Continuous Requirement for Host P10 Protein During Bacteriophage T4 Infection

ROBERT L. CAFFERATA AND ROBERT HASELKORN

Department of Biophysics, University of Chicago, Chicago, Illinois 60637

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The *Escherichia coli* 30S ribosomal protein P10, the product of the *str* gene, known to be involved only in the initiation of protein synthesis, is required for all bacteriophage T4 protein synthesis.

The program of gene expression during bacteriophage T4 infection of *Escherichia coli* is satisfactorily explained by regulation at the level of transcription, with two known exceptions. First, some messenger ribonucleic acid (RNA) molecules containing nucleotide sequences corresponding to the sense strand of the lysozyme gene are transcribed early in infection but are not translated (5). Second, T4 infection uncouples RNA and protein synthesis directed by RNA phages (2); a factor that could be responsible in part for this uncoupling has been found associated with ribosomes in T4-infected cells, and has been shown to inhibit MS-2 RNA-directed protein synthesis in vitro (3).

The complexity of the ribosome and ignorance of the detailed function of its parts have made it an object for speculation about translational regulation. Such speculation seems warranted by the isolation from ribosomes of message-discriminating factors active in vitro (3), and the observation of several new proteins firmly bound to *E. coli* ribosomes in T4-infected cells (7).

The 30S ribosomal protein specified by the *str* gene of *E. coli* seemed a likely candidate for involvement in translational control. The *str* gene product, termed P10 by Ozaki et al. (6), had been shown to be dispensable for poly U-directed polyphenylalanine synthesis, but required for RNA-directed protein synthesis, and for the binding of F-met transfer RNA (5a). The availability of a temperature-sensitive (*ts*) mutation in the *str* gene product (4) permitted us to test the hypothesis that T4 directs the synthesis of a replacement for the P10 protein, thereby modifying translation at the initiation step. The results reported here disprove this hypothesis. The *E. coli* P10 protein is required for all T4 protein synthesis throughout T4 infection.

The isolation of a double mutant (C1714)

carrying both streptomycin resistance and a temperature-sensitive marker within the *str* gene has been described elsewhere (4). Because T4 adsorbed poorly to this particular strain as well as its parental strain (Hfr H3000), the streptomycin-resistant, temperature-sensitive phenotype was transduced by P1 into the strain AB 259. The strain now called KC4000 (*str* A-1714) was prepared in this manner by S.-S. Kang to whom we are indebted.

When strain KC4000 is shifted from 30 to 42 C, protein synthesis, measured by ¹⁴C-leucine incorporation, stops within 3 min, as shown in Fig. 1a. (The temperature shift in all experiments includes a brief period during which the temperature of the culture reaches 45 C; without this mild shock, the loss of function at 42 C is very slow in strain KC4000.) If T4 directed the synthesis of a new P10 protein, we would expect protein synthesis in T4-infected KC4000 to become insensitive to the temperature shift at a time corresponding to the time of synthesis of the new thermostable protein. When strain KC4000 is shifted to 42 C at 15 min after T4 infection, ¹⁴C-leucine incorporation ceases completely within 3 to 4 min (Fig. 1c). [It has been known since the earliest experiments of Hershey that the global rate of isotope incorporation into protein is approximately halved after T-even virus infection of *E. coli* (Fig. 1b and 1d). There are trivial explanations of this result, but a particularly interesting one is that ribosomes travel half as fast on T4 messages as on *E. coli* messages. In such a case, the capacity for subsequent protein synthesis after inactivation of P10 in T4-infected cells would be twice that in uninfected cells, when the capacity is expressed in terms of the extent of incorporation per unit time. The results shown in Fig. 1a and 1c are consistent with this prediction.]

FIG. 1. Incorporation of ^{14}C -leucine into hot trichloroacetic acid-precipitable material by uninfected and T4-infected strains KC4000 and AB259. Cultures $\circ$, the two strains were grown aerobically at 30°C in medium M9 supplemented with 1 mg of Casamino Acids per ml (1). At a cell density of 3×10^8 /ml, tryptophan was added to a final concentration of 20 $\mu\text{g}/\text{ml}$, and each culture was divided into two parts. Three minutes later, one part was infected with T4, at a multiplicity of 5. Under these conditions, 88% of the KC4000 and 97% of the AB259 were infected within 5 min. Fifteen minutes after the addition of phage, 7-ml samples from each portion of cells were transferred to 14 ml of labeled medium (0.25 μCi of ^{14}C -leucine per ml) that had been preincubated at either 30 or 42°C. The temperature shift was facilitated by a 10-sec period at 70°C. At the times indicated, 1.0-ml samples were withdrawn and added to 2.0 ml of 7.5% trichloroacetic acid containing 200 μg of L-leucine per ml at 0°C. These mixtures were heated for 15 min in a boiling-water bath, collected on membrane filters, washed, and dried. The radioactivity on each filter was measured in a Packard liquid scintillation counter with the use of a toluene base scintillator. The background $\circ$, ^{14}C -leucine adsorbed by cold trichloroacetic acid-precipitated cells (53 to 94 counts/min) has been subtracted. ^{14}C -leucine incorporation by uninfected (a) and T4-infected (c) strain KC4000; ^{14}C -leucine incorporation by uninfected (b) and T4-infected (d) strain AB259; $\circ$, 30°C; $\bullet$, 42°C.

A similar result is observed when strain KC4000 is shifted to 42°C at either 4 or 20 min after T4 infection. However, ^{14}C -leucine incorporation in uninfected and T4-infected cells of the wild-type strain AB 259 continues, at 42°C, at nearly twice the rate observed at 30°C (Fig. 1b and 1d).

Evidence that the *ts* mutation in strain KC4000 (C1714) is in the P10 protein is strong. The *ts* mutation is co-transduced with *str^r* by P1 with a frequency of 99.8%; small polysomes accumulate after shift-up *in vivo*, and protein-synthesizing extracts prepared from C1714 are temperature sensitive with respect to RNA-directed protein synthesis, but not with respect to poly U-directed polyphenylalanine synthesis (4).

We conclude that, since T4 fails to rescue the temperature-sensitive function in strain KC4000, the host P10 protein is required for all T4 protein synthesis.

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