INITIATION OF TRANSCRIPTION IS TEMPERATURE-DEPENDENT IN AN E. coli MUTANT WITH ts ADENYLATE KINASE

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SUMMARY: In a mutant of E. coli with temperature-sensitive adenylate kinase (AK) activity the synthesis of β-galactosidase or ornithine transcarbamylase cannot be induced at the restrictive temperature of 40°, but is induced normally at 27°. However, bulk protein synthesis, including the synthesis of pre-induced β-galactosidase, continues for several minutes after a temperature shift from 27° to 40°. These effects of temperature shift mimic the effects of rifampin on a related strain with normal AK activity. The results are interpreted as indicating that the mutant bacteria at the restrictive temperature are blocked in initiation of transcription.

Adenylate kinase (AK) is an ubiquitous enzyme that serves to replenish the adenosine diphosphate pool. A conditionally lethal mutant of Escherichia coli with temperature-sensitive AK activity has been described (1, 2). Within a few minutes after a culture of this mutant is transferred to a non-permissive temperature, the syntheses of protein, RNA and DNA cease and the bacteria die. We report here findings that indicate that the observed arrest in the synthesis of protein is due to an arrest in initiation in gene transcription.

Figure 1 illustrates the results of a typical experiment in which a culture of the AK_{ts} mutant CR341T28 (1, 2) was transferred from 27° to 40° and protein synthesis was followed at intervals by pulse incorporation of [^{14}C]proline. ATP levels were also followed by the firefly assay method. The observed changes in rate of protein synthesis and in ATP levels closely resemble those that have been reported for this mutant (1, 2). The rate of protein synthesis began to decline after about 5 minutes and decreased to less than 10 percent of the initial value by 15 minutes.

A very different picture is observed if one measures the ability to

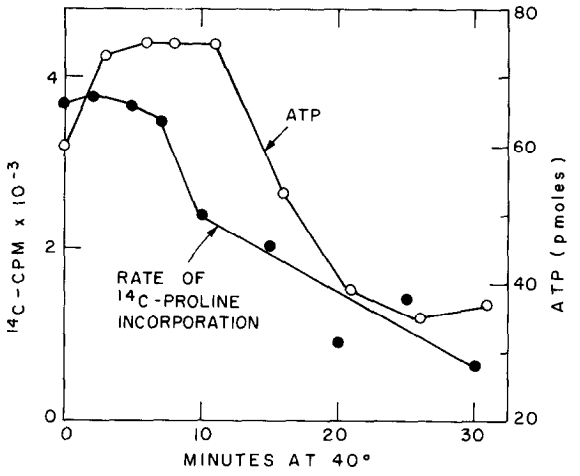

Figure 1. Levels of ATP and rate of protein synthesis in E. coli CR341T28 after shift to 40°. A culture grown at 27° was sampled immediately before (0 time) and at the indicated times after shift to 40°. For ATP assay aliquots (0.5 ml) were removed, heated in a boiling water bath for 10 minutes, and assayed using dried firefly lanterns. The rate of protein synthesis was measured by transferring 0.5 ml aliquots to tubes at 40° containing 0.48 µCi [^{14}C]proline (10.6 µCi/µmole). After 3 minutes incorporation was terminated by addition of cold 10% TCA. The precipitates were collected on 0.6 µm nitrocellulose filters, washed with cold 5% TCA, dried, and counted in a Beckman liquid scintillation counter.

TABLE 1

Enzyme Synthesis at Permissive and Non-Permissive Temperatures in E. coli Strains CR34 and CR341T28[a]

Period of Induction or Derepression (minutes)	Amount of Enzyme Synthesized					
	β-Galactosidase[b] (units/ml)				Ornithine Transcarbamylase[c] (units/ml)	
	27°		40°		27°	40°
	CR34	CR341T28	CR34	CR341T28	CR341T28	CR341T28
10	16.5	19.2	64.8	2.1	0.05	<0.04
15	37.2	48.1	122.5	2.1	0.35	"
20	57.7	81.4	184.0	2.1	4.7	"
25	76.4	89.5	220.5	2.0	8.3	"

[a]Strain CR341T28 was a gift from Dr. D. Cousin. Strain CR34, closely related to the mutant but with normal AK activity, is from our stock collection. Cultures were grown at 27° in Ozeki minimal glucose medium (3).

respond to derepression of specific enzymes (Tables 1 and 2). Within less than 3 minutes after raising the temperature to 40°, the induction of either β-galactosidase or ornithine transcarbamylase (the former a catabolite-repressible, the latter a non-catabolite-repressible enzyme) is virtually abolished in strain CR341T28. That this does not represent a peculiar behavior of these enzymes as compared with other proteins is shown by the fact that, if an inducer of β-galactosidase is added before the temperature shift, the synthesis of β-galactosidase continues for several minutes (Figure 2A) as does the synthesis of bulk protein (Figure 1).

We interpret these findings as indicative of an almost immediate block in initiation of transcription or translation upon placement of the mutant bacteria at the higher temperature (at which AK is inactive). The amino acid incorporation and the increase in pre-induced enzyme activity observed at the non-permissive temperature are assumed to represent completion of polypeptides already started and, if inhibition occurs only at the initiation of transcription, possibly also repeated translations of existing messenger RNA molecules.

The relatively slow arrest in protein synthesis suggests that the block produced by the temperature shift is at initiation of transcription rather than of translation. This appears to be confirmed by a comparison of the results in Figure 2A with those of Figure 2B. In the experiment of Figure 2B bacteria

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TABLE 1, continued.

[b] Synthesis of β-galactosidase was induced by the addition of isopropyl-thio-β-D-galactoside (IPTG) (10^{-3} M) to 20 ml cultures incubated in a shaking water bath at the indicated temperatures. For the 40° induction the cultures were shifted from 27° to 40° and pre-incubated for 3 minutes before addition of IPTG. At the indicated times aliquots (0.5 ml) were removed and toluenized. β-galactosidase was assayed by the procedure of Pardee et al. (4), except that o-nitrophenyl-β-D-galactoside hydrolysis was carried out at 37°.

[c] Synthesis of ornithine transcarbamylase (OTC) was derepressed by removal of arginine from a culture grown with 200 μg/ml arginine. The cells were harvested, washed by filtration at room temperature and at 0 time diluted 1:5 into medium without arginine prewarmed to the indicated temperature. At the indicated times aliquots (1.0 ml) were removed and rapidly chilled by dilution into cold buffer. OTC was assayed as described by Gorini (5).

TABLE 2

Induction of β-Galactosidase in E. coli CR341T28 Starting at Various Times After Temperature Shift

Time of IPTG Addition After a 27°→40° Shift (minutes)	β-Galactosidase Formed in 10 minutes at 40° (units/ml)
0	8.1
0.5	6.0
1.5	4.6
3	2.1

IPTG (10^{-3} M) was added either immediately before (0 minutes) or at the indicated times after shifting a 20 ml culture from 27° to 40°.

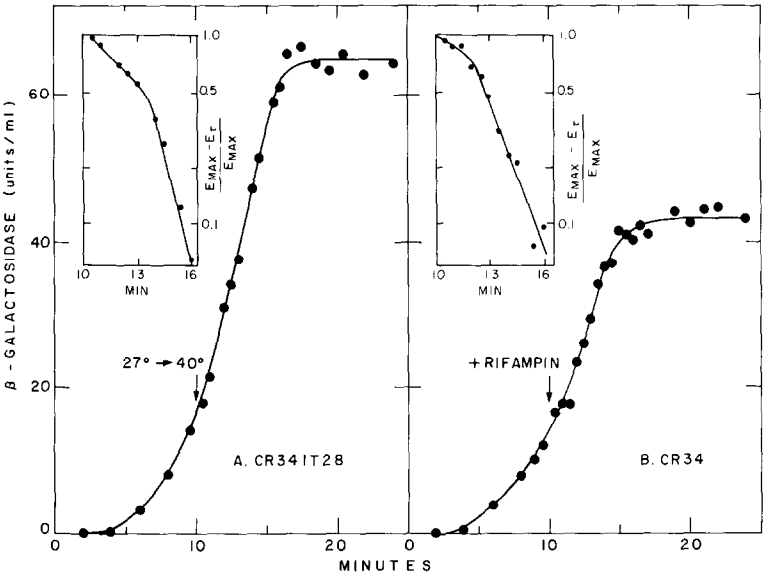

Figure 2. β-galactosidase synthesis in E. coli CR341T28 after a shift from 27° to 40°, and in E. coli CR34 after addition of rifampin. At 0 time IPTG (10^{-3} M) was added to cultures (20 ml) growing at 27°. Aliquots (0.5 ml) were removed for β-galactosidase assay at the indicated times.

A. CR341T28 shifted at 10 minutes to 40°. B. CR34 with rifampin (Ciba, 100 μg/ml) added at 10 minutes, then shifted to 40°.

The insets describe the decrease in β-galactosidase synthetic rate after the treatment initiated at 10 minutes. E_{max} is the final level of enzyme reached minus the level at 10 minutes; E_t, the level of enzyme at any time t minus the 10 minute level.

of the strain with normal AK activity (CR34) were exposed to rifampin, a selective inhibitor of initiation of transcription. The effects of rifampin on β-galactosidase synthesis in the normal strain resemble the effects of thermal shift alone on the AK_{ts} strain (Figure 2A).

A temperature shift to 40° results in a rapid reduction in the rate of RNA synthesis in CR341T28 bacteria, comparable to the reduction produced by rifampin in cells of strain CR34 (data not presented). DNA synthesis, however, is inhibited much more slowly by rifampin than by the temperature shift. Apparently, the effect of the temperature shift on DNA synthesis is not mediated only through the inhibition of transcription.

Whether the present results indicate that AK plays a role in the initiation of transcription, and what such role might be, can at present be only a subject for speculation. According to a recent report (6) AK_{ts} mutations in strain CR341T28 and in another E. coli mutant, CV15, confer temperature sensitivity also to another enzyme, sn-glycerol-3-phosphate acyltransferase. This, and the report that the AK enzyme of CR341T28 regains partial heat stability in the course of purification (7), make it plausible that the ts mutation in question affects a gene coding for a subunit common to several enzymes. Yet, it may be worth keeping in mind the possibility that a physiological connection exists between AK activity and initiation of transcription. The initiation process has an apparent in vitro requirement for high levels of nucleoside triphosphates (8), which might reflect, for example, sensitivity to an inhibitory action of monophosphates. It is apparent that a regulatory role of AMP on initiation of transcription would constitute a pleasingly elegant feedback for tying the rate of initiation to the overall rate of macromolecular syntheses. Experiments to test this suggestion are being undertaken.

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