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# Priming and inhibitory activities of RNAs for the influenza viral transcriptase do not require base pairing with the virion template RNA

(capped and uncapped ribopolymers/secondary structure effects)

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**ABSTRACT** **Capped ribopolymers lacking a sequence complementary to the common 3′ end of the influenza virion RNA segments effectively stimulated transcription of these RNAs by the virion-associated transcriptase. Thus, stimulation of transcription results not from hydrogen bonding between the capped RNA and the 3′ end of the virion RNA but presumably from a specific interaction of the capped RNA with protein(s) in the transcriptase complex. Although no specific nucleotide sequence was required for priming activity, capped mRNAs with diminished secondary structure were preferred as primers. Inosine-substituted or bisulfite-modified capped reovirus mRNAs were at least 3- to 5-fold more effective as primers than were the native capped mRNAs. On the other hand, inosine substitution or bisulfite treatment of the uncapped form of reovirus mRNAs converted them from essentially inactive species to potent inhibitors of the transcriptase reaction primed by either ApG or globin mRNA. These effects of reduced secondary structure also most probably reflect an interaction of the exogenous RNAs with transcriptase protein(s). The results obtained from screening a series of native uncapped ribopolymers were consistent with inhibitory activity requiring the absence of most hydrogen bonding in the ribopolymer and also suggested that specific structural feature(s) of the nucleotides in the chain were important.**

Capped eukaryotic mRNAs strongly stimulate influenza viral RNA transcription *in vitro* (1, 2). During this reaction, the methylated cap structure and a short stretch of 5′-terminal nucleotides are transferred from the stimulating primer mRNA to the influenza viral RNA transcripts (2–4). Although the presence of a methylated cap structure in the exogenous mRNA clearly is required for priming activity (2, 3, 5), other RNA structural features also may affect priming. In terms of understanding the mechanism of priming, it is of particular interest to determine whether the capped primer must also contain one or more sequences complementary to the 3′ terminus [3′ U-C-G-U (6, 7)] of the virion RNA (vRNA) in order to allow hydrogen bonding between the primer and template RNAs.

To answer this question, we tested the priming activity of various capped ribopolymers of different base composition. In this report we show that capped ribopolymers lacking a sequence complementary to the 3′ terminus of vRNA are effective primers for influenza viral RNA transcription. This indicates that stimulation of the initiation of transcription does not require specific hydrogen bonding between the primer and template RNAs. Although no specific nucleotide sequence is apparently necessary, sequence alterations that affect the secondary structure of capped RNAs markedly influence priming activity. Experiments involving inosine-substituted and bisulfite-modified reovirus mRNAs indicate that the influenza virion transcriptase prefers capped RNAs with diminished secondary structure as primers. In the course of these experiments, it was also found that decreasing the secondary structure of the uncapped form of reovirus mRNA converted it from an essentially inactive species to a potent inhibitor of the transcriptase reaction. Some uncapped polyribonucleotides with minimal secondary structure were also strong inhibitors. These results indicate that both the stimulation by capped RNAs and the inhibition by uncapped RNAs most likely result from an interaction between these RNAs and one or more virion-associated transciptase proteins.

## MATERIALS AND METHODS

**Preparation of Reovirus and Rabbit Globin mRNAs.** Purified reovirus (Dearing strain, type 3) was digested with chymotrypsin, and the resulting viral cores were used to synthesize capped viral mRNAs *in vitro* as described (8, 9). Uncapped mRNAs with 5′-terminal ppG were synthesized in reaction mixtures containing 0.2 mM *S*-adenosylhomocysteine (AdoHcy) in place of *S*-adenosylmethionine (AdoMet), 1 mM inorganic pyrophosphate, and 0.4 mM GTP (9); $m^7I$ caps were added to these molecules by a subsequent incubation in the presence of 4 mM ITP and 20 μM [*methyl*-$^3$H]AdoMet (10, 11). Uncapped, IMP-substituted mRNAs were obtained by replacing GTP with ITP in the incubation mixtures, but inorganic pyrophosphate was not added and the ITP concentration was not reduced from 4 mM because these changes resulted in a lower yield of mRNA. Because of the absence of inorganic pyrophosphate, some (≈35%) of the molecules contained IpppI instead of ppI 5′ ends. The IMP-containing mRNAs were subsequently incubated in the presence of 4 mM GTP and 20 μM [*methyl*-$^3$H]AdoMet to yield predominantly $m^7GpppI^m$-terminated mRNA and some $m^7IpppI^m$-terminated mRNA. Bisulfite modification of capped and uncapped (GMP-containing) reovirus mRNA was done under denaturing conditions essentially as described (12) but without the addition of carrier tRNA. Globin mRNA was purified from rabbit reticulocytes as described (1).

**Ribopolymer Preparations.** Capped A-, C-, and U-containing ribopolymers were synthesized from ribonucleoside diphosphates in the presence of the primer [*methyl*-$^3$H]-$m^7GpppG^mpC$ with *Micrococcus luteus* polynucleotide phosphorylase (kindly provided by C. Klee or purchased from P-L Biochemicals) under primer-dependent conditions (13). The $m^7GpppG^mpC$ primer (specific activity, $10^5$ cpm/pmol) was prepared by incubating reovirus cores in an incomplete

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Abbreviations: vRNA, virion RNA; AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine.

transcription reaction mixture containing 3 μM [*methyl*-$^3$H]-AdoMet, GTP, and CTP. The amount (pmol) of capped ribopolymers synthesized during the polynucleotide phosphorylase reaction was determined from the amount of [*methyl*-$^3$H]-$m^7GpppG^mpC$ incorporated into high molecular weight products isolated by exclusion filtration through Sephadex G-50.

To monitor the primer dependence of the polymerization, parallel reactions with labeled ribonucleoside diphosphate precursors were carried out in the presence and absence of the primer. In the presence of CDP or ADP as the single ribonucleoside diphosphate precursor, primer dependence was almost complete, whereas with UDP there was little or no dependence. Despite the lack of primer dependence, capped poly(U) and uncapped polymer were synthesized as shown by the incorporation of [$^3$H]methyl-labeled primer into high molecular weight products. Polymerization of UDP became largely primer-dependent when ADP was also present in the reaction mixture at the same concentration (13).

For the synthesis of capped poly(A,G) (1:1 input ratio of ADP to GDP), *Escherichia coli* polynucleotide phosphorylase (from P-L Biochemicals) was used (14). Minimal primer dependence was observed, and both capped and uncapped poly(A,G) were synthesized. The size of the polymers was determined, after 3′ labeling with 5′-[$^{32}$P]pCp (1000 Ci/mmol; 1 Ci = $3.7 \times 10^{10}$ becquerels) (15), by electrophoresis on 12% (wt/vol) polyacrylamide gels containing 6 M urea with *E. coli* tRNA as a size marker. The polymers were heterogeneous in size, ranging from about 40 to 200 nucleotides.

Uncapped ribopolymers were obtained from Sigma and Collaborative Research, Waltham, MA, and were purified by sucrose density gradient centrifugation. All polymers had a sedimentation coefficient of at least 4 S. The deoxyhomopolymers poly(dU) and poly(dI) were purchased from P-L Biochemicals.

**Influenza Viral RNA Transcription.** The synthesis of influenza viral RNA transcripts *in vitro* by detergent-treated purified influenza virus was carried out as described (1–5). The primer (capped RNA or ApG) and labeled ribonucleoside triphosphate that were added are indicated in each table and figure. *E. coli* tRNA was added at a concentration of 2 mg/ml to the reaction mixtures (4), which increased viral RNA transcription about 3- to 5-fold. Reactions in a 25-μl volume were incubated for 60 min at 31°C, and incorporation of CMP into viral RNA transcripts in an ApG- or globin mRNA-primed reaction ranged from 14 to 32 nmol per mg of viral protein per hr with different virus preparations.

## RESULTS

**Priming Activity of Capped Ribopolymers Does Not Require Complementarity to the 3′ End of Template RNA.** Several capped ribopolymers were effective primers for influenza viral RNA transcription (Table 1). Capped poly(A) and capped poly(A,U), neither of which contain a sequence (A-G or A-G-C) complementary to the 3′ terminus of influenza vRNA [3′ U-C-G-U (6, 7)], were about as effective as globin mRNA. The size of the capped ribopolymers apparently did not strongly influence their activity because capped poly(A) with median chain lengths of 50 and 150 nucleotides had similar priming activities. Capped poly(U) was also active, although the 3′-terminal 12-nucleotide sequence common to the eight influenza vRNA segments contains no A residues (6, 7). Capped poly(A) and capped poly(A,U) were more active than capped poly(U) and capped poly(A,G), but the activity of the latter two may be underestimated because the uncapped polymers present in these two preparations [poly(U) and poly(A,G)] have inhibitory activity (see below). Of the various polymers tested as primer, capped poly(C) was the least effective. These results indicate that whereas there may be some effect of sequence on the efficiency of transcriptase priming by an exogenous capped ribopolymer, there is no requirement for the presence of a sequence complementary to the 3′ terminus of the vRNA template.

Table 1. Priming activity of capped synthetic ribopolymers

| Exp. | Primer added: Type | Primer added: Amt. pmol | GMP incorp., pmol |
|---|---|---|---|
| 1 | None | | 1.9 |
| | Globin mRNA | 6 | 84.4 |
| | Capped poly(U) | 3 | 29.1 |
| | Capped poly(A) | 3 | 59.1 |
| | Capped poly(AU) | 3 | 62.7 |
| 2 | None | | 1.1 |
| | Capped poly(A) | 3 | 33.0 |
| | Capped poly(A,G) | 3 | 16.3 |
| 3 | None | | 1.2 |
| | Globin mRNA | 6 | 31.6 |
| | Capped poly(A,U) | 3 | 27.0 |
| | Capped poly(C) | 3 | 5.5 |

In the three experiments shown, different virus preparations with different specific transcriptase activities were used.

In contrast, ribopolymer priming activity was dependent on the presence of a 5′-terminal cap structure (Table 2), as reported for natural mRNA primers (2, 3, 5). Removal of the terminal $m^7G$ of capped poly(A,U) by β-elimination or by treatment with tobacco acid pyrophosphatase (3, 5) drastically reduced priming activity. After the polymer underwent β-elimination followed by enzymatic recapping by partially purified vaccinia enzymes (3, 5), priming activity was restored to a level greater than that of the starting material. This may be because the starting material contained a fraction of molecules with caps that were not fully methylated (5).

Gel electrophoretic analyses of the viral RNA transcripts indicated that capped poly(A,U), a representative capped ribopolymer, donated about 10–15 nucleotides to the viral RNA transcripts (data not shown), as observed with globin mRNA and other capped mRNAs (2–4).

**Loss of Secondary Structure in Capped mRNA Is Accompanied by Increased Priming Activity.** To assess the possible influence of secondary structure on priming, we compared native reovirus mRNA to the same RNA after modification to decrease its secondary structure. One of the modifications employed was the substitution of IMP for GMP. Uncapped reovirus mRNAs were synthesized in reaction mixtures con-

Table 2. Priming activity of synthetic ribopolymers requires a 5′-terminal methylated cap structure

| Capped poly(A,U) added (3 pmol)* | UMP incorp., pmol |
|---|---|
| None | 1.0 |
| Untreated | 33.6 |
| β-Elimination | 3.5 |
| $TAPP_i$ase | 8.0 |
| β-Elimination and enzymatic recapping | 41.3 |

* β-elimination, treatment with tobacco acid pyrophosphatase ($TAPP_i$ase), and enzymatic recapping (with vaccinia virus capping and methylating enzymes) of the polymer that underwent β-elimination were carried out as described (3, 5).

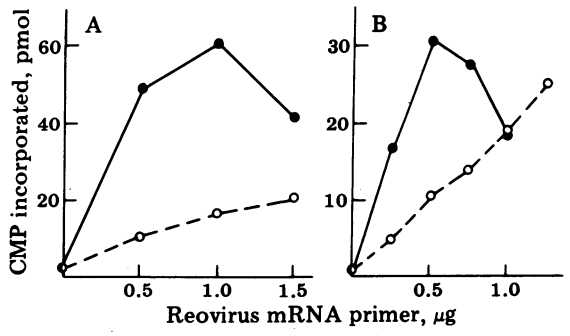


FIG. 1. Effect of reducing the secondary structure of reovirus mRNA on its activity as a primer for influenza viral RNA transcription. (*A*) IMP-substituted reovirus mRNA (●) and native reovirus mRNA (○). (*B*) Bisulfite-treated reovirus mRNA (●) and native reovirus mRNA (○).

taining ITP in place of GTP. IMP-substituted mRNAs have been shown to be almost completely sensitive to nuclease S1 digestion (12) and to migrate in polyacrylamide gels with a mobility similar to that of unsubstituted glyoxal-treated samples (11), consistent with minimal secondary structure. The uncapped IMP-substituted mRNAs were capped after transcription by incubation with GTP and AdoMet to yield predominantly (≈55%) molecules with 5′-terminal $m^7GpppI^m$ and IMP at internal positions. This modification greatly enhanced the priming activity of reovirus mRNA Fig. 1*A*. At levels of 0.5 and 1.0 μg, the IMP-substituted reovirus mRNA was about 4- to 5-fold more effective as a primer than was native (GMP-containing) mRNA. The lower stimulation seen at the highest level (1.5 μg) of IMP-substituted mRNA was presumably due to inhibition by the small amount (≈10%) of uncapped, IMP-substituted molecules in the preparation (see below).

Because some (≈35%) of the IMP-substituted reovirus mRNA molecules contained $m^7I$ caps, it was conceivable that the higher priming activity was due to the presence of an altered cap structure rather than to a diminished secondary structure resulting from internal IMP substitutions. This appears not to be the case because natural (GMP-containing) reovirus mRNA capped after transcription in the presence of ITP and AdoMet to form $m^7I$ caps was about 40–50% as active as the same GMP-containing RNA with $m^7G$ caps—i.e., 1.5 μg each of $m^7GpppG^m$- and $m^7IpppG^m$-treated mRNAs primed the incorporation of 25.9 and 12.8 pmol of CMP into influenza viral RNA transcripts, respectively. These results suggest that a mRNA with a "normal" $m^7G$ cap is preferred for priming.

The other approach employed for eliminating secondary structure in reovirus mRNA was the conversion of its cytosine residues to uridine by deamination with bisulfite in the presence of urea (12). Reovirus mRNA modified by this procedure was also more effective as a primer; at low levels (0.25 and 0.5 μg), the modified reovirus mRNA stimulated influenza viral RNA transcription 3- to 4-fold more than the native mRNA (Fig. 1*B*). The priming activity at higher levels (>0.5 μg) of the modified mRNA decreased relative to that of the native mRNA, again presumably due to the presence of inhibitory molecules (see below).

**Influenza Viral RNA Transcription Is Inhibited by Certain Uncapped RNAs.** *Modified, uncapped reovirus mRNAs.* The fact that the addition of increasing amounts of IMP-substituted or bisulfite-treated reovirus mRNAs resulted in a decrease in the stimulation of transcription (Fig. 2) suggested that inhibitory molecules were also present in these preparations. Prime candidates as inhibitors were the small numbers of molecules that contained diphosphorylated (ppG, ppI) or blocked, unmethylated (IpppI, GpppI, GpppG) 5′ ends. As shown in Table 3,

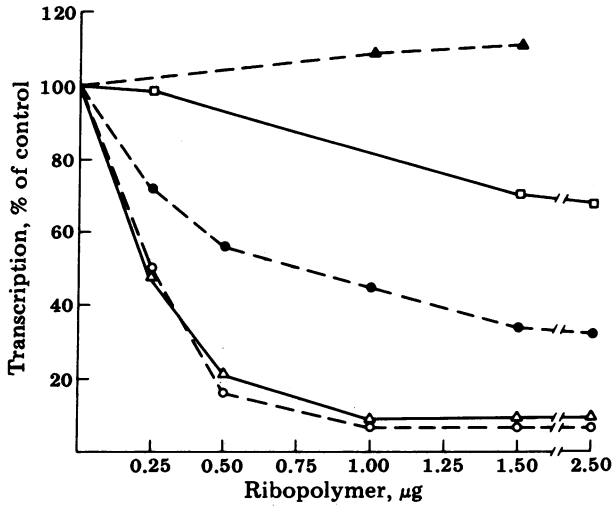


FIG. 2. Effect of various uncapped ribopolymers on globin mRNA-primed influenza viral RNA transcription. The indicated amounts of the various ribopolymers were added to a 25-μl transcriptase assay containing 6 pmol (1.2 μg) of globin mRNA as primer. The amount of CMP incorporation after 60 min at 31°C was determined. In the control reaction (without the ribopolymer) 40 pmol of CMP were incorporated. ▲, Poly(C); □, poly(G); ●, poly(U); △, poly(A,G); ○, poly($s^4U$).

uncapped, IMP-substituted or bisulfite-treated reovirus mRNAs at a level of 1.5 μg inhibited influenza viral RNA transcription by about 80%. A similar inhibition occurred with either globin mRNA or ApG as primer. In contrast, the same amount of uncapped, unmodified reovirus mRNA caused either a slight stimulation (globin mRNA-primed) or only a small inhibition (ApG-primed) of influenza viral RNA transcription. Thus, modifications that diminish the secondary structure of uncapped reovirus mRNAs convert these RNAs into potent inhibitors of influenza viral RNA transcription.

*Uncapped ribopolymers.* Because of the above results, we tested various synthetic ribopolymers for their ability to inhibit influenza viral RNA transcription primed by globin mRNA or by ApG (Fig. 2 and Table 4). The effects on globin mRNA and ApG-primed transcription were similar. Of the homopolymers tested, poly(4-thiouridylic acid) [poly($s^4U$)] was the most effective inhibitor, 1 μg reducing transcription by 95%. Poly(U) was less effective, 1 μg inhibiting transcription by about 60%, followed in order by poly(I) and poly(G). Poly(A) and poly(C)

Table 3. Inhibition of globin mRNA- and ApG-primed transcription by uncapped reovirus mRNAs with diminished secondary structure

| Exp. | Primer* | Uncapped mRNA (1.5 μg)† | CMP incorp., pmol |
|---|---|---|---|
| 1 | Globin mRNA | None | 40.7 |
| | | Native reovirus | 56.4 |
| | | IMP-substituted reovirus | 8.0 |
| | ApG | None | 45.5 |
| | | Native reovirus | 35.7 |
| | | IMP-substituted reovirus | 6.0 |
| 2 | Globin mRNA | None | 28.2 |
| | | Bisulfite-treated reovirus | 7.1 |
| | ApG | None | 55.9 |
| | | Bisulfite-treated reovirus | 9.0 |

* The amount of globin mRNA and ApG added were 6 pmol (1.2 μg) and 7500 pmol, respectively.

† 1.5 μg of uncapped reovirus mRNA corresponds to about 2.5 pmol.

Table 4. Inhibition of globin mRNA- and ApG-primed transcription by uncapped ribopolymers

| Ribopolymers (1.5 μg) | % inhibition of transcription* | |
|---|---|---|
| | Globin mRNA primer | ApG primer |
| Poly($s^4$U) | 96 | 95 |
| Poly(U) | 62 | 63 |
| Poly(I) | 42 | 48 |
| Poly(G) | 33 | 37 |
| Poly(C) | 0 | 0 |
| Poly(A) | 0 | 0 |
| Poly(U)·poly(A)† | 0 | 0 |
| Poly(A,U) | 0 | 0 |
| Poly(C,G) | 0 | 0 |
| Poly($U_2$,G) | 81 | 78 |
| Poly(A,G) | 94 | 91 |
| Poly(A,I) | 67 | 70 |

* The percentage inhibition shown for each ribopolymer represents the average of at least three assays. The variations between assays were 10% or less. Globin mRNA and ApG were added at 6 and 7500 pmol, respectively. In the control reactions (in the absence of the ribopolymer), the incorporation of CMP into viral RNA transcripts was 30–80 pmol.

† Poly(A) and poly(U), each at 100 μg/ml, were hybridized by incubation in 0.30 M NaCl/5 mM EDTA/0.05 M Tris·HCl, pH 7.4, at 37°C (or 65°C) for 3 hr.

had no inhibitory activity. Inhibition required the absence of hydrogen bonding in the ribopolymer because, after hybridization to poly(A), poly(U) no longer inhibited transcription. At least one synthetic deoxyhomopolymer, poly(dU), also inhibited viral RNA transcription; 1.5 μg inhibited transcription by about 50%, which was only slightly less than that caused by poly(U). Poly(dI), however, had no inhibitory activity.

Among the hetero(ribo)polymers tested, the two with highly organized helical structure, poly(A,U) and poly(C,G), did not inhibit viral RNA transcription. In contrast, poly($U_2$,G) was a strong inhibitor—better in fact than poly(U). Poly(A,G) was the most effective inhibitor, with an activity similar to that of poly($s^4$U). Poly(A,I) was somewhat less effective.

## DISCUSSION

The results presented here indicate that capped ribopolymers lacking a sequence complementary to the common 3′ end of the influenza vRNA segments effectively stimulate transcription of these RNAs by the virion-associated transcriptase. Consequently, the stimulation observed does not result from hydrogen bonding between the added capped RNA and the 3′ end of the vRNA. As further evidence for this conclusion, it has been found that 5′-terminal fragments of alfalfa mosaic virus RNA 4 that are too short to contain a sequence complementary to the 3′ end of vRNA are also effective primers (unpublished data).

A different type of interaction must therefore be involved in the stimulation of influenza viral RNA transcription *in vitro*. The most likely possibility is a specific interaction between the capped RNA and one or more proteins associated with the transcriptase complex. As a result the transcriptase acquires the ability to initiate transcription. Experiments to be reported elsewhere show that the first step of the transcriptase reaction primed by capped RNAs is the cleavage of these RNAs to produce 5′-terminal fragments 10–14 nucleotides in length, followed by the addition to these capped fragments of a G residue (most probably directed by the 3′-penultimate C of the vRNA). Presumably, it is the initiation with a G residue that cannot be carried out by the transcriptase until it interacts with a capped RNA. This putative specific RNA–protein interaction is apparently mediated primarily through the 5′-terminal methylated cap structure because the priming activity of synthetic ribopolymers (as shown here) and of native RNAs requires the presence of this cap structure (2, 3, 5). In fact, both the 7-methyl group in the 5′-terminal G and the 2′-*O*-methyl group of the penultimate nucleotide in the cap strongly influence priming activity (16). An interaction between mRNA cap structure and protein(s) associated with eukaryotic ribosomes is also involved in the initiation of eukaryotic protein synthesis (17).

In apparent contrast to the lack of a requirement for hydrogen bonding with capped RNAs as primers, the only dinucleotides that stimulate viral RNA transcription are those that are capable of hydrogen bonding to the 3′ end of the vRNA, namely ApG, GpG, and GpC (18, 19). However, it is most likely that priming by these dinucleotides is not the normal mechanism for the initiation of influenza viral RNA transcription because (*i*) optimal priming by these dinucleotides requires a molar concentration 1000 times higher than that needed for optimal priming by capped RNAs (1, 18) and (*ii*) the viral mRNAs synthesized *in vivo* are apparently initiated by capped RNAs (20). Presumably these dinucleotides allow the transcriptase to avoid the normal initiation step *in vitro* by providing a high concentration of the initiating G residue in a form in which it can interact with the 3′ end of the vRNA.

Although no specific sequence in the capped RNA is required for priming, our results indicate that reduction in its secondary structure is accompanied by enhancement of activity. We were not able to establish this point by using synthetic capped ribopolymers of different base composition because these ribopolymer preparations included various amounts of the corresponding uncapped polymers, some of which inhibited the transcriptase reaction. As an alternative approach, we showed that two different modifications of reovirus mRNA that abolished G·C base pairing, and thus reduced secondary structure, increased priming activity by at least 3- to 5-fold. The same increase was observed when purified viral cores were used to catalyze transcription (unpublished data), which indicates that the preference for reduced secondary structure in a primer RNA is most likely an intrinsic property of the transcriptase complex and does not simply reflect differential penetration of these RNAs into detergent-treated virions. In fact, the preference may actually be greater than that observed because the preparations of modified reovirus mRNA also contained some uncapped, inhibitory molecules.

Diminished secondary structure in a capped RNA, particularly in its 5′-terminal region, probably facilitates the first step of the transcriptase reaction (i.e., cleavage of these RNAs by the virion-associated endonuclease). This may be one of the reasons that 2′-*O*-methylated alfalfa mosaic virus RNA 4 is the best mRNA primer for the influenza transcriptase so far examined (5). Of the first 18 5′-terminal nucleotides in this RNA, 14 are U, a sequence which should be free of most secondary structure (21). A similar explanation may hold for the observation that capped, 5′-terminal, RNase T1-derived fragments of mRNAs are actually 4- to 8-fold better primers on a molar basis than the intact mRNAs from which they were derived (unpublished data).

Reduced secondary structure also was shown to be one of the major features in an uncapped RNA or ribopolymer that is needed for inhibitory activity. The same modifications that reduced secondary structure and thus increased priming activity of capped mRNAs converted the corresponding uncapped mRNAs from essentially inert forms to potent inhibitors of the transcriptase. In addition, various uncapped ribopolymers were found to inhibit the virion transcriptase, and one of the requirements for inhibitory activity was the absence of most

hydrogen bonding in the ribopolymer. Thus, poly(A,U) and poly(C,G), which have highly ordered secondary structures (22, 23), and multistranded poly(A)·poly(U) did not inhibit the transcriptase, whereas poly(U) and especially poly($s^4$U) [which probably has less secondary structure than poly(U) (24)] were effective inhibitors of both mRNA and ApG-primed RNA synthesis. Inhibition of ApG-primed influenza viral RNA transcription by poly(U) and poly($s^4$U) was reported also by others (25).

However, the absence of hydrogen bonding in a ribopolymer was apparently by itself not sufficient for conferring inhibitory activity. In the transcriptase assay mixture (neutral pH, 0.1 M KCl/5 mM $Mg^{2+}$), both poly(A) and poly(C) should be free of most hydrogen bonding (22), yet these two homopolymers did not inhibit transcription. This suggests that nucleotide specificity also may be involved in inhibition. Such specificity would explain why poly(A,G), which should also be free of hydrogen bonding (22), is an extremely effective inhibitor in contrast to poly(A)—i.e., it is the localized G-containing regions in poly(A,G) that confer inhibitory activity. The much weaker inhibitory activity of poly(G) is presumably due to the fact that it is predominantly in the form of multistranded complexes (22). In contrast, poly(A,I) and poly(I) differ only slightly in inhibitory activity, probably because hydrogen bonding interactions are possible in both of these polymers (22). Thus, U, $s^4$U, G, and I (but not A and C) apparently contribute to the inhibitory activity of a ribopolymer when these nucleotides are not base-paired in the polymer. These nucleotides contain an oxygen or sulphur (rather than an amino) group at equivalent sites on the purine (6 position) or pyrimidine (4 position) ring. Further work clearly is needed to define the exact structural feature(s) of the nucleotides in the ribopolymer that are important for inhibitory activity.

Experiments to be published elsewhere indicate that poly($s^4$U) and poly(A,G), the two ribopolymers with the highest inhibitory activity, specifically inhibit the first step in mRNA-primed transcription, the endonucleolytic cleavage of the mRNA primer. The fact that ApG-primed transcription, which does not require the action of this nuclease, is also inhibited by these ribopolymers would then suggest that the activity of the endonuclease and the transcriptase are somehow coupled, possibly because they reside in the same protein or protein complex.

The present study as well as further elucidation of the mechanism of mRNA-primed influenza viral RNA transcription should be of interest not only for understanding the molecular biology of influenza virus but also for the development of compounds that may be potent and clinically useful anti-influenza agents.

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