poly(A) content of vegetative and sporulating mRNA has yet to be carried out.

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Identification of the Transcribing DNA Strand for the Deoxynucleotide Kinase Gene of Bacteriophage T2†

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ABSTRACT: A modified procedure was developed which allows RNA–DNA hybridization reactions to be performed without the loss in translational capacity of mRNA which accompanies hybridization at elevated temperatures or in the presence of the denaturing agent formamide. Separated l and r strands of bacteriophage T2 DNA were hybridized in the presence of 4 M sodium perchlorate at 37 °C with total RNA from infected cells. After passage of the hybridization mixture through a nitrocellulose column to remove single-strand DNA and DNA–RNA hybrids, the eluent was measured for its capacity to promote deoxynucleotide kinase (gene 1) synthesis in an in vitro protein-synthesizing system derived from uninfected *Escherichia coli.* With this procedure, which should be of general use for any message whose product can be measured either enzymatically, immunologically, or by location in an acrylamide gel, it was demonstrated that deoxynucleotide kinase mRNA is transcribed from the l strand of bacteriophage T2 DNA. By titrating with l strand DNA, the number of deoxynucleotide kinase transcripts present 9 min after T2 phage infection at 30 °C was estimated to be about 38 copies per cell.

RNA–DNA hybridization techniques and cell-free translation systems have added immeasurably to our knowledge about the synthesis and regulation of specific mRNAs in numerous biological systems. Each methodology, however, possesses a limited capacity to provide information about a given mRNA. For example, translational assays can indicate the presence or absence of a specific mRNA, providing an assay for its product is available, but it cannot reveal more than a qualitative estimate of the abundance of the mRNA. Hybridization methods can provide an accurate measure of mRNA abundance, but this may require either the isolation of nonlethal mutants deficient in the gene transcript or the laborious purification of the mRNA and preparation of its DNA complement to serve as a probe.

By coupling the strengths of both cell-free translation assays and hybridization techniques, we have developed a system for

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identifying and quantitating specific mRNA species in a complex mixture of RNAs and have applied this method as a means of identifying the T-even phage DNA strand which contains the information for the gene 1 product, deoxynucleotide kinase. While it might be implied that this gene is located on the l strand of T-even DNA, based on previous studies (Kasai et al., 1968; Jayaraman and Goldberg, 1970; Guha et al., 1971) demonstrating that the early or prereplicative mRNA is transcribed predominantly from this strand, to date, no firm evidence to this effect has been presented. Through this procedure we can clearly demonstrate that gene 1 mRNA is transcribed from the l strand of T2 DNA.

Experimental Procedures

Microorganisms. Escherichia coli B and T2r+ bacteriophage were used exclusively in this study.

Materials and General Methods. Poly(G-U) with a minimum molecular weight of 100 000 was obtained from Miles Laboratories. Sarkosyl NL97 was a gift from Geigy Pharmaceuticals, and DNase-free bovine pancreatic RNase was supplied by Dr. Thomas H. Plummer, Jr., of this laboratory. Nitrocellulose was purchased from Serva. Siliclad was from Clay Adams, and [^{14}C]uridine (50 μCi/μmol) and L-[4,5-^{3}H]leucine (300 μCi/μmol) were from Schwarz/Mann. All other chemicals were either reagent grade or the highest purity available.

To minimize the loss of nucleic acids due to contaminating nucleases and through adsorption, glassware was treated with Siliclad and heat sterilized prior to use. In addition, all buffers were boiled for 15 min and passed through a 0.2 μm Nalgene sterile filter.

Prior to use, Sephadex G-25 (coarse) was treated for several hours in 10 mM sodium hydroxide to remove any traces of RNase. After washing with several volumes of sterile distilled water, the gel beads were poured into a 1 × 25 cm glass column containing an integral glass frit (coarse) bed support. T2 phage stocks were prepared as described earlier (Maley et al., 1972).

Preparation of RNA and in Vitro Protein Synthesis. Since most of these procedures have been detailed elsewhere (Trimble and Maley, 1975, 1976), their description is only briefly presented here. For phage RNA, 300–500-mL cultures of *E. coli* B were grown to 2 × 10^8 cells/mL in M-9 medium containing 0.4% glucose and infected with T2 phage at a multiplicity of six to eight viruses/cell. After 10 min, chloramphenicol was added to a concentration of 100 μg/mL and 30 s later the culture was poured onto chipped ice. RNA was phenol extracted at room temperature from the harvested cells and then ethanol precipitated (Young and Van Houwe, 1970). After washing twice in 70% ethanol and drying in vacuo, the RNA was dissolved in sterile distilled water at a concentration of 8 to 10 mg/mL and stored unfrozen at 0 °C.

To label RNA, a 75-mL culture of T2-infected *E. coli* B was supplemented with 20 μCi of [^{14}C]uridine from 3 to 9 min postinfection. Cells were harvested at 9 min and the RNA was extracted as above. Less than 0.3% of this RNA, which had a specific activity of 3700 counts min^{-1} μg^{-1}, was RNase stable.

Uninfected S-30 extracts of *E. coli* B were used to promote mRNA-directed protein synthesis by employing recently described modifications which maximize RNA-directed product formation (Trimble and Maley, 1976). Rifampicin was included at a concentration of 2 μg/mL to prevent possible RNA synthesis. The template capacity of the RNAs was directly proportional to the input amount up to about 1.5 mg/mL. Translation assays were allowed to proceed for 20 min at 37 °C using an equivalent amount of total RNA[1] both before and after hybridization. The actual level of RNA added is indicated in the separate table and figure legends and was always in the range of linear response. The measurement of deoxynucleotide kinase activity synthesized in vitro has been described (Trimble et al., 1972a). Incorporation of [^{3}H]leucine, which was included at a final specific activity of 25 μCi/μmol, has also been described (Trimble et al., 1972b).

DNA Strand Separation. T2 phage were purified by centrifugation in a CsCl step gradient (Thomas and Abelson, 1966) and dialyzed overnight against 1 mM sodium ethylenediaminetetraacetate (EDTA[2]) (pH 7.2). Strands were separated at pH 8.5 in the presence of 0.13% Sarkosyl NL97 by the method of Szybalski et al. (1971). Duplicate samples containing approximately 400 μg of T2 phage DNA (10 A_{260} units) and 200 μg of poly(G-U) were heated in 1.5 mL of 1 mM EDTA for 5 min at 100 °C and quenched in ice water. Room-temperature saturated CsCl was added (about 6.5 mL) to give a final density of 1.73 g/mL, and four-mL aliquots were placed in each of four $\frac{5}{8}$ × 3 in. polyallomer tubes. Samples were overlayed with paraffin oil and centrifuged at 30 000 rpm for 70 h at 10 °C in a Beckman Type 40 angle rotor (Flamm et al., 1966). Fractions of 0.1 mL were collected from the top using an ISCO density gradient fractionator. Those samples representing the central 80% of the symmetrical absorbance peak of each separated strand were combined from each of four gradients and centrifuged at 15 000*g* for 10 min to bring any residual paraffin oil to the surface. The l and r strand pools were made 0.1 N in NaOH and incubated for 8 h at 37 °C to hydrolyze the poly(G-U) copolymer. After dialysis against several changes of 0.05 mM EDTA, pH 8.0, NaCl was added to a final concentration of 0.1 M, and the DNA was precipitated with ethanol as described above for RNA. The DNA strands were centrifuged, dried in vacuo, and dissolved in about 1 mL of sterile distilled water. From 800 μg of phage DNA, 220 to 280 μg of each strand possessing an A_{260}/A_{280} absorbance ratio of 1.75 was routinely obtained.

Hybridization Conditions. Phage mRNA and separated T2 DNA strands were hybridized at 37 °C in 4 M sodium perchlorate–0.18 M sodium phosphate, pH 7.1, or in 2 × SSC (SSC = 0.15 M sodium chloride–0.015 M sodium citrate) at 62 °C (Guha et al., 1971) for the times indicated. The perchlorate-containing reactions were prepared by adding 225 mg of solid sodium perchlorate to 70 μL of 1 M sodium phosphate, pH 7.1, in a small conical tube followed by appropriate amounts of phage RNA and separated DNA strands. The final volume of each reaction was adjusted to 0.4 mL with sterile water.

For hybridization with labeled mRNA, reactions were reduced to either 50 or 100 μL and overlayed with a drop of paraffin oil to minimize evaporation. Sampling was performed through the paraffin oil with a microsyringe (Precision Sampling Corp.). To determine total acid-precipitable counts and counts in the RNA–DNA hybrids, a 20- or 40-μL sample was withdrawn at various times and diluted into 1.0 mL of 1× SSC. This sample was divided; to one half was added 50 μg of *E. coli* ribosomal RNA as carrier (Trimble and Maley, 1973) followed by 2 mL of ice-cold 5% trichloroacetic acid; to the other half was added 10 μg of RNase and, after incubation at 37 °C for 30 min, trichloroacetic acid was added in the presence of carrier RNA as above. Precipitates were collected on GF/C

[1] In this paper, total RNA refers to the phenol-extracted RNAs from T2-infected cells which consists largely of ribosomal and transfer RNAs in addition to the phage mRNA.

[2] Abbreviation used: EDTA, ethylenediaminetetraacetic acid.

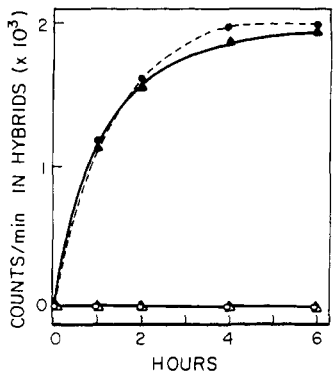

FIGURE 1: Hybridization kinetics of T2 phage [^{14}C]RNA to T2 DNA l and r strands. The 100-μL reactions contained 4.8 μg of l (●, ▲) or r (○, △) DNA strands and 48 μg of T2 10-min RNA (177 000 cpm) in 2× SSC at 62 °C (▲, △) or 4 M sodium perchlorate–0.18 M sodium phosphate, pH 7.1, at 37 °C (●, ○). A 20-μL aliquot was removed at the times indicated, half of which was assayed for total acid-precipitable radioactivity and the other half for RNase-stable radioactivity as described under Experimental Procedures.

glass-fiber filters (Whatman) and counted in a toluene based fluor (Trimble and Maley, 1973).

Recovery of RNA from Hybridization Reactions. Subsequent to hybridization, the nucleic acid fraction consisting of DNA, RNA, and hybrids was desalted by passage through a column of Sephadex G-25 equilibrated in and eluted with 0.5 M sodium acetate, pH 6.5. The effluent was monitored in a flow cell at 254 nm (ISCO UA-4) to obtain the optical peak (4 mL) in a single tube. Separation of nucleic acids from sodium perchlorate was ensured by testing each sample with silver nitrate in the presence of 1 N nitric acid.

Unhybridized phage mRNA, as well as ribosomal and transfer RNAs, were separated from single-strand DNA and DNA–RNA hybrids by passing the Sephadex G-25 eluent dropwise through a tightly packed 1 × 2 cm column of nitrocellulose equilibrated with 0.5 M sodium acetate, pH 6.5. This procedure retains both the single-strand DNA and DNA–RNA hybrids (Boezi and Armstrong, 1967). After washing the nitrocellulose column with two 1-mL portions of 0.5 M sodium acetate, which were combined with the initial effluent, the RNA was ethanol precipitated as described above. The final dried RNA pellet was dissolved in a volume of sterile distilled water equivalent to the volume of the RNA sample initially added to the original hybridization reaction. The recovery, based on absorbance at 260 nm, was routinely 85 to 90%. A portion of this solution was then added to the *E. coli* cell-free system for translation.

Results

Since the strand-identification experiments were based on the premise that mRNA when subjected to the conditions of hybridization could still be recovered and translated in vitro, it was essential to first demonstrate the validity of this assumption. In preliminary studies, it was determined that RNA from T2-infected *E. coli* was recovered quantitatively (based on A_{260} units) following a 12-h incubation with either 50% formamide at 35 °C (Gillespie and Gillespie, 1971), 8 M urea at 40 °C (Kourilsky et al., 1968), or 2× SSC at 62 °C (Guha et al., 1971) but was only 30 to 60% as efficient a template for in vitro synthesis as RNA maintained at 0 °C under the above hybridization conditions (data not shown). Since these losses varied from experiment to experiment, an alternative hybridization procedure was sought to improve the translational capacity of the mRNA.

Hybridization in 4 M Sodium Perchlorate Solution. Sodium perchlorate has been shown to be a potent denaturant for double-stranded DNA (Hamaguchi and Geiduschek, 1962), and at a concentration of 4 M the melting temperature of sheared T4 DNA is lowered by nearly 33 °C (Wetmur and Davidson, 1968). Hoyer and van de Velde (1974) found that the reassociation rate of denatured *E. coli* DNA in 4 M sodium perchlorate–0.14 M sodium phosphate, pH 7.1, at 41 °C was not only 3.5-fold faster than in the 0.14 M phosphate at 60 °C but that RNase was completely inactive in the presence of this chaotropic agent. Thus, the increased reassociation rates, decreased hybridization temperatures, and inhibition of RNase activity provided by sodium perchlorate appeared to be of value in the present study, provided the translational capacity of this RNA was superior to those tested above.

Because the application of sodium perchlorate to RNA–DNA hybridization had not, to our knowledge, been described, it was necessary to establish parameters for the intended reactions. Conditions previously determined to optimize the kinetics of well-matched DNA–DNA reassociation should in theory also optimize the hybridization of *separated* DNA strands and their complimentary RNAs (Galua et al., 1977; Hutton and Wetmur, 1973). Sodium perchlorate at 4 M was employed, as this concentration impaired RNase activity (Hoyer and van de Velde, 1974) and decreased the melting temperature to about 62 °C (Wetmur and Davidson, 1968). Since the reassociation of well-matched nucleic acid strands is optimum at about 25 °C below their melting temperature under a given set of conditions (Britten et al., 1974), a temperature of 37 °C was chosen for the incubations. Sodium phosphate, pH 7.1, was included at 0.18 M to increase the sodium ion concentration to a level that maximized the nucleation rate (Britten et al., 1974). Under the experimental conditions described, sodium phosphate reaches the practical limit of solubility in 4 M sodium perchlorate at a concentration of about 0.2 M.

Before applying hybridization in perchlorate to the identification of the deoxynucleotide kinase transcribing strand, it was necessary to determine the stability of phage mRNA in 4 M sodium perchlorate–0.18 M sodium phosphate, pH 7.1. After 12 h at 37 °C, T2 RNA extracted 10 min postinfection was desalted on a column of Sephadex G-25 and precipitated with ethanol. The recovery of the product was quantitative (based on absorbance at 260 nm), and found, in contrast to the other hybridization conditions tested, to retain about 90% of its in vitro template capacity when measured by either labeled amino acid incorporation or deoxynucleotide kinase enzyme synthesis (data not shown).

To determine a suitable incubation time for RNA–DNA hybridization reactions, the association kinetics in 4 M sodium perchlorate of T2 phage RNA, labeled with [^{14}C]uridine from 3 to 9 min postinfection, and T2 DNA l and r strands were measured. Figure 1 shows that at an RNA/DNA weight ratio of 10, essentially all of the RNase-stable count was bound to the DNA l strand and that the reaction was complete in 4 h at 37 °C. Hybridization at this ratio of DNA to RNA (Figure 1) in the presence of 2× SSC at 62 °C (Guha et al., 1971) revealed that, although the perchlorate-containing reaction approached completion somewhat sooner, about 12% of the input count was ultimately retained in hybrids under both conditions.

In order to ascertain the binding capacity of the T2 DNA l strands, a saturation experiment was performed using constant DNA and increasing levels of RNA. After 6 h at 37 °C in 4 M sodium perchlorate, RNA/DNA weight ratios of up to 5 yielded a constant proportion of the input radioactivity (14%) in RNase-stable counts (Figure 2). This condition is

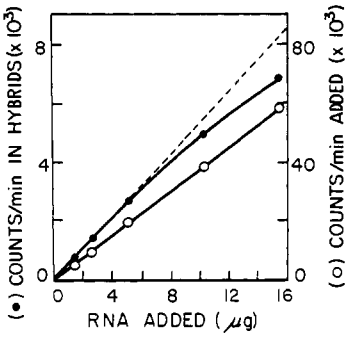

FIGURE 2: Hybridization saturation of T2 DNA l strands by phage mRNA. Each reaction contained 4.0 M sodium perchlorate–0.18 M sodium phosphate (pH 7.1), 2.4 μg of T2 DNA l strands, and increasing amounts of T2 RNA labeled with [^{14}C]uridine from 3 to 9 min postinfection. After hybridization at 37 °C for 6 h, 40 μL was removed and half processed for total acid-precipitable radioactivity (○); the other half was assayed for RNase-stable radioactivity in hybrids (●). The dashed line represents extrapolation of the initial three points where 14% of the input radioactivity was captured in hybrids.

TABLE I: Effect of Separated T2 DNA Strands on the Translation of T2 10-min RNA in Vitro.

template added[a]	deoxynucleotide kinase act. (milliunits/mg)	[^{3}H]Leu incorp (cts min^{-1} 25 μL^{-1})
none	0	96
10-min RNA	0.38	1697
10-min RNA + DNA l strand	0.13	1179
10-min RNA + DNA r strand	0.14	1277

[a] Each 0.2-mL *E. coli* S-30 translation assay contained 165 μg of phage RNA and 15 μg of T2 DNA l or r strands where indicated.

characteristic of hybridization of RNA with excess DNA (Bolle et al., 1968a). Above this ratio, the proportion of RNA label in hybrids decreased progressively, indicating that an increasing number of sites on the DNA had become saturated by their complementary RNAs.

Removing Single-Strand DNA and Hybrids Prior to Translation. Since single-strand DNA forms tight initiation complexes with bacterial ribosomes (Condit et al., 1973), it was necessary to determine whether the separated DNA strands and hybrids present in the hybridization reactions would impair the translation of free mRNA in the *E. coli* cell-free system. As shown in Table I, the addition of about 10% by weight of separated T2 DNA l or r strands to phage mRNA isolated 10 min postinfection resulted in a 30% inhibition of overall peptide synthesis and a striking 65% reduction in deoxynucleotide kinase enzyme synthesis in the cell-free protein-synthesizing system. This interference could be prevented, however, by passage of the hybridization mixture, after G-25 chromatography, through a small nitrocellulose column as described in the Experimental Procedures. As shown in Table II, about 85% of the single-strand DNA and RNA–DNA hybrids was retained by the nitrocellulose, while only 10 to 15% of the RNA and its associated translational capacity was removed by this treatment.

T2 l Strand Codes for Deoxynucleotide Kinase mRNA. Having ascertained that translatable RNA was recoverable in high yields from perchlorate-containing hybridization reactions, the identity of the DNA strand coding for the gene 1 message was investigated. The results of hybridization reactions performed with 10-min T2 RNA in a tenfold weight excess over T2 DNA l and r strands are presented in Table III.

TABLE II: Retention by Nitrocellulose of RNA, Single-Strand DNA, and RNA–DNA Hybrids.

condition	l strand DNA[a] (A_{260})	RNA–DNA hybrids[b] (cpm)	RNA[c] A_{260}	RNA[c] kinase translated (milliunits/mg)
applied	1.250	15 700	6.4	0.410
eluted	0.153	2 400	6.0	0.357

[a] T2 l strand DNA (1.25 A_{260}) in 1.0 mL of 0.5 M sodium acetate, pH 6.5, was applied to nitrocellulose and eluted with sodium acetate as described under Experimental Procedures. [b] T2 [^{14}C]RNA (40 μg; 148 000 cpm) was hybridized for 5 h at 62 °C with 5 μg of l strand DNA in 0.1 mL of 2× SSC, diluted into 1.0 mL of 0.5 M sodium acetate, pH 6.5, and passed through nitrocellulose. Samples were taken before and after the nitrocellulose step, treated with RNase, and analyzed for acid-precipitable radioactivity in the hybrids. [c] T2 10-min RNA (6.4 A_{260}) in 1.0 mL of 0.5 M sodium acetate, pH 6.5, was passed through nitrocellulose, ethanol precipitated, and dissolved in 0.1 mL of water. Two hundred micrograms of the RNA, before and after nitrocellulose chromatography, was translated in vitro.

TABLE III: Translation of 10-min T2 RNA after Hybridization to Separated T2 DNA l and r Strands.[a]

template added	synthesis in vitro: deoxynucleotide kinase (milliunits/mg)	synthesis in vitro: [^{3}H]Leu (cts min^{-1} 25 μL^{-1})
none	0	56
untreated RNA	0.35	1618
treated RNA	0.30	1590
RNA hybridized to DNA l strand	0.07	675
RNA hybridized to DNA r strand	0.33	1660

[a] Where present, 500 μg of RNA and 50 μg of separated DNA strands were incubated for 6 h at 37 °C in 4 M sodium perchlorate–0.18 M sodium phosphate, pH 7.1. The untreated RNA was kept in ice and the treated RNA was incubated in perchlorate without DNA. RNA (200 μg) isolated from the incubation mixtures as described under Experimental Procedures served as template in the 0.2-mL cell-free incubations.

Translation of 200 μg of RNA in each case, after the desalting and nitrocellulose steps, clearly shows (Table III) that 80% of the deoxynucleotide kinase messenger activity present at 10-min in T2-infected *E. coli* was removed by the DNA l strands. It is important to note that hybridization with the r strands did not diminish the capacity of the isolated RNA to direct either deoxynucleotide kinase or general protein synthesis relative to the RNA from control incubations. The experimental results clearly indicate, therefore, that deoxynucleotide kinase mRNA is complementary to the T2 DNA l strand.

Estimation of Gene 1 mRNA Copies Per Cell. As shown in Table III, nearly all of the deoxynucleotide kinase mRNA was depleted from 500 μg of 10-min T2 RNA by using as a specific affinity absorbant 50 μg of the l-strand T2 DNA. By using lesser amounts of DNA l strands in separate hybridization reactions, a titration curve for kinase mRNA should theoretically be obtained. This curve might provide an estimate of the number of kinase mRNA copies present in the RNA sample, and thus the number of transcripts present per infected cell at the time the RNA was isolated.

To test this concept, separate 500-μg portions of T2 9-min RNA were hybridized in the 4 M sodium perchlorate system with different amounts of T2 DNA l strands (Figure 3). The

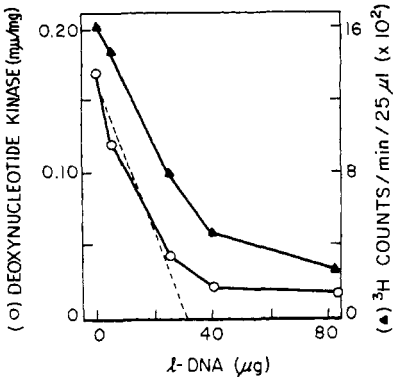

FIGURE 3: Titration of deoxynucleotide kinase mRNA from 9-min T2 RNA with increasing amounts of T2 DNA l strands. Each 0.4-mL hybridization solution contained 4 M sodium perchlorate–0.18 M sodium phosphate (pH 7.1), 500 μg of 9-min T2 RNA, and the indicated amount of DNA l strands. After hybridization for 6 h at 37 °C, RNA was isolated as described under Experimental Procedures and dissolved in 50 μL of water. Twenty microliters of each RNA sample was translated in 0.2-mL of an *E. coli* cell-free system. The enzyme activity synthesized (○) and labeled leucine incorporated (▲) are plotted against the actual amount of DNA added to the hybridization reaction mixture. Extrapolation of the kinase synthesis curve to the abscissa indicates the approximate level of DNA l strands which theoretically remove all of the kinase message from the RNA sample.

RNAs recovered after passage through nitrocellulose were translated in the *E. coli* cell-free system, and the extent of deoxynucleotide kinase synthesis was measured. As shown in Figure 3, 30% of the kinase transcripts present in 500 μg of 9-min RNA was removed by 5 μg of l-strand DNA, while 75% was removed by 25 μg. By increasing the level of l strands further, over 90% of the kinase mRNA could be depleted from the RNA sample.

At low DNA levels, which represent hybridization in RNA excess, a linear relationship should exist between the DNA added and the kinase mRNA removed, since under these conditions all of the available gene 1 sites on the DNA should be saturated with their complementary RNAs (Bolle et al., 1968a). Extrapolating the linear portion of this curve to the abscissa provides an intercept which approximates the amount of DNA theoretically required to deplete all of the kinase messages from the 500 μg of RNA hybridized. While a broad range of DNA was chosen in Figure 3 to display the entire titration curve, a more accurate initial slope might be expected with DNA levels which deplete less than 50% of the kinase mRNA, as this would ensure conditions of RNA excess. Nevertheless, from the initial slope in Figure 3 (dashed line) an intercept of about 30 μg of DNA l strands was obtained. Assuming that the T2 genome consists of 160 kilobase pairs (Kim and Davidson, 1974) which is equivalent to 1.08×10^8 daltons, each of the separated strands should approximate half of this mass or 5.4×10^7 daltons. From this value it is determined that each strand weighs 9×10^{-9} μg and thus 30 μg of strands, the amount theoretically required to deplete all the kinase mRNA from the RNA sample employed (Figure 3), should contain 3.34×10^{11} l-strand copies. Since each strand possesses only one kinase gene, 3.34×10^{11} must also be the number of kinase mRNA transcripts present in the 500 μg of RNA hybridized with the DNA. As this amount of RNA was isolated from 8.7×10^9 cells at 9 min postinfection, we calculate that there should be 38 kinase transcript copies per cell at this time period.

Discussion

T-even phage development in *Escherichia coli* is associated with the temporal expression of specific "classes" of viral RNA which can be broadly divided into early (prereplicative) and late (postreplicative) periods of synthesis (Hall et al., 1964; Bolle et al., 1968a, 1968b). By separating the complementary strands of DNA (Guha and Szybalski, 1968), it has been possible to show that most of the true early RNA is transcribed from the l strand of the DNA (Kasai et al., 1968; Jayaraman and Goldberg, 1970; Guha et al., 1971), while the majority of the late RNA is copied from the r strand (Kasai et al., 1968; Jayaraman and Goldberg, 1970).

The early RNA appears to be generated from two different types of promoter sites, distinguished by their different temporal sensitivities to rifampicin (O'Farrell and Gold, 1973), an inhibitor of RNA synthesis initiation (Wherli and Staehelin, 1971). One site termed "early" (P_E) is *insensitive* to rifampicin almost from the start of infection, while the other site termed delayed or "quasi-late" (P_Q) is *sensitive* to rifampicin until about 2-min postinfection. In most cases, the P_Q promoters are positioned within reading units of cotranscribed genes which are initiated at P_E promoters (O'Farrell and Gold, 1973; Hercules and Sauerbier, 1973, 1974; Schmidt and Mazaitis, 1970), but limited number of genes, including 32 (O'Farrell and Gold, 1973), 1 (Cohen et al., 1974; Trimble and Maley, 1975), and perhaps 40 and 41 (Jayaraman, 1972) appear to be transcribed solely from P_Q promoters.

Gene 32 was shown recently, by comparing the size of amber peptides with their genetic mapping position, to be transcribed in a counterclockwise manner from the T-even DNA l-strand (Mosig and Bock, 1976). By combining the specificity of hybridization with the resolving power of in vitro translation, we have provided evidence for the anticipated result that the T2 phage DNA l strand is that which codes for the deoxynucleotide kinase gene (Table III). Thus, like the other genes initiated from P_E and P_Q promoters, gene 1 is also transcribed in a counterclockwise direction.

Although several hybridization systems were tested (Guha et al., 1971; Gillespie and Gillespie, 1971; Kourilsky et al., 1970), none were as effective as 4 M sodium perchlorate in maintaining the template capacity of the mRNA. Strand scission has been shown to be a problem with high-temperature hybridization techniques (Kourilsky et al., 1970) and we have observed that RNA aggregates in 50% formamide (Gillespie and Gillespie, 1971), as evidenced by increased *s* values on sucrose gradient centrifugation (unpublished data). Both factors would be expected to diminish the translational capacity of an RNA preparation. An additional complication, that of single-strand DNA interfering with cell-free protein synthesis (Table I), was overcome in our study by using nitrocellulose (Boezi and Armstrong, 1967) to remove the DNA single strands and hybrids without appreciably altering the template capacity of the residual mRNA fraction (Table III). The interference in translation by DNA may be a characteristic of the in vitro prokaryote translating system used in these studies, since as shown recently (Paterson et al., 1977) with a hybrid-arrest eukaryote translating system similar in concept to that described here, high levels of DNA were not inhibitory. This finding may reflect the difference in efficiencies of translation of capped eukaryote mRNA vs. uncapped prokaryote mRNA in the presence of DNA.

With this procedure, we have been able to obtain an estimate of the number of deoxynucleotide kinase transcripts present in a complex pool of T2 phage and *E. coli* RNAs (Figure 3). Thus, at 9 min postinfection, the approximate peak of kinase message production at 30 °C (unpublished observations), the T2 mRNA pool contains about 38 kinase mRNA copies per cell. Since the yield of RNA from the cells and efficiency of translation may have been less than 100%, 38 copies per cell

would represent a lower limit. Using a different hybridization procedure, Kasai and Bautz (1969) found that there are 63 copies of T4 rII message per cell at 4 to 6 min postinfection and 20 copies of lysozyme message per cell at 12 to 15 min postinfection, which coincides well with the value reported here for the number of kinase messages present in T2-infected cells.

The advantage of the methodology described in this paper is that it offers a relatively simple means for obtaining the l- or r-strand location of various genes and for determining specific mRNA transcript numbers without the necessity of using purified mRNA and cDNA probes. If the DNA of the test organism can be separated into complementary strands, quantitation of any mRNA should be possible, providing it yields a translation product which can be assayed enzymatically, as a radioactive band on acrylamide gel, or as a radioactive immune precipitate.

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