JOURNAL OF BACTERIOLOGY, July 1985, p. 407–409
0021-9193/85/070407-03$02.00/0

Vol. 163, No. 1

Evidence that the *recA441* (*tif-1*) Mutant of *Escherichia coli* K-12 Contains a Thermosensitive Intragenic Suppressor of RecA Constitutive Protease Activity

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Received 14 January 1985/Accepted 1 April 1985

The *recA441* mutant of *Escherichia coli*, which has been thought to have thermoinducible constitutive RecA protease activity, is known to have two mutations within *recA*. We show here that the mutation that alters codon 38 actually confers temperature-independent constitutive protease activity; the second mutation in *recA441*, which is at codon 298, appears to be acting as a temperature-sensitive suppressor of the protease activity.

The *recA441* mutant of *Escherichia coli*, formerly called *tif-1*, was first recognized by its property of thermally dependent induction of prophage lambda (3). Later it was found to show thermally induced lethal filamentation (7), which gave rise to the acronym *tif*. The phenotypic properties of *recA441* are due to constitutive RecA protease activity at elevated temperatures. It is shown here that this mutant is anomalous.

In a recent study by E. S. Tessman and P. K. Peterson (unpublished data), approximately 150 *recA* (Prt^c) mutants were isolated, in which the phenotypic designation Prt^c denotes constitutive protease *recA* mutants, i.e., mutants whose RecA protein is always in the activated protease state without the usual need for activation by damaged DNA. We use the term Prt^c to denote RecA constitutive protease activity at any growth temperature, without any implications of thermal inducibility. Thus, the *recA441* mutant is a Prt^c mutant at high temperature. It has recently been reported that RecA protein may not be a true protease but may merely enhance autodigestion of its substrate (9). Until this point is settled we will use the term RecA protease for convenience.

The new Prt^c mutants were isolated by mutagenesis of a λ *recA cIind* phage. The mutant phages were detected by their blue plaque color upon plating on a Δ*recA* strain that carried a *lac* fusion in a RecA-inducible gene. The Prt^c phenotype was measured quantitatively in terms of β-galactosidase specific activity after lysogenization of Δ*recA dinD*::*lac* (EST1515) with each λ *recA* (Prt^c) mutant phage. These new Prt^c mutants all differ from the original Prt^c mutant, *recA441*, in having constitutive protease activity at any growth temperature, whereas *recA441* is singular in requiring a shift to 41°C to express its constitutive protease activity (3, 7). In addition, many of the new Prt^c mutants have a constitutive protease activity that is considerably greater than that of *recA441* as measured by several indices of protease strength (Tessman and Peterson, unpublished data).

The *recA441* mutant has recently been shown by Knight et al. (8) by peptide analysis of its RecA protein to have two mutations in the *recA* gene. In the work of these authors it was not yet known which of the two mutations in *recA441* conferred constitutive protease activity or whether both mutations contributed to the *recA441* phenotype. The aim of the present report was to provide answers to these questions.

We have begun a DNA sequence analysis of a selected number of the new *recA* (Prt^c) alleles to identify effector-binding sites in the RecA protein. Initial results with six mutants show that each is altered in a single amino acid, namely amino acid 25, 38, 158, or 179, since each has only a single base-pair change from the wild type (unpublished data). The entire *recA* DNA was sequenced for each mutant and, except for the single base change, the sequence of each was identical with the sequence previously reported for

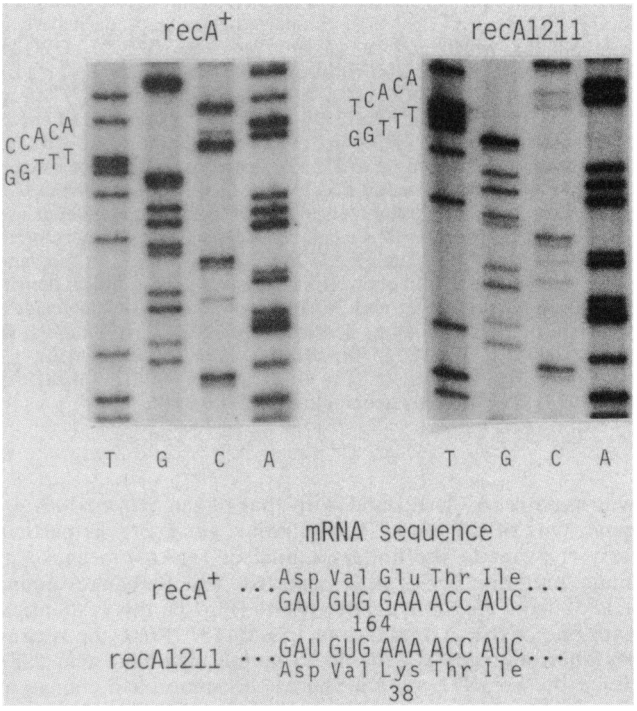

FIG. 1. Comparison of the portions of DNA-sequencing gels for *recA1211* (Prt^c) and *recA*$^+$ that show the change in base 164 (codon 38) that occurred in the DNA of *recA1211*. The standard Sanger dideoxy method was used.

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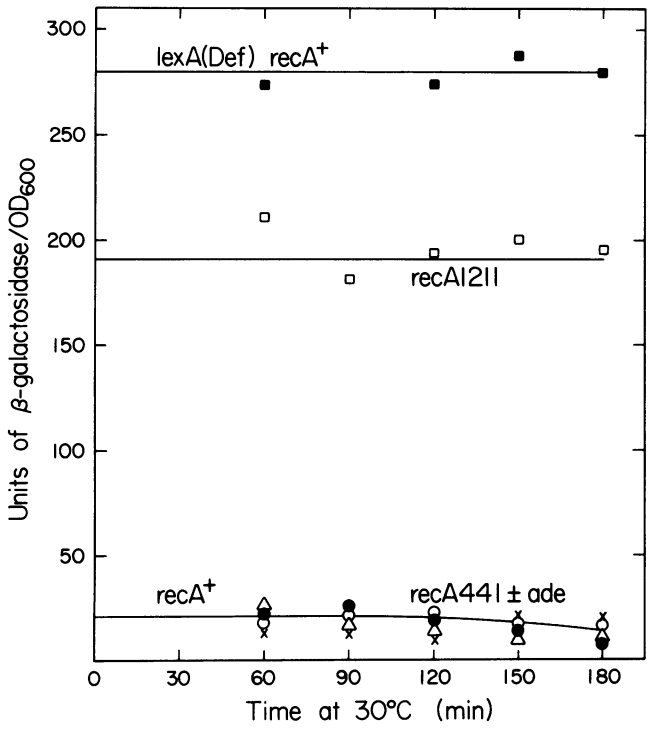

FIG. 2. Comparison of the RecA constitutive protease activity of *recA441* and *recA1211* measured by β-galactosidase specific activity expressed from the difficult-to-induce SOS gene *dinD* (6). Two *recA441 dinD* strains, almost completely isogenic except for the source of their *recA441* alleles, were used to ensure that the quantitative aspects of the *recA441* phenotype were reproducible. One of the *recA441* strains (EST1130) was derived by minor modifications of strain GC3217 *recA441 sulA11* (2), which is also the parent of the RecA-overproducing *recA441* strain used by Knight et al. (8). Strain EST1130 is a temperature-resistant derivative of GW1040 (6), which in turn was derived from GC3217. GW1040 carries *recA441 sfiA11 dinD*::Mu *cts* d(Ap *lac*). Strain EST1515 is the same as EST1130 but Δ*recA*. A second *recA441* strain (EST1018) is the same as EST1130 except that its *recA* gene comes from λ *recA441* cI857 (10) which was integrated into the chromosome of EST1515. Our *recA1211* (Prt^c) mutant is a stable lysogen constructed by lysogenizing EST1515 with λ *recA1211 cInd*. Cultures were grown overnight at 30°C in M9 minimal medium (11) supplemented with 0.5% Casamino Acids (Difco Laboratories, Detroit, Mich.) and thiamine and then diluted 50-fold in the same medium and grown until exponential phase, with the optical density at 600 nm (OD_{600}) being about 0.10 at time zero. β-Galactosidase was measured essentially as described by Miller (11). Symbols: ■, *lexA* (Def) *recA*$^+$ (EST1550); ×, *lexA*$^+$ *recA*$^+$ (EST1450); □, *recA1211*; ●, *recA441* (EST1130); ○, *recA441* (EST1018); △, *recA441* (EST1018) plus adenine (ade) (100 μg/ml).

wild-type *recA* (4, 12) and with that of our parental *recA*$^+$ gene. One of the *recA* (Prt^c) mutants, *recA1211*, is particularly relevant to the understanding of *recA441* because its single mutation is G → A at base 164, which changes amino acid 38 from glutamic acid to lysine (Fig. 1); this is identical with one of the two mutations in *recA441* (5, 8) (the second mutation changes isoleucine to valine at amino acid 298). Since the *recA441* mutant shares the same base change as the single mutant *recA1211*, it follows that the change in amino acid 38 must confer a constitutive protease phenotype on the *recA441* strain and that the change in amino acid 298 confers a temperature-sensitive modification of that phenotype. An independently isolated Prt^c mutant, *recA1249*, was also found to have the G → A mutation at base 164, suggesting that this site may be particularly vulnerable to mutagenesis.

There are clear qualitative differences between the phenotypes of *recA441* and *recA1211*. The *recA441* strain had no constitutive expression of *dinD* at 30°C, whereas the *recA1211* strain showed strong constitutive protease activity at this temperature (Fig. 2). Comparison of the strains at 41°C (Fig. 3) showed that the *recA441* strain had inducible protease activity, whereas the *recA1211* strain had constitutive protease activity.

Since the double mutant, *recA441*, differs from the single mutant, *recA1211*, by a single mutation that affects amino acid 298, we conclude that this mutation is a temperature-sensitive suppressor of the common mutant allele. At low temperatures the mutation in codon 298 eliminates the constitutive protease activity conferred by the change in codon 38; at high temperatures the suppressing activity disappears and the *recA441* strain becomes a constitutive protease mutant, similar to the single mutant but still showing marked phenotypic differences. The RecA regions around amino acids 38 and 298 may together form part of a single effector-binding site. Evidence has been presented that the N-terminal region of RecA protein is required for binding of single-stranded DNA (5).

It appears now that thermal activation of the *recA441* strain is an exceptional condition produced by the mutation which changes amino acid 298. This conclusion is further supported by the fact that when we isolated 50 λ *recA* (Prt^c) mutants by screening for a constitutive protease phenotype at 40°C, all turned out to have strong constitutive protease activity at 30°C also. The apparent thermal inducibility of *recA441* is therefore not typical of constitutive protease *recA* mutants, which is understandable in view of the occurrence of the second mutation that suppressed the constitutive protease activity at low temperature. It should be noted that at the time the *recA441* strain was isolated (3) the available conditions would not have permitted detection of constitutive protease *recA* mutants that are active at any growth temperature. This is because activated RecA protein induces

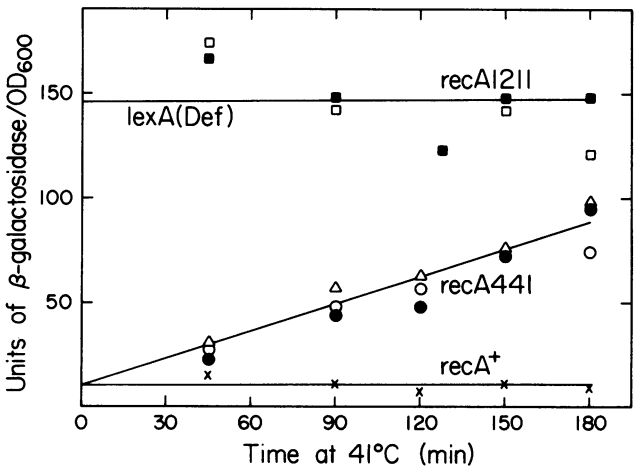

FIG. 3. Comparison of the RecA constitutive protease activity of *recA441* and *recA1211* at 41°C. Cultures were grown at 30°C and then shifted to 41°C at time zero. Procedures were as described in the legend to Fig. 2. Symbols: ■, *lexA* (Def) *recA*$^+$ (EST1550); ×, *lexA*$^+$ *recA*$^+$ (EST1450); □, *recA1211*; ○, ●, *recA441* (EST1130); △, *recA441* (EST1018).

the *sulA* and *sulC* genes, resulting in lethal filamentation (1, 2). For a *recA* (Prt^c) mutant to grow normally it must be defective in *sulA* and *sulC* (1, 2). The strains used here contained the mutation *sulA11* (2) and were SulC$^-$. This genetic background was not available when the *recA441* mutant was isolated, so only conditional Prt^c mutants could have been obtained.

This work was supported by Public Health Service grant AI-17566 from the National Institutes of Health to E.S.T.

We thank Irwin Tessman for his advice, for reading the manuscript, and for preparing Fig. 1.

LITERATURE CITED

1. **D'Ari, R., and O. Huisman.** 1983. Novel mechanism of cell division inhibition associated with the SOS response in *Escherichia coli*. J. Bacteriol. **156**:243–250.
2. **George, J. M., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
3. **Goldthwait, D., and F. Jacob.** 1964. Sur le mécanisme de l'induction du développement du prophage chez les bacteries lysogènes. C. R. Acad. Sci. **259**:661–664.
4. **Horii, T., T. Ogawa, and H. Ogawa.** 1980. Organization of the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:313–317.
5. **Kawashima, H., T. Horii, T. Ogawa, and H. Ogawa.** 1984. Functional domains of *Escherichia coli* recA protein deduced from the mutational sites in the gene. Mol. Gen. Genet. **193**:288–292.
6. **Kenyon, C. J., and G. C. Walker.** 1980. DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:2819–2823.
7. **Kirby, E. P., F. Jacob, and D. A. Goldthwait.** 1967. Prophage induction and filament formation in a mutant strain of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **58**:1903–1910.
8. **Knight, K. L., K. H. Aoki, E. L. Ujita, and K. McEntee.** 1984. Identification of the amino acid substitutions in two mutant forms of the *recA* protein from *Escherichia coli*: *recA441* and *recA629*. J. Biol. Chem. **259**:11279–11283.
9. **Little, J. W.** 1984. Autodigestion of lexA and phage λ repressors. Proc. Natl. Acad. Sci. U.S.A. **81**:1375–1379.
10. **McEntee, K.** 1977. Protein X is the product of the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5275–5279.
11. **Miller, J. H. (ed.).** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Sancar, A., C. Stachelek, W. Konigsberg, and W. D. Rupp.** 1980. Sequences of the *recA* gene and protein. Proc. Natl. Acad. Sci. U.S.A. **77**:2611–2615.