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Escherichia coli Mutants in Which Transcription Is Dependent on *recA* Function

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A gene essential for viability in *recA* mutants of *Escherichia coli* K-12 was identified. This gene, *rdg* for *recA*-dependent growth, is near 16 min on the *E. coli* chromosome between *phr* and *gltA* and is 90% cotransduced with *gltA*. In a strain with an *rdg* deletion and the temperature-sensitive *recA* allele, *recA200*, growth stopped within 7 min after cells were shifted to the nonpermissive temperature (42°C). The cells remained viable for many hours at 42°C. The defect at the nonpermissive temperature is in ribonucleic acid synthesis, which was completely shut off within 20 min after the temperature shift. Protein synthesis was also shut off, but deoxyribonucleic acid synthesis continued for at least 2 h after the shift. The *rdg* mutation alone had no apparent effect on growth, deoxyribonucleic acid repair, or recombination.

The product of the *recA* gene of *Escherichia coli* is a multifunctional protein with several enzymatic activities. Activities on DNA, including renaturing activity and pairing of double-stranded DNA with single-stranded fragments, probably represent the role of the *recA* protein in homologous recombination and may be related to its role in DNA repair (3, 15, 20, 23). The protein has a protease activity which cleaves the λ repressor and its own repressor, the *lexA* protein (2, 11, 19). This effect on repressors accounts for the role of *recA* in the induction of prophages and is the basis for the *recA* dependence of a number of inducible DNA repair processes which have been referred to as SOS functions (for reviews, see references 9 and 24). Evidence has also been found for an effect of the *recA* protein on ribosomal ambiguity (4, 17).

We report here evidence for yet another role of the *recA* protein, namely, a role in transcription. We have identified a genetic locus near 16 min on the map of *E. coli* which is essential for viability in *recA* mutants. We call this locus *rdg*, for *recA*-dependent growth. The simultaneous loss of *rdg* and *recA* function blocks transcription. A preliminary report of some of this work has been presented previously (B. Froehlich and W. Epstein, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, H17, p. 111).

Most of the strains used were derivatives of strain BF1000, which is *alaS5*, *his*, *nadA*, *metB*, *argI*, *rpsL*, and *lacZ*. Strains were constructed by P1 transduction of strain BF1000, introducing desired *recA* alleles by cotransduction with *alaS* and introducing mutations in the region of *rdg* by cotransduction with *nadA*. P1 transductions were performed by standard methods (5). Deletions in the *rdg* region were obtained in a selection for growth at 42°C of a strain in which λcI857 is inserted in the *kdp* gene (18). Thymine-requiring strains used for [^{3}H]thymine incorporation were made by selecting for resistance to trimethoprim (21). The complex growth medium, KML, and minimal media, K115 and KO, were made as described previously (6).

An *rdg* point mutation was obtained by using the Hong and Ames method of localized mutagenesis (10). A phage P1 lysate of a strain carrying a *gltA* mutation was concentrated to about 10^{11} phage per ml by centrifugation and suspended in K115 medium. The mutagenesis mixture contained 1 part phage suspension, 2 parts 0.5 M $NaPO_4$–0.005 M NaEDTA (pH = 6), 4 parts 1 M $NH_2OH \cdot HCl$–0.4 M NaOH, and 3 parts water. After incubation for up to 70 h at 37°C, the phage were pelleted by ultracentrifugation and suspended in ML broth (6) containing 1 M NaCl and 1 mM EDTA. The mutagenized phage stock was used to transduce strain BF1001 (*recA200 nadA*) to *nadA*$^+$. The *rdg* mutant was scored by the inability of the *recA200 rdg* strain to grow at 42°C, the nonpermissive temperature for *recA200* (12). One mutation, *rdg-2*, was found after scoring 1,300 *nadA*$^+$ transductants.

The discovery of the *rdg* gene resulted from our inability to construct *recA* derivatives of a strain carrying the *101* deletion which extends

from *kdp* to past *gltA* (Fig. 1). To test the idea that such a combination is inviable we constructed strains in which deletion *101* was combined with the high-temperature-sensitive *recA* alleles *recA44* (8) or *recA200* (12) or the cold-sensitive mutation *recA629* (23). The temperature-sensitive mutations resulted in strains which grew at 30°C but not at 42°C, whereas the cold-sensitive mutation resulted in a strain which grew at 42°C but which grew very poorly at 30°C. Thus, a locus deleted in *101* is essential for growth in the absence of *recA* function. Since results with the *recA200* allele were the clearest, we chose this mutation for subsequent studies.

In the selection for deletions in the *kdp* region, we obtained some ending in the interval between *kdp* and *gltA* as well as others extending at least into *gltA*. Youngs and Smith (25) used these deletions to confirm the location of *phr* between *kdp* and *gltA*. Analysis showed that deletions *207* and *214* were *rdg*$^+$, and deletion *235* was mutant for *rdg* (Fig. 1). This result places *rdg* between *phr* and *gltA*. The presumed point mutation *rdg-2* is 90% cotransduced with *gltA*, showing that *rdg* is very close to the latter marker.

The effect of shifting strain BF1014 (*recA200* Δ*rdg-101*) from 30 to 42°C is shown in Fig. 2. Within 7 min, growth, as measured by turbidity, stopped. The cells remained viable at 42°C for several hours without a significant change in turbidity. The number of viable cells remained constant for at least 7 h (data not shown). Upon shifting back to 30°C, growth resumed with, at most, a short lag even after 5 h at 42°C (Fig. 2). Similar results were obtained with *recA200* strains carrying Δ*rdg-235* or the *rdg-2* mutation. Control experiments with isogenic *recA200 rdg*$^+$ and *recA*$^+$ Δ*rdg-101* strains showed that growth continued at 42°C (data not shown).

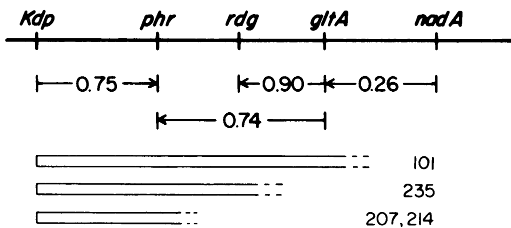

FIG. 1. *Map location of the rdg locus. Cotransduction frequencies of phr with kdp and with gltA are from Youngs and Smith (25). Other cotransduction data are from transduction of strain BF2000 (recA200 rdg-2 gltA) with a P1kc lysate of strain TKN2004 (nadA kdpA4 rha gal lacZ trkA). Arrows point from selected marker to unselected, scored markers. The rdg status of the deletions was scored by introducing them into a recA200 strain by cotransduction with nadA, testing for inheritance of the deletion by scoring for kdp, and testing for rdg by growth at 42°C.*

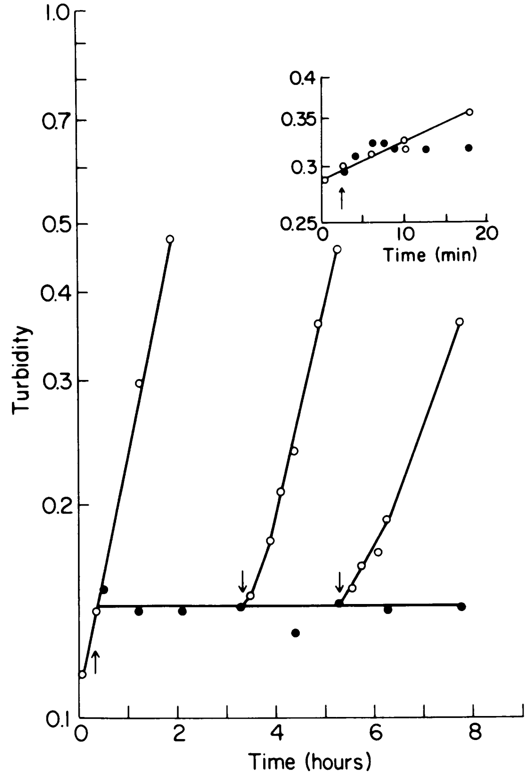

FIG. 2. *Growth of strain BF1014 (Δrdg-101 recA200) at 30 and 42°C. The strain was grown with shaking in KML medium at 30°C. At the time indicated by the upward-pointing arrow (↑), part of the culture was shifted to 42°C. At downward-pointing arrows (↓), portions were shifted back to 30°C. The inset shows in detail events immediately after the shift to 42°C. Turbidity was monitored as previously described (5). Symbols: ○, 30°C; ●, 42°C.*

The synthesis of DNA is not defective after the shift of strain BF1014 to 42°C. Incorporation of thymine continued for at least 2 h after the shift and at a slightly greater rate than at 30°C, even though growth stopped within 10 min (data not shown). The rate of RNA synthesis, measured as the amount of uridine incorporated in a 2-min pulse, began to drop in strain BF1014 without a perceptible lag after the shift to 42°C, and by 20 min, it had reached a plateau at approximately 30% that of the control (Fig. 3). This residual incorporation is over 90% alkali stable and therefore, at most, 10% RNA, indicating that RNA synthesis was almost completely arrested within 20 min of the shift to 42°C. As expected, protein synthesis in strain BF1014 also fell rapidly after the shift to 42°C (data not shown). RNA and protein synthesis

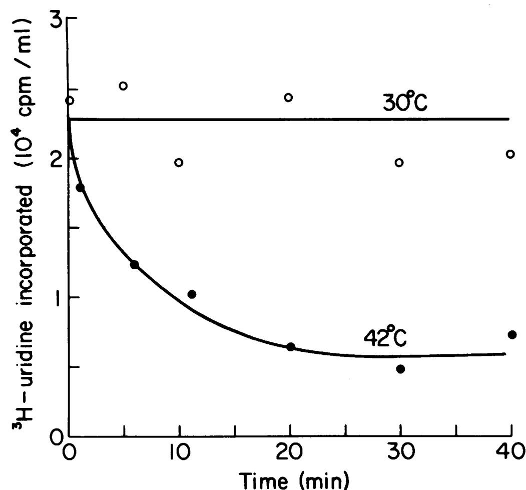

FIG. 3. *[^{3}H]uridine incorporation in strain BF1014 (Δrdg-101 recA200). The cells were grown at 30°C in minimal K115 medium containing 0.2% glucose and 0.2% Casamino Acids. When the culture reached 10^8 cells per ml, one-half was shifted to 42°C (0 min). [5-^{3}H]uridine (Schwartz/Mann) was added to samples of cells to achieve final concentrations of uridine of 5 μCi and 2 μg per ml. Incorporation was stopped after 2 min by spotting samples onto dried trichloroacetic acid-soaked Whatman 3-mm filters which were then washed in cold trichloroacetic acid, washed with ethanol, dried, and counted in a liquid scintillation counter. Symbols: ○, 30°C; ●, 42°C.*

were not inhibited by a shift to 42°C in an isogenic strain containing the shorter *rdg*$^+$ deletion *214* (data not shown).

We have not detected any phenotypic effect of *rdg* mutations in a *recA*$^+$ background. We compared *rdg* deletion mutants with isogenic *rdg*$^+$ strains and found no difference in growth rate, survival after UV irradiation, or proficiency in transductional and conjugal crosses (data not shown). Loss of *rdg* function alone is of little consequence to the cell.

Mutations at *rdg* result in a phenotype not previously described: no effect of *rdg* alone, but a block in transcription when the *recA* function is lost. The *rdg* locus is not near other known genes affecting transcription (1). The nearby *phr*, for photoreactivating enzyme (22), is almost certainly a distinct locus because we can separate it from *rdg* by deletions, and linkage of these two with *gltA* is different (Fig. 1).

At first glance, our results resemble those for the *polA* (7) and *dam* (13) mutations, which are also inviable when *recA* function is lost (12, 13, 16). However, the defect in the latter types of strains contrasts dramatically with ours. In the *polA* and *dam* cases, the primary defect appears to be in DNA metabolism. DNA synthesis continues for a time under the nonpermissive conditions, only to be overshadowed by DNA degradation (12, 14, 16). Protein synthesis and increase in cell mass also continue for a time; later, filamentation and extensive killing occur (12, 13, 16). In the *rdg* case, growth, protein synthesis, and RNA synthesis stop immediately upon the shift to the nonpermissive conditions, although the cells remain viable for many hours (Fig. 2 and 3). We infer that there is little, if any, breakdown of DNA because there was no loss of viability and because growth resumed without a lag upon return to the permissive temperature (Fig. 2).

To the known interaction of *recA* protein with DNA, we here add another role, that of promoting transcription. The specific nature of this interaction remains obscure. The formation of nicks in the DNA, a need for a specific type of helix-destabilizing function, or a specific interaction with RNA polymerase are among the possible functions that can be satisified by the *recA* or *rdg* products.

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