COUPLING OF PROCESSING OF α-GLUCOSYL TRANSFERASE mRNA WITH TRANSLATION

Herbert L. Ennis*, Marcia L. Walsh+,
Kevin R. Lynch+, Janet M. Hill* and Paul S. Cohen+

*Roche Institute of Molecular Biology, Nutley, New Jersey 07110

and

+University of Rhode Island, Kingston, Rhode Island 02881

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SUMMARY

Bacteriophage T4 α-glucosyl transferase mRNA is made as a polycistronic 21S molecule that is processed during normal infection to the commonly found 14.5S species. By using antibiotic inhibitors of protein synthesis, it is possible to distinguish two steps involved in the processing of the 21S polycistronic α-gt mRNA in T4-infected Escherichia coli. There is an initial cleavage to an 18S molecule that does not require protein synthesis. However, the next step, the conversion of the 18S into the 14.5S molecule, requires simultaneous protein synthesis.

Eukaryotic tRNA, rRNA and mRNA are made as precursors which are processed by nucleolytic cleavage to the respective smaller functional species (1-3). In prokaryotic cells there is evidence that tRNA and rRNA are made as transient precursors that are larger than the mature species commonly found in the cell (2,4). However, there is no evidence that bacterial mRNAs are made as precursors. The early mRNAs of phage T3 and T7 are apparently made as a single large molecule, which is subsequently cleaved by RNase III to the five individual messengers (5). There is a very close coupling of cleavage of the precursor with transcription, since no large molecules are found during normal infection of Escherichia coli. Inhibition of translation does not affect processing of these mRNAs.

Abbreviations: FA, fusidic acid; CM, chloramphenicol; VM, vernamycin A; PM, puromycin; and TET, tetracycline.

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The mRNA that codes for the synthesis of the early T4 enzyme, α-glucosyltransferase (EC 2.4.1.2;1,4-α-D-glucan:1,6-α-D-glucan 6-α-glucosyltransferase or dextrin dextranase, hereafter called α-gt), isolated 8 min after infection, is 14.5S (6). However, the α-gt mRNA made *in vitro* from T4 DNA is located at the promoter distal end of a 21S molecule (7). It has further been shown that *in vivo*, the 14.5S molecule is found predominantly on polysomes containing 6 ribosomes; the 21S mRNA is found on polysomes with at least 14 ribosomes; and an 18S species, is found on polysomes containing at least 9 ribosomes (8). However, the 18S and 21S mRNAs are a minor fraction *in vivo*. Since only a few discrete sizes of α-gt mRNA are found *in vivo*, the rapid processing of the larger α-gt mRNA species to the 14.5S form probably occurs by a very few endonucleolytic cleavages at specific sites (see also 6). The 21S molecule corresponds to approximately 2780 nucleotides and the 14.5S molecule to about 1220 nucleotides, but only about 700-800 nucleotides are required for the synthesis of the α-gt subunit of 23,000 daltons (9). The 14.5S molecule probably has in addition to the nucleotides required for the structural portion of the α-gt mRNA, discrete initiation and termination sites. The 21S species is clearly polycistronic. The 18S intermediate corresponds to a size of approximately 1910 nucleotides.

We present evidence which suggests that cleavage of the 21S to the 18S α-gt messages occurs in the absence of protein synthesis, but the transition from 18S to 14.5S requires simultaneous protein synthesis (Buchanan, personal communication, has similar data).

RESULTS

During the course of experiments on the *in vivo* effects of inhibitors of protein synthesis on phage T4 mRNA decay we found that processing of α-gt mRNA was inhibited and that an intermediate 18S molecule was found.

Cells growing at 37° were infected with T4, and 4 min later (when α-gt mRNA is beginning to be transcribed) protein synthesis was inhibited. RNA

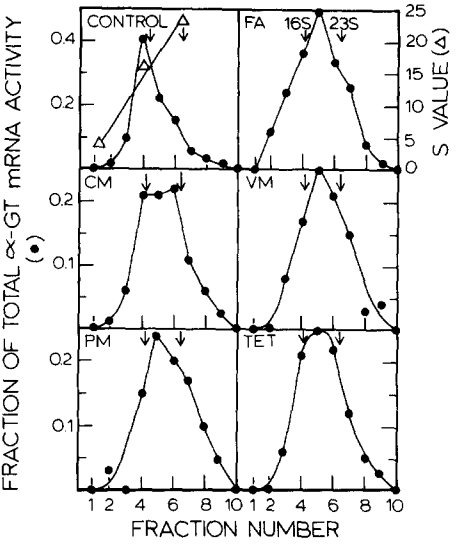

Fig. 1: Size of α-gt mRNA made during normal infection and during infection in the presence of antibiotics. Cells (5 x 10^8/ml) of E. coli AS19 growing at 37° were infected with wild-type T4D at a multiplicity of 25 as previously described (12). Four min later antibiotic was added at a concentration that rapidly and completely inhibits protein synthesis, and the infection was continued for an additional 5 min (9 min total). (All antibiotics were added to a final concentration of 100 µg/ml, except kasugamycin which was added to a final concentration of 3.4 mg/ml.) No antibiotic was added to the control, which was also infected for a total of 9 min. The cells were rapidly cooled and RNA was isolated as previously described (13). Approximately 100 µg samples of RNA in 0.2 ml of H_2O were layered onto 4.8 ml linear 5-20% (w/v) sucrose gradients (0.01 M Tris-HCl, pH 7.5, 0.1 M KCl) and centrifuged in a Spinco SW 50L rotor at 48,000 rpm for 210 min at 2°. Ten 0.5 ml fractions were collected using an ISCO density gradient fractionator connected to a Sargent Welsh SRLG recorder. Each fraction then received 50 µg of E. coli tRNA, 0.14 ml of 1 M sodium acetate, pH 5.2, and 0.72 ml of 95% ethanol. Following overnight precipitation at -15°, the samples were centrifuged at 5000 xg for 20 min at 4° and the tubes were inverted and allowed to dry over anhydrous $CaSO_4$, for 2 h. Water (0.2 ml) was added to each tube, and the contents were then assayed for α-gt mRNA activities as described previously (12). In control experiments it was found that between 90 and 100% of the RNA in each fraction is recoverable by these methods (data not shown). Heat denaturing the RNA (2 min at 80°, in 1.0 mM sodium acetate, pH 5.2) prior to sucrose gradient centrifugation had no effect on mRNA size and therefore this step was routinely omitted. The media used and conditions for growth and infection have been previously described (12).

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was isolated 5 min later (9 min after infection) and subjected to sucrose density gradient centrifugation. The RNA in each fraction of the gradient was added to an in vitro protein synthesizing system and the resultant α-gt

synthesized was assayed. Since the amount of enzyme synthesized is proportional to the relative concentration of mRNA present in each sample, this is a reflection of the mRNA content in each gradient fraction. The following antibiotics were used to stop protein synthesis: amicetin, clindamycin, chloramphenicol, erythromycin, fusidic acid, kasugamycin, puromycin, spiramycin III, tetracycline and vernamycin A. The results obtained with several of the inhibitors are presented in Fig. 1. Clearly, at 9 min after normal T4 infection, the majority of the α-gt mRNA has been processed to the 14.5S form, although a small shoulder at 21S mRNA is also present. In contrast to this finding, at 9 min after T4 infection of cells in which protein synthesis was inhibited at 4 min after infection, little of the polycistronic α-gt mRNA accumulated has been completely processes to the monocistronic form, except in cells treated with chloramphenicol. In these cells, some of the mRNA is 14.5S. With all the other antibiotics, the cleavage that occurs reduces the size of the polycistronic 21S messenger to a species that sediments with a broad peak at an average sedimentation value of approximately 18S. In contrast, the mRNA for another early enzyme, deoxynucleotide kinase, which is known to be synthesized as a monocistronic messenger (10), is found as a 14.5S molecule in the control as well as in the antibiotic-treated cells (data not shown).

From this study we are able to distinguish at least two steps that are involved in the processing of the 21S α-gt mRNA to its commonly found 14.5S form. There is an initial cleavage of the 21S molecule to a species of approximately 18S. This step does not require protein synthesis. The next step, the conversion of the 18S form into the 14.5S molecule, apparently requires protein synthesis for its elicitation. These cleavages are presumably endonucleolytic (6,8). Since α-gt mRNA is in the promoter distal position on the 21S mRNA, (7), the cleavages that are observed are in the promoter proximal portion of the cistron.

Inhibition of processing of the 18S RNA in the absence of protein synthesis could occur either because protein synthesis is required for the formation of

a T4 DNA-encoded processing enzyme or because the translation process itself provides the proper ribosome mRNA configuration for an already present processing enzyme to function. It was seen (Fig. 1) that in the presence of chloramphenicol a large portion of the α-gt mRNA was processed to the 14.5S form. We know, from independent experiments, that essentially all protein synthesis is inhibited in these cells in the presence of this concentration of chloramphenicol. Because rapid cleavage to 14.5S mRNA is observed in the presence of chloramphenicol, which is known to allow some ribosome movement on and off the mRNA (11), we favor the idea that during protein synthesis, the site(s) susceptible to the already present processing enzyme are made available as the ribosome travels along the mRNA. In this case the nuclease responsible for processing may be associated with the ribosome.

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