

lon Incompatibility Associated with Mutations Causing SOS Induction: Null *uvrD* Alleles Induce an SOS Response in *Escherichia coli*

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The *uvrD* gene in *Escherichia coli* encodes a 720-amino-acid 3'-5' DNA helicase which, although nonessential for viability, is required for methyl-directed mismatch repair and nucleotide excision repair and furthermore is believed to participate in recombination and DNA replication. We have shown in this study that null mutations in *uvrD* are incompatible with *lon*, the incompatibility being a consequence of the chronic induction of SOS in *uvrD* strains and the resultant accumulation of the cell septation inhibitor Sula (which is a normal target for degradation by Lon protease). *uvrD-lon* incompatibility was suppressed by *sulA*, *lexA3*(Ind⁻), or *recA* (Def) mutations. Other mutations, such as *priA*, *dam*, *polA*, and *dnaQ* (*mutD*) mutations, which lead to persistent SOS induction, were also *lon* incompatible. SOS induction was not observed in *uvrC* and *mutH* (or *mutS*) mutants defective, respectively, in excision repair and mismatch repair. Nor was *uvrD*-mediated SOS induction abolished by mutations in genes that affect mismatch repair (*mutH*), excision repair (*uvrC*), or recombination (*recB* and *recF*). These data suggest that SOS induction in *uvrD* mutants is not a consequence of defects in these three pathways. We propose that the UvrD helicase participates in DNA replication to unwind secondary structures on the lagging strand immediately behind the progressing replication fork, and that it is the absence of this function which contributes to SOS induction in *uvrD* strains.

DNA helicases are enzymes involved in a variety of processes such as DNA replication, repair, and recombination, and at least 12 have been identified in *Escherichia coli* (29, 30). Of these, only DnaB helicase (with 5'-3' polarity) is essential for cell viability, being associated with movement of the chromosome replication fork (27). Three other helicases, Rep, PriA, and UvrD, each with 3'-5' polarity, have also been suggested to participate in chromosome replication, but their roles are not as well established.

The UvrD helicase (also called helicase II) is the product of the *uvrD* gene and is a polypeptide of 720 amino acids. UvrD is required in the pathways of both methyl-directed mismatch repair (34, 35) and UvrABC-mediated excision repair (46, 47), and *uvrD* mutants exhibit a spontaneous mutator phenotype as well as increased sensitivity to UV irradiation. Genetic evidence has implicated UvrD in homologous recombination (21, 23, 26, 32). The suggestion has also been made that UvrD participates in DNA replication, based on (i) experiments with cell-free systems (17, 22), (ii) the observation that *uvrD* null mutations are incompatible with *rep* (encoding the Rep helicase) or *polA* (encoding DNA polymerase I) mutations (56), and (iii) identification of dominant-lethal *uvrD* mutations (8, 59).

In the present study, we discovered that *lon* (encoding the Lon protease) represents a third locus, mutations in which are incompatible with *uvrD* null alleles. Chronic induction of the SOS response (reviewed in references 23 and 55) in the *uvrD* null strains was shown to be the basis for *lon* incompatibility; other mutations (in *dam*, *priA*, *mutD*, and *polA*) that exhibited persistent SOS induction were also *lon* incompatible. We propose a model suggesting a role for the UvrD helicase in removing regions of secondary structure in the lagging strand

during replication fork progression, which could account for chronic generation of an SOS-inducing signal in *uvrD* mutants.

MATERIALS AND METHODS

Growth media and conditions. The defined and nutrient media were, respectively, glucose-minimal A and Luria-Bertani (LB) medium (33). *cpsB-lac* expression was monitored on MacConkey-lactose agar (Difco). Unless otherwise indicated, the growth temperature was 37°C. Isopropyl β-D-thiogalactopyranoside (IPTG) was added at a final concentration of 1 mM. Concentrations of antibiotics and 5-bromo-4-chloro 3-indolyl-β-D-galactoside (X-Gal) used were as described previously (43, 44).

Bacterial strains and phages. The *E. coli* strains used are listed in Tables 1 and 2. Phage P1kc was from our laboratory stock. The transposon vehicle phages λ1105, λ1323, and λ1324 for transposition, respectively, of Tn10dKan, Tn10dTet, and Tn10dCm, have been described elsewhere (16). The ordered λ phage library of the *E. coli* genome (19) was obtained from K. Isono.

Plasmids. The plasmid vectors pCL1920 (pSC101 based, encoding Sm^r Sp^r, for general cloning [24]) and pMAK705 (pSC101 based, encoding Cm^r, temperature-sensitive replicon [10]) have been described previously. Plasmids pHYD130 and pHYD141 were constructed in this study and carry the *lon*⁺ gene subcloned from the λ phage 148 of the ordered library of Kohara et al. (19): the former bears a 6-kb insert comprising two contiguous *Pst*I chromosomal segments in the *Pst*I site of vector pCL1920, and the latter bears a 4.1-kb *Bgl*II-*Kpn*I chromosomal segment in the *Bam*HI-*Kpn*I sites of vector pMAK705 (see Fig. 1). Plasmids pHYD136 and pHYD138 were also constructed in this study and are described below.

Isolation and characterization of the *uvrD400::Tn10dTet* mutant. Strain GJ1901, carrying a *lacZ*(Am) mutation, was subjected to random insertion mutagenesis with the Tn10dTet transposon following infection with λ1323, as described elsewhere (16). Tet^r colonies exhibiting increased Lac⁺ papillation frequency were identified on LB agar plates supplemented with 0.1% lactose and X-Gal, as described earlier (43). One such mutant clone was designated GJ1904, and the following characteristics led to the conclusion that it carried a *uvrD* insertion, designated *uvrD400::Tn10dTet*. (i) Strain GJ1904 both was UV sensitive and exhibited a high frequency of spontaneous mutations to Lac⁺, nalidixic acid resistance, or rifampin resistance (data not shown). (ii) The Tn10dTet insertion in GJ1904 was linked 30% to the *ilv* locus in P1 transduction. (iii) Lastly, the *uvrD400::Tn10dTet* insertion was cloned from the chromosome of GJ1904 as part of a 6.6-kb *Pst*I fragment (size inclusive of the 2.9-kb Tn10dTet element) in the plasmid vector pCL1920, and the resulting plasmid was designated pHYD136. The nucleotide sequence at either junction of Tn10dTet with the *E. coli* chromosomal DNA was determined by using pHYD136 DNA as a template and the oligonucleotides 5'-TGGTCACCAACGCTTTCCCGAG-3' and 5'-CTGTTGACAAAGGAATCATAG-3' as primers (designed so as to

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TABLE 1. List of *E. coli* strains^a

Strain	Genotype ^b	Source or reference
BL21	<i>hsdS gal</i>	33
JL2301 ^c	<i>thr leu arg his ΔlexA300::(Sm^r Sp^r) sulA (λsulA::lac)</i>	J. Little via M. Radman
MG1655	Wild type	Lab stock
SG20780	<i>araD139 Δ(argF-lac)U169 rpsL150 relA1 flbB5301 Δlon-510 cpsB10::lac-Mu-λ-imm</i>	57
SG20781	SG20780 <i>lon</i> ⁺	57
GJ1823	MG1655 <i>zbh-900::Tn10dKan(Ts)1 lacZ4525::Tn10dKan lon-103::IS186</i>	44
GJ1901	<i>ara zbh-900::Tn10dKan(Ts)1 lacZ(Am) lacI3098::Tn10Kan</i>	From CSH142 (33), in several steps
GJ1904	GJ1901 <i>uvrD400::Tn10dTet</i>	This study
GJ1907	MG1655 <i>zbh-900::Tn10dKan(Ts)1 lon-103::IS186</i>	This study
GJ1912	MG1655 <i>lon-103::IS186 zba-901::Tn10dKan</i>	This study
GJ1913	MG1655 <i>zba-901::Tn10dKan</i>	This study
GJ1916	MG1655 <i>lon-104::IS186::Tn10dCm zba-901::Tn10dKan</i>	This study
GJ1922	JL2301 <i>lexA⁺ malB::Tn9</i>	This study
GJ1925	JL2301 <i>lexA⁺ malF3180::Tn10Kan</i>	This study
GJ1988	GJ1907 <i>sulA::Tn5 uvrD400::Tn10dTet</i>	This study
GJ1989	GJ1907 <i>lexA3 malB::Tn9 uvrD400::Tn10dTet</i>	This study
GJ1992	JL2301 <i>lexA⁺</i>	This study
GJ1997	GJ1907 <i>sulA::Tn5 dam::Tn9</i>	This study
GJ1998	GJ1907 <i>sulA::Tn5 dnaQ (mutD5) zae-502::Tn10</i>	This study
GJ1999	GJ1907 <i>lexA3 malB::Tn9 priA1::Kan</i>	This study
GJ2101	SG20780 <i>ΔuvrD288::Kan/pHYD141</i>	This study
GJ2102	SG20780 <i>ΔuvrD288::Kan recA56 srl-300::Tn10</i>	This study
GJ2122	MG1655 <i>ΔpolA::Kan lon-146::Tn10dTet/F'[polA(Klenow⁺)Cm^r]</i>	This study
GJ2126	SG20780 <i>uvrD3 ihv-500::Tn10</i>	This study
GJ2127	SG20780 <i>Δrep::Cm</i>	This study

^a Strain BL21 is an *E. coli* B derivative. All other strains are derivatives of *E. coli* K-12. Genotypes of additional *sulA-lac* strains used in this study are described in Table 2.

^b Genotype designations are as in the work of Berlyn (2). All strains are F⁻ unless otherwise indicated. Allele numbers are given where they are known. Listed below in parentheses are references or sources for various mutations introduced into the GJ strains: *ihv-500::Tn10*, *lacI3098::Tn10Kan*, and *malF3180::Tn10Kan* (52); *lacZ(Am)*, *dnaQ*, and *zae-502::Tn10* (33); *ΔpolA::Kan* and F'[*polA*(Klenow⁺)Cm^r] (12); *lon-146::Tn10dTet* (31); *dam::Tn9* (N. Kleckner); *ΔuvrD288::Kan* (56); *priA1::Kan* (36); *lexA3*, *sulA::Tn5*, and *malB::Tn9* (M. Radman); *Δrep::Cm* (B. Michel); *recA56* and *srl-300::Tn10* (44); and *uvrD3* (*E. coli* Genetic Stock Center).

^c Genotype markers of JL2301 relevant to this study are listed.

read outward from sites immediately internal to the inverted terminal repeat at either end of Tn10dTet; comparison of our results with the *E. coli* genome sequence (4) permitted the conclusion that the insertion had disrupted the (720-residue) *uvrD* open reading frame immediately after the second base of codon 476 (data not shown).

Transposon tagging and genetic mapping of *uvrD*-incompatible mutation in GJ1823. Strain GJ1907 (a spontaneous Lac⁺ revertant of GJ1823) is Kan^r at 30°C but Kan^s at 42°C, because of the presence in GJ1823 (and its retention in GJ1907) of a Kan^r(Ts) insertion near the *gal* locus (44). GJ1907 was initially transduced to Kan^r at 42°C with a P1 lysate prepared on a population of random Tn10dKan insertion clones of the wild-type strain MG1655. The pool of GJ1907 Kan^r cells was then used as a recipient for transduction to Tet^r with a P1 lysate prepared on the *uvrD400::Tn10dTet* strain GJ1904. Since the *uvrD* mutation is ordinarily lethal in GJ1823 and its derivatives (see below), only a small number of Tet^r transductants were recovered in this cross, and these were presumed to represent clones in which the locus conferring incompatibility in GJ1823 had been replaced with the wild-type allele from MG1655 in the first transduction to Kan^r. By this approach, a Kan^r insertion (designated *zba-901::Tn10dKan*) 83% linked to the locus governing *uvrD* incompatibility was identified, and it was mapped to the 10-min region (data not shown). Subsequently, the mutation conferring incompatibility with *uvrD* was itself shown to be 92% cotransducible with a *hupB::Kan* insertion (data not shown).

Transposon-generated mutation that suppresses *uvrD* incompatibility in GJ1912. Strain GJ1912 (which carries the *uvrD*-incompatible mutation) was subjected to random insertion mutagenesis with Tn10dCm following infection with λ1324, as described previously (16). The introduction of an *uvrD400::Tn10dTet* insertion into the pooled culture of Cm^r clones, by P1 transduction, was then attempted. One viable Tet^r transductant colony (GJ1916) so recovered was shown to have a Cm^r insertion that (i) was located in the same 10-min chromosomal region as the locus conferring *uvrD* incompatibility in GJ1912 (and GJ1823) and (ii) was 100% cotransducible with the phenotype of suppression of *uvrD* incompatibility. The chromosomal region bearing the Tn10dCm insertion was cloned from GJ1916 on a 3.2-kb *Pst*I fragment in the plasmid vector pCL1920, and the resultant plasmid was designated pHYD138. The oligonucleotide primer 5'-ATTCTGCCTCCAGAGCTGA-3', designed to read outward from a site immediately internal to the inverted terminal repeat at one end of Tn10dCm, was used to determine the sequence at the junction of the Tn10dCm insertion in the insert DNA of pHYD138.

Tests for *lon* incompatibility. Incompatibility of different mutations with *lon* was established by transduction experiments, wherein it was demonstrated that no colonies were recovered when an attempt was made to introduce an insertion mutation in the concerned locus into a *lon* strain, or vice versa, unless the recipient carried a plasmid such as pHYD130 bearing the *lon*⁺ gene. The demonstration in this study (see below) that the *lon* incompatibility of different mutations could be partially suppressed by growth at 42°C also allowed us to recover transductants from various crosses at the elevated temperature and then test them for a lethality phenotype at 37 or 30°C.

DNA methods. The standard protocols of Sambrook et al. (45) were followed for experiments involving recombinant DNA. Nucleotide sequence determinations were done with the aid of an automated DNA sequencer, using double-stranded plasmid DNAs as templates. The BLAST programs were employed for comparison of DNA sequences with the GenBank sequence database.

Other techniques. Procedures for conjugation (33), P1kc transduction (9), measurement of spontaneous mutation frequency to nalidixic acid or rifampin resistance (43), assay of cell survival following UV radiation (33), and measurement of β-galactosidase activity in cultures (33) were as described previously.

RESULTS

Identification of a cryptic *uvrD*-incompatible mutation in strain GJ1823 as *lon*. The starting point of this study was our observation that a Tn10dTet insertion in codon 476 of the *uvrD* gene (*uvrD400*), obtained as described above, could not be transduced into GJ1823, a strain that had been derived (44) by transduction from wild-type MG1655 and that was not expected to carry a *uvrD*-incompatible mutation. We also confirmed that GJ1823 had not acquired a *rep* or *polA* mutation (data not shown); these are the two loci known earlier to be *uvrD* incompatible.

A transposon-tagging strategy was employed to localize the cryptic mutation in GJ1823 to the 10-min chromosomal region, and the following lines of evidence indicated that the mutation

is in the *lon* locus. (i) Colonies of strains carrying the GJ1823 mutation were mucoid, and expression of *cpsB-lac* in strain SG20781 was markedly increased following introduction of the mutation by P1 transduction (data not shown); these are characteristic Lon phenotypes, since one of the substrates of the Lon protease is RcsA, an activator of the *cps* operon involved in capsular polysaccharide biosynthesis (31, 57). (ii) The *uvrD::Tn10dTet* insertion could not be introduced by transduction into strain SG20780, the isogenic Δlon derivative of SG20781. (iii) Strains GJ1823 and SG20780 could both be complemented for the *uvrD* incompatibility phenotype by plasmid pHYD130 carrying the cloned *lon*⁺ gene. (iv) Finally, DNA sequence determination of the insert cloned in plasmid pHYD138 (whose construction has been described above), and its comparison with the *E. coli* genome sequence (4), indicated, first, that the mutation in GJ1823 is an *IS186* insertion (6, 20) within a run of G's in the spacer sequence between the -10 and -35 regions of the *lon* promoter and, second, that a *Tn10dCm* insertion which suppressed the *UvrD* incompatibility phenotype of GJ1823 was situated within, and 116 bp from the right end of, *IS186* (as depicted in Fig. 1). The same *Cm*^r insertion was also able to suppress the phenotypes of mucoidy and *cpsB-lac* derepression associated with the GJ1823 mutation (data not shown). The simplest hypothesis to explain these observations is that the *IS186* insertion in GJ1823 disrupts the *lon* promoter and that the *Tn10dCm* suppressor restores *lon*⁺ expression by providing a new promoter (see Fig. 1). The original cryptic mutation in GJ1823 and the suppressor have been designated *lon-103::IS186* and *lon-104::IS186::Tn10dCm*, respectively.

These results therefore established *lon* as an additional locus in which mutations are inviable in combination with *uvrD*.

SOS induction in *uvrD400::Tn10dTet* as basis for *lon* incompatibility. Another substrate of the Lon protease is the cell separation inhibitor SulA, whose synthesis is induced during the SOS response; as a consequence, *lon* mutants are known to be sensitive to SOS-inducing agents (reviewed in references 23 and 55). At least some *uvrD* mutations have been shown earlier to induce the SOS response (3, 25, 38), and we therefore tested the hypothesis that the *uvrD-lon* combination is rendered inviable because Lon becomes essential to degrade the increased concentration of SulA in the *uvrD* mutant background. (i) Using *sulA-lac* expression as a measure of SOS induction, we found that the *uvrD400::Tn10dTet* insertion (which is *lon* incompatible) caused a moderate (5-fold) induction of the SOS regulon, as against the 70-fold increase observed in a fully derepressed ($\Delta lexA$) strain (Table 2). The expression value for a merodiploid strain bearing *uvrD*⁺ on the chromosome and *uvrD400* on plasmid pHYD136 was the same as that for haploid *uvrD*⁺ (Table 2; compare the first and second rows), indicating that the mutation is recessive to the wild-type gene. (ii) Contrarily, the *uvrD3* mutation, which does not induce the SOS response (38) (see Table 2), was *lon* compatible (strain GJ2126). (iii) Finally, the *uvrD400::Tn10dTet* incompatibility with *lon* could be suppressed by *sulA* and *lexA3* (Ind⁻) mutations in strains GJ1988 and GJ1989, respectively, in which the inhibitory accumulation of SulA is expected to be abolished (21, 23, 55); predictably, the *uvrD400*-mediated derepression of *sulA-lac* was not observed in the *lexA3* background (Table 2, GJ1991).

By these experiments, therefore, we could conclude (i) that the *lon* incompatibility of *uvrD400* is correlated with chronic SOS induction and the consequent accumulation of SulA in the latter strain and (ii) that such induction is not essential for the viability of the *uvrD* mutant (as the *uvrD lexA3* strains are viable).

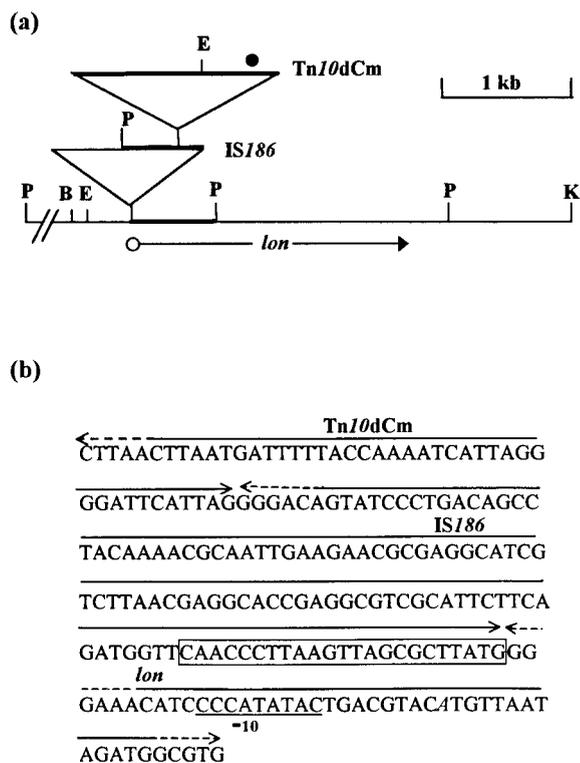


FIG. 1. Molecular characterization of *lon-103* and *lon-104* mutations. (a) The line at the bottom depicts the physical map of the wild-type *lon* locus (4, 19) for the enzymes *Bg*II (B), *Eco*RI (E), *Kpn*I (K), and *Pst*I (P); the open circle and arrow beneath the line denote, respectively, the promoter and transcribed region of the *lon* gene. Horizontal line segments of the lower and upper inverted triangles represent, respectively, *IS186* and *Tn10dCm*; the vertices denote sites of insertion of these two elements in the *lon-103* mutant and the *lon-104* suppressor-bearing strain, respectively. The figure is drawn to the scale marked; the distance between the *Pst*I site at the left and the *Bg*II site is 2.42 kb. The contiguous region of DNA (delimited by *Pst*I sites) corresponding to the insert cloned in plasmid pHYD138 is shown by heavy line segments. The recognition site for the *Tn10dCm*-specific sequencing primer (see the text) is marked by the solid circle. (b) Nucleotide sequence determined with a *Tn10dCm*-specific sequencing primer using pHYD138 DNA as a template. The extents of *Tn10dCm*, *IS186*, and *lon* sequences are indicated by double-headed arrows above the sequence; dashed lines have been used where the delimited ends do not represent the natural ends of the sequence in question. The right end of *IS186* is as specified in the work of Sengstag et al. (50), and the sequence corresponding to the 23-bp right inverted repeat is boxed. The -10 region of the *lon* promoter (4, 11) is underlined, and the base representing the transcription start site is italicized.

SOS induction is associated with null mutations in *uvrD*. In order to address the question of whether SOS induction in *uvrD* strains is the null phenotype, we tested two deletion-insertion *uvrD* mutations, constructed by Kushner and coworkers (56) and recessive to *uvrD*⁺, for their effects on reporter gene expression from SOS-responsive promoters and for *lon* incompatibility. The $\Delta uvrD288::Kan$ allele (in which more than two-thirds of the gene has been deleted from its 3' end) was associated with *sulA-lac* derepression (Table 2). Furthermore, both $\Delta uvrD288$ and the $\Delta uvrD290::Tet$ mutation (which represents a deletion of the *uvrD* promoter and the 5' region of the gene) were *lon-103::IS186* incompatible (data not shown). We therefore conclude that SOS induction by *uvrD* is the null phenotype.

We also employed the *uvrD288::Kan* mutation to construct a viable *uvrD lon recA* (Def) triple mutant (GJ2102), as follows. The *uvrD* allele was first introduced by P1 transduction at 30°C into SG20780/pHYD141, a chromosomal *lon* mutant strain

TABLE 2. Effects of mutations on *sulA::lac* expression^a

Strain	Mutation ^b	Enzyme sp act
GJ1922 derivatives		
GJ1922	None	49
With pHYD136		44
GJ1923	$\Delta lexA300::(Sm^r Sp^r)$	3,574
GJ1927	<i>dnaQ</i> (<i>mutD5</i>) <i>zae-502::Tn10</i>	138
GJ1930	<i>uvrD400::Tn10dTet</i>	264
Grown at 42°C		236
GJ1931	<i>priA1::Kan</i>	397
GJ1965	$\Delta uvrD288::Kan$	258
GJ1966	<i>uvrD3 ilv-500::Tn10</i>	36
GJ1991	<i>lexA3 malF3180::Tn10Kan uvrD400::Tn10dTet</i>	23
GJ2103	<i>mutH471::Kan</i>	51
GJ2104	<i>mutS::Tn10dTet</i>	62
GJ2105	<i>uvrC279::Tn10</i>	59
GJ2106	<i>mutH471::Kan uvrC279::Tn10</i>	65
GJ2107	<i>mutS::Tn10dTet $\Delta uvrD288::Kan$</i>	239
GJ2108	<i>uvrC279::Tn10 $\Delta uvrD288::Kan$</i>	271
GJ1925 derivatives		
GJ1925	None	52
GJ1929	<i>dam::Tn9</i>	237
GJ1992 derivatives		
GJ1992	None	52
GJ1993	$\Delta polA::Kan$	2,050
GJ1994	$\Delta polA::Kan/F'$ [<i>polA</i> ⁺ Cm ^r]	37
GJ1995	$\Delta polA::Kan/F'$ [<i>polA</i> (Klenow ⁺)Cm ^r]	1,488
With IPTG		41
GJ1996	$\Delta polA::Kan/F'$ [<i>polA</i> (5' Exo ⁺)Cm ^r]	197

^a The specific activity of β -galactosidase was determined in each of the cultures grown to exponential phase in glucose-minimal medium (for strains GJ1993 and GJ1995) or LB (for all other strains), and values (representing the means of at least three independent experiments) are given in Miller units (33).

^b Mutations in addition to those present in the parental strain GJ1922, GJ1925, or GJ1992 are listed for each derivative. The reference or source for each of the mutations not described in Table 1 is given below in parentheses: *mutH471::Kan* (*E. coli* Genetic Stock Center); *mutS::Tn10dTet* (33); *uvrC279::Tn10* (52); F' [*polA*⁺ Cm^r] and F' [*polA* (5' Exo⁺)Cm^r] (12).

carrying *lon*⁺ on a Ts plasmid replicon. The resulting strain (GJ2101) could be successfully cured of the plasmid after it was made *recA*(Def). This experiment demonstrated that *recA* (which also serves to abrogate the SOS response [21, 23, 55]) is an additional suppressor of *lon-uvrD* incompatibility.

***dam*, *mutD*, *polA*, and *priA* mutations are also *lon* incompatible.** Mutations in *dam*, *dnaQ* (*mutD*), *polA*, and *priA* have previously been shown (or suggested) to lead to chronic SOS induction (1, 36, 39, 40, 48, 53), and we examined whether they are also *lon* incompatible. We initially confirmed that each of the mutations did lead to a 3- to 40-fold derepression of *sulA-lac* (Table 2). We then showed, by the criteria listed above, that all four mutations were *lon* incompatible (*dam*, *dnaQ*, and *priA* were tested with *lon-103::IS186*; $\Delta polA$ was tested with *lon-146::Tn10dTet*) (data not shown). Suppression of *lon* incompatibility by *sulA* was demonstrated for *dam* and *dnaQ* (strains GJ1997 and GJ1998, respectively), and suppression of *lon* incompatibility by *lexA3* was demonstrated for *priA* (strain GJ1999).

By introducing F' plasmids carrying the appropriate *polA* variants into a chromosomal $\Delta polA$ strain, we also tested the effects of the *polA* (5' Exo⁺) and *polA* (Klenow⁺) mutations on SOS induction and *lon* incompatibility. The former encodes the isolated 5'-3' exonuclease activity, and the latter encodes the 5'-3' polymerase with associated 3'-5' exonuclease activities of DNA polymerase I (12). We found that the *polA* (5' Exo⁺) mutant exhibited fourfold chronic SOS induction (Table 2) and was also *lon* incompatible; the suggestion that *polA* (5' Exo⁺) is associated with SOS induction has also been made

earlier (1). On the other hand, the *polA* (Klenow⁺) allele (which is known to require IPTG for its expression [12]) was able to cause *sulA-lac* expression in a $\Delta polA$ strain to revert to basal levels (Table 2); correspondingly, the *lon polA* (Klenow⁺) strain GJ2122 was viable on IPTG-supplemented media.

Finally, mutations in *rep* were shown earlier to be associated with a low level of chronic SOS induction (38, 49), and we also observed that a *rep lon* mutant strain (GJ2127) is very sick. Taken together, these data serve to establish the general validity of the notion that *lon* mutations are lethal in strain backgrounds which exhibit chronic SOS induction and that the lethality is *sulA* and *lexA3* suppressible.

***lon* incompatibility of SOS-inducing mutations is partially suppressed at 42°C.** We noticed that the various SOS-inducing mutations such as *uvrD400::Tn10dTet*, *uvrD288*, *dam*, *priA*, and *mutD* could be introduced by transduction into a *lon* strain to give small colonies on plates incubated at 42°C and that the transductants could grow, albeit poorly, upon restreaking at 42°C but not at 30 or 37°C. The degree of *sulA-lac* derepression by *uvrD400::Tn10dTet* was unaffected at 42°C (Table 2). On the other hand, *cpsB-lac* expression in the *lon* mutant was less pronounced at 42°C (data not shown). Our results support the findings by other workers (13, 15, 51, 57) that alternative proteases such as HslVU (ClpYQ) whose synthesis is induced upon heat shock are able to substitute for Lon in degrading the substrates RcsA and SulA at the elevated temperature.

Dissociation of the *uvrD* role in SOS induction from its functions in mismatch repair, excision repair, and recombi-

nation. The following experiments indicated that SOS induction in *uvrD* strains is not the consequence of the absence of mismatch repair or nucleotide excision repair. Mutations in *mutH* or *mutS* that interfere with mismatch repair did not induce the SOS response, nor did a mutation in *uvrC* that renders defective the pathway of nucleotide excision repair (Table 2); similar results with *mutH*, *mutL*, *mutS*, and *uvrA* have been reported earlier (28, 38). A *mutH uvrC* double mutant in which both pathways are simultaneously nonfunctional (as is the case in *uvrD*) also was not derepressed for *sulA-lac* expression (Table 2).

We considered the possibility that SOS induction in the *uvrD* mutant is the result of accumulation of a trapped intermediate in either the mismatch repair or the excision repair pathway. For example, UvrD has been shown to be necessary for turnover of UvrBC during excision repair (37). However, the observation that strains that were *uvrD mutS* or *uvrD uvrC* continued to exhibit SOS induction to the same extent as the *uvrD* single mutant (Table 2) served to exclude this explanation.

In view of the possibility that UvrD participates in recombination, we tested the effects of mutations in several recombination genes (other than *recA*) on SOS induction in *uvrD* mutants. The expression of *sulA-lac* in a *uvrD* strain remained elevated despite introduction of mutations in *recB*, *recF*, *recQ*, or *ruvABC* (data not shown). Substantially similar results have also been described earlier (3).

***uvrD* derivatives of BL21 are very sick.** It is known that *E. coli* B strains are naturally Lon protease deficient. Consistent with the results described above was our observation that *uvrD400::Tn10dTet* transductants of the *E. coli* B strain derivative BL21 grow at 37°C as tiny colonies and that they are somewhat more healthy at 42°C. Other workers (8) have earlier reported the construction of UvrD-deficient derivatives of BL21(DE3), but it is possible that these derivatives may inadvertently also have been selected for suppressors of *uvrD-lon* incompatibility such as *sulA*.

DISCUSSION

We have identified in this study a new set of incompatible pairs of mutations involving the *lon* gene on the one hand and *uvrD*, *priA*, *mutD*, *dam*, or *polA* on the other. The common features that characterize this set are as follows: (i) each of the latter partners of the set results in moderate SOS induction, and (ii) the incompatibilities are suppressed by *sulA*, *lexA3* (Ind⁻), or *recA* mutations and are partially suppressed by growth at 42°C. Included in the category of *lon*-incompatible SOS-inducing mutations are both those (such as *uvrD*, *priA*, and *mutD*) for which SOS induction itself is not essential for viability and others (such as *dam* and *polA*) for which it is. The simplest interpretation to explain the incompatibility is that persistent SOS induction causes a moderate elevation in levels of SulA protein and that the Lon protease becomes essential for inactivating SulA under these conditions.

The demonstration of *lon* incompatibility for mutations such as *uvrD* and *priA* permits the inference that the latter mutations lead to moderate SOS induction in all (or a majority of) cells in the culture rather than to extreme SOS induction in a smaller subpopulation. The *rep* mutation appears to be associated with an even lower level of SOS induction than *uvrD* or *priA*, since a *lon rep* mutant is sick yet viable. Chronic SOS induction has also been reported in wild-type cells in the stationary-growth phase (54) but is probably mild enough not to be lethal in a *lon* mutant. Likewise, mutations in *recF* (40), *ruv* (40), or *recBC sbcBC* (14, 28, 39) have been reported to cause

SOS induction, but we found each of them to be *lon* compatible (data not shown).

It may be noted that the *IS186* insertion mutation in the promoter region of *lon* in strain GJ1823 had arisen spontaneously, in the absence of any imposition of ostensible selection. Ignatov and Chistyakova (11) have also independently reported the identification of a spontaneous *IS186* insertion at the same site in the *lon* promoter. Combined with the fact that wild-type *E. coli* B isolates are *lon* negative, our observations raise the question whether natural selection may operate for *lon*-defective strains under certain conditions.

SOS induction in *uvrD* mutants probably occurs through a defect in DNA replication. It has been established from earlier studies that the signal for SOS induction in vivo is single-stranded DNA (reviewed in references 23 and 55). Our results indicate that chronic SOS induction in *uvrD* mutants is the null phenotype. Nevertheless, identification of the precise mechanism is still complicated by the fact that UvrD is involved in several discrete functions and pathways, loss of any one of which could plausibly be modeled to account for SOS induction.

The findings that *mutH*, *mutS*, or *uvrC* strains are not SOS induced and that SOS induction continues to occur in *mutS uvrD* and *uvrC uvrD* double mutants indicate that the induction is not due to the loss of mismatch repair or excision repair pathways in the *uvrD* mutant, nor is it due to a trapped intermediate in either pathway. Likewise, our results render unlikely the possibility that it is the loss of a recombination function of UvrD which is responsible for SOS induction. One could therefore consider some defect in DNA replication to be the cause for spontaneous SOS induction in *uvrD* strains.

Role for UvrD in lagging-strand replication? In order to account for generation of a moderate SOS-inducing signal in all cells of a culture of a *uvrD* strain, we propose that UvrD functions as a 3'-5' helicase during lagging-strand replication. The chromosomal replication fork is expected to contain a loop of unreplicated lagging strand that is approximately 1 kb long (27). We suggest that the 3'-5' helicase activity of UvrD helps maintain this single-stranded DNA region free of secondary structure and that in the absence of UvrD, synthesis of some fraction of Okazaki fragments by DNA polymerase III is impeded so that a chronic, low-level SOS-inducing signal is generated by the unreplicated DNA segments on the lagging strand. A similar function was in fact postulated earlier for PriA (29); however, more recent findings have established that the preeminent role of this protein is in the reassembly of collapsed replication forks (7, 18, 58) and that loss of its helicase activity is not sufficient for generation of an SOS-inducing signal in vivo (18, 48).

Our model for UvrD's role in replication may explain the synthetic lethality of null *uvrD* mutants with *polA* (reference 56 and this study). Both the polymerase and 5'-3' exonuclease activities of DNA polymerase I have been implicated in efficient lagging-strand replication (27). Our own observations (data not shown) using the *polA* (Klenow⁺) or *polA* (5' Exo⁺) alleles in combination with *uvrD400::Tn10dTet* are consistent with earlier reports that null *uvrD* strains are rendered inviable upon loss of either catalytic activity of DNA polymerase I (56). It is also possible that Rep becomes essential for lagging-strand replication in a *uvrD* mutant, thereby accounting for *rep-uvrD* incompatibility (56).

Finally, we speculate that the availability of unreplicated stretches of DNA on the lagging strand might contribute to the hyperrecombinational phenotype of *uvrD* strains described earlier (21, 23), through the mechanism that has been referred to as daughter strand gap repair (7, 23, 55). Previous studies

have shown that *uvrD* mutants exhibit hyperrecombination as a consequence of the loss of mismatch repair (41, 42), and we suggest that the two mechanisms (loss of mismatch repair and daughter strand gap repair) operate independently. For example, the latter mechanism may explain the RecA- and RecF-dependent increase in the frequency of tandem repeat deletions (3) and of transposon precise excisions (5) in *uvrD* mutants, since such dependence is the hallmark of daughter strand gap repair. Lloyd also suggested earlier that the hyperrecombinational phenotype associated with *uvrD* operates through the RecF pathway.

In summary, therefore, we propose that several phenotypes associated with *uvrD* null mutations, including chronic SOS induction and the consequent *lon* incompatibility described in this study, may be explained by a model in which UvrD participates as a helicase in lagging-strand DNA replication in *E. coli*.

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