Stable RNA synthesis and its control in *Mycoplasma capricolum*

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ABSTRACT

The synthesis of stable RNA in *Mycoplasma capricolum* was studied by [³²P] labeling of cellular RNA of cells grown in a partially-defined medium in the presence or absence of an amino acid mixture supplement. The results indicate that *M. capricolum* employs the same stringent control mechanism used by *E. coli* cells, as judged by a decreased synthesis of stable RNA and accumulation of 5'-triphosphoguanosine-3'-diphosphate (pppGpp) and 5'-diphosphoguanosine-3'-diphosphate (ppGpp) in response to amino acid starvation. In addition, the results suggest that precursors of stable RNA accumulate and an intracellular pool of the precursors exists at all times under the growth conditions used by us. These findings may be interpreted to reflect a slow rate of RNA processing in *M. capricolum*.

INTRODUCTION

The mycoplasmas have the smallest genome recorded in prokaryotes (about one-sixth to one-third of that of *E. coli*). The minute genome size and its extremely low GC content (about 25 mol % in many mycoplasmas) suggest a relatively simple genome organization (1,2). These features of the mycoplasma genome provide a convenient system for studying regulation of gene expression.

One well known control mechanism of gene expression in bacteria is the stringent control of stable RNA synthesis. The phenomenon of stringent control in *E. coli* is characterized by a complete cessation of stable RNA synthesis and accumulation of ppGpp and pppGpp when the cells are deprived of amino acids (3-5). Mutation of *E. coli* in the *rel A* gene results in a relaxed strain that is not subject to this stringent control. Therefore *rel A E. coli* cells starved to amino acids will continue to synthesize stable RNA and ppGpp will not accumulate (6).

The present communication reports stringent control of stable RNA synthesis in *M. capricolum*. The major guanosine nucleotide which accumulates in response to amino acid starvation in these cells is pppGpp. In addition, a very slow rate of processing of rRNA and of other stable RNA species was

observed.

MATERIALS AND METHODS

Organisms and growth conditions. *Mycoplasma capricolum* (ATCC 27343) was grown for 24 h at 37°C in 40 ml of a modified Edward medium (7) supplemented with 4% (v/v) horse serum. The organisms were collected by centrifugation at 12,000 xg for 15 min and were washed once in a cold salt solution consisting of (g/l) 6.8 NaCl, 0.4 KCl, 0.2 MgSO₄·7H₂O and 6.0 Tris·HCl, pH 8.0. The washed cells were resuspended in 5 ml of the salt solution and then inoculated into 100 ml of a partially-defined medium consisting of the above salt solution supplemented with all 20 L-amino acids (20 µg/ml of each), the vitamins thiamine, riboflavin, pyridoxine·HCl, pyridoxal·HCl, nicotinamide, nicotinic acid, pantothenic acid (Ca salt), folic acid, choline·HCl, m-inositol (3.3 µg/ml of each), glucose (7.5 mg/ml), ribonucleic acid and deoxyribonucleic acid (50 µg/ml of each), penicillin G (2500 units/ml) and 10% (v/v) of dialyzed horse serum. Dialysis of serum was carried out in the cold for 24 h against 200 volumes of the salt solution with one change of solution. The organisms were grown in this medium for 48 h at 37°C when absorbance values of cultures reached 0.2-0.3 at 640 nm. The culture was divided into two equal portions and centrifuged. One of the resulting cell pellets was resuspended in the partially-defined medium devoid of the amino acid supplement and the other in the complete medium (cell density A₆₄₀ = 0.4). *Escherichia coli* strains CP78 and CP79 (stringent-relaxed pair obtained from M. Cashel) were grown to A₆₀₀ = 0.5 in a defined medium supplemented with 20 µg/ml of each of the 20 amino acids (8). The cells were collected by centrifugation and resuspended in the same medium devoid of amino acids. The suspension was then split into two equal portions, to one of which the 20 amino acid mixture was added.

Analysis of stable RNA synthesis. *M. capricolum* cell suspensions (A₆₄₀ = 0.4) in the partially-defined medium with and without amino acids, were incubated at 37°C in the presence of 100 µCi/ml carrier-free [³²P] phosphate (Amersham, England). The cells were harvested after 5, 10, 15 or 30 min and lysed by freezing and thawing (9). The lysates were analysed by gel electrophoresis on slab gels (3% acrylamide, 0.5% agarose) at 100 mA (9). The gels were dried and exposed to a RP2 Kodak X-ray film for 16 h.

Analysis of intracellular pppGpp and ppGpp. Aliquots (50 µl) of the [³²P] labelled cell suspensions withdrawn at the time periods described above, were added to 50 µl of 5 M formic acid. The formic acid extracts were directly

applied to polyethyleneimine (PEI) cellulose thin layer sheets (Macherey-Nagel Co.) and chromatographed by ascending chromatography in 1.5 M KH_2PO_4 , pH 3.5. The dried chromatograms were exposed for 16 h to RP2 Kodak X-ray film. Two dimensional chromatography of the formic acid extract was performed using 1.5M KH_2PO_4 , pH 3.5 and 1.25 M guanidine-HCl in the first dimension and 1.5 M NaCl in 0.2 M acetic acid in the second dimension.

RESULTS

Inhibition of stable RNA synthesis by amino acid starvation. As can be seen in Fig. 1, ribosomal RNA synthesis by *M. capricolum* was markedly affected by the omission of the amino acid supplement from the partially-defined medium. The major labeled bands on the chromatogram were identified as 23S and 16S rRNAs by comparison with the electrophoretic migration of *E. coli* 23S and 16S and of eukaryotic 28S and 18S rRNAs used as markers (data not shown). The band above the 16S band may represent a precursor of 16S rRNA as it appeared after a short pulse when the amount of 16S rRNA was minimal (lane B after 5 min in Fig. 1). The other faint bands seen in Fig. 1 have not been identified as yet.

Fig. 1. Stable RNA synthesis in *M. capricolum* in the absence (A) and presence (B) of amino acids. [^{32}P] labeling conditions and gel electrophoretic analysis are described under Materials and Methods.

Appearance of pppGpp and ppGpp in *M. capricolum* starved for amino acids.

As can be seen in Fig. 2, both pppGpp and ppGpp accumulated in *M. capricolum* cells starved for amino acids. However, in contrast to the findings with the amino acid-starved *E. coli*, the major nucleotide accumulated in *M. capricolum* was pppGpp.

To ensure identification of the labeled spots in the mycoplasma cell extract, a mixture of cell extracts from *M. capricolum* and *E. coli* starved for amino acids was subjected to two-dimensional chromatography. As can be seen in Fig. 3, the [32 P] labeled spots from both organisms comigrated supporting their identification with pppGpp and ppGpp. It should be noted that pppGpp and ppGpp accumulated slowly also in *M. capricolum* cells incubated with amino acids (Fig. 2, 15 min).

DISCUSSION

The present effort to study the synthesis of stable RNA in *M. capricolum* yielded several interesting results. One major conclusion that can be drawn from our experiments is that the synthesis of stable RNA in this mycoplasma is subject to stringent control, resembling that described for other prokaryotes. This control mechanism is characterized by a decrease in the rate of stable RNA synthesis and the concomitant accumulation of pppGpp in response to amino acid starvation. However, the lack of a totally-defined growth medium

Fig. 2. Accumulation of pppGpp and ppGpp in *M. capricolum* in response to amino acid starvation. A - with amino acids, B - without amino acids.

Fig. 3. Two-dimensional chromatographic analysis of the mixture of guanine nucleotides accumulated in amino acid starved *M. capricolum* and *E. coli*. The mixture consisted of 100 μ l of mycoplasma extract and 1 μ l of the *E. coli* extract.

enabling optimal growth of *M. capricolum* blurs somewhat the picture. Thus, the most prominent differences in RNA synthesis and pppGpp accumulation between cells incubated with or without the amino acid supplement could be observed after short periods of labeling (5 min). When analysis was done on cells labeled for 15 min, RNA synthesis was observed even in the starved cells and pppGpp accumulated in cells incubated in the presence of amino acids. The reason for the slow RNA synthesis in the absence of the amino acid supplement may depend on provision of some amino acids by the dialyzed serum component. More extensive dialysis of the serum caused a further decrease in RNA synthesis in the amino acid-deficient medium (data not shown). On the other hand, the partially-defined medium used by us is far from being optimal for *M. capricolum* growth, even when supplemented by amino acids, as evidenced by the much slower growth rate and lower yield of cells as compared to growth in the rich Edward medium. Hence, we may consider the organisms grown in this medium to be partially starved, a fact which may explain the production of small amounts of pppGpp even in the presence of the amino acid supplement.

The discovery of stringent control in *M. capricolum* raises the possibility that the promoter of rRNA of *E. coli*, which is sensitive to this control mechanism, might cross hybridize with the rRNA promoter of *M. capricolum*. Preliminary experiments in our laboratory indicate that the cloned promoter

of the *rrnB* cistron of *E. coli* (pGG1) (9) did not cross hybridize with *M. capricolum* DNA. Neither was there any cross hybridization between 16S and 23S RNA of *M. capricolum* with *EcoR* I fragments of *E. coli* DNA. An unambiguous answer to the question of what determines the sensitivity of the *M. capricolum* rRNA gene to stringent control must await experiments with a cloned promoter of the rRNA gene. Attempts in this direction are currently underway.

The almost identical banding pattern obtained by gel electrophoresis of stable RNA extracted from [³²P] labeled and unlabeled *M. capricolum* cells, and the pattern itself, as seen in Fig. 1, suggest that the enzymatic reactions involved in processing of stable RNA in *M. capricolum* are slow. This fact provides a convenient system for studying RNA processing without having to resort to mutants in processing functions as is the case when processing is rapid like in *E. coli*.

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