# STABILITY OF PHAGE T7 GENE I mRNA IN *E. COLI* CELLS INFECTED WITH WILD TYPE PHAGE AND WITH GENE I AMBER MUTANTS

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## 1. Introduction

The polarity effect which many nonsense mutations exert on the expression of genes distal to the operator region has prompted several investigators to study the cause for polarity at the level of transcription or of messenger degradation. The most widely accepted view is that failure to translate a messenger RNA molecule past the nonsense codon results in exposure of the operator-distal regions of the message to nucleases and consequently in rapid breakdown of the RNA chain. While this situation may hold for bacterial operons such as *tryp* [1] and *lac* [2], it had been shown by one of us [3] that in the case of the phage T4 rII messenger a nonsense codon located near the beginning of a cistron does not seem to affect the rate of breakdown of this message. Thus, the data on the bacterial operons cited should not be overinterpreted to mean that the occurrence of a nonsense triplet will always lead to an increased rate of message breakdown. As the mRNA of phage T7 was found to be degraded rather slowly [4] it seems to lend itself as an excellent test system to study differences in messenger stability between wild type and amber mutants. Such studies have led Siegel and Summers [5] to conclude that very little intact gene I message can be found after infection with a gene I amber mutant of T7 compared with wild type phage, and that normal amounts of gene I mRNA were found if the amber mutant was allowed to infect SuA, a strain of *E. coli* known to suppress polarity [6].

Scrutinizing Summers' data, we had come to the conclusion that the messenger fraction he was looking at in his work with SuA did not in size correspond with the gene I product and we therefore decided to reinvestigate this question by more thoroughly identifying the gene I RNA product through i) hybridization of all pulse labeled RNA fractions with T7 DNA and ii) synthesis of enzymatically active gene I product in a cell-free system. The results summarized in this communication have forced us to conclude that an amber codon in gene I has no effect on the rate of either synthesis or breakdown of the gene I messenger.

## 2. Materials and methods

T7 wild type phage, the T7 gene I mutants am 342 and am 44/73 were used. They were grown on *E. coli* BA (non-permissive) and BBW/1 (permissive), respectively.

Ribosomes and supernatant for the protein synthesis *in vitro* were prepared from *E. coli* 1200 as described by Gold and Schweiger [7]. Suppressor tRNA was isolated from *E. coli* BBW/1 according to Capecchi [8]. Incubations and assays for gene I activity produced *in vitro* were done as described elsewhere [9].

RNA was isolated from T7 infected cells by lysing the cells with 1% SDS in the presence of 300 μg/ml of predigested proteinase K [10] in $10^{-2}$ M Tris-HCl buffer pH 7.5 for 60 min at 37°, followed by fractional precipitation of the RNA according to Kirby [11].

RNA pulse labeled with [$^3$H]uridine and isolated by the proteinase K method was fractionated by acrylamide gel electrophoresis according to Summers [12]. After electrophoresis, the gels (5 × 0.6 cm) were cut into discs of 1.5 mm thickness. The discs were

directly incubated with nitrocellulose filters containing 15 μg of denatured T7 DNA in 2 ml 0.3 M NaCl + 0.03 M sodium citrate for 34 hr at 65°, washed and counted. During this prolonged time of hybridization, the RNA was eluted quantitatively from the gel discs.

## 3. Results

RNA, pulse labeled after infection of *E. coli* BA with T7 wild type phage in the presence of chloramphenicol was fractionated by gel electrophoresis and each fraction was hybridized with T7 DNA. The profile of hybridizable RNA obtained is shown in fig. 1. There are four distinct high molecular weight RNA species observed; the largest moving directly under the 23 S rRNA species is of the size expected of the gene I message. This RNA species cannot be discerned without hybridization as it is completely covered by the ribosomal RNA abundantly produced early after infection. A more stringent way of identifying the gene I product is to assay the RNA fraction for its capacity to code for active T7 RNA polymerase in a

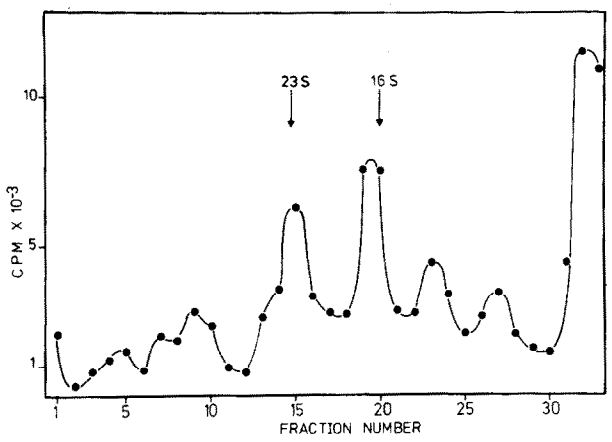


Fig. 1. A 10 ml culture of *E. coli* cells was infected with T7 wild type phage at a multiplicity of 10 in the presence of 100 μg/ml chloramphenicol. The cells were pulse labeled from 0 to 5 min after infection with 150 μCi [$^3$H]uridine (specific activity 5 Ci/mmole). The extracted RNA (7 X10$^5$ cpm) was subjected to acrylamide gel electrophoresis; subsequently, the gel was cut into thin slices which were incubated with T7 DNA filters. The ordinate gives the number of counts which hybridized with T7 DNA. Electrophoresis was from left to right and the mobilities of the two large ribosomal RNA species are indicated.

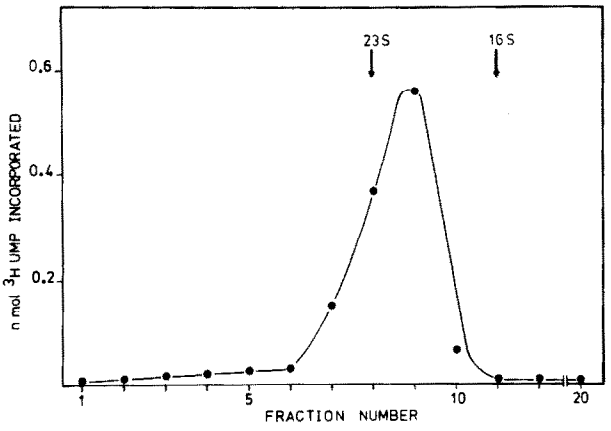


Fig. 2. Sucrose gradient centrifugation of RNA coding for T7 RNA polymerase activity. Cold RNA (2 mg) isolated from T7 infected cells which were harvested 6 min after infection was applied to a 5–30% sucrose gradient. After centrifugation at 87 000 *g* for 18.5 hr in a SW27 rotor, 1.9 ml fractions were collected, concentrated and tested for T7 RNA polymerase activity in the cell-free system.

protein synthesizing system. For that purpose, fractionation of the RNA by sucrose gradient centrifugation was employed as it affords a better recovery of RNA active in protein synthesis. As shown in fig. 2, the coding activity for gene I protein trails the 23 S rRNA marker, the peak of activity sedimenting with an S value of 21 to 22. While the leading edge of the peak seems to be skewed, there is no evidence for a distinct class of polycistronic messenger. A similar peak of activity is observed if RNA is isolated from cells infected with a gene I amber mutant when the template activity is tested in the presence of suppressor tRNA.

In order to answer the question whether the gene I messenger RNA of an amber mutant is degraded faster than that of wild type phage, we have measured the half life of messenger activity for both in the following way: Initiation of transcription by *E. coli* polymerase at the early promoter was stopped 4 min after infection by the addition of rifampicin. Gene I messenger activity was found to rise until minute six, a minimum of 2 min being required for the poymerase to read to the end of gene I, followed by exponential decay of activity (fig. 3). The slope of this decay was found to be identical for both RNA extracted from cells infected with wild type phage and with the gene I

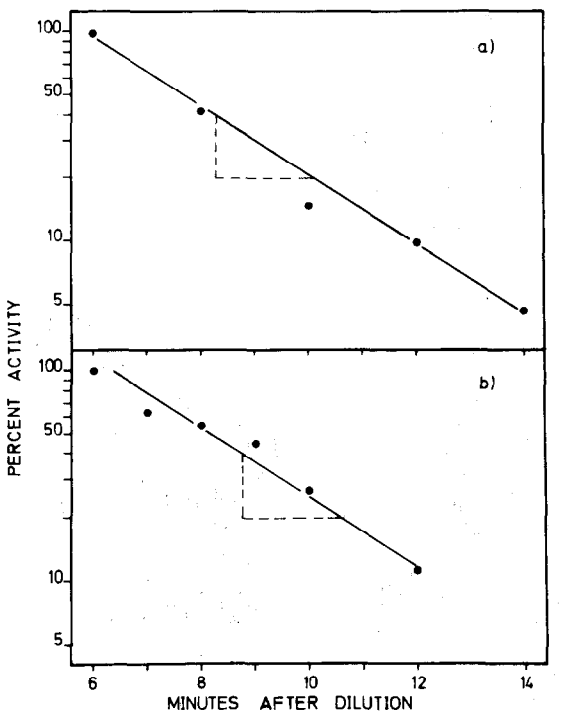


Fig. 3. Decay of gene I messenger activity in a) T7 wild type and b) T7 am342 infected *E. coli* cells. Batches of 400 ml of *E. coli* cells were infected at multiplicities of 10 and after 4 min, rifampicin was added to a final conc. of 40 μg/ml. At the times indicated 50 ml aliquots were harvested by pouring over 30 ml of crushed ice. RNA was extracted and 80 μg was assayed in a cell-free system containing suppressor tRNA for production of T7 RNA polymerase. The 100% values represent 57 and 9.5 pmoles [$^3$H]UMP incorporated for infection with wild type and am342 phage, respectively.

mutant am342. In a coupled system, involving both *in vitro* transcription and translation from added DNA, DNA of the amber mutant gives, in the presence of suppressor tRNA, about 17% of the gene I activity the wild type DNA yields. We assume that the efficiency of suppression in the experiments using isolated DNA is of the same order and we therefore conclude that there is no difference in either the rate of synthesis or the stability of the gene I messenger between wild type and amber mutants in the non-permissive host.

Thus, in the case of phage T7, the introduction of a nonsense triplet has no effect on the stability of the messenger RNA distal to that triplet.

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