Recombination-Dependent Growth in Exonuclease-Depleted *recBC sbcBC* Strains of *Escherichia coli* K-12

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ABSTRACT

Analysis of the *aroLM-sbcCD* interval of the *Escherichia coli* K-12 chromosome revealed a new gene (*rdgC*) encoding a function required for growth in recombination-deficient *recBC sbcBC* strains. Deletion of *rdgC* does not reduce viability, conjugational recombination, or DNA repair in *rec*$^+$, *recA*, *recB*, *recF*, or *recJ* mutants. However, it makes the growth of *recBC sbcBC* strains reliant on the RecA, RecF, and RuvC proteins and, to a large extent, on RuvAB. The *recBC sbcBC* Δ*rdgC ruvAB* construct forms colonies, but cell viability is reduced to <5%. A *recBC sbcBC* Δ*rdgC* derivative carrying the temperature-sensitive *recA200* allele grows at 32° but not 42°. Multicopy *rdgC*$^+$ plasmids reduce the growth rate of *recBC sbcBC* strains, while multicopy *sbcC*$^+$ plasmids that reactivate SbcCD nuclease cannot be maintained without RdgC protein. The data presented are interpreted to suggest that exonuclease-depleted *recBC sbcBC* strains have difficulty removing the displaced arm of a collapsed replication fork and that this problem is compounded in the absence of RdgC. Recombination then becomes necessary to repair the fork and allow chromosome duplication to be completed. The possibility that RdgC is an exonuclease is discussed.

THE genome of an organism has to be duplicated once per cell cycle and accurately so as to preserve genetic integrity. Errors are made, and these fuel evolution, but they are kept in check by repair systems that detect and eliminate damage. Recombination is vital, as evident from the reduced viability of mutants defective in this process. Exchanges between homologous sequences provide the replication machinery with a means to bypass lesions in DNA and to repair double-chain breaks. Loss of viability is especially acute in *recB* or *recC* null mutants of *Escherichia coli* (CAPALDO-KIMBALL and BARBOUR 1971). These strains lack RecBCD enzyme, the product of the *recB*, *recC* and *recD* genes. RecBCD is a potent DNA helicase and exonuclease (ExoV) that unwinds DNA from a duplex end and simultaneously degrades the single strands (TAYLOR 1988). Its ExoV activity is modulated by asymmetric octanucleotide sequences called Chi at which RecD is inactivated or eliminated, leaving RecBC(D) to unwind the DNA without further degradation. The most obvious consequence is to convert RecBCD to a recombinase capable of initiating genetic exchange since the single-strand tails generated can recruit RecA to promote homologous pairing (DIXON *et al.* 1994; KUZMINOV *et al.* 1994; MYERS *et al.* 1995).

Chi sequences are more common in the *E. coli* chromosome than would be predicted by chance. They are also distributed nonrandomly. The majority (90%) are in the orientation recognized by RecBCD if it were to travel along the DNA in the opposite direction to the replisome (BURLAND *et al.* 1993). This polarity, together with the reduced viability of *recB(C)* mutants, has been taken as evidence that RecBCD-Chi interactions play a vital role in the repair of collapsed replication forks (KUZMINOV 1995). When a fork encounters a single-strand interruption in the DNA template and collapses, RecBCD is proposed to gain access to the displaced duplex end and degrade the newly replicated DNA back toward the origin (*oriC*) until a Chi converts it to a recombinase. The single strands exposed by RecBC(D) helicase attract RecA and invade the intact sister duplex to set up a Holliday junction linked to a forked-structure that can be used to prime both leading- and lagging-strand synthesis (ASAI *et al.* 1994). All that is needed to complete repair is for a resolvase like RuvC to cleave the junction, leaving the chromosome with a new replication fork located upstream of where the original collapsed (KUZMINOV 1995).

A cell lacking RecBCD will have two problems. Without the recombinase it will be unable to repair the fork and resume replication. Without ExoV, it will also have difficulty removing the displaced arm, which means a new round of replication initiated from *oriC* is unlikely to yield viable chromosomes. This double jeopardy probably explains why *recB(C)* strains are less viable than *recA* mutants and cannot tolerate additional genetic defects that increase the probability of fork collapse (KUZMINOV 1995).

The poor viability of *recB* and *recC* strains is suppressed in derivatives with additional mutations in both *sbcB* and *sbcC* or *sbcD*, as are most other aspects of the *recBC* mutant phenotype. The *sbcB* mutation alone im-

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proves recombination and DNA repair, but viability remains a problem, so much so that cultures of *recBC sbcB* strains accumulate fast-growing, fully recombination-proficient variants mutant for *sbcC* or *sbcD* (LLOYD and BUCKMAN 1985; GIBSON *et al.* 1992). These two genes form an operon and encode polypeptides of 118 and 45 kDa, respectively (NAOM *et al.* 1989; GIBSON *et al.* 1992). The SbcC and SbcD proteins copurify and together provide an ATP-dependent dsDNA exonuclease activity and an ATP-independent ssDNA endonuclease activity that can incise DNA near the apex of hairpin structures (CONNELLY and LEACH 1996; D. R. L. LEACH, personal communication). SbcCD activity *in vivo* interferes with recombination in *recBC sbcB* strains and also with the replication of palindromic DNA (LLOYD and BUCKMAN 1985; CHALKER *et al.* 1988; GIBSON *et al.* 1992). CONNELLY and LEACH (1996) have suggested that it may attack hairpins formed through replication slippage at palindromes, or other repeated sequences, and thus force collapse of replication forks. By collapsing a fork deliberately, SbcCD provides another chance to replicate the repeat. Since slippage is rare, this second attempt is likely to proceed without error. The net effect would be to reduce deletion or duplication triggered by repeated sequences (LEACH 1994). However, as with ExoV, the dsDNA exonuclease activity of SbcCD would be expected to destroy potential substrates for recombination that could be used to restore collapsed forks. The absence of this nuclease and the reduced probability of fork collapse may explain why *sbcC(D)* mutations improve both recombination and viability in *recBC sbcB* strains.

Recombination in *recBC sbcBC(D)* strains is mediated by RecA and an array of other proteins, many of which are dispensable in the wild type (KOWALCZYKOWSKI *et al.* 1994). Some of these other proteins are likely to generate single-stranded DNA for RecA. RecQ and RecJ are the obvious candidates to act at DNA ends since the helicase and 5′–3′ single-stranded DNA exonuclease activities, respectively, of these two proteins would generate duplex molecules with 3′ single-strand tails to recruit RecA and prime DNA replication (LOVETT and KOLODNER 1989; UMEZU and NAKAYAMA 1993). The fact that *sbcB* encodes Exonuclease I (KUSHNER *et al.* 1971), which digests single-strands from 3′ ends, may explain why RecQ and RecJ are unable to promote recombination in *recBC* mutants.

Because *recBC sbcBC* strains are as viable as the wild type, we assume the recombination made possible by *sbcB* mutations and the loss of SbcCD must provide an efficient means of repairing collapsed replication forks. In this paper we describe a new gene located close to *sbcCD*, mutation of which causes the growth of *recBC sbcBC* strains to become especially vulnerable to SbcCD nuclease and dependent on functions necessary for recombination. The data reported also complete the genomic sequence between *aroLM* and *sbcCD*.

MATERIALS AND METHODS

Strains and plasmids: *E. coli* K-12 strains are listed in Table 1. pJP77 carries a >20-kb insert of *E. coli* DNA extending from *proC* to *phoBR* cloned in pACYC184 (Figure 1) (LLOYD and BUCKMAN 1985; NAOM *et al.* 1989). pBL121 carries a 10.2-kb *Eco*RI insert that spans *araJ* to *proC*, while pBL118 carries a 4.3-kb *Eco*RI-*Bam*HI subfragment of this region, both cloned in pBR322 (LLOYD and BUCKMAN 1985; NAOM *et al.* 1989). pIN507 and pMJ101 are pJP77 derivatives with Tn*1000* ($\gamma\delta$) insertions in *sbcC* and *rdgC*, respectively (Figure 1) (GIBSON *et al.* 1992). pLR110/111, pLR117/118, pLR122/123, pGS792/793, pGS794/795, and pGS805/806 carry inserts from pBL118 or pBL121 cloned in pGEM-7Zf (+)/(−), as shown in Figure 1. pLR124 and pLR125 are derivatives of pLR110 carrying 3- and 4-bp insertions, respectively, at the *Nhe*I site within *rdgC* (Figure 2 and RESULTS). pGS830 carries a 1.3-kb *Bam*HI-*Cla*I fragment inserted into pACYC184. pGS831 is a *Bam*HI-*Hpa*I deletion of pGS830. The *Bam*HI end was filled in using Klenow and dNTPs before ligation. pFG101 carries *sbcC*$^+$ fused to the *tac* promoter of pKK223-3 (GIBSON *et al.* 1992). pDL761 is an SbcCD overproducing plasmid provided by D. R. L. LEACH. It carries the *sbcDC* operon under control of the *trc* promoter in p*Trc*99A, a derivative of pKK223-2 (Pharmacia). Phage DRL130 is a λ derivative carrying a 571-bp DNA palindrome (CHALKER *et al.* 1988).

Media and general methods: LB broth and 56/2 salts media were as described (LLOYD *et al.* 1974). They were supplemented with 50 μg/ml ampicillin (Ap), 25 μg/ml chloramphenicol (Cm), 25 μg/ml kanamycin (Km), 20 μg/ml of tetracycline (Tc), and 100 μg/ml streptomycin (Str), as required. Methods for transduction with phage P1*vir* and for measuring growth in liquid culture, recombination in Hfr crosses, and sensitivity to UV light and mitomycin C have been cited (LLOYD and BUCKMAN 1985). Growth rate and viability were measured in LB media, using at least two independent cultures. The growth curves shown in Figures 6 and 7 are from representative single experiments, with strains grown in parallel. Total cell counts were determined by phase contrast microscopy. DNA analysis used the recipes and protocols described (SAMBROOK *et al.* 1989). Tn*1000* mutagenesis of pJP77 was achieved by conjugational mobilization of the plasmid from F-prime strain NH4104 to the F$^-$ recipient, JC7623, as described (LLOYD and BUCKMAN 1985).

DNA sequencing: Nucleotide sequencing was by the dideoxy chain-termination method, using T7 sequencing kits from Pharmacia and ssDNA from pGEM-7Zf clones in strain JM101 infected with M13 KO7 helper phage. Synthetic oligonucleotides (18-mers) were used as primers to complete gaps and provide overlaps. An overlap at the *Bam*HI site downstream of *rdgC* was obtained by sequencing a 430-bp *Pvu*II-*Eco*RV fragment spanning this region. The sequence of the 2642-bp *Nru*I fragment shown in Figure 2 was determined on both strands.

Disruption of *rdgC*: A 1.2-kb *Sma*I fragment carrying the kanamycin-resistance gene of pUC4-KIXX (Pharmacia) was inserted into pBL118 cut with *Hpa*I. The resulting plasmid, pLR112, has a 231-bp internal region of *rdgC* deleted and replaced with 1.2-kb *kan* insertion (Figure 1). pLR112 was digested with *Eco*RI and *Bam*HI and transformed into strain N2525, selecting for kanamycin resistance as described (RUSSELL *et al.* 1989). Km^r, Ap^s transformants were purified, and one of these was used as a donor to transduce the kanamycin-resistance marker to AB1157 to give strain LR274 (Δ*rdgC*::*kan*). Three-factor transductional crosses confirmed the location of the Km^r insertion between *proC* and *sbcC*.

Labeling plasmid-encoded proteins: Plasmid-encoded proteins were labeled with [^{35}S] methionine (Amersham) using the maxicell technique as described (GIBSON *et al.* 1992). Proteins were separated on 12.5% SDS-PAGE and visualized by

TABLE 1

***E. coli* K-12 strains**

Strain	Relevant genotype	Source of reference
W3110	*IN (rrnD-rrnE)1*	BACHMANN (1987)
AB1157	*rec*$^+$ *sbc*$^+$ *lex*$^+$ *ruv*$^+$ *rdgC*$^+$	BACHMANN (1987)
JC7623	*recB21 recC22 sbcB15 sbcC201*	KUSHNER *et al.* (1971)
JC8679	*recB21 recC22 sbcA23*	A. J. CLARK
JC12334	*tna-300*::Tn*10 recF143*	A. J. CLARK
CS81	*ruvB52 eda-51*::Tn*10*	SHARPLES *et al.* (1990)
CS85	*ruvC53 eda-51*	SHARPLES *et al.* (1990)
CS86	*ruvB54 eda-51*	SHARPLES *et al.* (1990)
SP256	*recN262 tyrA16*::Tn*10*	PICKSLEY *et al.* (1984)
LR218	*recD1009* Δ*rdgC*::*kan*	pLR112 × N2525 to Km^r Ap^s
LR258	*sbcC201* Δ*rdgC phoR79*::Tn*10*	P1.LR218 × N2364 to Km^r
LR263	*recBC sbcBC* Δ*rdgC phoR79*	P1.LR258 × JC7623 to Km^r
LR274	Δ*rdgC*	P1.LR218 × AB1157 to Km^r
N1627	*srl-1300*::Tn*10 recA200*	LLOYD *et al.* (1987)
N1642	*lexA3 malE*::Tn*10*	LLOYD *et al.* (1987)
N2057	*ruvA60*::Tn*10*	SHARPLES *et al.* (1990)
N2232	*recBC sbcBC lexA3 malE*::Tn*10*	LLOYD *et al.* (1987)
N2271	*recBC sbcBC thr*$^+$ *leu*$^+$ *ara*$^+$ *proA*$^+$ *proC29*	LLOYD and BUCKMAN (1985)
N2309	*recB21 recC22 sbcB15 phoR79*	LLOYD and BUCKMAN (1985)
N2364	*sbcC201 phoR79*	GIBSON *et al.* (1992)
N2525	*recD1009*	LLOYD *et al.* (1988)
N3072	*recA269*::Tn*10 IN(rnD-rrnE)1*	LLOYD *et al.* (1987)
N3865	*recBC sbcBC* Δ*rdgC*	P1.LR263 × JC7623 to Km^r
N3885	*recBC sbcBC* Δ*rdgC srl-1300 recA200*	P1.N1627 × N3865 to Tc^r
N3886	*recBC sbcBC* Δ*rdgC srl-1300*	P1.N1627 × N3865 to Tc^r
N3997	*recBC sbcBC recN262 tyrA16*	P1.SP256 × JC7623 to Tc^r
N3999	*recBC sbcBC* Δ*rdgC recN262 tyrA16*	P1.SP256 × N3865 to Tc^r
N4003	*recBC sbcBC* Δ*rdgC lexA3 malE*	P1.N1642 × N3865 to Tc^r
N4004	*recBC sbcBC* Δ*rdgC ruvA60*	P1.N2057 × N3865 to Tc^r
N4005	*recBC sbcBC ruvA60*	P1.N2057 × JC7623 to Tc^r
N4006	*recBC sbcB* Δ*rdgC*	P1.LR274 × JC7623 to Km^r
N4028	*recB21 recC22 sbcA23* Δ*rdgC*	P1.LR274 × JC8679 to Km^r
N4029	*recB21 recC22 sbcA23* Δ*rdgC sbcC201*	P1.N3865 × JC8679 to Km^r
NH4104	F′ (F42) *lac*$^+$	SHARPLES *et al.* (1990)
JM101	F′ (F128) *proAB*$^+$ *lacI*^{q} *ZΔM15*	YANISCH-PERRON *et al.* (1985)

Strains AB1157 through LR274, except N3072, are closely related and, except as shown, are also 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.* After strain JC7623, *recB21 recC22 sbcB15 sbcC201* is abbreviated to *recBC sbcBC.* Alleles marked by Tn*10* or *kan* insertions are shown in full only on the first listing.

fluorography. ^{14}C-labeled molecular weight markers were from Amersham.

RESULTS

Tn*1000* mutagenesis of the *sbcC*$^+$ plasmid pJP77: The *sbcDC* operon is located in the *proC-phoR* interval of the chromosome cloned in pJP77 (Figure 1) (LLOYD and BUCKMAN 1985; NAOM *et al.* 1989; GIBSON *et al.* 1992). This plasmid makes a *recBC sbcBC* strain grow very slowly, as might be expected from restoration of *sbcC*$^+$ and the known low viability of *recBC sbcB* strains. LLOYD and BUCKMAN (1985) predicted that inactivation of the plasmid *sbcC*$^+$ allele would improve growth and identified Tn*1000* insertions that had this effect. However, although the insertions were clustered within a short section of the insert, they were not in *sbcC* (NAOM *et al.* 1989). We reexamined this paradox by mobilizing pJP77 to the *recBC sbcBC* recipient, JC7623. Most (~90%) of the Cm^r (Str^r) transconjugants formed tiny colonies just visible after 24 hr at 37°. The remainder formed much larger colonies visible after 12 hr. Plasmid DNA was extracted from several large colonies selected in independent crosses and the Tn*1000* insertions were located. All fell within a 1-kb region between *aroLM* and *sbcCD*, ~3 kb downstream from *sbcC* (Figure 1). This finding confirms the observation made by LLOYD and BUCKMAN (1985).

Molecular analysis of the *aroLM-sbcCD* region: The *aroLM-sbcCD* region must contain a locus other than *sbcC*$^+$ that is detrimental to the growth of *recBC sbcBC* strains when present in multiple copies. The DNA sequence of the region tagged by the Tn*1000* insertions was determined (Figure 2). It overlaps at the 5′ end with *araJ* (REEDER and SCHLEIF 1991) and at the 3′ end with *aroLM* (DEFEYTER *et al.* 1986). Three of the Tn*1000*

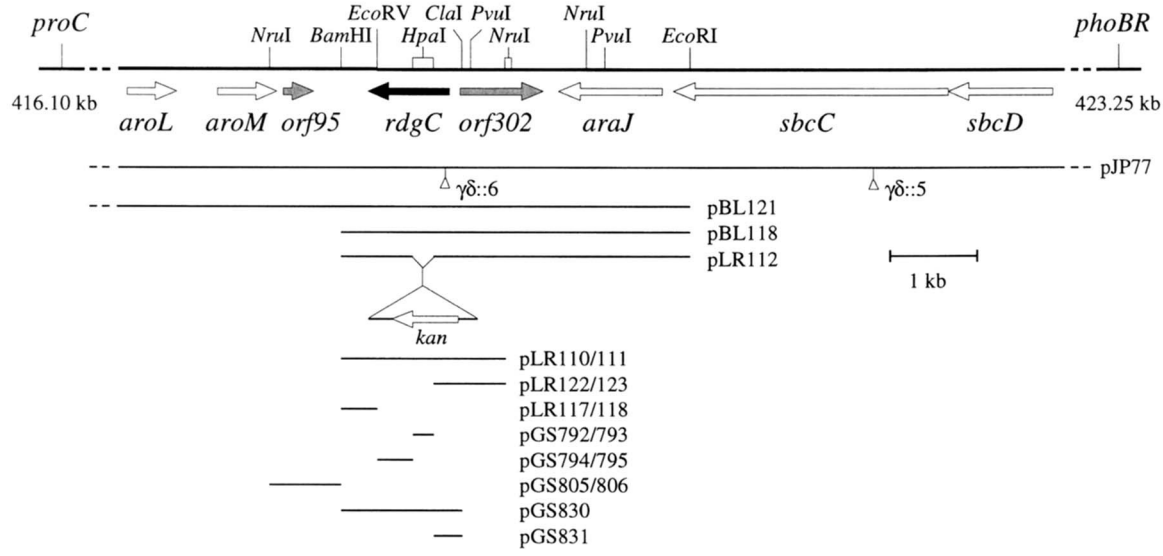


FIGURE 1.—Molecular organization of the *aroLM-sbcCD* region and plasmid construction. The chromosomal region is shown from *proC* to *phoBR*, between coordinates 416.10–423.25 kb (8.9–9.1 min) on the physical map (KOHARA *et al.* 1987; MÉDIGUE *et al.* 1991). The flanking regions are abbreviated for reasons of scale. The *Pvu*I restriction sites correspond to those identified by Reeder and Schleif (1991). Only restriction enzymes used for cloning are labeled. DNA inserts in the plasmids identified are aligned with the restriction map. Plasmid constructions are detailed in MATERIALS AND METHODS. Tn*1000* insertions in pJP77 are indicated by open triangles; the *rdgC* insertion (γδ::6) is in pMJ101, and the *sbcC* insertion (γδ::5) is in pIN507. An additional *orf* of 63 amino acids located between *aroL*, and *aroM* is not shown on the map (DEFEYTER *et al.* 1986).

insertions were located by sequencing. All interrupt an open reading frame (*orf*) of 303 amino acids (1042–1950) that could produce a 33,997-Da protein (Figure 2, γδ insertions 2, 6 and 7). For reasons that will become clear, *orf303* is named *rdgC*, for *r*ecombination-*d*ependent *g*rowth, after the convention established by CLYMAN and CUNNINGHAM (1987).

The *rdgC* gene is flanked 5′ by the divergently transcribed *orf302* and 3′ by the convergently transcribed *orf95*. Immediately 3′ is a REP sequence (1969-2010) and a possible Rho-independent terminator (2029-2058). The insertions are therefore unlikely to have a polar effect, from which we conclude that *rdgC* is most probably responsible for the negative effect of pJP77 on growth.

Translation of *rdgC* is assumed to initiate with the ATG at bp 1042. There is a possible ribosome-binding site upstream. The ATG codon at bp 1168 is a feasible alternative but would generate a product much smaller than is observed (see below). The putative 34-kDa RdgC product of initiation at bp 1042 would be expected to have an isoelectric point of 5.2. It is homologous to the product of an *orf* of 302 amino acids (HI0306) in the genome of *Haemophilus influenzae* (FLEISCHMANN *et al.* 1995). The two putative proteins show 52% identity and 64% similarity over their entire lengths (Figure 3A). The method of LUPAS *et al.* (1991) predicts a region of alpha-helical, coiled-coil common to both (residues 81–112 of *E. coli* RdgC). This particular region contains several conserved basic amino acids (Figure 3A).

The divergently transcribed *orf302* gene corresponds to the incomplete *orf260* identified previously and shown to be similar to *xylR* from *Bacillus subtilis* (REEDER and SCHLEIF 1991). Searches with the complete gene revealed better matches to HI0182 from *H. influenzae* (40% identity) and to Glk (glucose kinase) from *Streptomyces coelicolor* (28% identity) (ANGELL *et al.* 1992; FLEISCHMANN *et al.* 1995). Orf302 is even more related (63% identity) to a partial *orf* upstream of the *pmi* gene of *Rhizobium meliloti* (data not shown) (SCHMIDT *et al.* 1992). The high degree of similarity with Glk suggests Orf302 protein is also a sugar kinase. A number of sugar kinases and transcriptional repressors of sugar catabolite operons have been grouped together as the ROK family (TITGEMEYER *et al.* 1994). Orf302 contains most of the residues characteristic of the family, though neither Orf302 nor its close homologues have the N-terminal DNA-binding helix-turn-helix motif present in several members.

orf95 is immediately downstream of *aroM* and has potential transcriptional and translational start signals. It shares 69% identity with an *orf* located immediately 3′ of the *aroL* gene of *Erwinia chrysanthemi* (Figure 3B) (MINTON *et al.* 1989). The DNA structure of the two organisms is therefore similar in this region, although *aroM* is absent from Erwinia. A region of possible secondary structure overlapping the proposed −10 and ribosome-binding site of *orf95* may be a signal for protein recognition, or may terminate transcription from *aroLM* (Figure 2).

The intergenic region between the divergently transcribed *orf302* and *rdgC* contains several possible RNA polymerase binding sites and sequences that may regulate transcription (Figure 2). Two direct repeats (5′-GCATGATAA-3′) form part of two putative AraC half-sites identified at positions 1008-989 and 1047-1028.

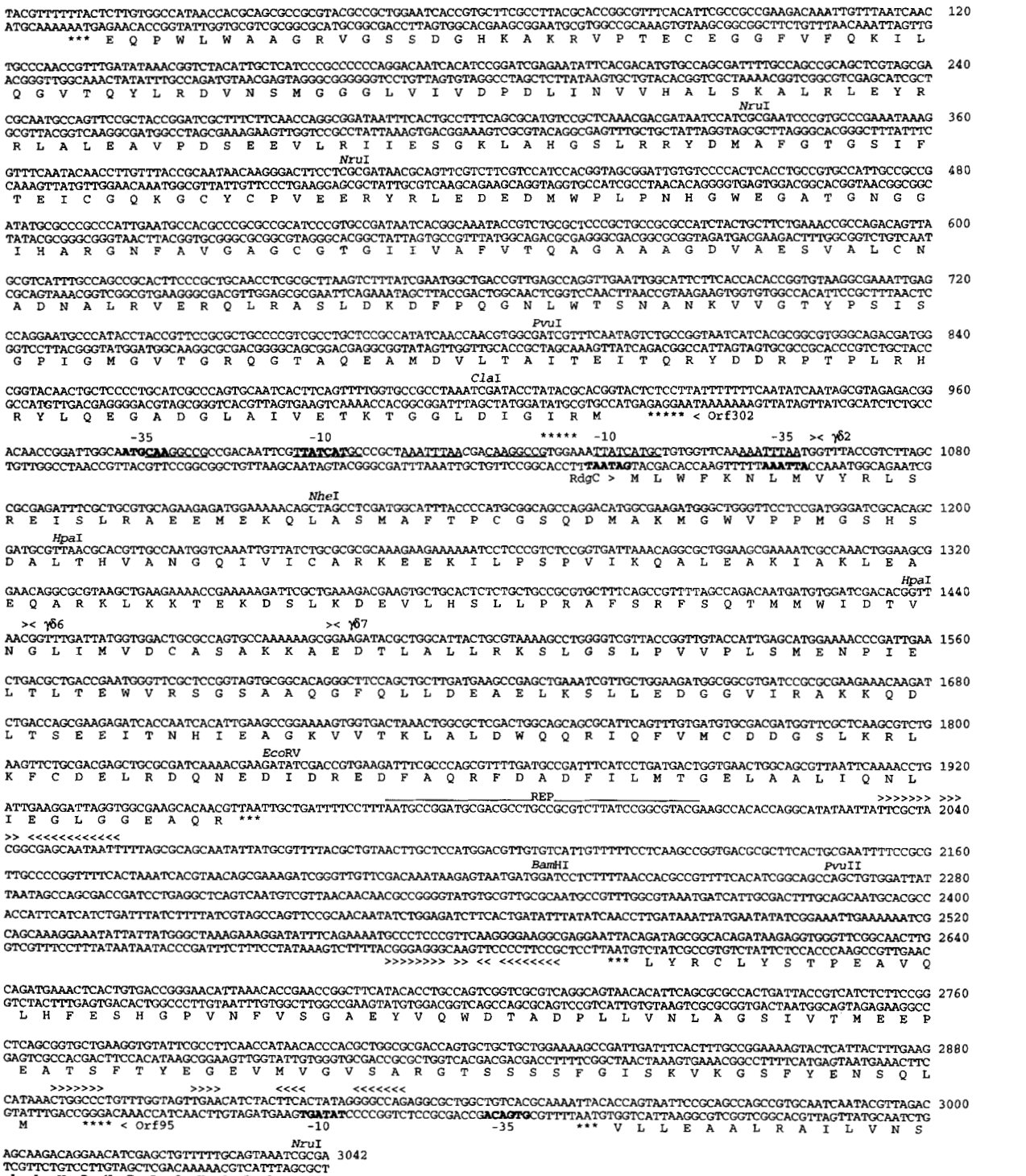


FIGURE 2.—Nucleotide sequence of the *rdgC* region. The 2640-bp *Nru*I (403) to *Nru*I (3042) was determined in this work. The first 408 bp of the sequence is taken from that determined by REEDER and SCHLEIF (1991) and is included to show the complete Orf302 open reading frame (previously called Orf260). Both strands of the sequence are shown for *orf*s translated in the other orientation. Restriction enzyme sites used in cloning are shown. Ribosome-binding sites are indicated by an asterisk. Putative promoter regions are boldface and labeled −35 and −10, chevrons indicate possible inverted repeats, and ><, Tn*1000* insertions γδ2, 6 and 7 in pJP77. A single REP sequence is overlined. Three sets of direct repeats of the sequences CAAGGCCG, TTATCATGC, and AAATTTAA are underlined. The entire nucleotide sequence can be found in the EMBL/Genbank data library under the accession number X76979.

They are both very similar to the known AraC binding site for the adjacent *araJ* gene. However, the two half-sites are not correctly positioned to provide binding sites for AraC and are therefore probably nonfunctional (CARRA and SCHLEIF 1993). The promoter region also contains another two sets of direct repeats. These se-

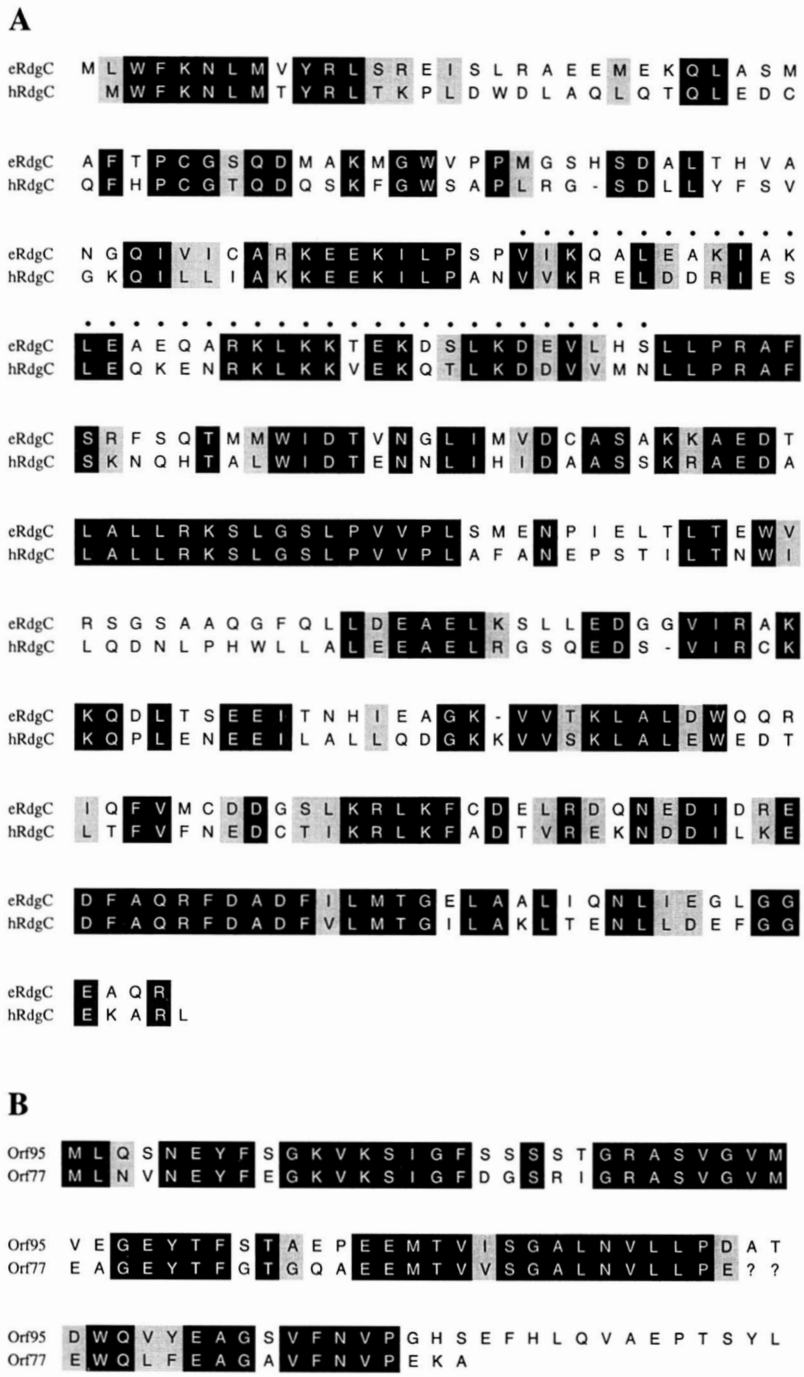


FIGURE 3.—Alignments. (A) Alignment of *E. coli* (e) and *H. influenzae* (h) RdgC. The proteins are aligned over their entire lengths. Gray shading is used to identify functionally similar amino acids (A and G; D and E; K and R; Q and N; S and T; F, Y and W; I, V, L and M). Residues predicted to form a coiled-coil structure are identified (●). (B) Alignment of *E. coli* Orf95 with Orf77 from the *E. chrysanthemi aroL* region (Genbank accession number A14577). The incomplete *orf77* nucleotide sequence has a frameshift between bp 2780 and 2785. The amino acids encoded by this region are therefore unknown.

quences are 5′-CAAGGCCG-3′ (979-986, 1023-1030) and 5′-AAATTTAA-3′ (1012-1019, 1056-63). Each set of direct repeats is separated by 25 bases (Figure 2). They may be protein binding sites, though they do not match sequences bound by known regulatory proteins.

Identification of RdgC: pLR110 and pGS830, in which only *rdgC* is intact, strongly express a protein of 33 kDa that is absent in the vector controls (Figure 4, lane b, and data not shown). Its mobility matches the 34-kDa product predicted by the sequence of *rdgC*. pLR112, which carries a *kan* insert in *rdgC*, does not produce a protein of this size but gives two products of ~30 and 6 kDa (Figure 4, lane c). The 6-kDa protein is probably an N-terminal fragment of RdgC. Translation of *rdgC* up to the *Hpa*I/*kan* junction would be expected to generate a protein of 6295 Da. The 30-kDa polypeptide is most likely a fusion product from sequences in the *kan* insert joined to the sequences remaining at the 3′ end of *rdgC*.

To confirm the identity of the selected start codon for *rdgC*, pLR110 was cut with *Nhe*I (Figure 2), end-filled, and religated. If the first start codon (bp 1042) is used, then a frameshift should occur in the *rdgC* reading frame, whereas the product would be unaffected if translation was initiated at the second AUG (bp 1168). On sequencing of the ligated products, two mutant plasmids were found. One (pLR125) carries a 3-bp insertion resulting in a valine insertion, and the other (pLR124)

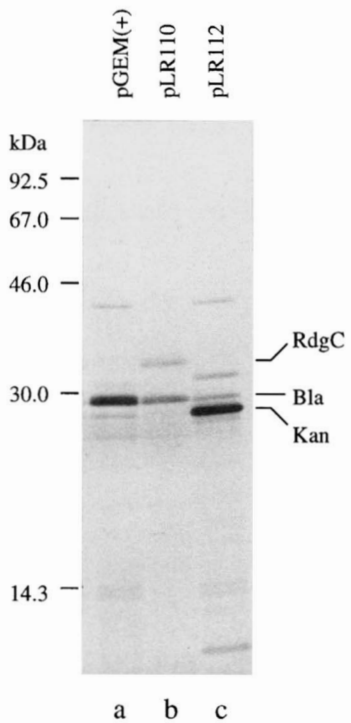


FIGURE 4.—Autoradiograph showing [^{35}S]-labeled plasmid-encoded proteins separated on a 12.5% SDS-polyacrylamide gel. Lane a, pGEM-7Zf (+); lane b, pLR110 (*rdgC*$^+$); lane c, pLR112 (Δ*rdgC*::*kan*). Molecular weight markers and protein products are labeled.

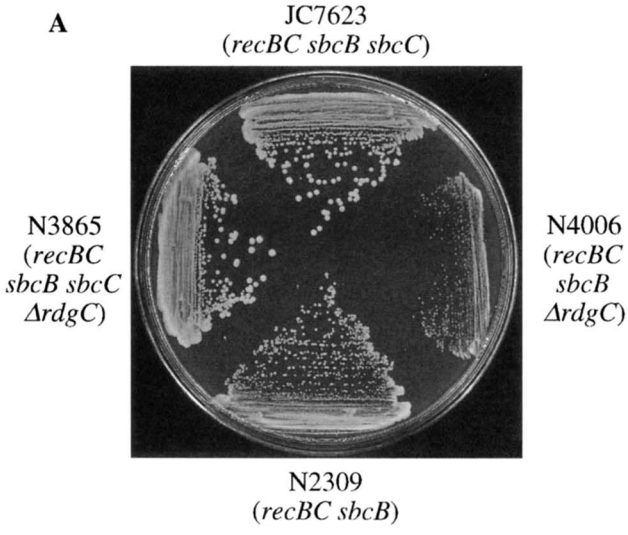
a 4-bp insertion that gives a frameshift. pLR124 fails to produce the 33-kDa protein found in pLR110 but does express a truncated product (data not shown). This 10-kDa species matches the polypeptide predicted if translation initiated at the start codon identified in Figure 2 and proceeded to a stop codon at bp 1281 after the frameshift. pLR125 gives a product of similar size to that from pLR110, consistent with a single amino acid insertion. These data support the choice of start codon for *rdgC*.

Disruption of the chromosomal *rdgC* locus and analysis of the mutant phenotype: A chromosomal disruption of *rdgC* was made by replacing an internal *Hpa*I fragment with a 1.2-kb *kan* insert. The Δ*rdgC* single mutant, LR274, proved indistinguishable from its *rdgC*$^+$ parent with respect to growth rate, sensitivity to mitomycin C, UV light and γ-radiation, the frequency of recombinant formation in conjugational and transductional crosses, and its failure to propagate a phage, λDRL130, with a long palindrome in its DNA (data not shown). The *rdgC* deletion was also introduced into a *recBC sbcBC* background. Strain N2271 (*recBC sbcB sbcC proC*) was transduced to Pro$^+$ with P1 phage from either W3110 (*rdgC*$^+$ *sbcC*$^+$) or LR218 (Δ*rdgC*::*kan sbcC*$^+$). Both *rdgC* and *sbcC* are linked to *proC* and were expected to segregate in these crosses. With P1 from W3110, ~50% of the transductants formed small, MC^s UV^s colonies, which is the phenotype expected from inheritance of the donor *sbcC*$^+$ allele (LLOYD and BUCKMAN 1985). A similar result was obtained with P1 from strain LR218, but we were also able to monitor *rdgC* by scoring resistance to kanamycin. All of the small, MC^s UV^s (*sbcC*$^+$) transductants were Km^r, as would be expected from the gene order (Figure 1). Evidently, Δ*rdgC* does not help *sbcB15* to suppress *recBC*.

From the latter cross and similar crosses with other *recBC sbcBC* strains, we isolated both Δ*rdgC recBC sbcB sbcC* and Δ*rdgC recBC sbcB* genotypes. The former proved quite healthy and, compared with the corresponding *rdgC*$^+$ control, did not show any obvious differences in growth rate, cell morphology, sensitivity to UV light or γ-irradiation, or conjugational recombination. It proved more sensitive to mitomycin C, but the effect was modest (data not shown). The latter formed smaller colonies on LB agar than was expected from its *recBC sbcB* genotype (Figure 5A), as if the inactivation of *rdgC* had made the cells exceptionally vulnerable to SbcCD nuclease. Cells of this genotype grown in liquid medium were rapidly overtaken by fast-growing *sbcC(D)* mutants (Figure 5B), much more rapidly than happens with *recBC sbcB* strains (data not shown). The unusually strong selection for these mutants confirms that SbcCD is particularly harmful to viability.

Growth of *recBC sbcBC rdgC* strains depends on recombination: We introduced *recA269*::Tn*10* into the Δ*rdgC recBC sbcBC* strain N3865 by transduction from strain N3072. However, no Tc^r colonies were recovered, whereas a good yield was obtained within 24 hr in a parallel cross with the *rdgC*$^+$ recipient, JC7623. Tiny colonies were visible after 5 days incubation, but these could not be subcultured. A similar problem was encountered when *recF143* was introduced from strain JC12334 by cotransduction with the very closely linked *tna*::Tn*10* marker. Ninety-five percent of the Tc^r transductants took 3 days to form colonies just visible to the naked eye. These were presumed to be *recF143* since the remaining 5% were UV^r. They proved very difficult to subculture by streaking on LB agar. A very poor yield of exceedingly slow-growing colonies was obtained. A few faster-growing variants emerged from some of the streaks, but these were resistant to mitomycin C and UV light, and presumably had either reverted to *recF*$^+$ or had acquired suppressors of *recF143*.

To confirm that RecA is needed to support growth, the temperature-sensitive *recA200* allele (LLOYD *et al.* 1974) was introduced from strain N1627 by cotransduction with *srl*::Tn*10*. The majority (22/28 tested) of the Tc^r transductants selected at 32° were unable to form colonies when restreaked at 42°. Backcrosses confirmed the presence of *recA200* in these clones. They grow rather slowly at 32°, which is to be expected given the reduced activity of RecA200 at this temperature. Doubling time in broth was 78 min, compared with 53 min for the *recA*$^+$ control (Figure 6). Growth slowed dramatically after a shift to 42°, and only one doubling in cell density was observed over the next 5 hr. The cells also became smaller than those incubated at 32°. However,



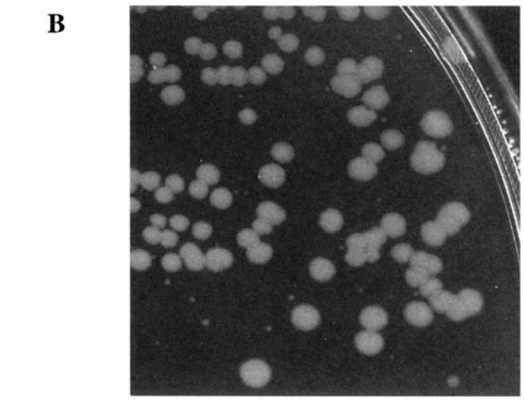


FIGURE 5.—Effect of Δ*rdgC* and *sbcC* on growth of *recBC sbcB* strains. (A) Colony size on LB agar. The agar plate shown was streaked with an inoculum from colonies of the same age for each strain identified, incubated for 20 hr, and photographed. (B) Accumulation of fast-growing *sbcC(D)* variants during subculture of the Δ*rdgC recBC sbcB* strain, N4006. A small colony of N4006 was picked into LB broth and incubated at 37° until growth was visible by eye, at which stage a sample was streaked on LB agar, incubated for 20 hr, and photographed. Variants forming large colonies are clearly visible. Cultures grown to saturation are completely overtaken by these variants.

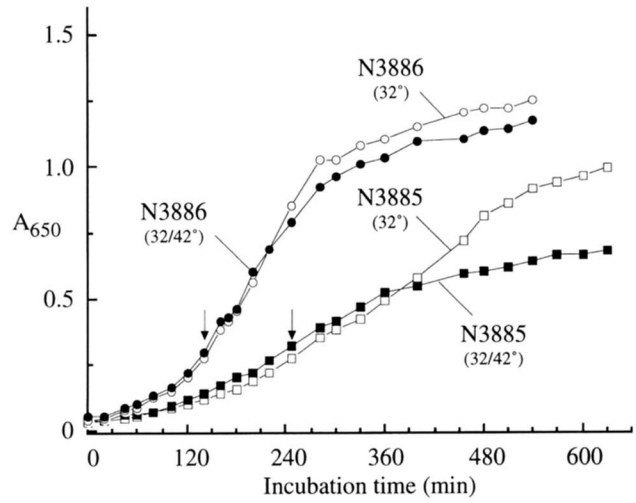


FIGURE 6.—Effect of temperature on growth of *recA200 recBC sbcBC rdgC* strains in liquid culture. Strains N3885 (*recBC sbcBC* Δ*rdgC recA200*) and N3886 (*recBC sbcBC* Δ*rdgC recA*$^+$) were grown in duplicate in LB broth at 32°. One culture of each strain (closed symbols) was transferred to 42° once the A_{650} reached 0.3 (denoted by arrows). Doubling times at 30° were 78 min for N3885 and 53 min for N3886.

they remained viable in that they could be rescued at 32°. We conclude that *recA* and *recF* are needed for growth of a *recBC sbcBC* Δ*rdgC* strain.

The very low viability of the *recA* and *recF* derivatives of strain N3865 could reflect an inability either to carry out recombination or to induce the SOS response (WALKER 1984). To distinguish between these possibilities, we introduced the SOS-noninducible *lexA3* allele into N3865 by cotransduction with *malE*::Tn*10* from strain N1642. None of the Tc^r colonies visible on the selection plates after 24 hr had the expected UV^s MC^s phenotype, whereas in a parallel cross with strain JC7623, 29% (40/136 tested) were of this type. After 4 days at 37°, a few tiny colonies appeared. In two separate crosses, they accounted for 4.5% (13/286) of the transductants detected after 8 days incubation. They could be subcultured on LB agar but took 48 hr or more to form colonies visible to the naked eye.

One putative *lexA3* construct, strain N4003, was studied in greater detail. Its doubling time in broth was 66 min (Table 2), which is surprisingly swift considering how long it takes cells to form colonies on plates. The reason for this paradox became clear when we found only one cell in 40 could form a colony. This reduced viability was associated with an increase in the number of elongated cells and short filaments compared with the *lexA3 rdgC*$^+$ control, N2232, though the majority of the cells were still quite small, as is typical of *lexA3* cells because of the inability to induce the SulA division inhibitor (WALKER 1984). Strain N4003 is very sensitive to UV light and mitomycin C (data not shown). However, saturated cultures contain rare variants able to form colonies ~0.5 mm in diameter within 24 hr on plates supplemented with 0.2 μg/ml mitomycin C. Phage P1 lysates could be grown on these variants, and they were used to transduce *malE*::Tn*10* to strain AB1157. In two of three cases tested, 45 and 60% of the Tc^r transductant selected proved very sensitive to UV. This confirms that N4003 carries *lexA3,* from which we conclude that increased expression of at least one LexA-regulated gene is necessary for normal growth.

Two loci, *recN* and *ruvAB,* regulated by LexA (PICKSLEY *et al.* 1984; SHINAGAWA *et al.* 1988) were investigated for their effect on the growth of strain N3865. Both specify activities linked with recombination. The role of RecN is not known, but RuvAB has been shown to drive branch migration of Holliday junctions and to act with RuvC protein to catalyze their resolution (MANDAL *et al.* 1993; KOWALCZYKOWSKI *et al.* 1994). We had no difficulty introducing a *recN* mutation into N3865. The

TABLE 2

Effect of *rdgC*, *lexA*, *recN*, and *ruvA* on growth and viability of a *recBC sbcBC* strain

Strain	Genotype (*recBC sbcB sbcC*)	Total cells/ml at A_{650} 0.4[a] ($\times 10^8$)	Doubling time (min)[b]	Viability[c]	Cell morphology[d]
JC7623		1.94	44	0.71	90% normal, 10% elongated
N3865	Δ*rdgC*	1.68	45	0.73	80% normal, 20% elongated
N2232	*lexA3*	3.13	50	0.6	98% short cells, 2% filaments
N4003	Δ*rdgC lexA3*	2.07	66	0.023	70% short cells, 20% elongated, 10% short filaments
N3997	*recN262*	1.49	49	0.63	90% normal, 10% elongated
N3999	Δ*rdgC recN262*	1.04	63	0.27	10% normal, 30% elongated, 60% filaments
N4005	*ruvA60*	1.42	50	0.48	40% normal, 40% elongated, 20% filaments
N4004	Δ*rdgC ruvA60*	1.11	69	0.034	30% normal, 70% long filaments

Cultures were grown to saturation in LB broth at 37°, diluted ~50-fold in fresh medium and incubated with vigorous aeration. Growth was monitored by following absorbance at 650 nm (A_{650}). The values are means based on measurements in two independent cultures, both of which gave very similar results.

[a] Number of cells/filaments visualized by phase contrast microscopy.

[b] As determined by following the increase in A_{650}.

[c] Fraction of total cell forms at A_{650} of 0.4 able to form colonies on agar plates.

[d] Normal = 1–2 cell bacilli, slightly longer than the average for the *rec*$^+$ *sbc*$^+$ ancestor, AB1157; short = mainly 1–2 cell coccobacilli; elongated = cells 3–10 × normal length; filaments = cell >10 ×, often >25 × normal cell length.

construct grew slowly compared with the *rdgC*$^+$ control and showed a tendency to form snake-like filaments (Table 2). Viability was reduced, but not as much as in the *lexA3* (Δ*rdgC*) strain. RuvAB was inactivated by introducing *ruvA60*::Tn*10* from strain N2057. In this case, the construct (strain N4004) behaved much like the *lexA3* derivative described above (Table 2). Viability was marginally better, but it still took 48 hr for cells to form colonies on LB agar. Cell filamentation was also much more pronounced than in the *rdgC*$^+$ control, as expected from the *lexA*$^+$ genotype and the consequent ability to induce SulA.

Because RuvC depends on RuvAB *in vivo* (MANDAL *et al.* 1993), *ruvA60* cells could have difficulty resolving Holliday junctions. We therefore attempted to introduce *ruvC53* into N3865 by cotransduction with *eda-51*::Tn*10* from strain CS85. No *ruvC53* transductants were recovered. Out of 253 Tc^r colonies tested, none had the expected UV^s MC^s phenotype, whereas 67% (44/66 tested) of those from a control cross with the *rdgC*$^+$ strain, JC7623, were of this type. Incubation of the selection plates for 8 days failed to reveal any slow-growing transductants of N3865. Similar crosses were conducted to introduce *ruvB* alleles from strains CS81 and CS86. In these two cases, 38.5% (25/72) and 42.2%, respectively, of the Tc^r (*eda-51*) colonies inherited *ruvB*. As with *ruvA60*, the *ruvB* constructs had very low viability and required 48 hr to form visible colonies on LB agar. The apparent linkage of *ruvB* to *eda* was lower than in crosses with the *rdgC*$^+$ strain, JC7623, but not by very much. We assume therefore that *ruvC53* can be introduced to N3865 by linkage to *eda* but reduces viability below the level needed to form a colony. Since *ruvC* is not regulated by LexA (TAKAHAGI *et al.* 1991), we conclude that the viability of a *recBC sbcBC* Δ*rdgC* strain depends on recombination.

Recombination-dependent growth is restricted to *recBC sbcBC* strains: We had no difficulty making *recA*, *recB*, *recF*, *recJ*, *ruvC*, or *sbcC* derivatives of the Δ*rdgC* single mutant, LR274. The double mutants proved indistinguishable from *rdgC*$^+$ controls in growth rate, cell viability, sensitivity to the DNA-damaging agents mitomycin C, UV light, and γ-radiation, and in their capacity for conjugational recombination (data not shown). *recB sbcC* Δ*rdgC* and *ruvC sbcC* Δ*rdgC* strains were also made. Only 10–20% of the total cells present in cultures of these constructs grown to an A_{650} of 0.4 were able to form colonies. However, this is typical of *recB* and *ruvC* single mutants. We also made Δ*rdgC* (N4028) and Δ*rdgC sbcC201* (N4029) derivatives of the *recBC sbcA* strain, JC8679. These grew just as well as the parent strain. Furthermore, we had no trouble introducing *recA269* or *recF143* into either construct. The recombination-deficient derivatives grew just as well as their *rdgC*$^+$ *sbcC*$^+$ counterparts in liquid culture (data not shown). The fraction of cells able to form colonies was between 5 and 10% of the total in different cultures, a reduction of only two- to threefold compared with the *recBC sbcA recA* and *recBC sbcA recF* controls. We conclude that the recombination-dependent growth caused by Δ*rdgC* is restricted to the *recBC sbcBC* background.

Effect of increased RdgC on viability of *recBC sbcBC* strains: By mobilizing the *sbcC*$^+$*D*$^+$ *rdgC*$^+$ construct, pJP77, to an *sbcC* single mutant, NAOM *et al.* (1989) succeeded in identifying insertions in the plasmid *sbcC* gene, leaving *rdgC* and the upstream *sbcD* gene intact. We made use of one of these constructs, pIN507, together with pJP77 and its *rdgC*::Tn*1000* derivative,

TABLE 3

Effect of *rdgC*$^+$ and *sbcC*$^+$ plasmids on growth and viability of *recBC sbcBC* strains

Plasmid (relevant genotype)	Transformant colonies[a]			
	JC7623 (*recBC sbcBC*)		LR263 (*recBC sbcBC* Δ*rdgC*)	
	Relative no.	Size	Relative no.	Size
pACYC184 constructs				
pACYC184	0.57 ± 0.13	0.5–1	0.57 ± 0.27	0.5–1
pJP77 (*sbcC*$^+$ *rdgC*$^+$)	0.14 ± 0.01	<0.25	0.12 ± 0.03	<0.25
pIN507 (*sbcC*::Tn*1000 rdgC*$^+$)	<0.00006		<0.00006	
pMJ101 (*sbcC*$^+$ *rdgC*::Tn*1000*)	0.12 ± 0.04	0.25–0.5	<0.00007	
pGS830 (*rdgC*$^+$)	0.41 ± 0.06	<0.25		
pGS831 (Δ*rdgC*)	0.56 ± 0.14	0.5–1		
pBR322 constructs				
pBR322	0.30 ± 0.20	0.5–1		
pBL118 (*rdgC*$^+$)	0.09 ± 0.04	<0.25		
pBL121 (*rdgC*$^+$)	0.06 ± 0.03	<0.25		
pGEM constructs				
pGEM-7Zf	0.17	0.5–1		
pLR110 (*rdgC*$^+$)	0.06 ± 0.04	<0.25		
pLR124 (*rdgC* frameshift)	0.30	0.25–0.5		
pKK223-2 constructs				
pKK223-2	0.46 ± 0.02	0.5–1	0.26 ± 0.02	0.5–1
pFG101 (*sbcC*$^+$)	0.55 ± 0.12	<0.25	<0.0002[b]	
pDL761 (*sbcC*$^+$ *sbcD*$^+$)	0.05 ± 0.005	<0.25	<0.0002[b]	

[a] Mean (±SE) number of transformants relative to the yield with strain AB1157. Selection plates were scored after 17 hr at 37°. Colony sizes are in mm. All plasmids transformed with high efficiency into AB1157 and the colonies selected were uniformly 1–2 mm in diameter.

[b] Small colonies containing *sbcC* mutant plasmids were visible after several days incubation.

pMJ101, to compare the effect of increased RdgC on growth in the presence and absence of SbcCD nuclease. These constructs were transformed into the *recBC sbcBC* strain, JC7623, and the relative yields of Cm^r transformants and colony size were used as indicators of growth and viability. The *rdgC* mutant (pMJ101) gave much larger colonies than pJP77, as expected from its original isolation (Table 3). This difference was due to growth rate rather than any lag in recovery of the transformed cells (Figure 7). However, the transformants obtained with the *rdgC* mutant (pMJ101) did not grow as well as those carrying the vector, pACYC184. Presumably, its *sbcC*$^+$ allele restores SbcCD activity, thereby reducing viability. No Cm^r colonies could be recovered with *sbcC* mutant (pIN507), which is expected to increase RdgC without restoring SbcCD nuclease (Table 3). We had no difficulty recovering transformants with the *rec*$^+$ *sbc*$^+$ strain, AB1157, nor with its SbcCD deficient derivative, N2364, and colony size was the same as the vector control.

The inviability of *recBC sbcBC* cells transformed with the *rdgC*$^+$ *sbcC* construct, pIN507, explains our earlier failure to recover Tn*1000* insertions in *sbcC* following mobilization of pJP77 into JC7623. It suggests that amplification of RdgC is lethal in the absence of SbcCD. However, it also highlights a rather puzzling contradiction. pJP77 must be maintained in *recBC sbcBC* strains, despite the increase in RdgC, through its ability to restore SbcCD activity. How can this be if SbcCD itself is detrimental to growth? To try and answer this question, we studied the effects of RdgC and SbcCD individually.

To eliminate SbcCD, strain JC7623 was transformed with the *rdgC*$^+$ constructs, pBL118 and pBL121, both of which lack the *sbcCD* region of pJP77, and also with pLR110 and pGS830, in which only *rdgC* is intact (Figure 1). As with pJP77, yields of transformants were reduced compared with vector controls, and the colonies were very small. Yields were better and colonies much larger with constructs (pLR124 and pGS831) deleted for *rdgC* (Table 3). We conclude that overexpression of RdgC inhibits the growth of a *recBC sbcBC* strain.

To eliminate RdgC, we used strain LR263 (*recBC sbcBC* Δ*rdgC*). In this case, both the *rdgC*$^+$ *sbcC* (pIN507) and *rdgC sbcC*$^+$ (pMJ101) constructs failed to give transformants. Identical findings were obtained with the *recBC sbcBC* Δ*rdgC* strain, N3865, and also with different preparations of plasmid DNA. pMJ101 could be transformed into the Δ*rdgC* single mutant, LR274, and the Cm^r colonies obtained were of the same size as those produced by the pACYC184 vector.

The failure to recover pMJ101 transformants of LR263 and N3865 confirms that restoration of SbcCD activity is particularly harmful in the absence of RdgC, as was suggested by our earlier discovery of the reduced viability of plasmid-free *recBC sbcB* Δ*rdgC* strains (Figure 5). To eliminate any possible effect of other factors

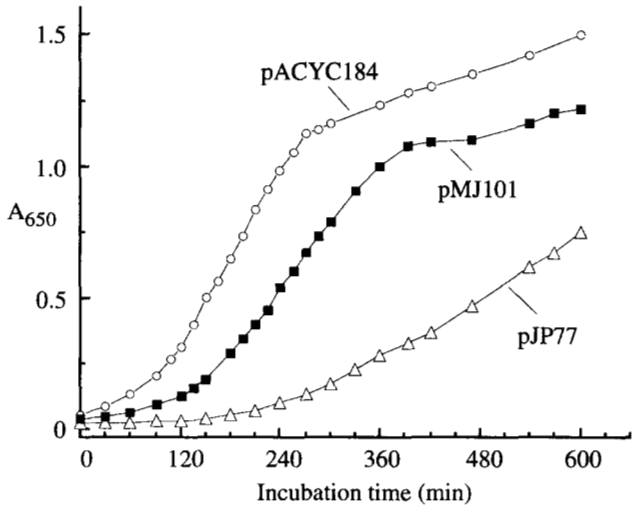


FIGURE 7.—Growth of *recBC sbcBC* strains carrying multicopy *sbcC*$^+$ and *rdgC*$^+$ *sbcC*$^+$ plasmids. pACYC184, pJP77 (*rdgC*$^+$ *sbcC*$^+$) and pMJ101 (*rdgC*::$\gamma\delta$ *sbcC*$^+$) were transformed into strain JC7623, and Cm^r colonies were inoculated into LB broth and incubated under selection. Doubling times were 46, 55, and 80 min for pACYC184, pMJ101, and pJP77, respectively.

cloned in pMJ101, we tested constructs that carry *sbcC*$^+$ alone (pFG101), or *sbcC*$^+$ and *sbcD*$^+$ (pDL761). Both plasmids were able to transform the *rdgC*$^+$ strain, JC7623, though the colonies obtained were very small. Neither was able to transform LR263 or N3865 (Table 3 and data not shown). Plasmid-free *recBC sbcB* Δ*rdgC* strains do form colonies, despite their reduced viability (Figure 5). The failure to recover transformants carrying pFG101 or pDL761 must therefore reflect the overproduction of SbcCD nuclease. Although pFG101 carries *sbcC*$^+$ alone, this should be sufficient to increase the level of SbcCD since *sbcD* is intact in the chromosome and is the more highly expressed of the two genes in the *sbcDC* operon (NAOM *et al.* 1989). We also transformed pDL761 into recombination-deficient *recA* and *ruvAB recG* strains. In both cases, the transformants formed tiny colonies relative to vector controls, which provides further evidence that overexpression of SbcCD causes damage to DNA.

From these results it is clear that both RdgC and SbcCD have an adverse effect on a *recBC sbcBC* strain when overproduced. Yet it remains that the *rdgC*$^+$ *sbcC*$^+$ construct, pJP77, can be maintained only as long as both activities are available. If either is missing as a result of mutations in the plasmid or in the chromosome, the plasmid-carrying cells are inviable. In the absence of SbcCD, the size of plasmid insert carrying *rdgC*$^+$ is also a significant factor in determining viability. As measured by the efficiency of transformation, viability decreases as the size of the insert increases (Table 3, Figure 1). Presumably, the detrimental effect of increased RdgC is exacerbated by some feature of plasmid biology that is affected by DNA length, or by the extent of homology with the chromosome. It is perhaps significant that vector plasmids are maintained without difficulty in the absence of SbcCD alone, or of both SbcCD and RdgC (Table 3).

DISCUSSION

We have described a new *E. coli* gene, *rdgC*, in the *aroLM-sbcCD* region of the chromosome that encodes a previously unknown protein of 34 kDa. The amino acid sequence predicted for RdgC gives no clue as to its function. However, its inactivation through deletion of an internal fragment of *rdgC* causes the viability of *recBC sbcBC* strains to be dependent on proteins necessary for recombination. These Δ*rdgC* strains repair damage to DNA caused by UV light or ionizing radiation, and are proficient in conjugational and transductional recombination. However, they do not grow, or grow very poorly, if recombination is disabled through mutation of *recA, recF, ruvA, ruvB,* or *ruvC*. Viability is also reduced severely by a mutation of *lexA* (*lexA3*) known to prevent induction of the SOS response and to block recombination in the *recBC sbcBC* background (LLOYD *et al.* 1987; LOVETT and CLARK 1983). We assume this is due to the combined effect of reducing the levels of RecA, RuvAB, RecN, and RecQ proteins, all of which are regulated by LexA protein (PICKSLEY *et al.* 1984; NAKAYAMA *et al.* 1985; SHINAGAWA *et al.* 1988).

The need for recombination to sustain growth implies some form of chromosomal damage. Because viability is reduced whether recombination is blocked early (*recA, recF*) or late (*ruvA, ruvB, ruvC*), the damage must be of the type repairable only by recombination. If it could be repaired by other means, but provoked recombination, a block at a late stage might reduce viability by trapping intermediates. However, a block at initiation would avoid this problem and should have no more effect on viability than is seen in an *rdgC*$^+$ strain.

A clue to what this damage might be emerged when we reactivated SbcCD nuclease. When *sbcC*$^+$ was restored to the chromosome, the resulting construct formed tiny colonies with such low cell viability that *sbcC(D)* mutant derivatives reappeared very rapidly. When *sbcC*$^+$ was introduced on a multicopy plasmid, viability was reduced to the extent that the transformants could not form colonies. SbcCD nuclease is clearly detrimental to viability, much more so than in the isogenic *rdgC*$^+$ strain. It has an endonuclease activity that nicks DNA hairpins near the apex of the loop and an exonuclease activity that degrades DNA from duplex ends (CONNELLY and LEACH 1996; D. R. L. LEACH, personal communication). LEACH (1994) has suggested that it may attack a hairpin formed by replication slippage within a repeated sequence and thereby collapse the replication fork. Could it be that a *recBC sbcB(C)* cell has difficulty coping with a collapsed fork, and that the problem is exacerbated by Δ*rdgC* to the extent that it is forced to rely on recombination to complete chromosome duplication? The exceptionally low viability of

a Δ*rdgC recBC sbcB* strain could be explained if SbcCD not only increased the frequency of fork collapse but compounded the injury by degrading the displaced arm, thus reducing the opportunity for recombination. Previous studies showed that SbcCD does inhibit recombination in *recBC sbcB* strains (LLOYD and BUCKMAN 1985).

It has been suggested that chromosome duplication resumes after a replication fork has collapsed either by degrading the displaced arm and reinitiating DNA synthesis from *oriC* (UZEST *et al.* 1995), or by recombining this arm with the intact sister duplex to restore the fork and continue the current round (KUZMINOV 1995). The latter route is the more economical since it conserves the DNA already replicated. But why should Δ*rdgC* force a *recBC sbcBC* cell to rely on recombination? One possibility is that it causes most forks initiated at *oriC* to collapse before they reach *Ter*, the site for termination of replication. Recombination then becomes necessary to complete the cycle. A similar argument has been used to explain why recombination is needed to maintain viability in certain repair-defective strains (*lig, polA,* and *dam* mutants) that accumulate single-strand interruptions in their DNA (KUZMINOV 1995). However, we found *recA, recB, recF, recJ,* and *ruvC* derivatives of a Δ*rdgC* single mutant to be as viable as their *rdgC*$^+$ counterparts. If Δ*rdgC* increased fork collapse sufficiently to prevent the completion of replication, we would have expected at least *recA* and *recB* derivatives to be inviable. RecA is absolutely essential for recombination while, in the absence of *sbcB* and *sbcC* mutations, RecBCD enzyme is required to initiate an exchange at a duplex DNA end. The fact that these strains can form colonies argues strongly against any general increase in fork collapse.

An alternative is that reinitiation from *oriC* is blocked. Since recombination-proficient Δ*rdgC recBC sbcBC* strains grow perfectly well, they cannot have a problem with initiation itself. Perhaps they have difficulty removing the displaced arm of the fork. Such a possibility would arise if RdgC was an exonuclease or provided an activity needed for an exonuclease to act on the DNA. In its absence, the DNA would persist, thus reducing the chances of making viable products. The already depleted nuclease activity in *recBC sbcBC* strains makes this an attractive possibility. It can explain why Δ*rdgC* does not affect viability in *recA* or *recB* mutants, or in recombination-deficient derivatives of *recBC sbcA* strains. The rampant ExoV activity evident in *recA* mutants from their "reckless" DNA breakdown phenotype would quickly remove any displaced DNA. The absence of ExoV would make the task more difficult for *recB* mutants, which may explain their lower viability (KUZMINOV 1995). However they do have the exonuclease activities of RecJ and ExoI, which degrade single-stranded DNA from 5′ and 3′ ends, respectively. When coupled with RecQ, or some other helicase that can unwind DNA from a duplex end, these two enzymes may be sufficient to remove any impediment to replication from *oriC*. They have been shown to degrade linear duplex DNA in ExoV-deficient *recD* strains, though this degradation seems to rely on *recBC* (RINKEN *et al.* 1992). SbcCD is also available but may not be so critical as we found a Δ*rdgC sbcC recB* strain to be as viable as the *sbcC*$^+$ control. The *sbcA* mutation in *recBC sbcA* strains activates the *recE* product, exonuclease VIII, which resects linear duplex DNA to expose 3′ single-strand tails (JOSEPH and KOLODNER 1983; KOWALCZYKOWSKI *et al.* 1994). Combined with ExoI, this activity should enable recombination-deficient *recBC sbcA* cells to remove the displaced arm of a collapsed replication fork quite effectively.

The Δ*rdgC recBC sbcBC* strains we studied retain both RecQ and RecJ. These two proteins should suffice to expose a 3′ single-strand tail to provoke recombination and prime replication, especially since ExoI, the *sbcB* product, is missing. However, when recombination is disabled, a 3′–5′ single-strand exonuclease would be needed to remove this tail and allow reinitiation from *oriC*. We are currently testing the possibility that RdgC may provide such an activity. Strains carrying *sbcB15* or *xonA* alleles of *sbcB* that eliminate exonuclease I, show ≤25% of residual single-strand exonuclease activity in crude cell extracts (KUSHNER *et al.* 1971; PHILLIPS *et al.* 1988). *E. coli* may therefore have two or more enzymes of this type (KUSHNER *et al.* 1971).

If RdgC proves to have 3′–5′ single-strand exonuclease activity, it would explain why multicopy *rdgC*$^+$ plasmids reduce or inhibit the growth of *recBC sbcBC* strains. In these strains, replication primed by recombination from a 3′ end provides the most efficient route to chromosome duplication after collapse of a replication fork. Increased synthesis of RdgC would therefore impede recovery by removing 3′ single-strand ends. It also helps us to understand why at least some RdgC activity is needed to maintain an *sbcC*$^+$ plasmid in a *recBC sbcBC* strain. If the reactivated SbcCD nuclease increases fork collapse and interferes with recombination, RdgC will be needed to help remove the dislodged DNA and enable new replication initiated from *oriC* to produce viable products. However, growth would be expected to be very slow, as observed.

Multicopy plasmids are generally unstable in the *recBC sbcBC* background because they tend to form multimeric species and other DNA forms that interfere with plasmid segregation at cell division (KUSANO *et al.* 1989). These multimers are thought to arise from rolling-circle intermediates, which could well form through collapse of forks. The duplex ends provide entry sites for recombination enzymes and can therefore provoke homologous exchanges with other molecules to form more complex multimer species. Multimer formation is reduced in recombination-deficient *recBC sbcBC* strains (KUSANO *et al.* 1989). If 3′ single-strand tails are conserved in the absence of RdgC, and these provoke a higher level of recombination, plasmids may be even

more unstable in a Δ*rdgC* derivative of a *recBC sbcBC* strain. Inserts of chromosomal DNA would be expected to pose an additional problem since they would allow exchanges with the chromosome. While interplasmid exchanges may reduce plasmid stability, plasmid-chromosome exchanges would threaten cell viability. This possibility may provide an additional reason why transformants carrying an *sbcC*$^+$ plasmid cannot be recovered in a Δ*rdgC recBC sbcBC* background. We are testing this possibility by monitoring the relative stability of plasmids with and without homologous chromosomal inserts.

How often do forks collapse? If we are correct in concluding that Δ*rdgC recBC sbcBC* strains are incapable of removing the dislodged arm of a collapsed fork and are forced to rely on recombination to sustain growth, the probability of collapse per round of replication must be quite high. A value close to 0.5 would account for the <5% cell viability of the recombination-deficient constructs we made. This value is in line with estimates of double-chain breaks in *recA* mutant cells based on viability and the formation of anucleate cells (HORIUCHI and FUJIMURA 1995). A higher value could be accommodated if a partially replicated chromosome with one intact fork could be rescued. The lack of exonuclease activity in (Δ*rdgC*) *recBC sbcBC* cells would make it very difficult for DNA degradation from the exposed duplex end to keep up with, let alone catch, the remaining fork. This active fork is therefore likely to reach *Ter* where it will pause because of the failure to complete replication, thus increasing the probability of its collapse (HORIUCHI *et al.* 1994; HORIUCHI and FUJIMURA 1995). Depending on which template strand gave way, this second collapse would either linearize the DNA or free the collapsed arm. While the latter would solve the problem for exonuclease depleted cells, it is an uneconomical solution. Nevertheless, it may explain why recombination-deficient Δ*rdgC recBC sbcBC* cells grow and divide to some extent despite their low viability overall. Presumably the signals needed to maintain cell division continue to be generated for at least a few cell cycles.

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