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Biochemistry

Bacteriophage T3 and T7 early RNAs are translated by eukaryotic 80S ribosomes: Active phage T3 coded *S*-adenosylmethionine cleaving enzyme* is synthesized

(fractionated mammalian system/wheat germ S-30 cell fraction/polycistronic RNA/7-methylguanosine/gene transfer)

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ABSTRACT **RNA transcribed *in vitro* from the early region of bacteriophage T3 or T7 was translated by cytoplasmic ribosomes which synthesized protein in cell-free systems prepared from mammalian cells and wheat germ. The proteins synthesized *in vitro* and their counterparts prepared from infected *Escherichia coli* comigrate by polyacrylamide gel electrophoresis with sodium dodecyl sulfate and are similarly affected by deletion or amber bacteriophage mutations. Bacteriophage T3 codes for an enzyme that cleaves *S*-adenosylmethionine and this activity was detected among the products of the mammalian cell-free system. Bacteriophage T3 or T7 RNA, after endoribonuclease III (EC 3.1.4.24) cleavage, gave higher levels of incorporation into phage T3 or T7 polypeptides than when an equivalent amount of the uncleaved RNA was added to the eukaryotic cell-free systems. Methylation of phage T3 or T7 RNAs is apparently not required for translation in either the wheat germ or mammalian cell-free system. The ability of T3 and T7 RNA to be translated in the presence of saturating amounts of natural eukaryotic mRNAs suggests that many prokaryotic genes introduced into mammalian cells might be expressed if they were transcribed in an appropriate form.**

Protein synthesis on eukaryotic ribosomes (80S) differs in several respects from protein synthesis on bacterial ribosomes. Transcription and translation are not coupled in eukaryotes, and there is no direct evidence that more than one protein can be independently initiated from one mRNA. Many eukaryotic mRNAs have 7-methylguanosine in 5′- to 5′-linkage with the first encoded base (1, 2) and this "cap" is required for the efficient translation of at least some natural eukaryotic mRNAs in cell-free systems (see ref. 3 for a review). No similar structures are known for prokaryotic mRNAs. Mutations (e.g., nonsense, ribosome binding, etc.) that affect the synthesis of specific eukaryotic proteins are not readily available, but many are available for specific prokaryotic messages. Often prokaryotic mRNAs can be readily obtained in relatively pure form and the initiation region of several such mRNAs have been sequenced.

It has been shown that RNA bacteriophage genomes can be translated in mammalian and in wheat germ cell-free systems synthesizing protein so as to produce coat and synthetase proteins (4–7). This paper reports the translation of mRNAs which were transcribed *in vitro* from the early regions of bacteriophage T3 and T7 DNAs. It provides additional data on the proposed interaction between ribosome binding sites on mRNA and the 3′ end of a ribosomal RNA (8, 9). We have investigated whether these prokaryotic mRNAs require the 7-methylguanosine group of the "cap" structure for their translation and the effect of adding the mRNAs in monocistronic [endoribonuclease III, EC 3.1.4.24 (RNase III-cleaved)] or polycistronic forms. We show that active T3 *S*-adenosylmethionine cleaving enzyme, an enzyme that degrades *S*-adenosylmethionine (SAMet), is synthesized on the 80S ribosomes of a mammalian cell-free system in response to early-synthesized phage T3 mRNA. This suggests that translation *per se* is unlikely to be a difficulty in experiments now underway to introduce certain prokaryotic genes into eukaryotic cells. In fact this process may occur in nature, for example in the induction of crown gall in plants by *Agrobacterium tumefaciens* (10).

Abbreviations: cap, 7-methylguanine added in 5′- to 5′-linkage with the first encoded base of an mRNA; M_r, molecular weight; SAMet, *S*-adenosylmethionine; $NaDodSO_4$, sodium dodecyl sulfate; SAHcy, *S*-adenosylhomocysteine.

* *S*-Adenosylmethionine cleavage enzyme has previously been noted as SAMase.

METHODS

***In Vitro* RNA Synthesis.** CsCl purified phage stocks were dialyzed against 10 mM Tris at pH 7.9, 20 mM KCl, and 1 mM EDTA and heated to 65° for 5 min to release phage DNA. The released DNA was used directly as template for *Escherichia coli* RNA polymerase otherwise as previously described (11). The RNA was labeled by including [^{14}C]ATP (5 mCi/mol) in the reaction mixture to facilitate adjustment of concentrations and analysis of the products. Optimal amounts of highly purified RNase III were added to some reactions to produce cleaved early RNAs (12, 13). After synthesis, the RNA was phenol extracted, chromatographed on Whatmann CF-11 cellulose, ethanol precipitated, and dissolved in H_2O. Portions of each RNA used for translation were analyzed on 2% polyacrylamide-0.5% agarose gels (11).

***In Vitro* Protein Synthesis.** The fractionated mammalian cell-free system is a modification (7, 14) of that described by Schreier and Staehelin (15). Twenty-five microliter reactions were incubated at 37° for 90 min unless otherwise noted.

The wheat germ cell-free system was similar to that described previously (16). It was improved from that which we used earlier (14) by the addition of polyamines (7). Reactions of 25 μl contained 7.5 μl of wheat germ extract (A_{260} = 40), RNA as indicated, and had final concentrations of: 20 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (Hepes) at pH 7.6, 56 mM KCl, 2.0 mM $Mg(OAc)_2$, 2 mM dithiothreitol, 1 mM ATP, 20 μM CTP, 8 mM creatine phosphate, 40 μg/ml creatine kinase, 86 μM spermine, 25 μM each of 19 amino acids (minus methionine), and 5–15 μCi [^{35}S]methionine (*ca* 400 Ci/mmol, New England Nuclear). Reactions were incubated for 90 min at 30° unless otherwise indicated.

Other Materials. The mammalian translation components were kindly provided by J. Lewis and R. F. Gesteland (Cold Spring Harbor Laboratory). Reovirus RNA was a gift of A. Shatkin (Roche Institute), T3 and T7 bacteriophages were kindly provided by F. W. Studier and T7 RNA polymerase was donated by W. Summers (Yale University).

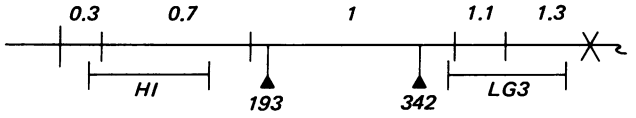

FIG. 1. The early transcribed region of T7 DNA (25, 26). The left hand 20% of the T7 (and T3) genome is transcribed by *E. coli* RNA polymerase starting at three separate sites to the left of gene *0.3* and stopping just to the right of gene *1.3* (cross). T7 RNA polymerase also transcribes from left to right, starting between genes *1* and *1.3* (27, 28). *E. coli* RNase III cleaves RNA from this region at five specific sites, between each of the genes and at the left of gene *0.3* (vertical bar). The five early gene products are: *0.3*, T7 = 8700 M_r, T3 = 11,500 M_r (SAMet cleavage enzyme); 0.7 (protein kinase) T7 = 42,000 M_r, T3 = 40,000 M_r; *1* (RNA polymerase) T7 = 100,000 M_r, T3 = 97,000 M_r; 1.1 (function unknown) T7 = 8,000 M_r, no known T3 product; *1.3* (DNA ligase) T7 = 40,000 M_r, T3 = 37,000 M_r. T7 *0.3* product overcomes host restriction but does not exhibit cleavage enzyme activity *in vitro*. The relative position of the T7 mutants, deletions H1 and LG3 and amber mutants *193* and *342*, are indicated below the horizontal line.

RESULTS

Synthesis of Early Phage T3 and T7 Polypeptides in a Cell-Free System Derived from Mammalian Cells. The early region of phage T3 or T7 DNA (Fig. 1) was transcribed *in vitro* with *E. coli* RNA polymerase (RNA nucleotidyltransferase, EC 2.7.7.6) in the presence or absence of RNase III. In the absence of RNase III, the primary product is a set of high-molecular-weight RNAs containing the coding information for all of the early genes. In the presence of RNase III, these polycistronic RNAs of high molecular weight are cleaved to yield individual mRNAs for each of the early-synthesized gene products (12, 13). Both cleaved and uncleaved RNA were used as template in a cell-free protein synthesizing system derived from mammalian cells. This system contained partially purified initiation factors from rabbit reticulocytes, ribosomes, tRNA, and other factors from Krebs II ascites cells.

Early T7 and T3 RNA, when added in its polycistronic form or as RNase III cleaved RNA to the mammalian cell-free system synthesizing protein, stimulated the incorporation of [^{35}S] methionine into high-molecular-weight polypeptides which, when analyzed by electrophoresis in polyacrylamide gels with sodium dodecyl sulfate ($NaDodSO_4$), closely resemble the pattern of polypeptides seen *in vivo* after infection of a UV-irradiated host (17). As shown in Fig. 2, polypeptides with the mobilities corresponding to T7 RNA polymerase [gene *1* product, 100,000 molecular weight (M_r)], ligase (gene *1.3* product, 40,000 M_r), and the *0.3* protein (8700 M_r) were among the products synthesized. Additional minor products are also observed; however, we did not observe products synthesized *in vitro* which correspond in mobility to the products of T7 genes *0.7* (42,000 M_r) and *1.1* (8000 M_r). Heating the T7 RNAs briefly to 60° and then quickly chilling, immediately prior to

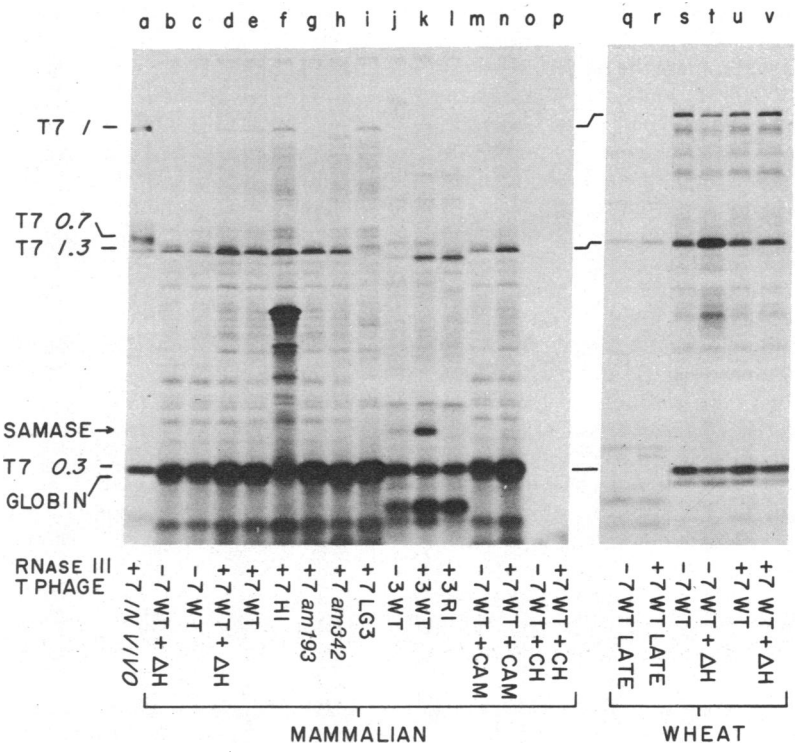

FIG. 2. Autoradiogram of the [^{35}S]methionine-labeled cell-free products examined on a 17.5% $NaDodSO_4$/polyacrylamide gel made as described (7). RNA was synthesized with *E. coli* RNA polymerase (i.e., early transcript) except where indicated to the contrary (see *Methods*). The extract of UV-irradiated, T7-infected *E. coli* labeled with [^{35}S]methionine *in vivo* 0–8 min after infection (a) was provided by F. W. Studier. Cell-free synthesis in the fractionated mammalian system was done with the following additional components per 25 μl reaction: (b) 1.7 μg of wild-type T7 polycistronic RNA, heated at 60° for 1 min; (c) as (b) but without heating; (d) 1.7 μg of RNase III-cleaved T7 RNA heated as in (b); (e) as (c) but without heating; (f) 1.6 μg of RNA from t7 H1; (g) 1.8 μg of RNA from T7 *am193;* (h) 1.9 μg of RNA from T7 *am342;* (i) 1.6 μg of RNA from T7 LG3; (j) 1.7 μg of polycistronic RNA from wild-type T3; (k) 2.6 μg of RNase III-cleaved RNA from T3; (l) 1.1 μg of RNA from T3 R1; (m) 1.7 μg of T7 polycistronic RNA, 1 mM chloramphenicol; (n) 1.7 μg of RNase III-cleaved T7 RNA, 1 mM chloramphenicol; (o) 1.7 μg of polycistronic T7 RNA, 0.3 mM cycloheximide; (p) 1.7 μg of RNase III-cleaved RNA, 0.3 mM cycloheximide. Wheat germ extracts were programmed with the following RNAs: (q) 1.6 μg of late-period T7 RNA synthesized *in vitro;* (r) 1.5 μg of RNase III-cleaved late-period T7 RNA synthesized *in vitro;* (s) 2 μg of T7 polycistronic RNA; (t) as (s) but heated 70° for 1 min before translation; (u) 1.9 μg of RNase III-cleaved T7 RNA; (v) as (u) but heated 70° for 1 min. Late-period T7 RNA was synthesized essentially as described (28). A major endogenous product of the mammalian system, globin, has a mobility similar to that of T7 gene *0.3* product; control samples using *E. coli* rRNA to protect endogenous synthesis are shown in Fig. 4.

translation, had no effect on the amounts of T7 products made (Fig. 2b and d). In the absence of any added RNA, or when *E. coli* ribosomal RNA was added (to protect endogenous mRNAs), the major product of the mammalian cell-free system is globin which has a mobility only slightly faster than T7 *0.3* product (Fig. 4g).

The polypeptides synthesized *in vitro* might fortuitously migrate like marker polypeptides. Therefore, we used RNA synthesized from T7 DNA carrying known amber or deletion mutations (see Fig. 1) as template for cell-free protein synthesis to unambiguously identify the T7 products (Fig. 2).

When RNA from the deletion mutant *H1* was used as template, a major polypeptide with a molecular weight of about 31,000 was produced and the intensity of the radioactivity in the globin region was substantially reduced (Fig. 2f). The *H1* deletion fuses the *0.3* and *0.7* genes and codes for a fusion polypeptide of about 31,000 (17). Also missing from the products of *H1* DNA directed synthesis is a minor polypeptide migrating slightly slower than T7 ligase (40,000) and a polypeptide of molecular weight *ca* 6000. The larger of these polypeptides is presumably related to the *0.7* gene product. This product is not efficiently synthesized in the bacterial cell-free system (11). The 6000 M_r polypeptide, which has run off the gel shown in Fig. 2, may result from incomplete synthesis of *0.3* or *0.7* gene products, or it may be related to peptides found *in vivo* which come from the right end of gene *0.3* RNA or the left end of gene *0.7* RNA (F. W. Studier, personal communication).

T7 deletion *LG3* removes the genes for the *1.1* (8000 M_r) and *1.3* (ligase, 40,000 M_r) products. RNA transcribed from DNA containing this deletion fails to stimulate the *in vitro* synthesis of the major polypeptide at 40,000 M_r which migrates with the mobility of T7 ligase (Fig. 2i). No polypeptide has been observed which exactly migrates with the gene *1.1* product. At least two minor polypeptides with mobilities between T7 ligase and gene *0.3* product are also missing from the products synthesized in response to T7 LG3 RNA: these (*ca* 18,000 M_r and 35,000 M_r) most likely result from incomplete synthesis of the T7 ligase product. Many additional minor polypeptides are found in this region of the polyacrylamide gel and the synthesis of some of these polypeptides is stimulated by RNase III cleavage of the template (compare Fig. 2b and c with 2d and e). Presumably these polypeptides result from incomplete synthesis of the products of genes *0.7* and *1*.

T7am193 has an amber mutation near the beginning of gene *1;* the nonsense fragment is not detected *in vivo* or *in vitro* by gel analysis (11, 17). T7 *am193* RNA fails to stimulate the synthesis of a large number of polypeptides which migrate more slowly than the *0.7* gene product (42,000 M_r) including the major polypeptide which migrates with the mobility of gene *1* product (RNA polymerase). Confirmation that this polypeptide does, in fact, correspond to gene *1* product comes from analysis of the products synthesized in response to T7 *am342* RNA. T7 *am342* contains an amber mutation near the distal end of gene *1* and RNA from this mutant stimulates the synthesis of a major polypeptide of *ca* 95,000 M_r, the expected size (17) of the gene *1* nonsense fragment.

Phage T3 and T7 code for similar but distinguishable products. Phage T3 RNA synthesized *in vitro*, as described above, stimulates the synthesis of at least four early T3 products in the mammalian cell-free system (Figs. 2j, k, and l). The only T3 product whose authenticity we have conclusively demonstrated by the use of RNAs transcribed from mutant phage DNAs is that of the T3 *0.3* gene product (SAMet cleavage enzyme). This product (11,500 M_r) is missing when RNA from the deletion mutant DNA T3 R1 is used as template. T3 R1 lacks all of gene *0.3* and the proximal portion of gene *0.7* (18). Also absent when T3 R1 RNA is used as template is a polypeptide of molecular weight 41,000, the approximate molecular weight of T3 gene *0.7* product. Although the T3 R1 deletion extends into the *0.7* gene, it does not remove the ribosome initiation site for this gene's product and *0.7* product is observed *in vivo* (18). Since the *0.7* RNA is not sufficiently large to encode two 41,000 M_r polypeptides, the peptide observed here is presumably gene *0.7* product although this remains to be conclusively demonstrated.

Synthesis Using a Wheat Germ System. A wheat germ system synthesized the *0.3*, *1*, and *1.3* gene products in response to added early T7 RNA (Fig. 2s–v). As was found with the mammalian system, RNA cleaved by RNase III is a better template both in terms of total incorporation and specific polypeptides synthesized than polycistronic RNA. Analysis of the products made in response to templates transcribed from mutant phage DNA (data not shown) demonstrated that the three major products synthesized in response to early T7 RNA are in fact the products of genes *0.3*, *1*, and *1.3*. A fourth major polypeptide, synthesized in wheat germ extracts but not in the mammalian cell free system, has a molecular weight close to that expected for T7 gene *1.1* product (8000 M_r). This product does not result from the translation of gene *1.1* RNA, as the product is present when synthesis was programmed with RNA from the LG3 deletion. It most likely results from incomplete synthesis of either gene *0.3* or *0.7*, as it is missing from the products encoded by the H1 deletion.

Phage T3 RNA, cleaved by RNase III, efficiently stimulates the synthesis of T3 gene *1* and *1.3* products, but in contrast to the mammalian system it was not an effective mRNA in wheat germ for the synthesis of T3 *0.3* product (SAMet cleavage enzyme) [data shown elsewhere (19)]. Added T3 polycistronic RNA was less effective in stimulating synthesis of all T3 early products and failed to program the synthesis of detectable amounts of *0.3* product.

When RNA synthesized by purified T7 polymerase was used as template in either the mammalian (data not shown) or wheat germ (Fig. 2q and r) system, several discrete polypeptides ranging in molecular weight from 6000 to 90,000 were produced. One of these had a mobility equal to that of gene *1.3* product. RNase III-cleaved RNA produced at least one peptide (M_r *ca* 10,000) not synthesized in response to uncleaved late T7 RNA. The products synthesized in the mammalian system were similar to those found with wheat germ extracts except that again more minor polypeptides were observed.

Characteristics of Phage T3 and T7 RNA Translation. The above results present compelling evidence that both bacteriophage T3 and T7 RNAs are translatable by cell-free extracts of mammalian cells and wheat. That synthesis occurs on cytoplasmic robosomes is shown by the fact that T7 RNA directed synthesis is inhibited by cycloheximide (0.3 mm), an inhibitor of protein synthesis on 80S ribosomes, but not by chloramphenicol (1 mM), an inhibitor of bacterial and mitochondrial protein synthesis (20) (Fig. 2m–p).

The monovalent (K^+) and divalent (Mg^{++}) optima for protein synthesis with T3 and T7 RNA are the same as we previously found for adenovirus type 2 RNA (7) (mammalian system: 100 mM KCl, 2.2 mM $MgCl_2$, 8 mM putrescine; wheat system: 70 mM KCl, 2.2 mM $MgCl_2$, 86 μM spermine).

The amount of T7 RNA required to saturate a 25 μl reaction, incubated for 90 min, is in excess of 2.7 μg, which is greater than that found for most natural mammalian mRNAs (*ca* 0.5 μg or less). With this amount of RNA (2.7 μg) up to 3×10^6 cpm of [^{35}S]methionine was incorporated, an amount equal to one-third the radioactivity incorporated with saturating amounts of adenovirus type 2 RNA. Both T3 polycistronic and T3 RNase

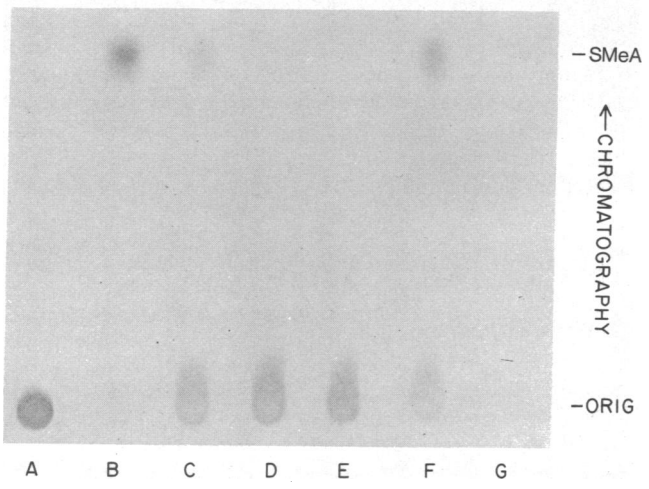

FIG. 3. Assay of *in vitro* synthesized product for SAMet cleavage enzyme activity. SAMet cleavage enzyme cleaves SAMet to thiomethyl adenosine (SMeA) and homoserine (29). Assays were performed essentially as described (21). Five microliter of cell-free extract was added to 20 μl of assay mixture containing 0.05 μCi of *S*-adenosyl[^{14}C]methionine (6.7 μCi/mmol) in 0.05 M Tris·HCl at pH 7.9. After incubation for 1 hr at 30°, 10 μl of assay mixture was spotted on a 20 × 20 cm of cellulose thin-layer sheet (Eastman Kodak no. 13255) and chromatographed with 65% (vol/vol) aqueous ethanol. The samples were: (A) assay mix, no extract; and (B) 2.5 μl extract of T3-infected *E. coli;* the mammalian cell-free reactions were programmed with: (C) 0.87 μg of uncleaved T3 RNA; (D) no exogenous RNA added; (E) RNA from T3 R1 deletion (SAMet cleavage enzyme-deficient); (F) 0.68 μg of RNase III-cleaved T3 RNA; (G) as (F) but without *S*-adenosyl[^{14}C]methionine. Autoradiography was done for 3 days. Analysis of the spots indicated that approximately 3.5 μmol of SAMet was cleaved per 5 μl of RNase III-cleaved T3 RNA programmed reaction per hour.

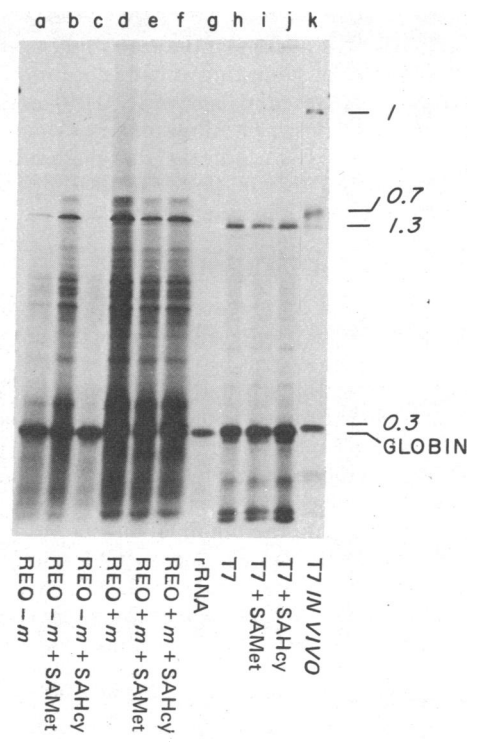

FIG. 4. Effect of SAMet and SAHcy on reovirus and T7 programmed synthesis in the fractionated mammalian cell-free system. Cell-free synthesis and NaDodSO$_4$/polyacrylamide gel analysis was performed as in *Methods* and Fig. 2. The reactions contained 500 μM SAHcy or 6 μM SAMet where indicated: synthesis was programmed with 1 μg of reovirus RNA synthesized in the presence of SAHcy (REO − *m*) or SAMet (REO + *m*) or 2 μg of RNase III-cleaved T7 RNA per reaction as indicated. Track g shows the products synthesized in the presence of 4 μg of *E. coli* ribosomal RNA to protect endogenous synthesis; (k) shows the same *in vivo* labeled T7 extract depicted in Fig. 2a.

III-cleaved RNA (0.73 μg/25 μl reaction) stimulated the incorporation of [^{35}S]methionine into trichloroacetic acid-insoluble products for at least 90 min. The half-life of T7 RNA in the wheat germ system was longer than in the mammalian system; nevertheless, little polycistronic RNA remained intact after 90 min incubation (unpublished results).

Synthesis of Functional T3 SAMet Cleavage Enzyme in the Mammalian Cell-Free System. Functional T3 SAMet cleavage enzyme can be synthesized in bacterial cell-free extracts (21). Fig. 3 shows that functional T3 SAMet cleavage enzyme is synthesized in the mammalian system in response to added T3 RNA. More SAMet cleavage enzyme activity was produced in response to RNA cleaved by RNase III (Fig. 3f) than with polycistronic T3 RNA (Fig. 3c), in agreement with the analysis of the products on NaDodSO$_4$/polyacrylamide gels. No SAMet cleavage enzyme activity was observed with endogenous RNA (Fig. 3d), with RNA from the T3 R1 deletion DNA (Fig. 3e), or with T7 RNA as templates for protein synthesis. The synthesis of SAMet cleavage enzyme activity was prevented by cycloheximide but not by chloramphenicol. No SAMet cleavage enzyme activity was observed at 0 min incubation and the level of SAMet cleavage enzyme synthesized increased substantially between 20 and 90 min of incubation, as expected from the time course of synthesis determined by incorporation of [^{35}S]methionine. Detection of active SAMet cleavage enzyme synthesis in wheat germ extracts was not attempted since preliminary experiments indicated that these extracts had a low level of SAMet cleavage enzyme activity.

Is "Capping" Required for T3 or T7 Translation by 80 S Ribosomes? Reovirus RNA, synthesized *in vitro* in the presence of *S*-adenosylhomocysteine (SAHcy), a competitive inhibitor of *S*-adenosylmethionine (SAMet)-dependent methylation, contains guanosine in 5′- to 5′-linkage with the first base of each mRNA species (but not each molecule); however, this guanosine is not methylated at the 7 position (22). Unmethylated reovirus RNA is not efficiently translated in wheat germ extracts unless SAMet is present. To determine if the "cap" structure was required for reovirus RNA translation in the fractionated mammalian cell-free system, and if this system was capable of methylating reovirus RNA, unmethylated reovirus RNA (REO − *m*) was used to program the mammalian system supplemented with either SAMet or SAHcy (Fig. 4). The incorporation of [^{35}S]methionine into reovirus polypeptides was stimulated by addition of SAMet and depressed by the addition of SAHcy. No effect of SAMet or SAHcy addition was observed on the translation of T7 RNA. Thus, the mammalian system is capable of SAMet-dependent methylation of reovirus RNA and this methylation is required for efficient translation of reovirus RNA. Similar results were obtained with wheat germ extracts. We conclude from the fact that SAMet and SAHcy had no effect on T7 RNA directed synthesis that a complete "cap" structure is not required for T7 translation by mammalian or wheat ribosomes.

Further evidence that T3 and T7 mRNAs are not capped *in vitro* prior to their translation in mammalian and wheat cell-free systems has been obtained by using 7-methylguanosine monophosphate (7mGMP) as an inhibitor of the translation of capped mRNAs (23): At a concentration of 0.25 mM 7mGMP, the translation of brome mosaic virus or globin mRNA was substantially inhibited in the wheat germ cell-free system while 7mGMP had little effect on the pattern of polypeptides made

in response to T7 early-synthesized mRNA (unpublished results). In fact, T3 and T7 early-synthesized mRNAs are translated in the presence of saturating amounts of brome mosaic virus (wheat) or globin (mammalian) mRNA (19), homologous mRNAs known to be "capped". Preliminary evidence using ^{32}P-labeled T3 and T7 RNA indicates that these mRNAs are not modified at their 5′ termini (less than 10% of the 5′ pNp) in the *in vitro* protein synthesizing systems.

DISCUSSION

Both wheat germ and mammalian cell-free systems which synthesize protein have been used to characterize many homologous and nonhomologous mRNAs. It is remarkable that both systems respond similarly to the different mRNAs which have been tried, e.g., brome mosaic virus, adenovirus type 2, and globin (9S) RNAs. However, with T3 and T7 RNAs as templates, two differences between the mammalian and wheat germ systems are found. (*i*) Polycistronic RNA was a better template when added to the mammalian system than when added to the wheat germ system. This result might reflect the more rapid degradation rate of polycistronic T3 or T7 RNA in the mammalian system if RNA cleavage is required for initiation of protein synthesis from internal sites within a polycistronic message. Alternatively, less efficient synthesis from polycistronic RNA may result from some other feature of this RNA, e.g., double stranded regions, than the absence of a free end near the initiation site for each protein. (*ii*) Although the mammalian system synthesized T3 *0.3* product (SAMet cleavage enzyme) with moderate efficiency and made detectable enzyme activity, little if any of this product was detected in wheat germ cell-free extracts programmed with the same RNA. Heating the RNA briefly before translation, RNase III cleavage, or addition of SAMet did not increase the amount of *0.3* product made in wheat germ extracts, yet T3 gene *1* and *1.3* products were synthesized with similar efficiencies in the two systems. Perhaps the wheat germ system rapidly and specifically degrades the T3 *0.3* product, or perhaps the T3 *0.3* gene contains a rare codon which is seldom used in wheat. Alternatively, the two systems differ slightly in their criteria for mRNA selection. The requirements for mRNA binding and initiation by 80S ribosomes is unclear but RNA sequence analysis has elucidated similarities between prokaryotic mRNAs which feature in their interaction with 70S ribosomes. It has been suggested that there is an interaction between the region of mRNA proximal to the initiation codon and either an (3′) AUUACUAG sequence at the 3′ end of 18S RNA in 80S ribosomes or a (3′) AUUCCUCC at the 3′ end of the 16S RNA in 70S ribosomes (8, 9). J. Steitz and R. Bryan (personal communication) have determined the binding site for *E. coli* (70S) ribosomes for the major polypeptide encoded by T7 *0.3* RNA. Five bases which occur eight bases 5′ (to the left as written) to the first AUG in this sequence are complementary to a sequence near the 3′ end of *E. coli* 16S ribosomal RNA, and four bases, one of which is separated by a noncomplementary base pair, are complementary to a sequence at the 3′ end of 18S ribosomal RNA. While the significance of this comparison is not clear, the use of additional characterized prokaryotic mRNAs as well as RNAs from appropriate mutants should yield further data on the proposed interaction.

Recently it has been shown that mammalian 80S ribosomes bind to the same sequences of phage f1 mRNA (S. Legon, P. Model, and H. D. Robertson, in preparation) as do *E. coli* ribosomes (24). This result taken together with the fact that the translation of T3 and T7 RNAs by 80S ribosomes is independent of methylation (see *Results*) is likely to mean that a nearby end, capped or uncapped, is not required for ribosome binding or initiation by 80S ribosomes. This interpretation suggests that translational problems do not preclude the existence of true eukaryotic polycistronic mRNAs although it remains to be shown that polycistronic mRNAs exist in higher organisms.

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