*rec$^+$ mut$^+$*	23.0	68.2	8.8	1
N2602	*mutH*	25.0	69.9	5.1	0.93
N2605	*mutS*	23.3	71.6	5.1	0.87
N2936	*mutY*	22.5	69.1	8.4	1.1
N2604	*recN mutH*	25.0	73.4	1.6	0.38
N2606	*recN mutS*	25.0	73.7	1.3	0.35
N2607	*recN recF mutS*	21.5	77.4	1.1	0.23
N2940	*recN mutY*	23.5	74.3	2.1	0.36

Mating was with Hfr KL226 for 60 min before interruption. Leu$^+$ Ara$^+$ (*rpsL*) recombinants were selected on minimal agar supplemented with X-gal to score the Lac phenotype. Yields of recombinants are relative to that obtained with the control strain N2419, which gave an average of 2.9 × 10^6 per ml of the mating mixture. Details of the recombinant yields, colonies scored and experimental variation are given in the APPENDIX. The arrangement of genetic markers is shown in Figure 2B.

be integrated into the chromosome. Previous studies showed that only a small fraction (0.5–2%) of *recA200* (*recD$^+$*) zygotes have completed recombination at 35° at the time of mating interruption, presumably because, in the majority of cases, transfer is still in progress and no DNA ends are available to initiate or complete the necessary exchanges (LLOYD and JOHNSON 1979). This explains why so many of the zygotes rapidly lose the ability to form recombinants at 35° after a short incubation at 42°. We assume that most of the RecA200 protein is very rapidly inactivated at 42° and that the kinetic differences observed are due to the slower degradation of the Hfr DNA fragment in the ExoV-deficient *recD* mutant, which would increase the chances of recovering recombinants upon subsequent transfer of the transconjugants to selective plates incubated at the permissive temperature of 35°. An alternative possibility is that the decline in the recovery of recombinants reflects the rate of reactivation of RecA200. In this case, the kinetic differences observed would require the assumption that recombination in *recD* mutants requires less RecA activity than in *recD$^+$* strains. At any one moment at 42°, there would be at least partial inactivation of RecA200 protein. Upon return to 35°, RecA activity slowly returns to the initial level. At some intermediate level, recombination occurs (at intermediate rate) in the *recD* mutant cells but not in the *recD$^+$* cells. Nonrecombined DNA is lost or degraded. We favor the former explanation. We know of no evidence for a lower requirement for RecA activity in *recD* mutants. Furthermore, *recA200* strains begin to degrade their DNA spontaneously on shifting from 35 to 42° at a rate that is typical of a *recA* null mutant (LLOYD *et al.* 1974), which suggests that most of the RecA200 protein is very rapidly inactivated. What is clear from the experiment de-

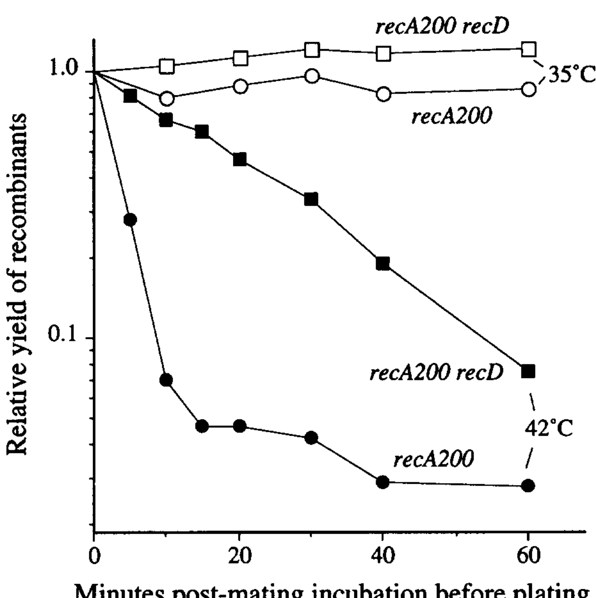

FIGURE 6.—Effect of *recD* on the ability of *recA* temperature-sensitive (*recA200*) transconjugants to retain the ability to form ProB$^+$ (*rpsL*) recombinant colonies at 35° after postmating incubation in broth medium at 35° (□ and ○) or 42° (■ and ●) for the indicated times before imposing selection. The recipient strains were N1332 (*recA200*) and N2888 (*recA200 recD*), whereas the Hfr donor was KL226. The yields of recombinants obtained per milliliter of the undiluted transconjugant mixture plated at 35° immediately after interruption were 1.27 × 10^5 (N1332) and 1.38 × 10^5 (N2888).

scribed in Figure 6 is that the Hfr DNA fragment released by the interrupion of mating is more stable in *recD* zygotes. We conclude that the opportunity for recombination is probably increased in *recD* transconjugants.

The frequency of sectoring is inversely correlated

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with the linkage between the selected and unselected marker: If sectored colonies arise from recombination between the initial recombinant chromosome and the excised fragment of the recipient chromosome, their frequency would be expected to increase with increasing distance between the selected and unselected markers. The probability that both excised markers will be destroyed before further exchanges can take place will be much higher when these are close together than when they are far apart. To test this possibility, we crossed strain N2419 with Hfr KL226 and used the X-gal assay to monitor segregation at *lacZ* among recombinants inheriting either *leu*$^+$ or *metE*$^+$ from the donor. We found that colonies sectored for *lacZ* formed 9.7% of the Leu$^+$ recombinants, which is consistent with previous results using the related recipient strain, N2415 (Table 7). However, they formed 14.5% of the recombinants when selection was for the more distal *metE*$^+$ marker (Table 11A). Similarly, we crossed strain N2419 or close relatives with Hfr AB259 and monitored segregation at *lacZ* among recombinants selected for *proC*$^+$, *purE*$^+$, *pyrD*$^+$, *pyrC*$^+$, *trp*$^+$ or *his*$^+$. These markers are located at increasing distances from the unselected *lacZ* marker (Figures 2A and 7). Again, the proportion of the recombinants sectored for *lacZ* increased as the distance between the selected marker and *lacZ* increased, at least as far as the *trp* locus (Table 11B). The values ranged from ~3% of ProC$^+$ recombinants to 25% of Trp$^+$ recombinants. Values for the His$^+$ selection averaged only 20%, despite the fact that *his*$^+$ was the most distal marker selected. We do not understand why the proportion of sectored colonies reaches a maximum with the Trp$^+$ selection and can suggest only that it may be related to the location of the terminus for DNA replication in this region of the chromosome. These data support the idea that sectored colonies arise from repeated exchanges between Hfr and F$^-$ DNA in the transconjugants before cell division.

Recombinants from crosses similar to those described in Table 11 were also analyzed independently for the inheritance of the unselected Hfr *leuB*$^+$, *lacZ*$^+$ and *proC*$^+$ markers. In this case, the colonies were picked with a needle and tested by replica plating. Colonies were taken from selective plates lacking X-gal to avoid any bias in sampling. The data obtained show that unselected markers are inherited with decreasing frequency as the distance to the selected marker increases (Table 12). Colonies of mixed genotype (sectored recombinants) show the opposite trend, as described above for the X-gal assay. However, the number of mixed colonies in each selection is lower than reported in Table 11 because of the possibility of picking a single genotype from a sectored colony. The numbers of recombinants showing multiple crossovers show a trend similar to that of the mixed colonies, which suggests that the events giving rise to these two categories are related. The combined effect of the events is to progressively reduce the number of recombinants showing just two exchanges, one in interval 1 and another in interval 5, from a value of ~50% in the case of PurE$^+$ selection to 25% in the case of His$^+$ selection. These data support the conclusions drawn earlier from the crosses reported in Tables 5 and 6.

Sectoring is correlated with the efficiency of recombination: If more than one pair of exchanges are needed to produce sectored recombinants, the frequency of such progeny should be lower in crosses with recombination-deficient recipients and higher in recombination proficient strains that degrade linear DNA less efficiently. Analysis of the data in Tables 7 and 8 revealed that most of the strains disabled for recombination genes show a reduced level of recombinant formation. The deficiencies noted are in line with those published previously with the same or similar constructs, though the absolute yields of recombinants were a little lower, which may be due to the selection of a more distal Hfr marker and the absence of any broth enrichment of the plate agar used for selection. The data show that the proportion of sectored colonies is indeed directly related to the efficiency of recombination. If recipients carrying mutations in both *recD* and other genes are excluded, the correlation is particularly strong (Figure 8a). Strains with mutations in *recB*, *recG*, *recN* or *ruv* have a greater deficiency in recombination and produce substantially fewer sectored colonies than those with mutations in *recF*, *recJ*, *recO* or *recQ*, which have rather modest effects. The correlation between sectored colonies and the efficiency of recombination is much less marked when these mutations are in a *recD* background (Figure 8b). In particular, the several *recD recJ* constructs (Table 7D) and the *recD recG* strain (Table 7F) are just as deficient in recombination as the *recD recN* strain (Table 8) and yet they produce sectored colonies at a much higher frequency, ~7–10%. These exceptions to the rule suggest that recombination can give rise to a high proportion of sectored progeny even when the efficiency of recombinant formation, and presumably the opportunity for repeated exchanges, is much reduced. The nature of the exchanges in these strains may therefore be rather different from those that predominate in the wild-type.

DISCUSSION

We used two related approaches to study the formation of recombinant chromosomes in conjugational crosses. Multiply marked strains were first used to try to locate crossovers relative to the Hfr origin of chromosomal DNA transfer in a recombination proficient recipient. Two clear conclusions could be drawn from crosses in which we selected either the *leuB* marker some 500 kb from the transfer origin of Hfr KL226 or the *proC* marker some 550 kb from the transfer origin of Hfr AB259: the majority of the recombinants recovered

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TABLE 11

Effect of map distance between selected and unselected markers on the frequency of sectoring at *lacZ* in Hfr × F⁻ crosses

Recipient strain	Selection (*rpsL*)	No tested	Lac phenotype (% of total)		
			Lac^-	Lac^+	Mixed
A. Hfr KL226 donor					
N2419	$LeuB^+$	2,265	29.7	60.6	9.7
N2419	$MetE^+$	3,470	55.8	29.7	14.5
B. Hfr AB259 donor					
N2419	$ProC^+$	4,435	6.9	89.3	3.8
N2415	$ProC^+$	2,342	10.4	87.2	2.4
N3842	$ProC^+$	2,626	7.4	89.7	2.9
N3842	$PurE^+$	2,662	23.8	67.3	8.9
N3854	$PyrD^+$	2,521	38.7	40.9	20.4
N3855	$PyrC^+$	2,677	38.8	36.9	24.2
N3856	Trp^+	2,405	42.5	32.4	25.1
N3857	His^+	2,079	53.8	25.5	20.7

Matings were for 60 min with Hfr KL226, and for 60 min ($ProC^+$, $PurE^+$), 70 min ($PyrD^+$, $PyrC^+$), 75 min (Trp^+) or 95 min (His^+) with Hfr AB259 before interruption. Recombinants were selected on minimal agar supplemented with X-gal to score the Lac phenotype. The Hfr KL226 crosses averaged 1.2×10^6 Leu^+ recombinants and 2.5×10^5 $MetE^+$ recombinants/ml of the mating mixture. Yields of recombinants with Hfr AB259 were in the range of 5.0×10^6 ($ProC^+$) to 6.5×10^5 (His^+) per ml of the mating mixture. The values given are the means of three or more independent crosses, except for Leu^+ selection in Hfr KL226 × N2419, in which case the values are based on two crosses. Standard errors range from 0.5 to 10% of the mean value. Markers are located in Figure 2A.

inherited Hfr markers in a single block and a disproportionate number of the crossovers proximal to the selected marker were located near the origin of chromosomal DNA transfer, as initially reported by LOW (1965) and PITTARD and WALKER (1967). These two facts support models in which recombinant chromosomes arise from just two exchanges, one located near each end of the transferred fragment, which integrate the Hfr DNA into the recipient chromosome as a single long segment (LLOYD and THOMAS 1984; SMITH 1991; TAYLOR and SMITH 1992). However, a rather different picture was painted by crosses with Hfr KL226 in which we selected markers at the more distant *argE* or *metE* loci, some 1088 and 1232 kb, respectively, from the origin. There was still a disproportionate number of exchanges near the origin, but fewer than half the recombinants had an exchange within the first 165–200 kb, and a substantial

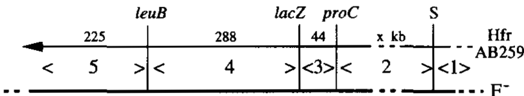

FIGURE 7.—Arrangement of markers and identification of genetic intervals for recombination in crosses between Hfr AB259 and F⁻ strains N3842 and N3854-N3857 (Tables 11 and 12). The relevant section of the transferred Hfr DNA is indicated by the arrowed line. The F⁻ strains are mutant for the markers shown, whereas the Hfr is wild type. Distances between markers, or between the first proximal marker and the Hfr origin, are indicated in kilobases. Selective marker S is *purE* (N3842), *pyrD* (N3854), *pyrC* (N3855), *trp* (N3856) or *his* (N3857), and the corresponding distance X in interval 2 is ~149, 580, 700, 910 or 1670 kb, respectively.

fraction of those that did had multiple exchanges. Recombinants with multiple exchanges were also a significantly larger fraction of the total, although the majority still appeared to have come from a single pair of exchanges. The actual number with multiple exchanges was probably higher than suggested by the data because the genetic markers available were restricted to about half the region monitored. We were able to draw the same conclusions from a series of crosses with Hfr AB259 in which we selected donor markers located at increasing distances from the Hfr origin. As the distance increased, the linkage of unselected proximal markers decreased, whereas the number showing multiple crossovers increased.

A survey of the literature revealed similar data. PITTARD and WALKER (1967) observed decreased linkage of a proximal marker (*tna*) with increasing distance to the selected marker (*mal* or *his*). DE HAAN et al. (1972) found that a substantial number (13.4%) of recombinants selected for a moderately distant marker showed evidence of multiple exchanges. JACOB and WOLLMAN (1961) analyzed 200 His^+ recombinants from a cross with Hfr Hayes for segregation of six reasonably well-spaced unselected proximal markers and found that 39% had multiples exchanges (Table XXXV, p. 227). The *his* locus is over 2000 kb from the Hfr Hayes origin of transfer. LLOYD and LOW (1976) observed a decreased linkage of proximal markers and an increased frequency of multiple exchanges with increasing distance to the selected marker in a variety of crosses with different Hfrs. These effects appear independent of the

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TABLE 12

Effect of map distance to selected marker on the segregation of unselected markers in Hfr crosses

Crossover intervals or recombinant category	% total (*rpsL*) recombinants analysed[a]				
	PurE+ ex N3842	PyrD+ ex N3854	PyrC+ ex N3855	Trp+ ex N3856	His+ ex N3857
Crossover interval[b]					
1 + 2	21.5	29.5	29.5	30.0	38.5
1 + 3	2.0	6.0	1.5	0	0.5
1 + 4	12.0	8.0	8.0	6.5	6.5
1 + 5	50.5	34.0	34.5	33.0	24.5
1 + 2 + 3 + 4	0	0	0.5	2.0	0
1 + 2 + 3 + 5	1.0	2.5	1.0	2.0	1.5
1 + 2 + 4 + 5	5.0	6.5	11.5	8.0	14.0
1 + 3 + 4 + 5	2.0	2.5	0.5	2.0	0
Mixed (sectored)	6.0	11.0	13.0	16.5	14.5
Total multiple exchanges	8.0	11.5	13.5	14.0	15.5
Linkage analysis[c]					
ProC+	72.3	57.1	51.4	48.3	37.3
Lac+	69.0	50.3	50.9	50.6	38.5
LeuB+	63.6	51.4	54.9	52.3	47.3

Mating was with Hfr AB259 for the times indicated in the footnote to Table 11 before blending to interrupt transfer and plating appropriate dilutions for selection of the recombinants indicated (all *rpsL*).

[a] Two-hundred recombinants were analyzed in each cross by replica-plating colonies picked with a needle and regrown on selective agar.

[b] The arrangement of genetic markers and crossover intervals for the two crosses are shown in Figure 7.

[c] Excluding recombinants in mixed category.

direction of Hfr DNA transfer relative to replication of the chromosome, though this was not tested systematically by LLOYD and LOW (1976). There seems little doubt, however, that the distance from the Hfr origin to the selected marker does have a substantial effect on the events observed.

The reduced inheritance of proximal markers, the increase in recombinants with multiple exchanges and

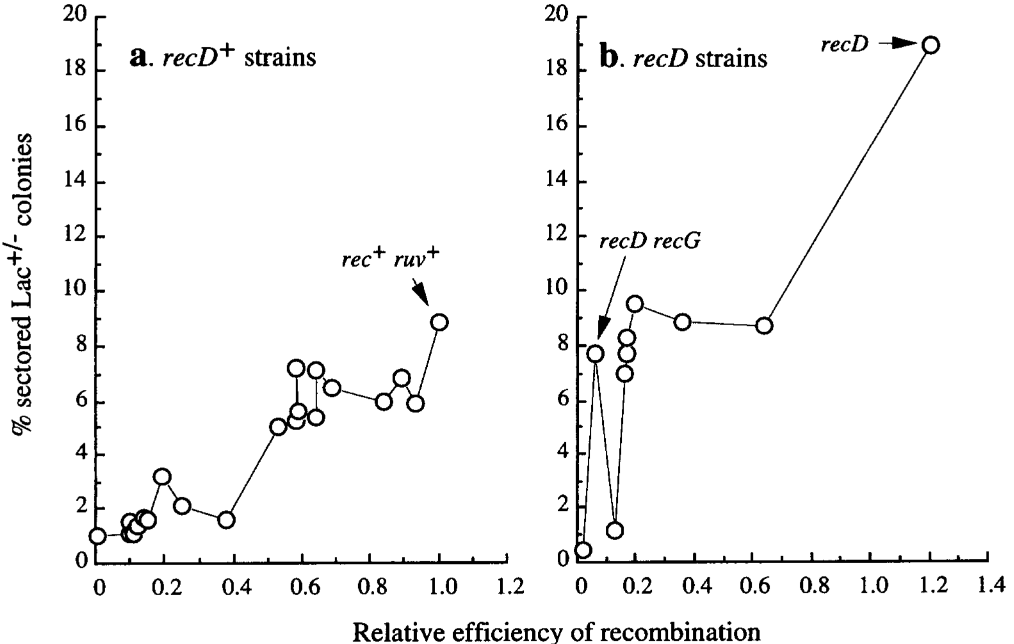

FIGURE 8.—Relationship between the frequency of sectored Lac$^{+/-}$ recombinants and the efficiency of recombination in Hfr KL226 × F$^-$ crosses selecting Leu$^+$ recombinants. The data are from Tables 7 and 8. Very similar values appear as a single point.

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the increase in the average distance from the origin to the first proximal exchange can each be explained in terms of the probability of exchange per unit length of DNA, that is, classical linkage. However, the last of these observations is at odds with models that propose RecBCD initiation at Chi. If the proximal exchange is initiated by RecBCD entering at the Hfr origin, its location should be determined by the location of Chi sequences and therefore should be largely independent of the distance to the selected marker. This would be particularly true for our crosses with Hfr KL226, which transfers DNA toward *oriC* (Figure 2A). DNA sequencing has revealed that 9 of 10 Chi sequences are oriented such that they would be recognized by RecBCD entering from the Hfr origin (BURLAND *et al.* 1993). Because Chi sequences occur every 5 kb or so, one would expect RecBCD to initiate an exchange well before reaching *proC* or *lac*, which are 165 and 209 kb, respectively, from the Hfr origin. The fact that more than half of the recombinants selected for the donor *argE* or *metE* marker (Tables 5 and 6) failed to show such an exchange is therefore surprising.

This apparent anomaly can be explained if some feature of conjugational recombination interferes with the spectrum of recombinants recovered in matings that transfer short segments of Hfr DNA but not in matings that transfer long segments. One possibility suggested by LLOYD and THOMAS (1984) is that recombination can initiate at a transient gap in the newly synthesized strand of the Hfr DNA while transfer is in progress, and the proximal and distal ends are not available for RecBCD to gain entry (Figure 1c). If much of the RecA is sequestered by this exchange, for instance by continued growth of the RecA filament in concert with the 5′-3′ transfer of the Hfr DNA, and DNA transfer terminates before RecA has been released, the exposed DNA ends may be degraded by the exoV activity of RecBCD. KUZMINOV et al. (1994) have recently shown that both RecA protein and Chi sequences in the DNA are needed to protect linear DNA from exonuclease degradation. The distal (3′) end would be particularly vulnerable in a KL226 cross as only 1 in 10 Chi sequences would be in the proper orientation to modulate the exonuclease activity of RecBCD. Consequently, the chances of recovering recombinants inheriting a marker distal to the first exchange would be very poor, especially if the exchange at a gap was initiated just before transfer of the marker. In matings where transfer continued long after the exchange at a gap was initiated, there would be more time to complete this exchange and release RecA before the ends of the Hfr DNA were released. More RecA would then be available to protect the distal end, thus favoring the recovery of recombinants in which the selected marker was coupled with a proximal exchange located some way from the Hfr origin. This would have the observed effect of reducing linkage of proximal markers with increasing distance to the selected marker.

From these studies we conclude that the model of RecBCD initiation at Chi does not apply in general to the exchanges initiated proximal to the selected marker. It may be true of the majority of matings in which the segment of Hfr DNA transferred is relatively short (up to 500 kb). However, it is not the main route for initiating the proximal exchange in matings that transfer fragments extending a fifth of the chromosome (~1000 kb) or more.

SMITH (1991) suggested that a category of recombinants comprising some 20% of the total arises from two close exchanges flanking the selected marker, which he described as "short-chunk integration." In our crosses with Hfr KL226 in which Leu$^+$ recombinants were selected, we did find that ~20–30% of the progeny inherited the selected donor *leuB* marker alone. However, the nearest unselected proximal marker in these crosses was *lacZ*, which is some 288 kb from *leuB*. When we reversed the direction of DNA transfer using Hfr AB259, only 6.7% of the recombinants obtained (with N2415) inherited the selected marker alone, in this case *proC*. The vast majority (86%) had also inherited the donor *lacZ* marker, some 44 kb proximal to *proC*. When we normalized the number of exchanges in any given interval to the length of DNA involved, we failed to detect in any of our crosses a particularly high frequency of recombination immediately proximal to the selected marker that would be consistent with the model of short-chunk integration. Instead, we found a sharp decline in the frequency of exchange with increasing distance from the Hfr origin. Short-chunk integration is therefore ambiguous. For instance, a number of the recombinants analyzed in Tables 4–6 and 11 had a single proximal exchange in intervals well away from both the selected marker and the Hfr origin. These could be regarded either as short-chunk integrations with a proximal exchange unusually far from the selected marker or as long-chunk integrations with a proximal exchange unusually far from the origin. We conclude that although some recombinants do inherit a short length of Hfr DNA, the idea that these arise from a special category of exchanges is probably misleading, except possibly in the sense that the exchanges may involve different subsets of recombination enzymes.

A second approach investigated this possibility by using recombination mutants as recipients. Most of the recombination genes identified in *E. coli* are redundant as far as Hfr crosses are concerned in the sense that they can be inactivated without causing a serious deficiency in the recovery of recombinants. The only exceptions are mutations that inactivate RecA protein, or the RecB or RecC subunits of RecBCD enzyme, which reduce recombination to 1% or less of the normal level. Why so many are redundant is not yet fully understood. Some genes may provide similar activities so that a re-

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duced yield of recombinants is observed only when mutations in these genes are combined (LLOYD *et al.* 1988; LOVETT *et al.* 1988; LLOYD 1991a; LLOYD and BUCKMAN 1991a). Another possibility is that the Hfr DNA fragment transferred to the recipient may provide a variety of locations for the initiation of recombination. If these are used by different subsets of proteins, the location of crossovers will vary with different mutant recipients. Our data support both possibilities in that all the mutations we studied reduced recombination or altered the distribution of crossovers, or both, either alone or in combination with other mutations.

The change in the location of crossovers was most marked in a *recB* mutant or with strains mutant for both *recD* and *recJ*. In both cases, recombination was reduced substantially and was correlated with a marked shift in the location of the proximal crossover away from the origin of transfer. The latter result was noted previously with a *recB* mutant (DE HAAN *et al.* 1972). These observations can be best explained by the selective reduction of a class of recombinants that have an exchange near the proximal end of the Hfr DNA fragment. They support the notion that RecBCD enzyme promotes crossing over near this end with high efficiency (CLARK 1991; SMITH 1991; TAYLOR and SMITH 1992). The fact that mutation of both *recD* and *recJ* reduces the yield of recombinants and the inheritance of an unselected proximal marker also suggests that exonuclease activity, or cutting at Chi, is necessary for this exchange. This would make sense if removal of the F DNA at the leading end, or Chi cutting within the non-F DNA, is needed to expose the homologous 3′ end favored by RecA for strand invasion. RecD is needed for the ATP-dependent exonuclease activity of RecBCD, whereas RecJ is a 5′-3′ exonuclease active on single-stranded DNA (CHAUDHURY and SMITH 1984; AMUNDSEN *et al.* 1986; LOVETT and KOLODNER 1989; TAYLOR 1988). In *recJ* strains, RecBCD enzyme would be sufficient to remove the F DNA, whereas in *recD* strains the helicase activity of RecBC, or perhaps RecQ, would expose the DNA to RecJ nuclease and also to Exonuclease I, which digests single-stranded DNA with a 3′ end (KUSHNER *et al.* 1971). Strains mutant for both *recD* and *recJ* would presumably have difficulty in generating the appropriate 3′ end, leaving recombination to occur by a less efficient exchange located further from the proximal region than is usually the case in a rec^+ strain (see below).

We previously hypothesized that the products of *recF* and other genes that can catalyze recombination independently of RecBCD enzyme may be able to initiate crossovers at a transient single-strand gap in the Hfr DNA (LLOYD and THOMAS 1984). As discussed above, exchanges initiated at gaps would be expected to produce crossovers located on average further from the origin than those initiated by RecBCD at Chi sites. Mutations that eliminate exchanges at gaps would tend therefore to favor recombinants with crossovers initiated by RecBCD in more proximal regions. We observed an increase in recombinants of this type with strains mutant for *recF*, *recO* or *recQ*. This effect was small but was observed repeatedly with several different recipients. Similar observations were reported by LANZOV *et al.* (1991), though the increase was greater than we observed. The reason for this difference is not clear. They used Hfr donors mutant for the *rec* genes tested to eliminate the possibility of making the zygotes rec^+. However, in the case of *recF*, we found that the use of such an Hfr made no difference to our crosses. Evidence that crossovers can be redirected toward RecBCD-dependent events by mutation of *recF*, *recO*, *recR* and *recQ* has also been reported by RYDER *et al.* (1994). Recent studies have shown that the RecO protein together with RecR, and possibly RecF, overcome the inhibitory effect of single-stranded DNA binding protein (SSB) on joint molecule formation by RecA (UMEZU *et al.* 1993). SSB protein would be expected to bind the single-stranded sections of the Hfr DNA remaining exposed in the recipient before completion of lagging strand synthesis. In the absence of RecF, RecO or RecR protein, RecA might initiate exchanges with more difficulty at these single-stranded regions. RecQ is a DNA helicase (UMEZU *et al.* 1990; UMEZU and NAKAYAMA 1993) and may normally help to promote recombination at gaps by unwinding adjacent duplex regions to extend strand exchange. The substantial reduction in recombinants (10% of the wild-type yield) observed when mutations in *recF*, *recO* or *recQ* were introduced into a *recN* background also provides some indication of the importance of these genes in recombination.

How often recombination initiates at a gap is difficult to assess because crossovers arising from initiation at a gap near the proximal end are not easily distinguished from those initiated by RecBCD entering the leading end of the DNA. Most of the proximal crossovers we recorded in crosses with Hfr KL226 were located ≥16 kb from the Hfr origin, with some 25–60% being beyond *proC*, ≥165 kb away. The actual number varied with the distance to the selected marker. As discussed already, RecBCD entering the leading end would be expected to have encountered an appropriately oriented Chi site much closer to the origin than *proC*. However, it is also clear that mutation of *recF*, *recO* or *recQ* eliminates only some of the crossovers distal to *proC* (Tables 3 and 7). We cannot rule out the possibility that these mutations present only a partial block to the initiation of recombination at gaps.

The mutations studied also affected the frequency of sectored recombinant colonies. Recombinants of this type are a well-documented feature of Hfr crosses, though how they arise has never been fully understood (LOTAN *et al.* 1972; MAHAJAN and DATTA 1979; BRESLER *et al.* 1981). By using plates containing X-gal, we found it particularly easy to score colonies sectored for *lacZ*.

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They comprised ~3–25% of the recombinant colonies in the crosses we conducted with *rec*$^+$ *ruv*$^+$ recipients, with the actual value being related directly to the distance between *lacZ* and the (distal) marker selected. MAHAJAN and DATTA (1979) suggested that colonies of this type result from integration of single-stranded Hfr DNA into the recipient chromosome followed by mismatch correction at the site of the selected marker but not of the unselected marker, which then segregates both donor and recipient genotypes on subsequent replication of the recombinant. Although mismatch repair operates in conjugational crosses (FEINSTEIN and LOW 1986; RAYSSIGUIER *et al.* 1989), our data suggest that the majority of sectored colonies arise when a fragment of recipient DNA excised during the integration of Hfr DNA recombines again with the recombinant chromosome as illustrated in Figure 9, a–e.

The model outlined proposes that RecA can initiate recombination in two ways. It can use a transient gap in the complementary strand of the Hfr DNA not far from the proximal end to initiate strand exchange during transfer and produce a Holliday junction that can be resolved by RuvC or an alternative resolvase (MANDAL *et al.* 1993). Resolution of the junction to a splice product will produce a linear chromosome with overlapping ends (Figure 9a(i)). If resolution occurs in the opposite orientation to produce a patch product or in the event that recombination failed to initiate at a gap, RecA would have another opportunity to initiate recombination after transfer had terminated when RecBCD enzyme enters an exposed duplex end, degrades the DNA to the first properly oriented Chi site and then proceeds to unwind the DNA, generating a 3′ single-strand end (TAYLOR and SMITH 1992; DIXON and KOWALCZYKOWSKI 1993). Because the ends of the Hfr DNA are probably inaccessible during transfer and the leading end is attached to a long segment of nonhomologous F DNA through which RecBCD will have to travel (Figure 1a), we suspect that the distal end will be engaged in an exchange first (Figure 9, a(ii)–b(ii)). Regardless of when, how or where the first exchange took place, the Hfr DNA ends will be exposed to RecBCD once mating has terminated. A second exchange initiated at an end by RecBCD will then integrate the Hfr DNA and displace the homologous section of the recipient chromosome (Figure 9c).

The probability of integrating the Hfr DNA with these first two exchanges will depend on whether the Holliday junctions are resolved to patch or splice products. If the proximal exchange produces a patch, RecBCD will be needed to reengage the end of the DNA to try again, especially if gaps have been closed. Repeated failures to produce the required splice product will foreshorten the proximal end of the Hfr DNA and reduce the chances of inheriting markers in this region in a manner that would reflect the incidence of Chi sites. Similar events will occur at the distal end but may not be noticed in genetic crosses because a crossover distal to the selected marker is obligatory for the recovery of recombinants.

Any excised linear fragment of recipient DNA is probably exposed to attack by exonucleases. However, if RecBCD enzyme enters the duplex ends, Chi and RecA will limit degradation (DABERT *et al.* 1992; KUZMINOV *et al.* 1994) and encourage further exchanges, this time with the recombinant chromosome, to modify the outcome of the initial exchanges. Assuming that the excised DNA is degraded from both ends, these modifications will be focused toward the middle of the DNA fragment transferred originally, though they may be distributed asymmetrically if RecBCD is responsible for most of the degradation of linear DNA and Chi sites are encountered with different frequencies from the two ends. Depending on the location of the available markers, a pair of additional exchanges will tend to produce more recombinants that inherit the selected marker alone or generate recombinants showing quadruple exchanges. Once the initial recombinant chromosome has been formed, it will be free to replicate. A pair of additional exchanges between the excised fragment and one of the two (or more) daughter chromosomes before division of the transconjugant cell will generate two types of recombinant chromosomes. Segregation of these two recombinant chromosomes during subsequent division will give rise to a colony that is sectored for one or more unselected markers (Figure 9, c–e). This model makes several predictions. (1) The frequency of sectored colonies should be correlated inversely with the distance between the selected marker and the unselected marker scored. (2) Recombinants sectored for one unselected marker should show a high frequency of cosectoring for a second marker located nearby. (3) The frequency of sectored colonies should increase when the half-life of linear DNA fragments in transconjugants is increased by reducing exonuclease activity. (4) The frequency of sectored colonies should be correlated with the efficiency of recombination. (5) The frequency of multiple exchanges should increase and the linkage of unselected proximal markers should decrease, with increasing distance from the Hfr origin to the selected marker. Our data support each of these predictions. The increased proportion of sectored recombinants in the absence of RecBCD exonuclease activity has also been seen in *recBC sbcBC* strains (LLOYD 1991b).

We have assumed that the recombination is initially conservative in that two products are generated from two substrates, though DNA ends are degraded and one product (the excised linear fragment) is ultimately removed. However, our experimental results with *recD* strains lead us to suggest that some exchanges may be nonconservative. Recombinational exchanges of this type have been described previously (TAKAHASHI *et al.* 1992; KOWALCZYKOWSKI *et al.* 1994). When mutations

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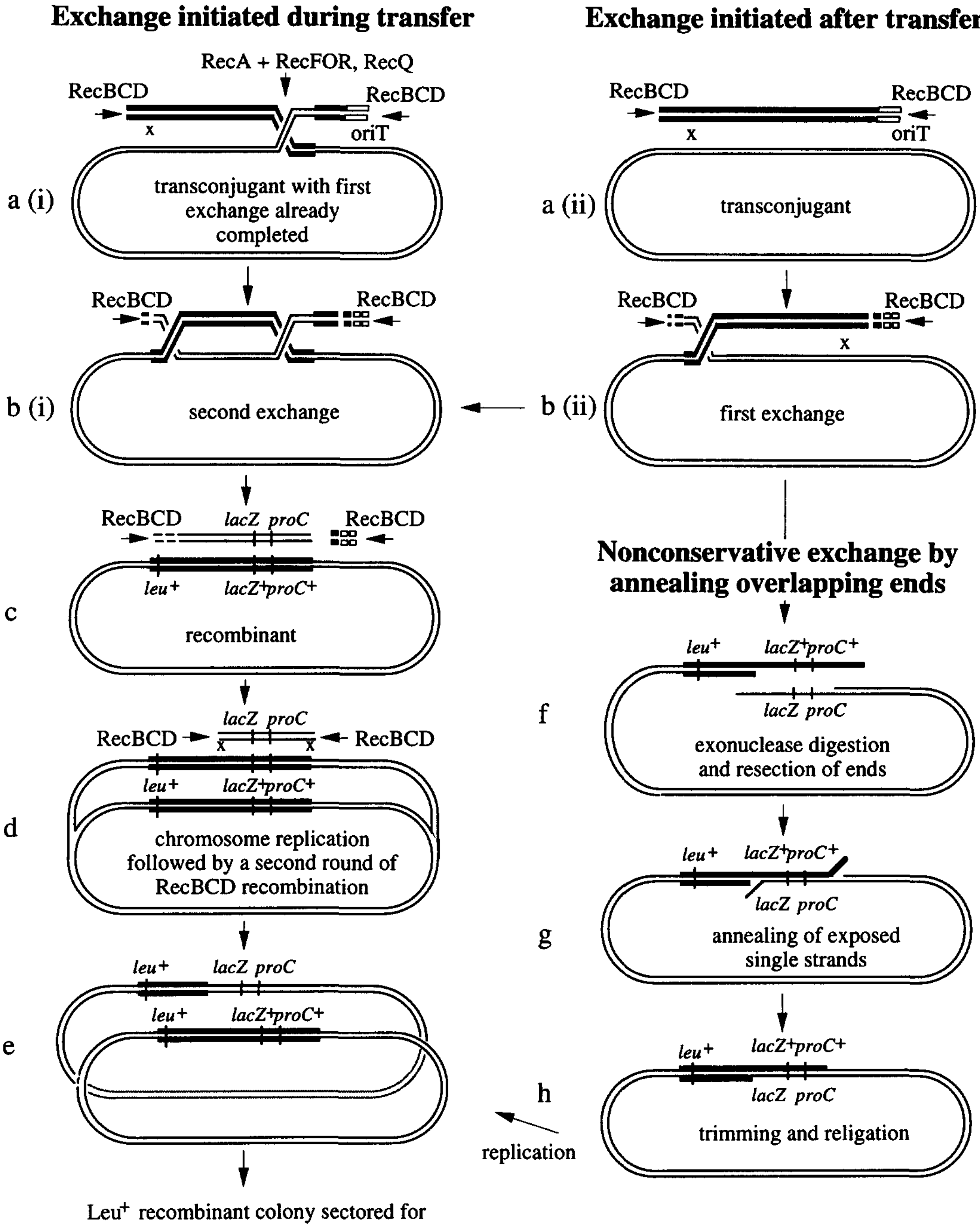

FIGURE 9.—Formation of Leu$^+$ recombinant colonies sectored for *lacZ* in Hfr KL226 × F$^-$ crosses by two cycles of DNA integration (a–e) or by formation of an extensive heteroduplex splice (f–h). The open rectangles labeled *oriT* represent the F DNA at the leading end of the transferred fragment of the Hfr chromosome. The Hfr fragment in a(ii) would have a heteroduplex section if it came via an exchange initiated during transfer (a(i)) that was resolved to a patch product. All duplex DNA ends in a–c are shown being attacked by RecBCD whether or not an exchange has already taken place. Details of the exchanges at each stage and the sequence of events are described in the text.

in *recG* or *recJ* were introduced into the *recD* background, the frequency of sectored colonies remained surprisingly high given the substantial deficiency in recombination, which is inconsistent with the model outlined in Figure 9, a–e. The reduced recovery of recombinants could not be attributed to reduced cell viability. The alternative is that the recombinants recovered arise via exchanges that are more prone to generating sectored colonies. A possible mechanism is outlined in Figure 9, f–h, where we assume the obligatory exchange distal to the selected marker has already taken place, leaving a molecule with overlapping ends. As already discussed, strains mutant for both *recD* and *recJ* may have difficulty in generating the appropriate 3′ end for RecA to initiate recombination. However, the exposed duplex ends may be unwound by the helicase activity of RecBC and

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or RecQ (MASTERSON *et al.* 1992; KORANGY and JULIN 1993; UMEZU and NAKAYAMA 1993), resected by Exonuclease I and annealed by RecA. Trimming of the ends and religation produces a recombinant with an extensive heteroduplex located some distance from the original ends of the DNA (Figure 9f). We imagine that the heteroduplex made this way is longer than the heteroduplex splice generated through resolution of a Holliday junction formed by an exchange initiated near the origin and therefore has a greater chance of extending across *lacZ*. Extensive splices could also explain the *recD recG* result, though we do not understand why in this case recombination should have to involve end annealing. Perhaps in this case branch migration of Holliday junctions generates longer heteroduplexes. There is evidence that RecG drives branch migration in the reverse direction to that initiated by RecA. In the absence of RecG, RuvAB may drive branch migration more freely (WHITBY *et al.* 1993; RYDER *et al.* 1994).

RecN protein appears to have a particular role in recombination that helps to promote the formation of sectored colonies. Because mutation of *recN* essentially eliminates sectored colonies in all the strains we examined, including the *recD recJ* background where it is also needed to promote recombination, we suspect that it may serve to protect the single-stranded DNA needed to initiate strand exchange or to anneal DNA ends. In the absence of RecN, DNA ends may be subjected to more rapid exonuclease digestion, thereby reducing the opportunity for the excised DNA to initiate further exchanges with the recombinant chromosome. The fact that *recN* is SOS inducible and DNA degradation is reduced in SOS-induced cells is consistent with this view, as is the fact that RecN is needed to regenerate high molecular weight DNA after radiation-induced double-strand breaks (PICKSLEY *et al.* 1984a,b; ROSTAS *et al.* 1987).

To conclude, we believe that recombinant chromosomes are formed initially in conjugational crosses by a pair of exchanges that integrates a single segment of duplex Hfr DNA into the recipient chromosome flanked by two splice joints. Further exchanges involving the excised recipient DNA can modify the initial product to generate the spectrum of recombinants normally observed in Hfr crosses. However, alternative models limiting recombination to a single pair of exchanges at the two ends of the Hfr DNA fragment that lead to replication forks (SMITH 1991) cannot be excluded. However, the high frequency of recombinants showing multiple exchanges in certain of the crosses reported here and in published data raises questions about how common such events would be.

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APPENDIX

Effect of *rec*, *ruv* and *mut* alleles on the production and Lac phenotype of Leu$^+$ or Leu$^+$ Ara$^+$ recombinant colonies in Hfr KL226 × F$^-$ crosses

Recipient strain	Genotype tested	No. of Expts	No. of colonies analyzed	Lac phenotype of selected colonies (% of total)			Relative viability of recipient cells	Relative yield of recombinants
				Lac$^-$	Lac$^+$	Mixed Lac$^{+/-}$		
A								
N2415	*rec$^+$ ruv$^+$ mut$^+$*	36	37,374	25.7 ± 0.7	65.2 ± 0.9	9.1 ± 0.3	1.0	1.0
N2420	*recB*	4	3,456	64.2 ± 2.2	34.8 ± 2.0	1.0 ± 0.36	0.32 ± 0.082	0.006 ± 0.0002
N2516	*recD*	10	11,381	25.9 ± 0.6	55.1 ± 1.1	19.0 ± 1.0	0.98 ± 0.14	1.22 ± 0.08
N2417	*recF*	8	7,283	22.1 ± 1.0	72.5 ± 1.2	5.4 ± 0.69	0.88 ± 0.13	0.64 ± 0.06
N2466	*recO*	8	6,762	18.8 ± 1.2	76.0 ± 1.8	5.2 ± 0.92	0.93 ± 0.14	0.58 ± 0.06
N3343	*recQ*	4	4,734	16.6 ± 1.1	76.6 ± 1.5	6.8 ± 0.66	0.89 ± 0.053	0.89 ± 0.088
N3345	*recF recQ*	3	2,346	17.7 ± 0.6	75.8 ± 0.9	6.5 ± 0.50	0.92 ± 0.018	0.69 ± 0.044
N3346	*recO recQ*	2	2,097	16.5 ± 0.9	76.4 ± 0.5	7.1 ± 0.45	0.76 ± 0.03	0.64 ± 0.035
N2517	*recD recF*	4	3,103	25.9 ± 0.4	65.2 ± 1.6	8.9 ± 1.6	1.11 ± 0.01	0.36 ± 0.02
N2518	*recD recO*	4	4,476	25.9 ± 2.0	65.4 ± 1.6	8.7 ± 1.1	0.76 ± 0.03	0.64 ± 0.1
N2468	*recJ*	8	10,011	27.8 ± 1.8	66.3 ± 2.3	5.9 ± 0.68	0.91 ± 0.097	0.93 ± 0.07
N2521	*recJ recF*	2	2,094	26.4 ± 1.0	66.5 ± 0.3	7.2 ± 0.65	0.86 ± 0.14	0.58 ± 0.07
N2523	*recJ recO*	2	2,284	22.0 ± 1.8	72.4 ± 1.7	5.6 ± 0.15	0.86 ± 0.15	0.59 ± 0.02
N2535	*recJ recF recO*	5	4,833	22.7 ± 1.4	72.3 ± 2.3	5.0 ± 0.10	0.95 ± 0.043	0.53 ± 0.06
N3349	*recJ recQ*	2	2,274	23.0 ± 0.8	71.0 ± 0.8	6.0 ± 0.05	0.87 ± 0.1	0.84 ± 0.015
N2519	*recJ recD*	4	2,308	42.8 ± 1.3	47.7 ± 1.4	9.5 ± 0.35	0.81 ± 0.06	0.20 ± 0.02
N2589	*recJ recD recF*	2	1,652	43.4 ± 1.3	49.6 ± 0.2	7.0 ± 1.0	0.58 ± 0.11	0.16 ± 0.03
N2588	*recJ recD recO*	2	1,506	41.3 ± 3.5	51.0 ± 3.3	7.7 ± 0.14	0.45 ± 0.09	0.17 ± 0.009
N2590	*recJ recD recF recO*	4	2,949	41.7 ± 1.6	50.0 ± 1.7	8.3 ± 0.16	0.55 ± 0.12	0.17 ± 0.02
N2488	*ruvA*	6	3,131	24.8 ± 1.8	72.0 ± 1.9	3.2 ± 0.56	0.75 ± 0.19	0.19 ± 0.03
N3755	*ruvC*	4	2,923	25.4 ± 1.5	72.9 ± 1.6	1.7 ± 0.20	0.31 ± 0.04	0.14 ± 0.01
N3756	*ΔruvAC*	2	2,488	24.4 ± 2.0	73.5 ± 1.9	2.1 ± 0.05	0.37 ± 0.13	0.25 ± 0.035
N2971	*recG*	5	4,230	28.8 ± 3.4	68.5 ± 4.0	2.7 ± 0.61	0.56 ± 0.05	0.11 ± 0.017
N2965	*recG recD*	4	2,014	33.5 ± 1.1	58.8 ± 2.1	7.7 ± 1.1	0.49 ± 0.037	0.061 ± 0.005
N3348	*recG recQ*	3	3,067	21.6 ± 1.7	76.4 ± 2.1	2.0 ± 0.40	0.73 ± 0.07	0.15 ± 0.027
N2477	*recN*	16	17,421	29.0 ± 1.2	69.6 ± 1.2	1.4 ± 0.15	0.81 ± 0.062	0.38 ± 0.04
N2485	*recN recF*	4	5,835	22.5 ± 2.7	76.4 ± 2.7	1.1 ± 0.05	0.88 ± 0.076	0.11 ± 0.02
N2498	*recN recF recO*	4	7,667	18.8 ± 3.3	80.1 ± 3.2	1.1 ± 0.07	0.92 ± 0.19	0.11 ± 0.01
N3347	*recN recQ*	5	2,278	27.4 ± 1.2	71.6 ± 1.1	1.0 ± 0.25	0.87 ± 0.08	0.10 ± 0.009
N2967	*recG recN*	4	1,391	37.6 ± 3.6	61.3 ± 3.4	1.1 ± 0.22	0.38 ± 0.05	0.10 ± 0.022
N2520	*recN recD*	4	2,514	31.8 ± 1.1	67.0 ± 1.2	1.2 ± 0.18	1.02 ± 0.22	0.13 ± 0.05
N2908	*recN recJ*	2	2,816	30.3 ± 1.4	68.3 ± 1.5	1.4 ± 0.1	0.75 ± 0.19	0.12 ± 0.005
N2914	*recN recJ recD*	5	1,232	40.6 ± 2.4	59.0 ± 2.3	0.42 ± 0.16	0.25 ± 0.029	0.02 ± 0.0006
B								
N2419	*rec$^+$ ruv$^+$ mut$^+$*	11	9,686	23.0 ± 1.2	68.2 ± 1.1	8.8 ± 0.32	1	1
N2602	*mutH*	6	4,800	25.0 ± 1.1	69.9 ± 1.3	5.1 ± 0.28	1.02[a]	0.93 ± 0.11[b]
N2605	*mutS*	6	2,959	23.3 ± 2.0	71.6 ± 2.2	5.1 ± 0.45	0.87[a]	0.56 ± 0.09[b]
N2936	*mutY*	3	2,149	22.5 ± 1.1	69.1 ± 1.3	8.4 ± 0.29	1.1 ± 0.15	0.97 ± 0.10
N2604	*recN mutH*	3	3,562	25.0 ± 2.5	73.4 ± 2.3	1.6 ± 0.23	ND	0.38 ± 0.03[b]
N2606	*recN mutS*	3	3,784	25.0 ± 1.3	73.7 ± 1.2	1.3 ± 0.09	ND	0.35 ± 0.07[b]
N2607	*recN recF mutS*	6	5,326	21.5 ± 0.7	77.4 ± 0.9	1.1 ± 0.22	ND	0.23 ± 0.03[b]
N2940	*recN mutY*	3	708	23.5 ± 1.5	74.3 ± 1.6	2.1 ± 0.24	0.72 ± 0.17	0.36 ± 0.11

Mating was with Hfr KL226 for 60 min before interuption. Recombinants were recovered on selective minimal agar supplemented with X-gal to score the Lac phenotype. Yields of recombinants, with their standard errors, are relative to the control strain and have been corrected for any deficiency in the viability of the recipient cells except as indicated. In A, selection was for Leu$^+$ (*rpsL*) recombinants and the average yield for the control strain N2415 was 4.7×10^6 per ml of the mating mixture. In B, selection was for Leu$^+$ Ara$^+$ (*rpsL*) and the average yield for the control strain N2419 was 2.9×10^6 per ml of the mating mixture. Viability is given relative to the number of colony forming units per ml in the cultures of the control recipient strain, which averaged 2.2×10^8 for N2415 and 1.8×10^8 for N2419. ND, not determined.

[a] Viability was measured in one experiment only.

[b] No correction has been made for viability.

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