RNA SYNTHESIS IN ESCHERICHIA COLI DURING K^+-DEPLETION

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SUMMARY

During K^+ depletion of a mutant of Escherichia coli which cannot concentrate this cation, protein synthesis is inhibited but RNA formation continues. The RNA produced during K^+ depletion was analyzed by gel electrophoresis. It was found that 4S, 5S and 23S RNA were synthesized by K^+-depleted cells whether uninfected or infected with phage T4. In addition, an RNA species moving close to 16S (presumably 17S) and material of about 6-10S were made during K^+ depletion. These species of RNA were not evident in growing cells. Methylation of RNA is severely inhibited during K^+ depletion.

INTRODUCTION

Microbial cells require K^+ for growth and protein synthesis. The isolation several years ago of an E. coli mutant which is unable to normally accumulate K^+ has enabled us to study the role of K^+ in cell metabolism (1). When this mutant is depleted of its intracellular K^+, protein synthesis is inhibited but RNA synthesis proceeds (2). When protein synthesis is allowed to proceed after phage infection, host RNA synthesis is rapidly terminated. On the other hand, when protein synthesis is inhibited prior to infection, host RNA synthesis can continue after infection (3,4,5,6,7). In the present study, the examination of the coupling between nucleic acid and protein synthesis was facilitated by the use of K^+ depletion, which results in a selective

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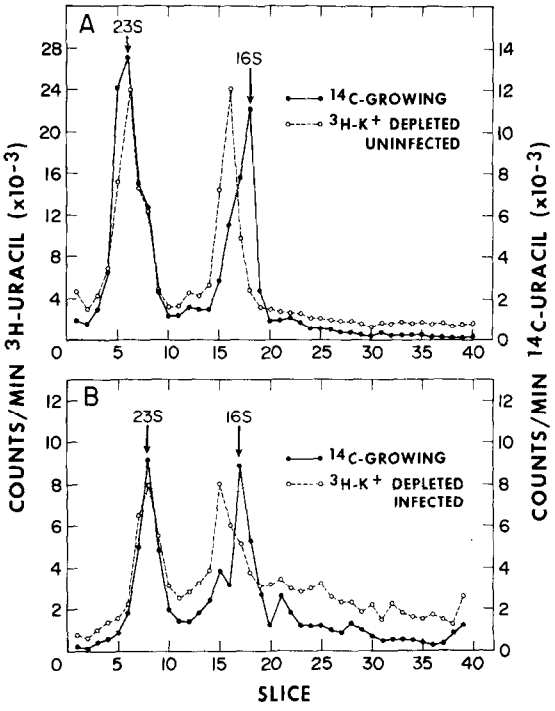

FIGURE 1: Polyacrylamide gel electrophoresis of RNA made in uninfected and infected cells incubated in the absence of K^+. The gels were composed of 3% acrylamide-bis-acrylamide-0.5% agarose. Cultures of strain B207 were labeled during growth for 3 hours with [^{14}C] uracil (0.2 μCi/ml; 0.4 μg/ml). The cells were washed free of K^+ and incubated in K^+-free medium. During 30 min K^+ starvation the cells were labeled with [^{3}H]uracil (1 μCi/ml; 2.5 μg/ml). 1A. culture starved for K^+ for 30 min, ●—● [^{14}C]RNA made during growth; 0---0 [^{3}H]RNA made in the absence of K^+. 1B. the culture was depleted of K^+ and infected with T4 in media lacking K^+ for 30 minutes, ●—● [^{14}C]RNA made during growth; 0---0 [^{3}H]RNA made in the absence of K^+. *E. coli* B207 is an *E. coli* B mutant that cannot accumulate or concentrate K^+ from its growth media (1). Wild-type T4 was used. For routine use, cells were grown in a glucose salts medium containing 33 mM K^+ with various other supplements including 0.3% casamino acids (13). K^+ phosphates were replaced by Na^+ phosphates during K^+ starvation. The method of T4 infection has been described previously (13). RNA was extracted by the method of Fry and Artman (14). The extracted RNA was left precipitated in 0.1 M NaCl, 60% ethanol at -20°C until subjected to electrophoresis no more than 24 hours after preparation. Electrophoresis was performed by the method of Corte *et al.* (8) with slight modification. Bis-acrylamide was the cross-linking agent, glycerol was omitted from the gels, 0.5% agarose was included in the 3% gels and 0.2% SDS was added to the tank buffer. After electrophoresis the gels were sliced in 2 mm pieces and analyzed as described by Peterson *et al.* (15). S values were determined by comparison with gels on which samples of known S value have been run under similar conditions.

inhibition of protein synthesis. The types of RNA synthesized during K^+ depletion of uninfected and phage T4 infected cells were studied.

RESULTS AND DISCUSSION

The following experiments were performed to determine the types of RNA made by cells incubated in the absence of K^+. In all cases this RNA is compared to that made during exponential growth. For this purpose, the RNA made during growth was labeled with [^{14}C]uracil and the RNA made during starvation with [^{3}H]uracil.

Figures 1 and 2 show the types of RNA made in the absence of K^+, and consequently in the absence of protein synthesis. Figure 1 shows an electropherogram of RNA using 3% acrylamide-agarose gels to display large RNA species in the ribosomal RNA class. As can be seen, in uninfected cells incubated in the absence of K^+ (1A), 23S and another species of RNA which runs more slowly than 16S (and is presumably 17S) are made. This same pattern is also seen in T4 infected cells incubated in the absence of K^+ (1B). 17S RNA may accumulate because RNase II which converts 17S to 16S is K^+ dependent (8,9). However, 17S RNA also accumulates in cells in the presence of K^+ treated with chloramphenicol or during amino acid starvation of relaxed mutants (10). The 17S species has been shown to be a precursor to 16S ribosomal RNA (8,9).

Figure 2 shows 6% gels using the same RNA preparations as in Figure 1. The 6% gels display low molecular weight RNA. Under both conditions of K^+ depletion investigated, 4 and 5S RNA are made. In addition, there is an increase in the fraction of the total radio-

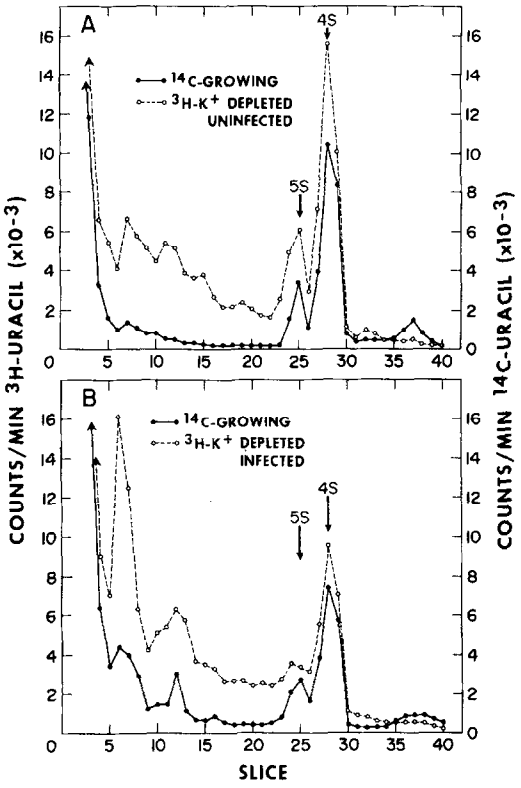

FIGURE 2: Polyacrylamide gel electrophoresis of RNA made in uninfected and infected cells incubated in the absence of K^+. The gels were composed of 6% acrylamide-bis-acrylamide. The RNA samples were the same as used in Fig. 1. 2A. culture starved for K^+ for 30 min, ●—● [^{14}C]RNA made during growth; 0---0 [^{3}H]RNA made in the absence of K^+. 2B. the culture was depleted of K^+ and infected with T4 in media lacking K^+ for 30 minutes, ●—● [^{14}C]RNA made during growth; 0---0 [^{3}H]RNA made in the absence of K^+.

activity found in about the 6-10S range (slice #6-#16) during K^+ depletion. This could represent messenger RNA accumulation or breakdown products of newly synthesized ribosomal RNA made during K^+ depletion. This does not represent breakdown of ribosomal RNA labeled before K^+ depletion because little [^{14}C]labeled RNA is observed in this region of the gel. The properties of the 6-10S RNA have not been further investigated.

Methylation during K^+ starvation is severely inhibited. As can

TABLE I

Incorporation of Methyl Groups into RNA

during Growth and K^+ depletion

Conditions of labeling	Counts/min/mg RNA	per cent
$+K^+$, [^{14}C]-methyl-methionine present for 20 min	36,930	100
$-K^+$, [^{14}C]-methyl-methionine present from		
0 to 20 min	1,970	5.3
20 to 40 min	2,180	5.9
40 to 60 min	1,990	5.3
0 to 60 min	6,410	17.3

Cells growing in glucose salts medium (11) supplemented with histidine and leucine (100 μg/ml each) and L-methionine (20 μg/ml) were labeled with [^{14}C]-methyl-methionine (1 μCi/ml) for 20 minute intervals before and after K^+-depletion. The cells were mixed with non-radioactive B207 cells and their RNA extracted. After purification the RNA was sedimented and dissolved in 0.1 M Tris buffer pH 7.8, an aliquot of known concentration mixed with scintillation solution (12) and counted using a Beckman LS-100 scintillation spectrometer.

be seen in Table I, the amount of methyl group incorporation during K^+ depletion decreases to approximately 5% of that observed in growing cells. Since the amount of incorporation during a 60 minute labeling period is not less than the total amount seen during three consecutive 20 minute labeling periods, one can assume there is no appreciable turnover of methyl groups during K^+ starvation.

Thus, under conditions of K^+ starvation one finds that although protein synthesis stops, RNA synthesis can continue (2). The types of RNA made include tRNA, 5S RNA, ribosomal RNA and probably mRNA. This synthesis also continues in K^+-depleted *E. coli* cells infected

with phage T4. We are at present analyzing the mRNA species made during K^+ depletion to determine whether functional mRNA is made.

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