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Escherichia coli Strains Lacking Protein HU Are UV Sensitive due to a Role for HU in Homologous Recombination

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***hupA* and *hupB* encode the α and β subunits of the *Escherichia coli* histone-like protein HU. Here we show that *E. coli hup* mutants are sensitive to UV in the *rec*$^+$ *sbc*$^+$, *recBC sbcA*, *recBC sbcBC*, *umuDC*, *recF*, and *recD* backgrounds. However, *hupAB* mutations do not enhance the UV sensitivity of resolvase-deficient *recG ruvA* strains. *hupAB uvrA* and *hupAB recG* strains are supersensitive to UV. *hup* mutations enhance the UV sensitivity of *ruvA* strains to a much lesser extent but enhance that of *rus-1 ruvA* strains to the same extent as for *rus*$^+$ *ruv*$^+$ strains. Our results suggest that HU plays a role in recombinational DNA repair that is not specifically limited to double-strand break repair or daughter strand gap repair; the lack of HU affects the RecG RusA and RuvABC pathways for Holliday junction processing equally if the two pathways are equally active in recombinational repair; the function of HU is not in the substrate processing step or in the RecFOR-directed synapsis action during recombinational repair. Furthermore, the UV sensitivity of *hup* mutants cannot be suppressed by overexpression of wild-type or mutant *gyrB*, which confers novobiocin resistance, or by different concentrations of a gyrase inhibitor that can increase or decrease the supercoiling of chromosomal DNA.**

HU is one of the most abundant DNA-binding proteins in *Escherichia coli*, and it contributes to the compaction of the genome into tight nucleosome-like structures (40). *E. coli* HU is a small, basic, heat-stable dimeric protein composed of two highly homologous subunits, HUα and HUβ, encoded by the *hupA* and *hupB* genes located at 90 and 10 min, respectively, on the *E. coli* chromosome (21, 22). Strains mutated in both *hupA* and *hupB* have reduced viability, perturbed cell division, and a number of other deficiencies (18, 50). The HU protein also participates in a number of cellular mechanisms such as modulating the expression of specific genes (36, 55), DNA melting at the initiation of replication (20, 45), DNA breaking/rejoining in transposition and inversion reactions (13, 25), and homologous recombination (7, 19). In addition, although HU does not recognize a particular DNA sequence, it can act at very precise locations on the chromosomal DNA through specific binding to particular DNA structures such as bulged DNA, four-way DNA junctions (2, 37), and single-strand breaks or gaps (5).

E. coli hup mutants are sensitive to γ irradiation, and in vitro studies show that HU protects DNA against cleavage by γ rays (3). This finding suggests that HU may play a role in DNA repair or in a mechanism of tolerance to DNA damage. There are several pathways for DNA repair or DNA damage tolerance in *E. coli*. The pathway(s) in which a specific gene is involved can be inferred by studying the phenotypic consequences of the interactions of the specific mutant gene with other mutant genes whose functions in DNA repair or damage tolerance have been well documented (4).

To determine in which pathway of DNA repair or damage tolerance the *hup* gene is involved, we examined the interactions between *hup* and other genes whose functions in DNA repair and/or damage tolerance have been well documented. It appears that a deficiency in homologous recombination renders *hup* mutants UV sensitive.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used are listed in Table 1. Standard phage P1 transduction, performed as described by Sternberg and Maurer (47), was used for the construction of different *hup* mutants. Plasmid transformation was performed by the cold $CaCl_2$ method as described by Sambrook et al. (42). Bacteria were grown in Luria broth (LB) medium and on LB agar. Tetracycline and chloramphenicol were used at 10 μg/ml. Kanamycin, erythromycin, and ampicillin were used at 50 μg/ml. Isopropyl-β-D-thiogalactopyranoside (IPTG) was used at 1 mM.

UV survival. Strains were grown in LB to mid-log phase (optical density at 600 nm [OD_{600}] ≃ 0.5) and serially 10-fold diluted in 1% NaCl; 50 μl of the diluted cell suspension was spread onto each of three LB agar plates per UV dose. To induce overexpression of wild-type or mutated plasmid-borne *gyrB*, 1 mM IPTG was included in the plates. To test the effect of the gyrase inhibitor novobiocin on UV sensitivity, 0 to 60 μg of novobiocin per ml was included in the plates. Under dimmed yellow light, the plates were irradiated with various doses of 254-nm UV and incubated in the dark for 24 or 48 h before the colonies were counted. The survival values given are the means of two to four independent experiments.

RESULTS AND DISCUSSION

UV sensitivity of *hup* strains. Although HU is not essential to *E. coli*, cells lacking HU have multiple deficiencies (18, 50). Moreover, it has been shown that *hup* mutants are sensitive to γ irradiation (3). As the types of damages induced by γ and UV irradiations are different, and mechanisms for repair of these damages are also different, we tested if *hup* mutations render cells UV sensitive. As shown in Fig. 1, *hup* mutations render the cells UV sensitive in *rec*$^+$ *sbc*$^+$, *recBC sbcA*, and *recBC sbcBC* backgrounds.

To test whether the UV sensitivity observed in *hupAB* mutants is directly due to the absence of HU and not due to the consequence of secondary mutations, which accumulate in the *hup* double mutants to compensate for the absence of HU (18), we introduced into *hupAB* mutants plasmid pYK20, carrying the *hupA* gene encoding HUα (21). It has been shown that plasmids bearing the *hupA* or *hupB* gene can restore the γ-ray resistance of *hupAB* strains to nearly the wild-type level (3). The production of α2 homodimers by plasmid pYK20 also

TABLE 1. *E. coli* strains and plasmids used

Strain or plasmid	Relevant genotype[a]	Other markers[b]	Source or reference
E. coli strains			
AB1157	*hup*$^+$	A	B. J. Bachmann
AQ9947	*recG162*	A	14
BH200	*uvrA*::Tn*10*	A	S. Boiteux
BT125	*recD1011*	B	39
EC8	*uvrA6*Δ(*umuDC*)*596*::*ermGT*	C	9
JR1669	*hup*$^+$	A	As AB1157; J. Rouviere-Yaniv
JR1670	*hupA*::Cm	A	J. Rouviere-Yaniv
JR1671	*hupB*::Km	A	J. Rouviere-Yaniv
JR1672	*hupA*::Cm *hupB*::Km	A	J. Rouviere-Yaniv
JC7623	*recBC sbcBC*	A	R. G. Lloyd
JC8679	*recBC sbcA*	A	R. G. Lloyd
N1234	*recF143*	A	R. G. Lloyd
N2057	*ruv60*::Tn*10*	A	R. G. Lloyd
SL1012	*recBC sbcBC hupA*	A	JR1670(P1) × JC7623 to Cm^r
SL1013	*recBC sbcBC hupB*	A	JR1671(P1) × JC7623 to Km^r
SL1014	*recBC sbcBC hupAB*	A	JR1671(P1) × SL1012 to Km^r
SL1015	*recBC sbcA hupA*	A	JR1670(P1) × JC8679 to Cm^r
SL1016	*recBC sbcA hupB*	A	JR1671(P1) × JC8679 to Km^r
SL1017	*recBC sbcA hupAB*	A	JR1671(P1) × SL1015 to Km^r
SL1018	Δ(*umuDC*)*596*	A	EC8(P1) × JR1669 to Ery^r
SL1019	Δ(*umuDC*)*596 hupA*	A	JR1670(P1) × SL1018 to Cm^r
SL1020	Δ(*umuDC*)*596 hupB*	A	JR1671(P1) × SL1018 to Km^r
SL1021	Δ(*umuDC*)*596 hupAB*	A	JR1671(P1) × SL1019 to Km^r
SL1022	*uvrA hupA*	A	JR1670(P1) × BH200 to Cm^r
SL1023	*uvrA hupB*	A	JR1671(P1) × BH200 to Km^r
SL1024	*uvrA hupAB*	A	JR1671(P1) × SL1022 to Km^r
SL1025	*recF143 hupA*	A	JR1670(P1) × N1234 to Cm^r
SL1026	*recF143 hupB*	A	JR1671(P1) × N1234 to Km^r
SL1027	*recF143 hupAB*	A	JR1671(P1) × SL1025 to Km^r
SL1028	*recD1011 hupA*	B	JR1670(P1) × BT125 to Cm^r
SL1029	*recD1011 hupB*	B	JR1671(P1) × BT125 to Km^r
SL1030	*recD1011 hupAB*	B	JR1671(P1) × SL1028 to Km^r
SL1031	*recG162 hupA*	A	JR1670(P1) × AQ9947 to Cm^r
SL1032	*recG162 hupB*	A	JR1671(P1) × AQ9947 to Km^r
SL1033	*recG162 hupAB*	A	JR1671(P1) × SL1031 to Km^r
SL1034	*ruvA60 hupA*	A	JR1670(P1) × N2057 to Cm^r
SL1035	*ruvA60 hupB*	A	JR1671(P1) × N2057 to Km^r
SL1036	*ruvA60 hupAB*	A	JR1671(P1) × SL1034 to Km^r
SL1037	*recG162 ruvA60*	A	N2057(P1) × AQ9947 to Tc^r
SL1038	*recG162 ruvA60 hupA*	A	N2057(P1) × SL1031 to Tc^r
SL1039	*recG162 ruvA60 hupB*	A	N2057(P1) × SL1032 to Tc^r
SL1040	*recG162 ruvA60 hupAB*	A	N2057(P1) × SL1033 to Tc^r
SL1041	*rus-1 ruvA60 hupA*	A	JR1670(P1) × TNM759 to Cm^r
SL1042	*rus-1 ruvA60 hupB*	A	JR1671(P1) × TNM759 to Km^r
SL1043	*rus-1 ruvA60 hupAB*	A	JR1671(P1) × SL1041 to Km^r
TNM759	*rus-1 ruvA60*	A	34
Plasmids			
pYK20	*hupA*		21
pAG111	*gyrB*		12
pCC205	$gyrB_{His\text{-}136}$		6
pCC206	$gyrB_{Cys\text{-}136}$		6

[a] After the first full listing, insertions are abbreviated to the gene symbol plus allele number.
[b] A, *thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2 hisG4 rpsL31 xyl-5 mtlL thi-1 argE3*; B, as A but *arg*$^+$; C, as A but *arg*$^+$ *ilv-325*.

increased the resistance of the *hupAB* mutants to UV to nearly the level of wild-type cells (Fig. 1a). The introduction of the same plasmid into wild-type cells caused no change in UV sensitivity (data not shown). This result suggests that the UV sensitivity of the *hup* mutants is directly caused by the absence of HU.

***hup* and *uvrA* interact synergistically.** HU is involved in the compaction of genomic DNA (40); the lack of HU can cause a topological change of DNA (1, 16, 17, 33, 41, 48). Therefore, it is possible that lack of HU can change the DNA UV photochemistry and the excision repair of UV photoproducts. We analyzed the induction and repair patterns of cyclobutane pyrimidine dimers (CPDs) at the nucleotide level in the replication origin *oriC*, in the mRNA genes *lacI* and *lacZ*, and in the tRNA gene *tyrT*. Almost identical patterns of CPD induction and removal were observed in wild-type and *hupAB* strains (references 26 and 27 and data not shown). Using a CPD-specific monoclonal antibody, we measured the induction and removal of CPDs in bulk genomic DNA. Again, no apparent difference was seen between wild-type and *hupAB* cells (data

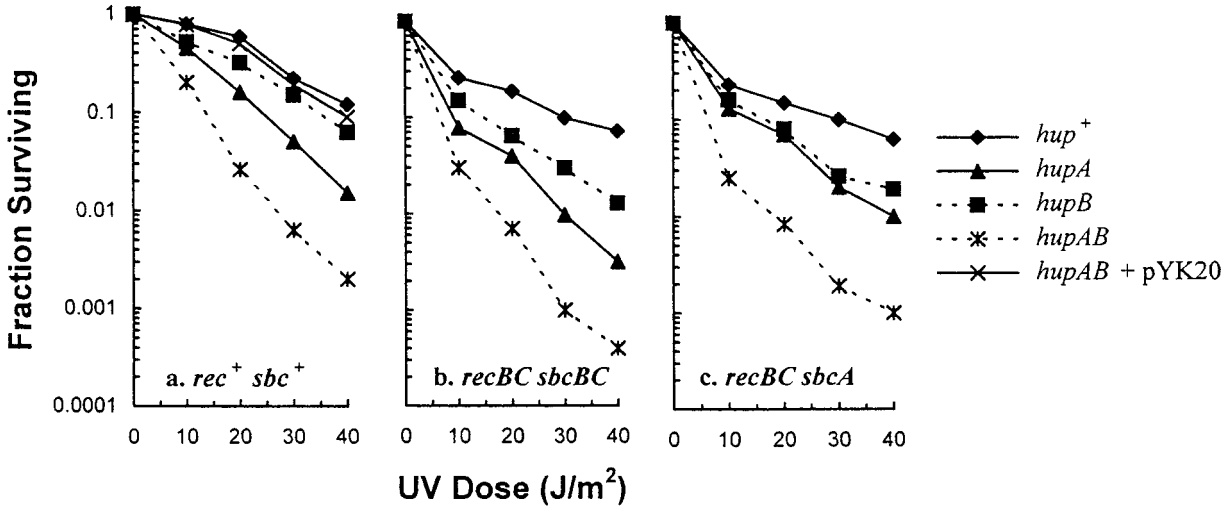

FIG. 1. Effects of *hup* mutations in different genetic backgrounds on sensitivity to UV. Strains were grown in LB to log phase ($OD_{600} \approx 0.5$), diluted in 1% NaCl, and irradiated on the surface of LB agar plates. Surviving colonies were scored after 24 h of incubation in the dark. The strains identified by genotype were JR1669, JR1670, JR1671, JR1672, and JR1672 transformed with plasmid pYK20 (a), JC7623, SL1012, SL1013, and SL1014 (b), and JC8679, SL1015, SL1016, and SL1017 (c).

not shown). These results suggest that the UV sensitivity of *hup* mutants is not caused by the change of UV photoproduct induction or a deficiency in nucleotide excision repair.

To further test if *hup* genes are involved in nucleotide excision repair, we examined the interaction between *hup* and *uvrA*, one of the genes essential for nucleotide excision repair in *E. coli* (for a review, see reference 43). As shown in Fig. 2d, the *hupAB uvrA* triple mutant is supersensitive to UV. It should be noted here that due to the high UV sensitivity of *uvrA* mutants, the applied UV doses are lower than those for the strains described in the adjacent graphs. The killing associated with the doses given to the triple mutant is much greater than the sum of the killing achieved by *uvrA* mutation plus the incremental killing by *hupAB* double mutations; i.e., *hup* and *uvrA* interact synergistically. Specifically, after a 3-J/m^2 dose of UV, the surviving fraction for *uvrA* is 0.0021, whereas for the *uvrA hupAB* strain it is 0.00017. Based on this result and those obtained from the direct measurement of CPD removal, we conclude that HU is not involved in nucleotide excision repair.

***hup* mutations markedly enhance the UV sensitivity of *umuDC* strains.** Reduced tolerance to DNA damage renders cells sensitive to the damaging agents. One of the known DNA damage tolerance mechanisms is via translesion synthesis, in which the *umuDC* operon has an indispensable role (reviewed in reference 11). We tested the interaction between mutations of *hup* and *umuDC*. As shown in Fig. 2c, the UV killing of the *hupAB umuDC* strain is roughly the sum of the killing in the *umuDC* mutant plus the increased killing by *hupAB* mutations over the level for wild-type strains. This finding suggests that *hup* and *umuDC* genes are involved in independent pathways for DNA damage tolerance or repair. In other words, the UV sensitivity caused by *hup* mutations is not due to the deficiency in translesion synthesis.

***hup* mutations do not enhance the UV sensitivity of resolvase-deficient *recG ruvA* strains.** *hupAB* mutations do not curtail the rapid SOS response (3). The γ-ray sensitivity of *hup* mutants may result from the lack of sufficient protection of the chromosomal DNA from radiation, as shown by in vitro experiments (3). Alternatively, this sensitivity may be due to the deficiency in the repair of γ-ray-induced double-strand breaks, which is achieved by homologous recombination (24). It has been shown that *hupAB* mutants are deficient in homologous recombination (7, 19). Our results, which are similar to those of Dri et al. (7), showed that *hupAB* caused a two- to fivefold reduction in P1 transduction and conjugational recombination in the *rec*$^+$ *sbc*$^+$, *recBC sbcA*, and *recBC sbcBC* backgrounds (data not shown). As shown above, *hupAB* interact with *uvrA* synergistically, as is typical for a mutation that blocks recombinational repair (15, 29, 30). The fact that *hupAB* mutations do not enhance the γ-ray sensitivity of *recA* strains (3) also supports the idea that HU is involved in recombinational repair.

To investigate the possible role of HU in recombinational repair, we first tested the effect of *hup* mutations on the UV sensitivity of resolvase-deficient *recG ruvA* strains. We constructed the *hup recG ruvA* strains by introducing the *ruvA60*::Tn*10* insertion into the *hup recG* strains, and substitution of the wild-type *ruvA* with *ruvA60*::Tn*10* was confirmed by Southern blot analysis (data not shown). As shown in Fig. 2h, *hup* mutations do not enhance the UV sensitivity of the resolvase-deficient *recG ruvA* strains. For example, after a 3-J/m^2 dose of UV, the surviving fraction of the *recG ruvA* strain is 0.009, whereas for the *recG ruvA hupAB* strain it is 0.01. This lack of synergism or additivity cannot be due to the high UV sensitivity of the *recG ruvA* strain. The result obtained with the even more UV sensitive *uvrA* strain described above, where synergism was observed, excludes this interpretation. Surprisingly, the *hupAB recG ruvA* strains are slightly more viable (data not shown) and slightly more UV resistant than *recG ruvA* strains (Fig. 2h). These results suggest that the UV sensitivity of *hup* mutants is indeed due to a defect in recombinational repair.

***hup* mutations confer different phenotypes on *recG* and *ruvA* strains.** RuvAB with RuvC and RecG with RusA provide two overlapping pathways for processing Holliday junctions (28, 32, 34, 44). To test the role of HU in the two pathways, we analyzed the interaction of *hup* with *recG* and *ruvA*. *hupAB recG* strains are far more sensitive to UV than *hupAB* or *recG* strains (Fig. 2f), while *hupAB ruvA* strains are slightly more sensitive than *ruvA* strains (Fig. 2g). This finding indicates that *hup* mutations mainly hinder the recombinational pathway in which Holliday junctions are processed by RuvABC. To exclude the

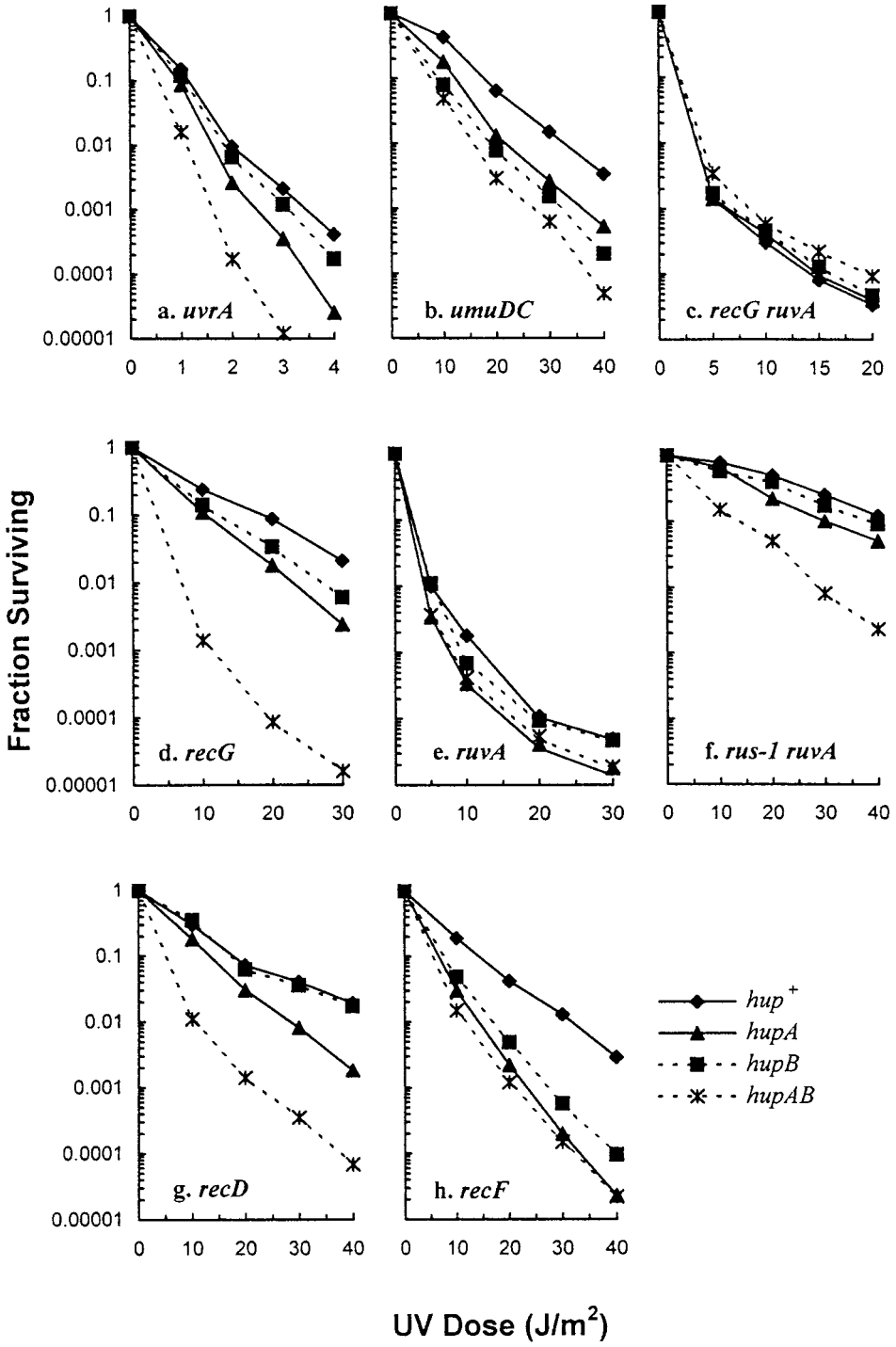

FIG. 2. Interactions in terms of UV sensitivity between *hup* and other genes. Strains were grown in LB to log phase ($OD_{600} \approx 0.5$), diluted in 1% NaCl, and irradiated on the surface of LB agar plates. Surviving colonies were scored after 24 h of incubation in the dark. The strains identified by genotype were BH200, SL1022, SL1023, and SL1024 (a), SL1018, SL1019, SL1020, and SL1021 (b), SL1037, SL1038, SL1039, and SL1040 (c), AQ9947, SL1031, SL1032, and SL1033 (d), N2057, SL1034, SL1035, and SL1036 (e), TNM759, SL1041, SL1042, and SL1043 (f), BT125, SL1028, SL1029, and SL1030 (g), and N1234, SL1025, SL1026, and SL1027 (h).

possibility that the interrupted genes were inserted into the recipient genomes rather than substituted for the normal genes during phage P1 transduction, we constructed the *hup ruvA* strains by introducing *ruvA60*::Tn*10* into the *hup* strains and by introducing *hupA*::Cm and *hupB*::Km into the *ruvA* strains. Substitutions of the wild-type genes with the interrupted genes were confirmed by Southern blot analysis (data not shown). The strains constructed showed the same interactions between *hup* and *ruvA*. Moreover, the *hupAB ruvA* strains were just as sensitive to UV as *hupA ruvA* strains (Fig. 2g). Additional *hupB* mutations in the *hupA ruvA* strains did not render the cells more UV sensitive. Presumably the β2 homodimers can-

not compensate for the function of the αβ heterodimers in *hupA ruvA* strains.

The fact that *hup* mutations hinder mainly the RuvABC pathway may result from the fact that *rusA* is poorly expressed in *rus*$^+$ strains in such a way that the RecG RusA pathway contributes to recombinational repair less than does the RuvABC pathway (32, 34, 44). To test this, we introduced the *hup* mutations into a *rus-1 ruvA* strain. By activating the expression of *rusA*, the *rus-1* mutation can completely suppress the recombinational deficiency of *ruvA* strains (32, 34, 44). As shown in Fig. 1 and 2e, *hup* mutations caused the same increase in UV sensitivity in the *rus-1 ruvA* strain as in *rus*$^+$ *ruv*$^+$ strains. This finding suggests that HU affects the RuvABC and RecG RusA pathways equally, provided that the two pathways are equally active in recombinational repair.

In which step(s) of recombinational repair is HU involved? The recombinational repair of UV-induced DNA damages is thought to occur by a mechanism termed postreplication repair. Two major types of postreplication repair processes exist; one repairs daughter strand DNA gaps, and the other repairs double-strand breaks generated from the unrepaired daughter strand gaps (51–53). Daughter strand gap repair depends on RecF (51, 52). Double-strand break repair depends mainly on RecB but to a minor extent on RecF (51–53). The RecBCD enzyme initiates DNA unwinding at double-strand DNA ends, and its nuclease activity is controlled by Chi sites in such a way that the enzyme produces a potent single-stranded DNA substrate for homologous pairing (for a review, see reference 46). However, the repair deficiency of *recB recC* mutants can be suppressed by secondary mutations in either the *sbcA* or *sbcB* locus, and in each case the suppression can be rationalized in terms of an effect on the generation of 3′ ends (for reviews, see references 23 and 54). *sbcA* mutations activate exonuclease VIII, which digests double-stranded DNA ends to produce long 3′ tails, and this could provide an alternative means of producing the invasive 3′ ends. *sbcBC* mutations inactivate exonuclease I, which digests single-stranded DNA from the 3′ end, so that its inactivation might leave 3′ ends available to initiate recombinational repair. The *hupAB* mutants do not degrade their DNA after UV irradiation any more extensively than wild-type strains (data not shown), although it has been proposed that the specific binding of HU to the DNA single-strand breaks or gaps may have a role in protecting these region from further degradation by endonucleases (5). The findings that *hupAB* mutations render *rec*$^+$ *sbc*$^+$, *recBC sbcA*, and *recBC sbcBC* (in which the recombinational substrates are generated by different mechanisms) strains sensitive to UV (Fig. 1) and that the UV sensitivity of the *recD* strain is also enhanced by the *hup* mutations (Fig. 2a) suggest that HU is unlikely to be involved in the substrate processing step of recombinational repair.

The products of *recF*, *recO*, and *recR* function together to facilitate synapsis during recombinational repair (49). Our results show that *hupAB* mutations greatly enhance the UV sensitivity of *recF* strains (Fig. 2b), indicating that HU is not involved in the synapsis action directed by RecFOR. Interestingly, in a *recF* background, *hupA* or *hupB* single mutations caused a considerable increase in UV sensitivity (Fig. 1 and 2), while *hupA hupB* double mutations led to no significant increase (Fig. 2b). Presumably, as in the *ruvA* background (see above), α2 or β2 homodimers cannot substitute for the function of the αβ heterodimers in the *recF* background. Experiments in vitro (38) showed that HU actually inhibits RecA-promoted pairing of homologous DNA molecules. Whether HU has a role in the synapsis actions that are not directed by RecFOR needs to be elucidated.

Our results concerning the interaction in terms of UV sensitivity between *hup* and the genes involved in homologous recombination, together with the fact that *hup* mutants are also sensitive to γ irradiation (3), suggest that the function of HU in recombinational repair lies in the common step(s) for double-strand break repair and daughter strand gap repair. It is quite likely that the common step is that of Holliday junction processing, since *hup* mutations do not cause an increase in the UV sensitivity of resolvase-deficient *recG ruvA* strains. If this is the case, the interaction between HU and the resolvases is unlikely to be a direct protein-protein contact, since *hup* mutations affect both RecG RusA and RuvABC pathways. Further work is needed to determine exactly in which step(s) or action(s) during recombinational repair HU is involved.

The UV sensitivity of *hup* mutants cannot be suppressed by overexpression of wild-type *gyrB* or *gyrB* mutations that confer novobiocin resistance. In prokaryotes, the degree of supercoiling is determined by the relative activities of at least two enzymes, DNA gyrase and topoisomerase I. DNA gyrase activity leads to increased negative supercoiling of the DNA, while topoisomerase I activity relaxes the DNA (for reviews, see references 8 and 31). Although lacking topoisomerase activity, HU may contribute to DNA topology. In vitro, HU bends DNA and wraps it into nucleosome-like structures (40). A small amount of relaxation was seen in DNA extracted from HU-deficient cells (41). *hupAB* mutants show a growth deficiency (18, 33, 50) and are hypersensitive to the gyrase inhibitor novobiocin. These phenotypes of *hupAB* strains may result from the relaxation of chromosomal DNA, as they can be suppressed by overexpression of the wild-type *gyrB* gene or by *gyrB* mutations that confer novobiocin resistance (33). This notion is supported by the observation that DNA supercoiling increased toward wild-type levels in the presence of *gyrB* suppressors (33). We wondered whether the UV sensitivity of *hup* mutants can also be suppressed by overexpression of the wild-type *gyrB* gene or by the *gyrB* mutations that confer novobiocin resistance. To test this, plasmid pAG111, which bears the *tac* promoter-controlled wild-type *gyrB* gene (12), was used to transform the wild-type and *hupAB* strains. The *hupAB* strains transformed with pAG111 formed large, uniform colonies if the cells were cultured on agar plates containing 1 mM IPTG. Variable sizes of colonies formed if the same cells were cultured on plates that did not contain IPTG (data not shown). These results indicate that the heterogeneous colony phenotype of *hupAB* strains is indeed suppressed by overexpression of the *gyrB* gene. However, UV sensitivity was virtually unchanged for both the wild-type and *hupAB* strains by inducing the overexpression of the wild-type *gyrB* gene borne on plasmid pAG111 (data not shown).

To test if *gyrB* mutations that confer resistance to novobiocin can suppress the UV sensitivity of *hupAB* strains, we isolated a number of novobiocin-resistant clones from *hupAB* strains by picking up large colonies from LB plates containing 150 μg of novobiocin per ml. The clones formed large, uniform colonies, but none showed increased resistance to UV (data not shown). We also transformed wild-type and *hupAB* strains with pAG111 derivatives pCC205 and pCC206 that bear mutant *gyrB* genes conferring novobiocin resistance (6). pCC205 bears the *gyrB* gene with a CG-TA transition at position 407, and pCC206 bears the *gyrB* gene with a GC-AT transition at position 406 (6). Again, no increased UV resistance was seen in the *hupAB* strains by inducing the overexpression of the mutant *gyrB* genes with IPTG (data not shown).

The UV sensitivity of *hup* mutants cannot be suppressed by different concentrations of gyrase inhibitor that can increase or decrease the supercoiling of the chromosomal DNA. Ben-

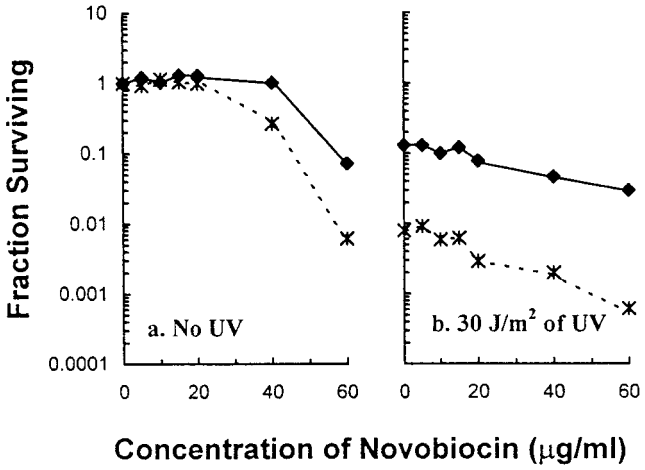

FIG. 3. Effects of the DNA gyrase inhibitor novobiocin on the UV sensitivity of wild-type (JR1669; ◆) and *hupAB* (JR1672; ∗) strains. Strains were grown in LB to log phase ($OD_{600} \approx 0.5$), diluted in 1% NaCl, and spread on the surface of LB agar plates containing 0 to 60 μg of novobiocin per ml. Surviving colonies were scored after 48 h of incubation in the dark. (a) Survival curves of unirradiated cells; (b) survival curves of UV-irradiated cells (the survival fractions are expressed as those obtained from the UV-irradiated plates divided by those obtained from the unirradiated plates containing the corresponding concentrations of novobiocin).

said et al. (1) showed that a decrease in the intracellular concentration of HU is accompanied by an increase in the relaxing activity of topoisomerase I; the ability to increase relaxing activity, or to decrease an excess of supercoiling, is important for cells to survive in the absence of HU. It is proposed that the absence of HU, like the removal of histones, results first in the excess of DNA supercoiling which must be removed by topoisomerase I activity for the cells to survive. Contrary to the scenario suggested by Malik et al. (33), the relaxation of the chromosomal DNA is proposed to be beneficial to the *hupAB* cells.

To determine whether the excess of unconstrained supercoiling due to the lack of HU is linked to UV sensitivity, we used the DNA gyrase inhibitor novobiocin. It has been shown that low levels (about 12.5 μg/ml for novobiocin) of gyrase inhibitors induce gyrase production, leading to a net increase in negative supercoiling of DNA, but higher levels reduce DNA supercoiling (10, 35). To measure the sensitivity of wild-type and *hupAB* strains to the gyrase inhibitor, cells were spread onto LB plates containing 0 to 60 μg of novobiocin per ml, and the plates were incubated for 48 h at 37°C before the colonies were counted. As shown in Fig. 3a, the *hupAB* strains were moderately sensitive to higher concentrations of novobiocin. To test the effect of DNA topological changes induced by novobiocin on UV sensitivity, the cells were also spread onto LB plates containing novobiocin (0 to 60 μg/ml), irradiated with UV (30 J/m^{-2}), and incubated for 48 h at 37°C. To determine the cell killing caused by novobiocin, the survival fractions were expressed as those obtained from the UV-irradiated plates divided by those obtained from the unirradiated plates containing the corresponding concentrations of novobiocin. As shown in Fig. 3b, the survival fraction for both wild-type and *hupAB* strains to the fixed dose of UV slightly decreased as the concentration of novobiocin increased. However, the curves of UV survival for the wild-type and *hupAB* strains are almost parallel within the range of novobiocin concentrations used (Fig. 3b), indicating that the UV sensitivity of *hupAB* strains cannot be suppressed by increasing or decreasing the supercoiling of the chromosomal DNA.

Negative supercoiling of intracellular DNA has been thought to be partitioned into two compartments, one of which comprises restrained supercoils and is different from the free superhelical tension affected by DNA gyrase (8). Part of the restrained compartment may be due to the action of HU in supercoiling and/or constraining supercoils by either direct or indirect interaction with DNA (48). It has been shown that a gyrase inhibitor or overexpression of gyrase can affect only the compartment of DNA supercoiling that is not restrained by HU (48). Our observations that the UV sensitivity of *hup* mutants cannot be suppressed by overexpression of *gyrB* or by different concentrations of novobiocin may be due to the compartment of DNA supercoiling restrained by HU being actually unchanged by these treatments. Alternatively, the deficiency in recombinational repair caused by *hup* mutations has nothing to do with the change of DNA supercoiling at all.

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