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Viability of *rep recA* Mutants Depends on Their Capacity To Cope with Spontaneous Oxidative Damage and on the DnaK Chaperone Protein

MARIE-FLORENCE BREDÈCHE,† S. DUSKO EHRLICH, AND BÉNÉDICTE MICHEL*

Laboratoire de Génétique Microbienne, Institut National de la Recherche Agronomique, Domaine de Vilvert, F-78352 Jouy en Josas Cedex, France

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Replication arrests due to the lack or the inhibition of replicative helicases are processed by recombination proteins. Consequently, cells deficient in the Rep helicase, in which replication pauses are frequent, require the RecBCD recombination complex for growth. *rep recA* mutants are viable and display no growth defect at 37 or 42°C. The putative role of chaperone proteins in *rep* and *rep recA* mutants was investigated by testing the effects of *dnaK* mutations. *dnaK756* and *dnaK306* mutations, which allow growth of otherwise wild-type *Escherichia coli* cells at 40°C, are lethal in *rep recA* mutants at this temperature. Furthermore, they affect the growth of *rep* mutants, and to a lesser extent, that of *recA* mutants. We conclude that both *rep* and *recA* mutants require DnaK for optimal growth, leading to low viability of the triple (*rep recA dnaK*) mutant. *rep recA* mutant cells form colonies at low efficiency when grown to exponential phase at 30°C. Although the plating defect is not observed at a high temperature, it is not suppressed by overexpression of heat shock proteins at 30°C. The plating defect of *rep recA* mutant cells is suppressed by the presence of catalase in the plates. The cryosensitivity of *rep recA* mutants therefore results from an increased sensitivity to oxidative damage upon propagation at low temperatures.

Interconnections between DNA replication and homologous recombination have been observed in a number of organisms and are likely to play an important role in the maintenance of genome integrity (reviewed in references 19, 21, and 29). An additional link was found with the observation that recombination enzymes act in *Escherichia coli* to rescue blocked replication forks (40). *rep* mutants were used to study the fate of replication forks upon blockage. The *rep* mutation causes a slow progression of chromosomal replication forks which suggests the occurrence of frequent pauses (6, 23). Because the Rep helicase is able to displace a DNA-bound protein in vitro, it was proposed that in vivo Rep could facilitate chromosomal replication by dislodging DNA-bound proteins from the path of the replication forks (28, 46).

rep mutants require the recombination complex RecBCD for viability (43), suggesting a link between replication fork arrest and homologous recombination. RecBCD initiates homologous recombination of linear DNA and is therefore essential for the repair of DNA double-strand breaks. It binds to DNA double-strand ends and opens while simultaneously degrading the DNA. Upon encounter with a specific sequence named CHI, RecBCD promotes the formation of single-stranded DNA recognized by RecA (reviewed in references 20, 25, and 33). *rep recBC* mutant lethality results from the occurrence of RuvABC-dependent DNA double-strand breaks (30, 40). The RuvAB proteins bind to Holliday junctions and catalyze branch migration. The RuvAB-bound DNA is cleaved by RuvC, which resolves the recombination intermediates by introducing nicks in strands of opposite polarity (reviewed in reference 44). To account for the action of RuvABC at blocked replication forks, a model was proposed in which, upon replication arrest, a Holliday junction forms by annealing of the two nascent strands (40). In the absence of RecBCD, resolution of the RuvAB-bound DNA by RuvC leads to chromosomal breakage. In cells proficient for homologous recombination, reincorporation of the double-strand tail formed by replication fork reversal into the chromosome allows replication restart from a recombination intermediate. However, *rep recA* mutants defective for homologous recombination are viable. The viability of *rep recA* mutants depends on the exonuclease V activity of RecBCD (40, 43); therefore, we proposed that in *rep recA* mutants RecBCD may degrade the double-strand tail formed by replication fork reversal, allowing replication restart from a Y-structure.

In this work we further analyzed the properties of the *rep recA* double mutant. We tested the effects of *dnaK* mutations on the viability of *rep recA* mutants. DnaK is a member of the Hsp70 family of stress-induced proteins which are highly conserved in procaryotes and eucaryotes (1). It is one of the major chaperone proteins induced by a shift to a high temperature in *E. coli*. Like several other chaperone proteins, DnaK is involved in processes that protect cells against various stresses and plays a role in DNA replication (reviewed in reference 12). We report that *dnaK* mutations that do not affect the viability of wild-type strains affect the growth of *rep* mutants and are lethal in *rep recA* double mutants. This indicates that *rep recA* mutants depend on DnaK for growth.

A peculiar property of the *rep recA* mutants remains unexplained. Liquid cultures grown to exponential phase at 30°C exhibit a defect in plating efficiency (43). A normal plating efficiency is spontaneously recovered when cells reach the end of exponential phase or if cells are grown at 37 or 42°C. The

* Corresponding author. Mailing address: Génétique Microbienne, INRA, 78352 Jouy en Josas Cedex, France. Phone: (33) 1 34 65 25 14. Fax: (33) 1 34 65 25 21. E-mail: bmichel@biotec.jouy.inra.fr.

† Present address: Génétique Moléculaire Evolutive et Médicale, Medical faculty-Necker Enfants Malades, E9916 INSERM, 75730 Paris Cedex 15, France.

TABLE 1. Strains

Strain	Relevant genotype	Construction or origin
CAG9270	Wild type (C600)	C. Gross
CAG9271	*dnaK756* derivative of CAG9270	C. Gross
GY9701	*recA938*::*cat miniF-kan-recA*$^+$	R. Devoret
K1019	*rep71 ilv*::Tn*10*	N. Zinder
MC1061	Wild type	M. Casadaban
TS154	*dnaK306* derivative of MC1061	C. Gross
JJC16	Δ(*recA-srl*)::Tn*10*	Laboratory stock
JJC40	Wild type (AB1157 *hsdR*)	Laboratory stock
JJC213	Δ*rep*::*kan*	Reference 38
JJC356	Δ(*recA-srl*)::Tn*10* Δ*rep*::*kan*	JJC213 ∗ P1 (JJC16)
JJC489	*rep71 ilv*::Tn*10*	JJC40 ∗ P1 (K1019)
JJC502	*recA938*::*cat rep71 ilv*::Tn*10*	JJC489 ∗ P1 (GY9701)
JJC526	Δ*rep*::*kan*	MC1061 ∗ P1(JJC213)
JJC527	Δ(*recA-srl*)::Tn*10*	MC1061 ∗ P1(JJC16)
JJC528	*dnaK306* Δ*rep*::*kan*	TS154 ∗ P1(JJC213)
JJC529	*dnaK306* Δ(*recA-srl*)::Tn*10*	TS154 ∗ P1(JJC16)
JJC530	Δ*rep*::*kan* Δ(*recA-srl*)::Tn*10*	JJC526 ∗ P1(JJC16)
JJC531	*dnaK306* Δ*rep*::*kan* Δ(*recA-srl*)::Tn*10*	JJC528 ∗ P1(JJC16)
JJC546	*dnaK756* Δ(*recA-srl*)::Tn*10*	CAG9271 ∗ P1(JJC16)
JJC547	*dnaK756* Δ*rep*::*kan*	CAG9271 ∗ P1(JJC213)
JJC633	Δ(*recA-srl*)::Tn*10*	C600 ∗ P1(JJC16)
JJC634	Δ*rep*::*kan*	C600 ∗ P1(JJC213)
JJC636	Δ*rep*::*kan* Δ(*recA-srl*)::Tn*10*	JJC634 ∗ P1(JJC16)
JJC677	*dnaK756* Δ*rep*::*kan* Δ(*recA-srl*)::Tn*10*	JJC547 ∗ P1(JJC16)

best characterized origin of double-strand breaks is the presence of oxidative compounds, a natural consequence of aerobic growth. DNA is the primary site of lethal damages (16, 17, 24). As a first line of defense against oxidative stress, bacterially encoded catalases and superoxide dismutases prevent the accumulation of reactive oxygen species (reviewed in references 8 and 10). A second line of defense is a set of DNA repair enzymes. DNA damages include mainly base modifications and DNA single-strand and double-strand breaks (24; reviewed in reference 17). Consequently, cells that lack enzymes required for recombinational or base-excision DNA repair pathways (RecA, RecB, PolA, Xth) are killed by low doses of H_2O_2 (16). In contrast, the *rep* mutants are not more sensitive to H_2O_2 than wild-type strains (16). We explored the reason for the plating defect of *rep recA* mutant cells grown at 30°C. The plating defect was not suppressed by overexpression of heat shock proteins at 30°C, whereas it was suppressed by the presence of catalase in plates, suggesting that it results from oxidative damage.

MATERIALS AND METHODS

Strains and media. Strains used in this work are described in Table 1. *dnaK756* carries three mutations in *dnaK* (31). *dnaK306* is a single mutant (45). Strains were constructed by P1 transduction. *rep, recA*, and *rep recA* derivatives of the *dnaK306* and *dnaK756* mutants were constructed at 30°C, and the isogenic *rep recA* double mutants were constructed at 37°C (with an MC1061 background for the *dnaK306* mutant and a C600 background for the *dnaK756* mutant, in contrast with the AB1157 background used for Fig. 3). Transductants obtained by introduction of the Δ*rep*::*kan* allele in the *dnaK756* mutant originally exhibited variable plating efficiencies at 40°C, but acquired during propagation at 30°C the capacity to form about 100% small colonies at 40°C in 24 to 48 h. One such clone, which was a *rep* (defective for M13 replication) and *dnaK756* (defective for λ growth) mutant but which may have acquired a compensatory mutation facilitating its propagation at 30°C, was used to perform the experiments reported below. All other double mutants were obtained with the expected efficiency and had the expected phenotype (see below). P1 transduction of the Δ(*recA-srl*)::Tn*10* mutation in the *rep dnaK306* mutant strain led to very few clones, and only one Δ(*recA-srl*)::Tn*10* clone was obtained with the *rep dnaK756* mutant strain. This may result from the poor plating efficiency of these strains (see below). Since *rep recA dnaK* mutants exhibited the expected phenotype (UV sensitive, with an M13 replication defect and a λ growth defect), they were used for further experiments. The Rep$^-$ phenotype was verified by transformation of $CaCl_2$ competent cells with M13mp2 DNA on a lawn of Hfr indicator strain, and *recA* mutants were verified by measuring their UV sensitivity. The *dnaK756* mutation was verified as preventing growth of wild-type λ phages and replication of the mini-F plasmid. The *dnaK306* mutation was verified as preventing mini-F replication. Plasmids pMob45 (*dnaKJ* carried by pMob vector; McMacken Laboratory) and pNRK416 (*dnaK* under lacUV5 promoter control; C. Gross Laboratory) were provided by Marie-Agnes Petit, and pCG179 (p*tac*12H*rpoH*$^+$) was provided by Philippe Bouloc (42). Plasmid pDWS2, a pBR322 derivative carrying the *recBCD* region (35), was provided by G. Smith. Cells were grown in LBT medium (Luria broth [LB] supplemented with 25 mg of thymidine per ml). Sigma catalase from bovine liver was used at a final concentration of 150 U per ml.

Micrographs of bacteria. Cell samples were fixed with 4% paraformaldehyde phenylindole (Sigma, St. Louis, Mo.), deposited on the slides, dried, colored with 4,6-diamidino-2-phenylindole (DAPI; Sigma) (2.5 μg/ml), and added directly to glycerol–phosphate-buffered saline mountant (Citifluor Ltd., Canterbury, United Kingdom). Photographs of cells were taken using an epifluorescence microscope (Nikon) with Sensia 400 film (Fuji). The slides were scanned and processed with the Adobe Illustrator, 7.0, program (Edinburgh, Scotland).

RESULTS

***dnaK* mutations decrease the plating efficiencies of *rep recA* mutants at 30°C.** We studied the effects of mutations of the major heat-induced chaperone protein, DnaK, in *rep, recA*, and *rep recA* mutants. Two *dnaK* mutants, TS214 (*dnaK306*) and CAG9271 (*dnaK756*), that grow at 40°C were used (45). Either *rep, recA*, or both *rep* and *recA* null mutations were introduced in these two strains as well as in isogenic *dnaK*$^+$ strains (see Material and Methods; Table 1). Cells were grown at 30°C to exponential phase and plated at the same temperature (Table 2). *dnaK306* and *dnaK756* mutations decreased the plating efficiency of *rep* mutant cells three- to fivefold in exponential and stationary phase. The term plating efficiency is used here as the ratio of CFU to optical density (OD). This ratio depends on (i) the average size of the cells (OD measures cell mass per ml) and (ii) the ability of individual cells to give rise to a colony. To further analyze the contribution of cell filamentation to the plating defect of these mutants, cells in exponential growth were examined by fluorescent microscopy and DAPI staining. Microscopic observations indicated that filamentation participates in the loss of plating efficiency for *rep dnaK756* mutant cells (Fig. 1, compare panel g with panels a and c), whereas *rep dnaK306* mutant cells were not significantly more elongated than *dnaK306* single mutants (data not shown). *recA* single mutants presented a 20 to 50% plating defect compared to isogenic *recA*$^+$ cells, as expected (3) (Table 2). In *recA* mutant cells, the *dnaK306* mutation decreased the plating efficiency twofold without inducing significant filamentation (Table 2; data not shown), whereas the *dnaK756* mutation had little effect (Table 2; Fig. 1e).

The combination of *rep* and *recA* mutations decreased the plating efficiency of C600 cells in exponential phase and not in stationary phase, as previously observed in an AB1157 background (43) (compare CAG9270 and JJC636 in Table 2). The plating efficiency of the MC1061 *rep recA* mutant (JJC530) was higher (23% of that of the isogenic wild-type strain MC1061 [Table 2]), suggesting that the plating defect of *rep recA* mutants grown to exponential phase may depend on the cellular background. The plating efficiency of *rep recA* mutant cells was further decreased three- to sevenfold by *dnaK306* or *dnaK756* mutations, in part because of filamentation, since *dnaK756* induced in *rep recA* mutant cells a level of filamentation similar

TABLE 2. *rep recA dnaK* mutant cells present a plating defect at 30°C[a]

Strain	Mutant genotype	Exponential cultures		Overnight cultures	
		CFU/ml	Relative value	CFU/ml	Relative value
MC1061	Wild type	$1.1 \times 10^8 \pm 3.5 \times 10^7$	1	$3.8 \times 10^9 \pm 7 \times 10^7$	1
JJC526	*rep*	$1.3 \times 10^8 \pm 1.1 \times 10^7$	1.2	$2.8 \times 10^9 \pm 4.7 \times 10^8$	0.74
JJC527	*recA*	$4.7 \times 10^7 \pm 1.5 \times 10^7$	0.43	$2.4 \times 10^9 \pm 2.5 \times 10^8$	0.63
TS154	*dnaK306*	$1.4 \times 10^8 \pm 5.8 \times 10^7$	1.3	$2 \times 10^9 \pm 2 \times 10^8$	0.5
JJC529	*dnaK306 recA*	$2 \times 10^7 \pm 1.3 \times 10^7$	0.18	$1.2 \times 10^9 \pm 8 \times 10^8$	0.3
JJC528	*dnaK306 rep*	$4 \times 10^7 \pm 3.7 \times 10^7$	0.36	$6.6 \times 10^8 \pm 2.5 \times 10^8$	0.17
JJC530	*rep recA*	$2.4 \times 10^7 \pm 1.8 \times 10^6$	0.23	$8.7 \times 10^8 \pm 3.7 \times 10^8$	0.22
JJC531	*dnaK306 rep recA*	$3.4 \times 10^6 \pm 4.4 \times 10^6$	0.031	$2.9 \times 10^8 \pm 1.4 \times 10^8$	0.07
CAG9270	Wild type	$1.3 \times 10^8 \pm 1.8 \times 10^7$	1	$1.8 \times 10^9 \pm 8 \times 10^8$	1
JJC634	*rep*	$8 \times 10^7 \pm 3.4 \times 10^7$	0.61	$1.6 \times 10^9 \pm 1.4 \times 10^8$	0.9
JJC633	*recA*	$1 \times 10^8 \pm 2.3 \times 10^7$	0.77	$2.5 \times 10^9 \pm 2 \times 10^8$	1.4
CAG9271	*dnaK756*	$1.4 \times 10^8 \pm 4.5 \times 10^7$	1.1	$1.3 \times 10^9 \pm 2 \times 10^8$	0.72
JJC546	*dnaK756 recA*	$7 \times 10^7 \pm 3.4 \times 10^7$	0.53	$9.5 \times 10^8 \pm 3.4 \times 10^8$	0.52
JJC547	*dnaK756 rep*	$2.8 \times 10^7 \pm 2.3 \times 10^7$	0.21	$5 \times 10^8 \pm 5 \times 10^7$	0.28
JJC636	*rep recA*	$4.5 \times 10^6 \pm 2 \times 10^6$	0.03	$4 \times 10^8 \pm 3 \times 10^8$	0.22
JJC677	*dnaK756 rep recA*	$1.2 \times 10^6 \pm 1.2 \times 10^6$	0.009	$1.4 \times 10^7 \pm 9 \times 10^6$	0.008

[a] Results are the average of three or four independent experiments. Cells were grown at 30°C and plated at the same temperature. Cells in exponential phase were harvested at an OD of 0.3 to 0.6 (most often at 0.4 to 0.5). Saturated cultures reached an OD of 1.5 to 4, depending on the strain. In order to compare the different cultures, results normalized for an OD of 0.4 (exponential cultures) or 2 (overnight cultures) were calculated.

to that observed in *rep* mutant cells (Fig. 1g) and *dnaK306* induced a lower level (data not shown). In *rep recA* double mutants, the defect in the ability to form colonies is not accompanied by filamentation (data not shown). Interestingly, plating efficiency was not improved in overnight cultures of the *rep recA dnaK756* mutant, suggesting that DnaK may participate in the recovery of plating efficiency of the *rep recA* mutant upon saturation (compare JJC636 and JJC677 overnight cultures in Table 2).

Taken together, these results show that (i) growth of *rep*, and to a lesser extent, *recA* mutant cells is affected at 30°C by *dnaK756* or *dnaK306* mutations (Fig. 1g; Table 2, compare *recA* with *recA dnaK306* mutants) and (ii) both *dnaK306* and *dnaK756* mutations affect the growth of *rep recA* mutant cells at 30°C.

***rep recA dnaK* mutants are thermosensitive for growth.** To measure the capacity of the various strains to form colonies at a high temperature, overnight cultures were plated at 30 and 40°C (Table 3). For all strains the number of colonies was similar at both temperatures, with the exception of the two *rep recA dnaK* mutants, which did not form colonies at 40°C (Table 3). This shows that in the two *dnaK* mutants tested, the *rep recA* combination of mutations renders cells thermosensitive for growth.

The plating efficiency of *rep dnaK* and *recA dnaK* mutants was not affected by temperature (Table 3). However, at 40°C colonies were smaller than those of single *dnaK* mutants (Fig. 2). Microscopic observations showed that *dnaK756* mutant cells were mildly elongated at 42°C (Fig. 1d). The *recA* mutation increased the number and the length of the filaments. For unknown reasons, it also increased the condensation of nucleoids (Fig. 1f). The *rep dnaK756* mutant was found to be strongly filamentous (Fig. 1h). In contrast, *dnaK306* single mutants (TS154) were more elongated at 42°C than *dnaK756* mutants (data not shown), and when introduced in the *dnaK306* mutant, the *rep* and *recA* mutations had a mild effect on cell morphology. In conclusion, our results indicate that both *recA* and *rep* mutants require a physiological concentration of chaperone proteins for normal growth and that this requirement is additive in *rep recA* mutant cells.

Loss of plating of the *rep recA* mutants at 30°C results from oxidative damage. The only noticeable growth defect of the *rep recA* mutant in laboratory conditions is a variable but significant defect in colony forming efficiency when cells in exponential phase at 30°C are plated on rich medium (43) (Table 2; Fig. 3). The plating defect is observed for liquid cultures propagated at 30°C regardless of the temperature at which the plates are then incubated. The low plating efficiency of the *rep recA* mutants is not observed when cells are grown at 37 or 42°C, suggesting that a higher temperature protects *rep recA* mutant cells. During growth at 42°C, the steady-state level of heat shock proteins is about twice as high as that at 30°C (12), which could be sufficient to restore a normal plating efficiency in *rep recA* mutants. In order to test whether the defect in the plating efficiency of AB1157 *rep recA* mutants at 30°C could be restored by an increased concentration in heat shock proteins, plasmids overexpressing DnaK (pNRK416) or DnaK and DnaJ (pMob-*dnaKJ*) were introduced in the *rep recA* mutant JJC356. Plating efficiency was not restored by the presence of either of these plasmids (data not shown). Hence, an increased level of DnaK and DnaJ is not sufficient to restore full viability of *rep recA* mutants. However, DnaK controls the expression of several other heat shock proteins (reviewed in reference 12). These proteins are repressed by overexpression of DnaK, and some of them may be required in *rep recA* mutant cells. The hypothesis that loss of colony-forming ability of *rep recA* mutant cells at 30°C is because of insufficient heat shock protein expression was therefore tested by overproduction of σ32, the *rpoH* gene product, which governs the heat shock response. The p*tac*12H*rpoH*$^+$ plasmid was used (42). Overexpression of σ32 did not restore the plasmid efficiency of the *rep recA* mutant (data not shown). In conclusion, increasing the level of heat shock proteins did not restore the plating efficiency of the *rep recA* mutant, suggesting that heat shock proteins are not at a limiting concentration at 30°C in the *rep recA* mutant cells.

The defect in plating of *rep recA* mutant cells could result

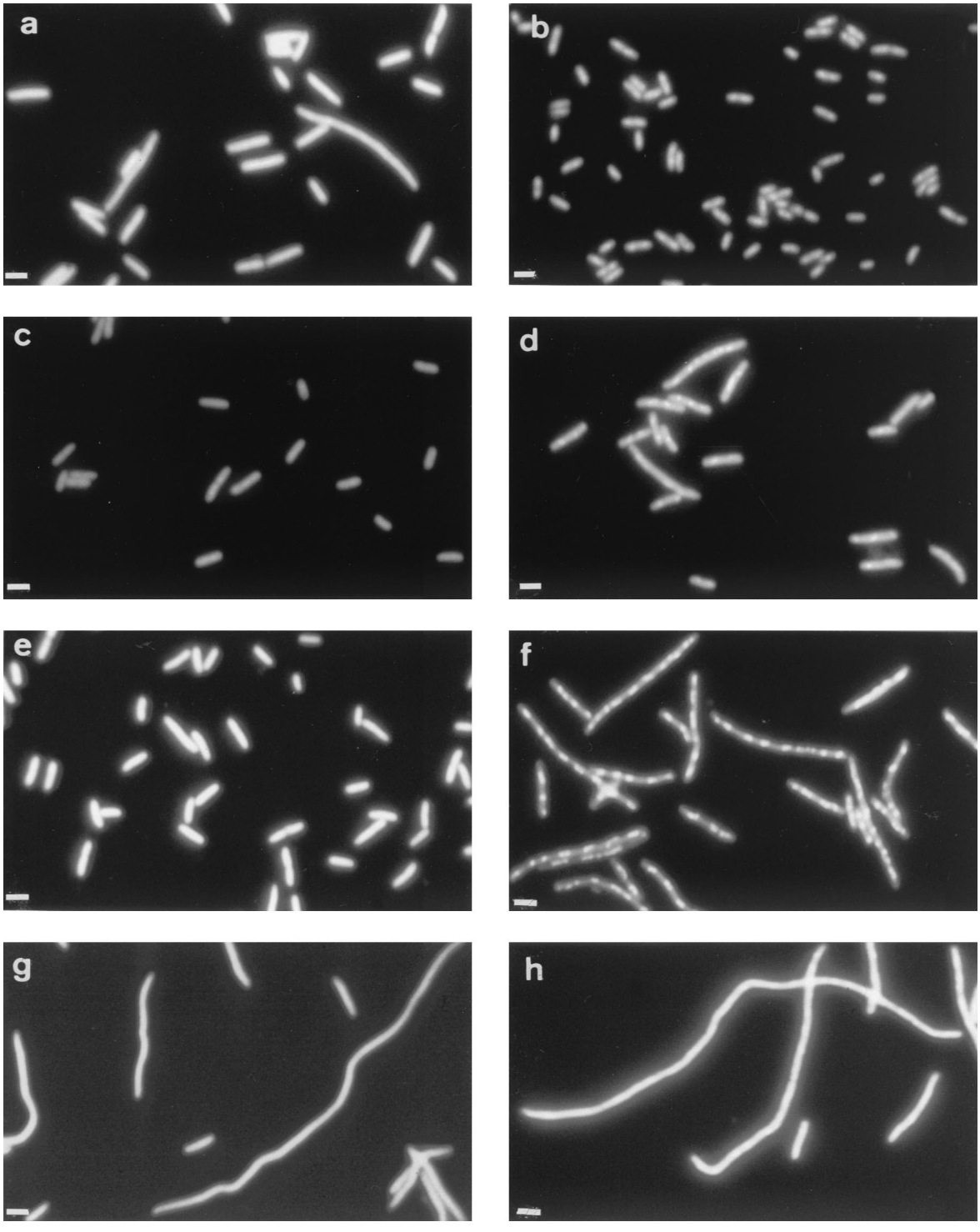

FIG. 1. Morphology of *dnaK756* derivatives. Cells were grown overnight at 30°C, diluted 100-fold, and grown for 3 h at either 30°C (a, c, e, g) or 42°C (b, d, f, h). JJC213 (*rep* mutant; a, b), CAG9271 (*dnaK756* mutants; c, d), JJC546 (*dnaK756 recA* mutant; e, f), and JJC547 (*dnaK756 rep* mutant; g, h) are shown. Wild-type cells were similar to JJC213 at 42°C and to CAG9271 at 30°C (data not shown). Bars, 1 μm.

from a limiting amount of an enzymatic activity essential for the viability of *rep recA* mutant cells other than that of heat shock proteins. RecBCD is a good candidate because (i) it is present in only 10 to 20 copies per cell (20), (ii) it is essential in *rep* mutants, and (iii) it is titrated out in cells defective for RecA due to the extensive DNA degradation that occurs in these cells (22, 41). A decrease in the amount of available RecBCD enzyme in vivo can be detected with the use of T4 phages deficient for the gene 2 product that protects linear T4 molecules against exonuclease V-mediated degradation upon

TABLE 3. *rep recA dnaK306* and *rep recA dnaK756* mutants are thermosensitive for growth

Strain	Mutant genotype	Plating efficiency[a]
MC1061	Wild type	0.9 ± 0.1
TS154	*dnaK306*	0.9 ± 0.1
JJC529	*dnaK306 recA*	0.6 ± 0.09
JJC528	*dnaK306 rep*	0.7 ± 0.3
JJC530	*rep recA*	1.1 ± 0.5
JJC531	*dnaK306 rep recA*	≤0.00046 ± 0.00012[b]
CAG9270	Wild type	0.8 ± 0.4
CAG9271	*dnaK756*	0.9 ± 0.09
JJC546	*dnaK756 recA*	1.1 ± 0.14
JJC547	*dnaK756 rep*	0.95 ± 0.03
JJC636	*rep recA*	1.1 ± 0.5
JJC677	*dnaK756 rep recA*	≤0.046 ± 0.046[b]

[a] Overnight cultures grown at 30°C were plated at 30 or 40°C. For each culture, the ratio of clones obtained at 40°C to those obtained at 30°C was calculated, and the results are expressed as the average of the ratios.

[b] A few clones were obtained at 40°C that were no longer thermosensitive, indicating that they probably have acquired a compensatory mutation that allows growth at a high temperature. In contrast, *rep recA dnaK* mutant clones obtained at 30°C were still thermosensitive.

infection (22, 38). As previously reported (22), we observed that infection by T4 2⁻ was increased by the presence of a *recA* mutation in the recipient cells. However, we found that it was not further modified by inactivation of *rep* and was not significantly influenced by temperature (data not shown). This suggests that *rep recA* mutant cells are not more deficient in exonuclease V activity at 30°C than at 42°C and are not more deficient than *recA* single mutants. To check more directly whether a decrease in the concentration of free RecBCD complexes could be responsible for the plating defect of *rep recA*

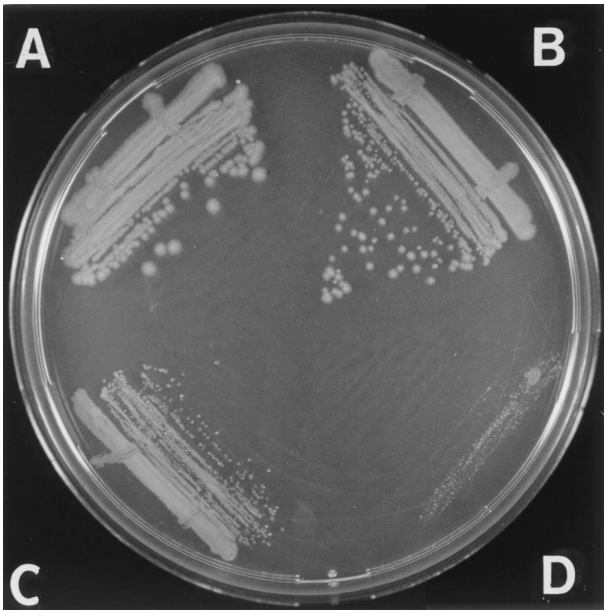

FIG. 2. Colony growth of *dnaK756* derivatives. CAG9271 (*dnaK756* mutant; A), JJC546 (*dnaK756 recA* mutant; B), JJC547 (*dnaK756 rep* mutant; C), and JJC677 (*dnaK756 rep recA* mutant; D) are shown. Overnight cultures grown at 30°C were streaked on LB agar thymine (LBAT), and plates were incubated overnight at 40°C. The size of *dnaK756* colonies was not significantly different from that of wild-type colonies at this temperature (data not shown).

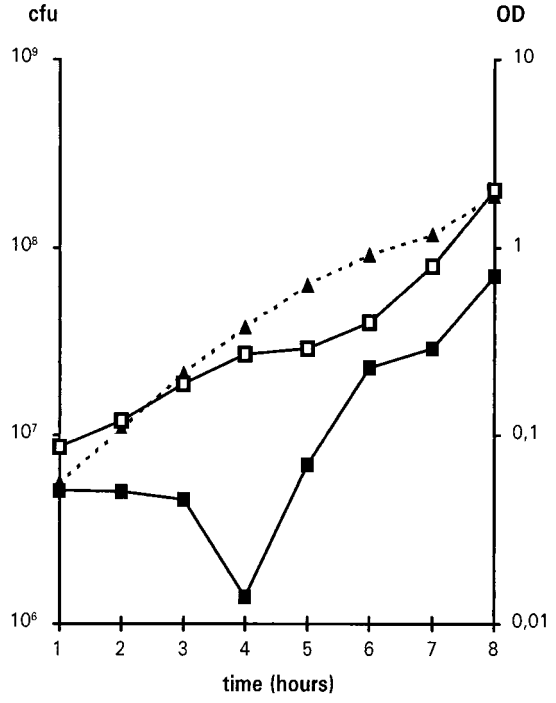

FIG. 3. Growth curves of *rep recA* mutant (JJC502) at 30°C. Overnight cultures grown at 37°C were diluted to an OD of 0.03, and cells were incubated in LB at 30°C with shaking. Every hour, the OD at 650 nm was measured, and 100 μl of an appropriate dilution was plated on LBAT plates with or without catalase to determine the number of CFU/ml. The defect of plating was variable, from a plateau to a 100-fold decrease in CFU, while the recovery on catalase-containing plates was always observed. An experiment in which the decrease was of intermediate level is shown. Triangles, OD at 650 nm; closed squares, CFU on LBAT; open squares, CFU on LBAT containing 150 U of catalase per ml.

mutants, we measured the plating efficiency of *rep recA* mutants containing the plasmid pDWS2, which carries the *recBCD* genes (35). Overexpression of RecBCD from this plasmid prevented growth of T4 2⁻ mutant phages on *rep recA* mutants as expected, while it did not revert the plating defect of the strain (data not shown). We conclude that a lack of RecBCD cannot be the reason for this plating defect.

Interestingly, exogenous catalase has been shown to improve the recovery of *E. coli* cells under various stress conditions, for example, heat-injured DNA-repair mutants (27). In addition, bacterial catalase does not protect isolated organisms but favors the survival of high-density and colonial *E. coli* (26). To test whether the low plating efficiency of the *rep recA* mutant could result from a defect in the repair of oxidative damage that occurs upon plating (26), we measured the capability of this strain to form colonies on catalase-containing plates (Fig. 3). The number of CFU was restored by the presence of 150 U of catalase/ml in the LB plates. This result indicates that the defect in colony formation results from oxidative damage due to the presence of compounds degraded by catalase. The oxidative damage occurred upon plating and not during exponential growth in liquid medium, as the presence of catalase in the culture did not rescue the cells plated on catalase-lacking plates and did not further enhance the plating efficiency on catalase-containing plates (data not shown). We conclude that

the low plating efficiency of the *rep recA* mutant propagated at low temperature could result from the additive effects of replication pauses due to the *rep* mutation and lesions due to the aerobic environment.

DISCUSSION

In this work, we studied the effects of *dnaK* mutations in *rep, recA*, and *rep recA* mutants and showed that two different *dnaK* mutations that do not prevent the growth of wild-type cells significantly impair the growth of *rep* and *rep recA* mutants. We also investigated the reasons for the cryosensitivity of *rep recA* mutants. We found that the presence of catalase in the plates relieves the plating defect of the strain, indicating that the defect in colony formation results from oxidative damage.

Role of DnaK in *rep* mutants. The combination of *rep* and *dnaK* mutations is sufficient to impair growth. *dnaK306* and *dnaK756* mutations decrease the plating efficiency of *rep* mutants three- to fivefold independently of the growth phase (Table 2) and independently of the temperature (Table 3). The strong filamentation induced by the combination of *rep* and *dnaK756* mutations is observed at low and high temperatures (Fig. 1g and h). It can be noted that the *rep* mutant cells are slightly elongated at 30°C, which may reflect their requirement for a high level of DnaK. The *rep* mutation affects the propagation of replication forks in *E. coli*, and the chaperone proteins DnaK and DnaJ play a role in the replication of several replicons. They are required for the initiation of F, P1, and lambda (reviewed in reference 5). In F and P1, they control the multimerization of the specific initiator protein encoded by the replicon. In λ, they appear to act by dissociating DnaB protein from the λ P protein, thereby allowing the helicase to act. They are also involved in *E. coli* chromosomal replication, where their role is less well understood. Deletion of DnaK causes temperature sensitivity for cell growth, abnormal cell division, and a reduced rate of replication at 30°C (2). *dnaK, dnaJ*, and *grpE* mutations cause a dramatic decrease in the level of epsilon, the proofreading subunit of DNA polymerase III, and a decrease in the apparent level of RNase H1 (9). DnaK and DnaJ are also required for the proper folding of UmuC, one of the subunits of DNA polymerase V (PolV) involved in replication restart from a lesion and lesion bypass (34, 37). The properties of the *dnaK756* and *dnaK306* mutants used in this work have been characterized (34, 45). These *dnaK* mutations prevent proteolysis (measured by degradation of the pyromycyl fragment) and mini-F plasmid replication. They differ in that the *dnaK756* mutant is deficient for λ growth and proficient for UmuC folding, whereas the *dnaK306* mutant has the opposite properties (34, 45). In *rep* mutants, chromosome replication is slowed down, which is compensated for by more replication forks per chromosome (6, 23). Replication pauses due to the Rep defect may be more frequent or longer in *dnaK* mutants, due to a role of chaperone proteins in the release of the obstacles that block replication forks in *rep* mutants. Alternatively, DnaK could facilitate replication restart after restoration of a replication fork by homologous recombination. In this case, as the defect due to the *rep dnaK* combination is also observed in *recA* mutants, DnaK would also participate in the restart of replication forks from arrested forks that have not recombined. Comparison of [^{3}H]thymidine incorporation under various conditions did not allow us to detect a defect in nucleotide incorporation in the *rep dnaK756* double mutant compared to the parental strains (data not shown). This suggests that the replication defect which causes the filamentation of the *rep dnaK756* mutant is, as in *rep* mutants, compensated by overinitiation at the origin. Altogether, this work supports the hypothesis that chaperone proteins play a role in the normal propagation of replication forks.

Plating defect and oxidative damage. The plating defect of the *rep recA* mutant indicates that lethal lesions occur in these cells upon cell isolation, and the recovery of plating efficiency upon addition of catalase in the plates shows that these lesions are caused by oxidative compounds. This is consistent with the observation that isolated cells are not protected against oxidative damage by their own catalase (26). This transient inactivation of the first line of defense, mediated by enzymes that destroy oxygen radicals, renders essential the action of the second line of defense, mediated by enzymes that repair lesions. Homologous recombination plays an essential role in this process. Oxidative lesions lead to the formation of single-strand and double-strand DNA breaks, known to be repaired by homologous recombination (4, 16). *recA* mutations also inactivate the induction of the SOS response, a set of genes involved in DNA repair. However, inactivation of SOS induction only in *rep* mutants has no effect on plating efficiency (43), suggesting that the defect in homologous recombination plays an essential role in the cryosensitivity of the *rep recA* mutant. In contrast with cells defective in homologous recombination, *rep* mutants are not more sensitive to H_2O_2 than wild-type strains (16); nevertheless, here *rep* mutations increase the sensitivity of *recA* mutants to oxidative stress. This suggests an additive effect of oxidative lesions and replication pauses due to the *rep* mutation. The *rep recA* mutant requires exonuclease V-mediated degradation for viability; however, we show here that the plating defect of *rep recA* mutant cells does not result from a lack of RecBCD. The plating defect was suppressed at 42°C; however, it was not suppressed by overexpression of heat shock proteins. The Rep helicase is very similar to the repair helicase UvrD (11), and *rep uvrD* double mutants are lethal. Interestingly, a mutation has been described in the *uvrD* gene (*uvrD307*) (47) that also specifically decreases the plating efficiency of certain wild-type strains of *E. coli* at 30°C. Whether the cryosensitivities of the *uvrD307* mutant and of *rep recA* mutants have a common origin remains to be determined.

DnaK and oxidative damage. A protective effect of heat shock proteins against oxidative damage has been previously documented in several studies. Heat shock proteins, including DnaK, are induced by H_2O_2 treatment (32; reviewed in reference 8). A *dnaK* null mutation increases the sensitivity of *E. coli* to H_2O_2 (7). We report here that *rep recA* mutant cells require the following for full viability: (i) DnaK and (ii) protection against oxidative damage. However, no direct link between these two observations could be found. If the low level of heat shock proteins at 30°C participates in the plating defect of *rep recA* mutant cells, it is not the only cause, since overproduction of heat shock proteins does not restore the plating efficiency. Conversely, the growth defect of *rep recA dnaK* mutant cells at high temperature does not result only from oxidative damage: plating on catalase-containing plates of either *rep recA dnaK* mutant increased 10- to 100-fold the number of

colonies at 40°C (data not shown); however, all colonies tested had acquired a suppressor mutation, suggesting that catalase only permits some residual growth that facilitates the acquisition of suppressor mutations, without restoring viability. Therefore, the observation that *rep* mutants are handicapped by either *recA* or *dnaK* mutations and that these handicaps are additive suggests that RecA and DnaK do different things that increase survival.

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