DNA REPLICATION BY PHAGE T_4 rII MUTANTS WITHOUT POLYNUCLEOTIDE LIGASE (GENE 30)

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Summary

In phage T4-infected E. coli, the polynucleotide ligase coded for by T4 gene 30 is not required for DNA replication and phage production when the infecting phage is an rII mutant. The wild-type T_4 rII gene product may be a nuclease or control a nuclease activity.

In phage T_4, gene 30 is the structural gene for polynucleotide ligase (1). This enzyme catalyses the repair of single-strand breaks in DNA duplexes (2-4). The role of the ligase *in vivo* is not clear; it may be involved in DNA replication (2,5,6), recombination (7,8), and repair of DNA after ultraviolet irradiation (9,10).

Under restrictive conditions, amber (am) and temperature-sensitive (ts) mutants of T_4 gene 30 show limited DNA synthesis (7,11). It will be shown that mutations in the rII gene of phage T_4 overcome the need for a gene 30 ligase. The results also suggest that the wild-type rII gene product is (or controls) a nuclease.

Materials and Methods

The E. coli strains BBstr^r, S/6, and K38(λ)str^r restrict the growth of T_4 am mutants. E. coli CR63 and E. coli K37(λ) are permissive (su^+_1, ser) for T_4 am mutants. The str^r strains are resistant to streptomycin.

The T_4 gene 30 mutations used were amH39X and tsA80. The mutation amB22 is in T_4 gene 43 (DNA polymerase). The rII system of phage T_4 and genetic mapping of rII mutations have been described by Benzer (12,13).

On E. coli S/6, T_4 rII mutants form large sharp-edged (r-type) plaques which can be easily distinguished from the smaller fuzzy-edged wild-type (r^+) plaques. The double mutants amH39X/rUV375, tsA80/rUV375, and amB22/rUV375 were constructed by genetic crosses and charactarized by genetic recombination and complementation. rUV375 is an ochre (UAA) mutation which maps in the early portion of the rIIB cistron.

Growth of bacterial and phage stocks and phage assays were as described by Steinberg and Edgar (14). The growth medium used was M9S supplemented with 20 μg tryptophan/ml (13). Thymidine-methyl-H^3 (H^3-TdR) was purchased from New England Nuclear Corp., Boston, Mass.

Results and Discussion

The T_4 gene 30 mutant amH39X plates at high efficiency on permissive (su^+) E. coli strains CR63 and K37(λ). The plating efficiency of this mutant on restrictive (su^-) hosts is very low (Table 1). Most of the plaques which do appear on E. coli S/6 are very small; of these, some (about 1%) are large r-type plaques. E. coli BBstr^r does not distinguish rII mutations, and all the plaques which appear on this strain have wild-type (r^+) morphology.

TABLE 1. Plating Efficiency of amH39X (Gene 30) on su^+ and su^- E. Coli Strains

The titre of an amH39X phage stock was determined on the various coli strains. The titre on E. coli CR63 was set at 100%. The values shown are based on plaque counts of at least 200 per assay.

E. coli Host	Plating Efficiency (%)
CR63	100
K37(λ)str^r	95
BBstr^r	0.02
S/6	0.01
K38(λ)str^r	0.002

Ten plaques picked at random from a plating of amH39X on E. coli BBstr^r were all amH39X/rII double mutants which had high replating efficiencies on both E. coli BBstr^r and E. coli CR63, but did not grow on E. coli K38(λ)str^r and E. coli K37(λ). On E. coli S/6 they produced mostly very small plaques and some (1-5%) large r-type plaques. Successive replating on E. coli S/6 resulted in the selection for large r-type plaques which had reverted at the amH39X site. This selection for am$^+$/rII does not occur on E. coli BBstr^r or E. coli CR63.

Thus, amH39X/rII double mutants can grow on certain bacterial strains (not lysogenic for λ) which normally restrict amH39X. The double mutants seem to occur at a frequency (Table 1) similar to the frequency of spontaneous r mutants in T_4 phage stocks (0.01-0.1%).

Fifty plaques (presumed amH39X/r) were picked at random from a plating of amH39X on E. coli BBstr^r and used to select am$^+$/r mutants by successive replating on E. coli S/6. Analysis indicated that one was rI (r-type plaques on E. coli K38(λ)str^r and S/6), two were rIII (r$^+$ plaques on K38 (λ)str^r and r-type plaques on S/6), and forty-seven were rII (no plaques on K38(λ)str^r and r-type plaques on S/6). The map pattern of the rII mutations resembled that of spontaneous rII mutants derived by Benzer (13); about 8% mapped in the rIIA spontaneous hot spot (r131) and 10% mapped in the rIIB spontaneous hot spot (r117). The rest consisted of two deletions, four UGA mutations (at three rIIB sites), one ochre (UAA) mutation in rIIB, and missense (or frameshift) mutations distributed between the two rII cistrons.

The growth of amH39X/rII double mutants on E. coli BBstr^r is not due to rII-related suppression of the am mutation since the double mutant tsA80/rUV375 will grow at 42°C whereas tsA80(rII$^+$) will not (Table 2). Also, a double mutant of rUV375 with amB22 (gene 43, DNA polymerase) will not grow on E. coli BBstr^r. These results suggest that the gene 30 polynucleotide ligase is dispensable when the rII gene is mutated. Possibly, rI and rIII mutations exert effects similar to those of rII.

TABLE 2. The Effect of Combining an rII Gene Mutation with Gene 30 Mutations.

The double mutants from crosses between rUV375 and gene 30 mutations form small plaques in spot tests on E. coli S/6 (at 42°C for tsA80/rUV375). They were further characterized by complementation mapping on E. coli K38(λ)str^r and by crossing back to wild-type phage and re-isolating the single mutations. The burst size (phage production per cell) was determined by the method of Champe and Benzer (15) except that NaCN was not used and M9S, instead of broth, was the growth medium. Growth temperatures were 30°C and 42°C. The values in parentheses are burst sizes at 42°C. Plaque assays were done on E. coli CR63 at 30°C.

	Burst Size on E. coli		
Phage Mutant	CR63	BBstr^r	K38(λ)str^r
amH39X(gene 30)	150	0.1	0.3
amH39X/rUV375	155	106	0.3
tsA80(gene 30)	140 (1.3)	200 (0.6)	86 (2)
tsA80/rUV375	170 (36)	190 (52)	0.1 (0.1)
amB22(gene 43)	110	0.1	0.1
amB22/rUV375	40	0.6	0.6
rUV375(rII gene)	188 (80)	116 (200)	0.3 (0.1)

Cultures of E. coli BBstr^r infected with amH39X/rUV375 were examined for abnormalities in phage DNA synthesis (Figure 1). The three mutants tested, amH39X/rUV375, amH39X(rII$^+$), and rUV375(am$^+$), all initiate DNA synthesis at about the same time after infection. However, amH39X/rUV375 and amH39X show slow synthesis for the first 20 min. Around this time, synthesis stops (DNA arrest) with amH39X, but rises to control (rUV375) rates with amH39X/rUV375. Similar results have been obtained on E. coli S/6. Preliminary experiments show that the rate increase with the double mutant can be abolished, if chloramphenicol (300 μg/ml) is added at 10 min (but not at 15 min) after infection.

Kozinski (16) showed that under restrictive conditions injected amH39X DNA undergoes nicking, double-strand scission and eventual

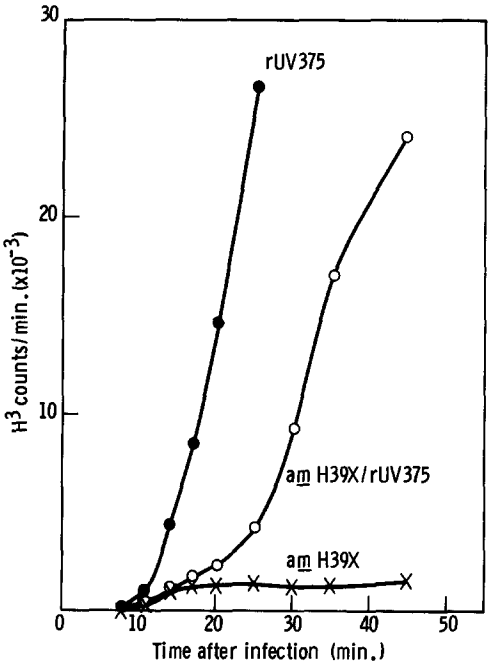

FIGURE 1. DNA Synthesis in amH39X/rUV375-Infected E. Coli BBstr^r.

Log phage cells ($1x10^8$) were infected with $1x10^9$ particles of the phage in question. After 5 min aeration at 30°C, the infected mixtures were diluted 6-fold into M9S containing 20 μc H^3-TdR and aerated at 30°C. The incorporation of H^3-TdR into DNA was measured as follows: samples (0.05 ml) of an incorporating culture were spotted onto 1 inch Whatman 3MM filter paper discs and the discs dropped into cold 10% trichloroacetic acid. They were then washed with 5% trichloroacetic acid, 95% ethyl alcohol, and ethyl ether, and the H^3 on the dried discs counted with a 1,4-Dioxane-based scintillator in a Beckman scintillation counter. The H^3-TdR was used at a specific activity of 20 μc H^3/μg TdR.

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breakdown to small fragments. Adding chloramphenicol at early times after infection prevents some of these events and results in productive DNA replication. Kozinski postulated that the gene 30 ligase compensates for the action of phage-induced nucleases.

The results here may mean that temporary damage to DNA occurs in amH39X/rUV375-infected cells and that this damage is repaired without a gene 30 ligase because the rII gene product is defective. The wild-type rII gene product may be a nuclease, or may control a nuclease activity, which damages the DNA further and beyond repair. Host and/or phage

derived functions may be involved in the repair.

Is polynucleotide ligase required in DNA replication? The growth of defective gene 30 phage may mean that (1) no ligase is involved in DNA replication; (2) a ligase (host or phage derived) other than the gene 30 ligase normally acts in T_4 DNA replication; or (3) rII mutations permit an alternate ligase (host or phage derived) to substitute for the gene 30 ligase.

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