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RNA Initiation with Dinucleoside Monophosphates during Transcription of Bacteriophage T4 DNA with RNA Polymerase of *Escherichia coli*

(dinucleoside monophosphate stimulation/sigma factor/dinucleoside monophosphate initiation)

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ABSTRACT **The effects of dinucleoside monophosphates on the transcription of phage T4 DNA by *E. coli* RNA polymerase have been examined at various concentrations of the sigma subunit and extremely low concentration of ribonucleoside triphosphate. The following conclusions were reached: (*i*) Labeled specific dinucleoside monophosphates are incorporated as chain initiators. (*ii*) When the ratio of sigma factor to core enzyme is small, there is a general stimulation by most 5′-guanosyl dinucleoside monophosphates. (*iii*) When the ratio is increased or holoenzyme is present, ApU, CpA, UpA, and GpU are the most effective stimulators. (*iv*) At high concentrations of sigma factor, only certain adenosine-containing dinucleoside monophosphates (ApU, CpA, UpA, and ApA) stimulate the reaction. (*v*) Competition hybridization studies indicate that the RNAs stimulated by dinucleoside monophosphates (ApU, CpA, UpA, and GpU) are of the T4 "early" type. (*vi*) Studies involving both combinations of stimulatory dinucleoside monophosphates and competitive effects of these compounds on chain initiation by ATP and GTP suggest that the stimulatory dinucleoside monophosphates act as chain initiators and may recognize part of a continuous sequence in a promoter region. Studies based on the incorporation of ^{3}H-labeled stimulatory dinucleoside monophosphates support the above conclusions.**

The discovery of the sigma (σ) subunit of bacterial RNA polymerase added strength to the concept of positive control of transcription *in vitro* (1–3). σ Factor is a necessary requirement for accurate initiation of RNA synthesis by core RNA polymerase *in vitro* on certain phage DNA templates (4–7). Our studies with well-defined templates have shown that the stimulatory effect of σ factor depends on both the base composition and secondary structure of the template, as well as the concentration of ribonucleoside triphosphate, and that the same type of stimulatory effect is obtained with oligoribonucleotides complementary to the templates (8). Earlier studies by Niyogi and Stevens (9) with holo RNA polymerase and synthetic polyribonucleotide templates indicated that *only complementary* oligoribonucleotides with a free 3′-hydroxyl group are stimulatory and preferentially incorporated into the 5′phosphomonoester chain end of the product, hence acting as chain initiators. Gros *et al.* (10), using *Escherichia coli* DNA as template and normal substrate concentrations, observed small stimulations by various oligoribonucleotides. Downey *et al.* (11) reported that certain dinucleoside monophosphates stimulate chain initiation and the overall rate of RNA synthesis at low substrate concentrations in reactions using either T4 DNA and holoenzyme or T5 DNA with core enzyme or holoenzyme. Our present studies with T4 DNA demonstrate that labeled dinucleoside monophosphates are incorporated as chain initiators and that the type of stimulatory dinucleoside monophosphates is selectively determined by the ratio of σ factor to core enzyme. A low ratio favors guanosine-starts, whereas at higher values adenosine-starts are predominant. With holoenzyme, ApU, CpA, GpU, and UpA are the most effective. RNAs stimulated by dinucleoside monophosphate (ApU, CpA, GpU, and UpA) are of the T4 "early" type, suggesting that these dinucleoside monophosphates act at T4 "early" promoter regions. Studies involving combinations of stimulatory dinucleoside monophosphates and incorporations of [γ-^{32}P]ATP, [γ-^{32}P]GTP, and ^{3}H-labeled stimulatory dinucleoside monophosphates suggest that the latter may recognize part of a continuous sequence in T4 DNA. A speculative model for T4 "early" RNA initiation (promoter) regions is proposed, based on previous studies (8, 9, 12) that RNA synthesis is stimulated only by oligoribonucleotides *complementary* to the template.

MATERIALS AND METHODS

Substrates and Enzymes. Unlabeled ribonucleoside triphosphates were products of P-L Biochemicals, and ^{14}C-labeled ribonucleoside triphosphates were purchased from Schwarz BioResearch. [γ-^{32}P]ATP and [γ-^{32}P]GTP were products of International Chemical and Nuclear Corp. All 16 unlabeled dinucleoside monophosphates were purchased from Sigma Chemical Co. Each of these, on alkaline hydrolysis and separation of the products by paper chromatography, yielded equivalent amounts of the expected nucleotide and nucleoside, thereby confirming both the purity and sequence of the compound. [^{3}H]ApU was prepared by the action of pancreatic RNase on [^{3}H]poly(A,U) of base ratio 1:1 that was prepared by polynucleotide phosphorylase (Worthington Biochemical Corp.). Products of the type ApUp, ApApUp, etc., were separated by paper chromatography with the solvent 1-propanol–concentrated NH_4OH–H_2O (55:10:35 by volume). The ApUp was then treated with *E. coli* alkaline phosphatase to produce ApU, which was purified by paper chromatography. Similarly, [^{3}H]GpU was made from [^{3}H]poly(G,U) of base ratio 1:1. Both dinucleosides contained 200 cpm/pmol.

T4 DNA was isolated by phenol extraction (13), dialyzed against, and stored in 20 mM Tris·HCl (pH 7.8)–25 mM KCl.

T4 DNA was denatured by heating in a boiling-water bath for 10 min and chilling the tube in an ice bath. Unlabeled 5-min and 20-min T4 RNAs were gifts from Dr. Audrey Stevens.

The purification of RNA polymerase from *E. coli* B was done according to Stevens (14), except that the final sucrose density-gradient centrifugation step was replaced by a glycerol-gradient centrifugation step (15). σ Factor and the "core" enzyme were separated by published procedures (1, 16).

Competition Hybridization. ^{14}C-Labeled T4 RNA was synthesized *in vitro* in a reaction mixture (3.0 ml) containing the following: 20 mM Tris·HCl (pH 8.1); 20 mM 2-mercaptoethanol; 10 mM $MgCl_2$; 10 μM each of [^{14}C]ATP, [^{14}C]CTP, [^{14}C]GTP, and [^{14}C]UTP; 150 μg of T4 DNA; 0.21 mM dinucleoside monophosphate as specified; and 100 μg of holo RNA polymerase. After incubation at 37° for 30 min, the synthesized RNA was isolated by the method of Bolle *et al.* (17), and competition hybridization was performed (17).

Enzymatic Assays. (*i*) The reaction mixture for measurement of the overall rate of RNA synthesis (0.2 ml) contained 20 mM Tris·HCl (pH 8.1); 20 mM 2-mercaptoethanol; 10 mM $MgCl_2$; 10 μM each of ATP, CTP, GTP, and [^{14}C]UTP; 10 μg of T4 DNA; and either core enzyme and σ factor or holoenzyme as specified in the tables and figures.

(*ii*) The reaction mixture for measurement of the initiation rate of RNA synthesis (0.2 ml) contained 20 mM Tris·HCl (pH 8.1); 20 mM 2-mercaptoethanol; 10 mM $MgCl_2$; 40 μM [γ-^{32}P]ATP or [γ-^{32}P]GTP; 0.40 mM of the remaining three ribonucleoside triphosphates; 10 μg of T4 DNA; 5 μg of core RNA polymerase; and 5 μg of σ factor.

The isotope incorporated into acid-insoluble material was measured after a 10-min incubation period of the above reaction mixtures at 37°. The reaction was stopped by addition of cold 5% trichloroacetic acid containing 10 mM sodium pyrophosphate, and the solution was filtered through a Whatman glass paper (GF/C) disc. The glass paper disc was then washed extensively with the same solvent, followed by cold ethanol, dried under an infrared heat lamp, placed in a scintillation vial, and counted with BBOT (2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene)-toluene solution (4 g of BBOT in 1 liter of toluene) in a Packard Tricarb spectrometer.

RESULTS

Effect of Dinucleoside Monophosphates on RNA Synthesis in the Presence and Absence of 0.2 M KCl. At low (10 μM) ribonucleoside triphosphate concentration and in the presence of 0.2 M KCl, RNA synthesis with holo RNA polymerase was reduced to one-tenth of that in the absence of KCl. The inhibitory effect of KCl at low substrate concentrations will be described in detail elsewhere. In the presence of 0.2 M KCl, various dinucleoside monophosphates produced rather high stimulations relative to the KCl-inhibited control value; of these, the highest, nearly 13-fold, was by UpA. This result agrees with the findings of Downey *et al.* (11), who found an almost 10-fold stimulation by UpA under similar conditions. CpA, ApU, GpU, UpG, and ApG stimulated 7.5-, 6.5-, 4.5-, 3.0-, and 2.5-fold, respectively. However, under noninhibitory conditions (when KCl was absent) more modest stimulations were obtained relative to the much higher control value. Rather similar stimulations were obtained selectively and consistently with ApU, CpA, UpA, and GpU, namely, 4.1-, 3.9-, 3.5-, and 2.9-fold, respectively.

Effects of Dinucleoside Monophosphates on RNA Synthesis with Various Amounts of σ Factor. Because of the aforementioned inhibitory effect of KCl at low substrate concentrations, subsequent experiments were done in the absence of KCl. In the first experiment, only core RNA polymerase was used. A large amount (20 μg) was added to each assay, since core enzyme alone transcribes T4 DNA very poorly. As shown (Table 1), GpG and GpA stimulated 2.7- and 2.1-fold, respectively. Under these conditions there appears to be some preference for 5′-guanosine initiations. When only 1 μg of σ factor was added to 10 μg of core enzyme, several dinucleoside monophosphates were found to stimulate over 3-fold. These include UpA, GpG, GpU, GpA, and GpC (Table 1). Here, with the exception of UpA, a preference for 5′-guanosine initiations is evident. When σ factor was increased to 5 μg in the presence of 10 μg of core enzyme, UpA, CpA, ApU, GpU, and UpG were the most effective stimulators (Table 1). With this increased amount of σ factor, there appears to be a transition away from predominantly guanosine "starts." With 5 μg of σ factor plus 5 μg of core enzyme, CpA, UpA, ApU, and GpU stimulated maximally (Table 1), indicating a further departure from guanosine "starts." This combination of core and σ factor gave results similar to holoenzyme in the absence of KCl. When a large excess of σ factor was added (20 μg of σ factor to 10 μg of core enzyme), CpA, UpA, ApU, and ApA were effective stimulators (not shown), indicating a total departure from guanosine "starts" and a predominance of adenosine-containing dinucleoside monophosphates as effective stimulators.

TABLE 1. *Effect of dinucleoside monophosphates on RNA synthesis*

Dinucleoside monophosphate added*	[^{14}C]-UMP incorporated (pmol)	Stimulation (-fold)	Dinucleoside monophosphate added	[^{14}C]-UMP incorporated (pmol)	Stimulation (-fold)
Core RNA polymerase (20 μg)			*Core RNA polymerase (10 μg) σ (1 μg)*		
None	6	—	None	6	—
GpG	16	2.7	UpA	46	7.6
GpA	13	2.1	GpG	42	7.0
			GpU	39	6.5
			GpA	26	4.3
			GpC	22	3.7
Core RNA polymerase (10 μg), σ (5 μg)			*Core RNA polymerase (5 μg), σ (5 μg)*		
None	21	—	None	15	—
UpA	84	4.0	CpA	72	4.8
CpA	74	3.5	ApU	69	4.6
ApU	65	3.1	UpA	68	4.5
GpU	53	2.5	GpU	47	3.1
UpG	47	2.3			

Dinucleoside monophosphates (where added) were 0.21 mM.
* The remaining dinucleoside monophosphates, not shown in this table, stimulated less than 2-fold.

Effects of Dinucleoside Monophosphate Combinations. The four most stimulating dinucleoside monophosphates (ApU, CpA, GpU, and UpA) were examined in all possible combinations of two to see whether there were negative, additive, or synergistic effects. These studies were done with different concentrations of T4 DNA and holoenzyme, since separate studies indicated that the stimulatory reactions are limited both by DNA and enzyme concentrations. With ApU, CpA, and UpA, the net effect with any combination of two was not additive, but usually approached the level of the more stimulatory of the two. Results with ApU and UpA are shown in Fig. 1. Additivity was found when GpU was com-

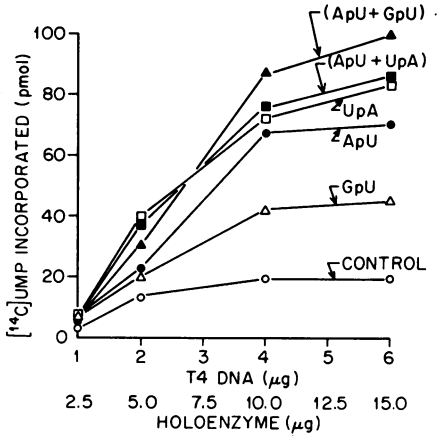

FIG. 1. Effects of dinucleoside monophosphate (0.21 mM, each) on [^{14}C]UMP incorporation with increasing concentrations of T4 DNA and holoenzyme.

bined with either ApU (Fig. 1) or CpA or UpA. These results would suggest that CpA, ApU, and UpA may recognize one continuous DNA sequence, whereas GpU and other guanosine-containing dinucleoside monophosphates may recognize another continuous DNA sequence in the promoter region(s).

Competition Hybridization Against 5- and 20-Min Unlabeled T4 RNA. It is well established that holo RNA polymerase transcribes only "early" phage T4 DNA genes (18). The following results (Table 2) indicate that the RNAs produced with holoenzyme without or with dinucleoside monophosphate (ApU, CpA, GpU, or UpA) stimulation fall in this category. All RNAs compete very well with 5-min "early" unlabeled RNA. Addition of 20-min "late" RNA has little additional competitive effect, demonstrating that these dinucleoside monophosphates act on T4 DNA regions recognized by RNA polymerase containing σ factor, namely, T4 "early" promoter regions.

TABLE 2. *Competition hybridization of dinucleoside monophosphate-stimulated T4 RNA against 5- and 20-min unlabeled T4 RNA*

	[^{14}C]RNA retained in hybrid (%)			
^{14}C-Labeled T4 RNA	0.6 mg/ml 5-min RNA	0.9 mg/ml 5-min RNA	1.35 mg/ml 5-min RNA	0.9 mg/ml 5-min RNA + 1.0 mg/ml 20-min RNA
Control (no dinucleoside monophosphate)	11	7	4	5
ApU-stimulated	15	10	8	7
CpA-stimulated	21	5	7	5
GpU-stimulated	12	10	8	7
UpA-stimulated	20	14	10	8

^{14}C-Labeled T4 RNAs were synthesized and isolated. Annealing was done at 60° in 0.3 M NaCl–0.03 M sodium citrate (pH 7.1) (total volume, 0.225 ml) according to the method of Bolle *et al.* (17). The ^{14}C-labeled RNAs were 7.5 μg/ml, 5- and 20-min unlabeled RNAs were at concentrations shown above, and the denatured T4 DNA was 11 μg/ml.

Dinucleoside Monophosphates as Competitors of ATP and GTP in Chain Initiation. RNA polymerase can initiate new RNA chains, almost exclusively with either ATP or GTP (19, 20). If dinucleoside monophosphates are incorporated at the 5′ terminus of the RNA chain, one would expect a reduction of [γ-^{32}P]ATP incorporation in the event of 5′-adenosine "starts" and a reduction of [γ-^{32}P]GTP incorporation in the event of 5′-guanosine "starts." Hence, the following experiments (Fig. 2) were designed to examine the effects of the highly stimulating dinucleoside monophosphates CpA, ApU, UpA, and GpU, and (not shown in Fig. 2) a mildly stimulating dinucleoside monophosphate, GpA, and a nonstimulating one, GpC. For ApU, at a maximum stimulatory concentration (as reflected by [^{14}C]UMP incorporation), [γ-^{32}P]ATP incorporation was reduced to less than 15% of that in the absence of ApU. [γ-^{32}P]GTP, however, remained stable at 5–10% above the control value. Thus, in this case it is clear that ApU is selectively competing with ATP for incorporation at the 5′ end. For UpA, [γ-^{32}P]ATP was almost immediately reduced to less than 35% of the initial control value. However, at higher concentrations of UpA there was also some effect on [γ-^{32}P]GTP, gradually reducing the incorporation from 80 to 55% of the control. At the maximum effective concentration of CpA, incorporation of [γ-^{32}P]ATP was reduced to less than 15% of the control, but that of [γ-^{32}P]GTP was reduced to about 60% of the control. For GpU, most of the effect was on [γ-^{32}P]GTP incorporation, with reduction to less than 40%, but there was some effect on [γ-^{32}P]ATP incorporation, with reduction to 70%. For GpA, which stimulated only modestly, [γ-^{32}P]ATP incorporation remained unaffected, and that of [γ-^{32}P]GTP was reduced to 35–50% of the control value (not shown). For GpC, which

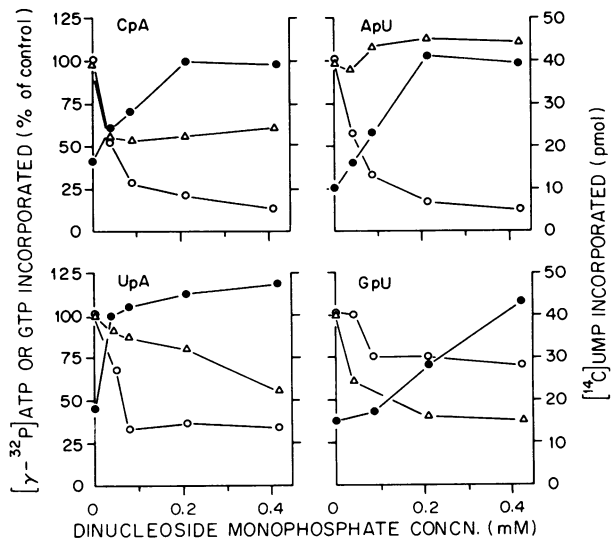

FIG. 2. Effect of increasing dinucleoside monophosphate concentration on [^{14}C]UMP, [γ-^{32}P]ATP, and [γ-^{32}P]GTP incorporations. Reaction conditions are described in *Methods* for measurement of the overall rate of RNA synthesis ([^{14}C]UMP incorporation) and the rate of initiation of RNA synthesis ([γ-^{32}P]-ATP and [γ-^{32}P]GTP incorporation). 5 μg of core enzyme and 5 μg of σ factor were used for each reaction mixture. O——O, [γ-^{32}P]ATP; Δ——Δ, [γ-^{32}P]GTP; ●——●, [^{14}C]UMP.

had no appreciable effect on overall synthesis, there was no appreciable reduction of [γ-^{32}P]ATP or [γ-^{32}P]GTP incorporation (not shown). Thus, ApU, UpA, and CpA seem to have a greater effect on [γ-^{32}P]ATP incorporation than on [γ-^{32}P]GTP incorporation, whereas with GpU and GpA the reverse is true. The fact that some dinucleoside monophosphates affect chain initiations by both ATP and GTP, although to different degrees, would suggest that these dinucleoside monophosphates may recognize adjoining DNA dinucleotide sequences.

Incorporation of Labeled Dinucieoside Monophosphates In order to show directly that stimulatory dinucleoside monophosphates act as chain initiators, it was necessary to show that these are incorporated into the RNA product. Thus, the following experiments were done with [^{3}H]ApU and [^{3}H]GpU. As shown in Fig. 3, incorporation of [^{3}H]ApU increased with its concentration, whereas incorporation of [γ-^{32}P]ATP gradually decreased. Thus, [^{3}H]ApU replaces [γ-^{32}P]ATP as an initiator during RNA synthesis. Separate studies confirm that ApU is incorporated at the 5′-terminus of the RNA product and not internally. Similar studies with [^{3}H]GpU and [γ-^{32}P]GTP suggest that GpU replaces GTP as an initiator during RNA synthesis. As a further examination of the possibility that the stimulatory dinucleoside monophosphates may recognize continuous DNA dinucleotide sequences, incorporation of [^{3}H]ApU was examined with increasing concentrations of unlabeled CpA or UpA. As seen in Fig. 4, either CpA or UpA very effectively reduces incorporation of [^{3}H]ApU, whereas compounds like GpU and GpC had little effect on incorporation. This would suggest that CpA, ApU, and UpA initiate synthesis of an RNA chain(s) by recognizing closely associated complementary DNA dinucleotide sequences in "early" promoter regions of T4 DNA. Similarly, from preliminary studies, incorporation of [^{3}H]GpU was effectively reduced by ApG or UpG, whereas ApU, UpA, or CpA had a smaller effect. Thus, ApG, GpU, and UpG may recognize adjoining DNA dinucleotide sequences in "early" promoter regions of T4 DNA.

DISCUSSION

Dinucleoside monophosphates selectively stimulate overall RNA synthesis, depending on the ratio of σ factor to core RNA polymerase. Guanosine-containing dinucleoside monophosphates are active at low ratios, whereas adenosine-containing ones are predominant at higher values. The results agree with our previous observations (unpublished) that σ factor preferentially stimulates initiation of chains beginning with ATP rather than with GTP. Competition experiments with dinucleoside monophosphates and either [γ-^{32}P]ATP or [γ-^{32}P]GTP suggest that the dinucleoside monophosphates are acting at the level of initiation in the presence of σ factor (Fig. 2). This conclusion was established by observation that [^{3}H]ApU incorporation competed effectively with [γ-^{32}P]ATP incorporation, depending on the concentration of the former (Fig. 3), whereas [^{3}H]GpU incorporation competed with [γ-^{32}P]GTP incorporation.

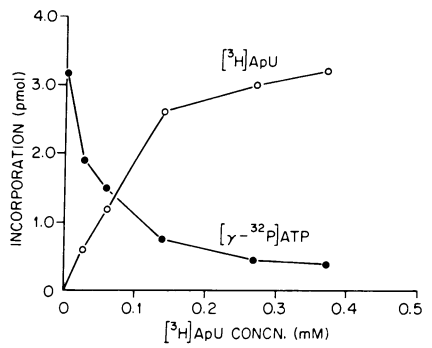

FIG. 3. Effect of increasing ApU concentration on chain initiation by either ApU or ATP. 10 μg Of holo RNA polymerase was used for each reaction mixture.

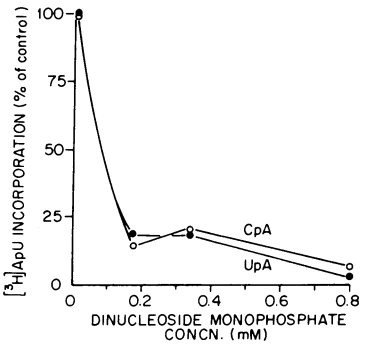

FIG. 4. Effect of increasing concentration of CpA or UpA on chain initiation by ApU. [^{3}H]ApU concentration was 0.10 mM. 10 μg of holo RNA polymerase was used for each reaction mixture.

Our observation that different combinations of stimulatory dinucleoside monophosphates (ApU, UpA, and CpA) exhibit little additivity in stimulatory effect suggests that the T4 DNA sequences complementary to these compounds may be in close proximity. Competition between [^{3}H]ApU and unlabeled dinucleoside monophosphates (CpA and UpA) in chain initiation (Fig. 4) supports this hypothesis. Similarly, GpU, ApG, and UpG may recognize another sequence in T4 DNA. In addition, competition hybridization experiments demonstrated that these dinucleoside monophosphates act by increasing the amount of "early" RNA synthesized, as does σ-containing RNA polymerase. The fact that higher concentrations of CpA and UpA cause some inhibition of GTP initiation as well as ATP, and that GpU in excess has similar effects on ATP initiation, further suggest a complex relation in which these compounds act in close proximity. It should be pointed out that these conclusions are based on previous studies (8, 9, 12) showing that stimulation of RNA synthesis is achieved only with oligoribonucleotides complementary to the template. For example, with poly(U) or poly(dT), only oligoadenylates are effective. Similarly, with poly[d(A-T)], only UpA and ApU are stimulatory. Confirmation of the purity and sequences (see *Methods*) of the dinucleoside monophosphates support the specificity of the stimulatory effects. The specificity of the stimulation is also corroborated by our observations (unpublished) that different dinucleoside monophosphates stimulate transcription of other DNAs, for example, T7 DNA. Similar findings have been reported by Downey *et al.* (11) with T5 DNA. Furthermore, our results (Table 1), showing the changes in specificity of the stimulation with changes in σ factor concentration, attest to the specificity of the stimulatory dinucleoside monophosphates. We suggest a possible, albeit speculative, T4 RNA initiation (promoter) sequence that is compatible with our results:

DNA-3′—G-T-A-T-$(N)_n$-A-T-C-A-C—5′

RNA-5′—C-A-U-A-$(N)_n$-U-A-G-U-G—3′

CpA, which stimulated well, was placed at the 5′ end of the RNA since no dinucleoside monophosphate of the type NpC was stimulatory. In this manner CpA would stimulate by initiating a start that would normally occur with ApU [since ApU is the most common starting sequence of T4 DNA (5)]. In a similar manner UpA, which stimulated well, would do so by initiating a start that would normally occur with ApU. The competition by CpA and UpA for ApU initiation (Fig. 4) would agree with the above. Of the dinucleoside monophosphates of the type NpG, ApG and UpG were the ones capable of stimulation; these would stimulate by initiating a start that would normally occur with GpU. Preliminary studies show that both ApG and UpG compete with GpU for RNA initiation. Some additional strength for this tentative model comes from our preliminary studies with some trinucleoside diphosphates, namely, ApApA, ApApC, ApApG, ApApU, ApUpU, ApUpG, ApUpA, and UpApG. Of these compounds, only ApUpA and UpApG are capable of significant stimulation, a result in agreement with our proposed model. One could propose that the DNA region complementary to C-A-U-A may represent a sequence for ATP starts, whereas that for A-G-U-G (based on preliminary data) may represent one for GTP starts. The fact that some dinucleoside monophosphates affect both ATP starts and GTP starts, although to different degrees, suggests that the distance between these two regions in the above model may comprise a small number of nucleotides.

Studies from this laboratory (unpublished) have shown RNA polymerase-bound promoter regions in T4 DNA to be about 22–25 nucleotides long, with a high (A+T)/(G+C) ratio. If all nucleotides are involved, one could postulate a multiple sequence of at least part of the above model. Lin and Riggs (21) have demonstrated that the *lac* repressor of *E. coli* binds quite well to poly[d(A−T)]. It is believed that the repressor-bound DNA segment comprises about 22–23 nucleotide pairs (Dr. W. Gilbert, personal communication). Adler *et al.* (22) postulated several sequences that are similar to ours. This seems plausible if the *lac* repressor inhibits RNA transcription by binding strongly to DNA sequences preferred by RNA polymerase. It is believed that there may be as many as 25 σ-recognized promoter regions per T4 genome (23). Our proposed model has features characteristic of a poly[d(A-T)] structure, which is known to be preferred by RNA polymerase (24, 25). Such a high (A+T) region should possess a low T_m and could have a partially "melted" structure that might serve as a target or recognition region for RNA polymerase on an otherwise duplex molecule. Downey *et al.* (11) indicated that a d(R←T←A) sequence was often recognized on T4 DNA by σ-containing holoenzyme, which is in agreement with our model. A conclusive proof of our tentative model will have to await a direct sequence analysis of T4 "early" promoter regions. However, our studies suggest the possibility of using dinucleoside monophosphates to probe RNA initiation (promoter) sequences *in vitro* and of using stimulatory dinucleoside monophosphates to initiate the synthesis of T4 (and other phage)-specific RNAs with "unique" starts.

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1. Burgess, R. R., Travers, A. A., Dunn, J. J. & Bautz, E. K. F. (1969) *Nature* **221**, 43–46.
2. Krakow, J. S., Daly, K. & Karstadt, M. (1969) *Proc. Nat. Acad. Sci. USA* **62**, 432–437.
3. Berg, D., Barrett, K. & Chamberlin, M. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21D, p. 506.
4. Travers, A. A. & Burgess, R. R. (1969) *Nature* **222**, 537–540.
5. Mauro, E., Snyder. L., Parino, P., Lamberti, A., Coppo, A. & Tocchini-Valentini, G. P. (1969) *Nature* **222**, 533–537.
6. Bautz, E. K. F., Bautz, F. A. & Dunn, J. J. (1969) *Nature* **223**, 1022–1024.
7. Sugiura, M., Okamoto, T. & Takanami, M. (1970) *Nature* **225**, 598–600.
8. Niyogi, S. K. (1972) *J.Mol. Biol.* **64**, 609–618.
9. Niyogi, S. K. & Stevens, A. (1965) *J. Biol. Chem.* **240**, 2593–2598.
10. Gros, F., Dubert, J., Tissières, A., Burgeois, S., Michelson, M., Soffer, R. & Legault, L. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28**, 299–213.
11. Downey, K. M., Jurmark, B. S. & So, A. G. (1971) *Biochemistry* **10**, 4970–4975.
12. Downey, K. M. & So, A. G. (1970) *Biochemistry* **9**, 2520.
13. Thomas, C. A., Jr., & Abelson, J. (1966) in *Procedures in Nucleic Acid Research*, eds. Cantoni, G. L. & Davies, D. R. (Harper and Row, New York), p. 553.
14. Stevens, A. (1969) *J. Biol. Chem.* **244**, 425–429.
15. Burgess, R. R.(1969) *J. Biol. Chem.* **244**, 6160–6167.
16. Burgess, R. R. & Travers, A. A. (1970) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21D, p. 500.
17. Bolle, A., Epstein, R. H., Salser, W. & Geiduschek, E. P. (1968) *J. Mol. Biol.* **31**, 325–340.
18. Brody, E. N. & Geiduschek, E. P. (1970) *Biochemistry* **9**, 1300–1309.
19. Maitra, U. & Hurwitz, J. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 815–822.
20. Bremer, H., Konrad, M. W., Gaines, K. & Stent, G. S. (1965) *J. Mol. Biol.* **13**, 540–553.
21. Lin, S. Y. & Riggs, A. D. (1970) *Nature* **228**, 1184–1186.
22. Adler, K., Beyreuther, K., Fanning, E., Geisler, N., Gronenborn, B., Klemm, A., Müller-Hill, B., Pfahl, M. & Schmitz, A. (1972) *Nature* **237**, 322–327.
23. Bautz, E. K. F. & Bautz, F. A. (1970) *Nature* **226**, 1219–1222.
24. Jones, O. W. & Berg, P. (1966) *J. Mol. Biol.* **22**, 199–209.
25. Shishido, K. & Ikeda, Y. (1971) *Biochem. Biophys. Res. Commun.* **44**, 1420–1428.