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Effect of Capping upon the mRNA Properties of Satellite Tobacco Necrosis Virus Ribonucleic Acid†

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ABSTRACT: The mRNA guanylyltransferase–mRNA methyltransferases of vaccinia virions can be used to introduce a 5′-terminal m^{7}G(5′)ppp(5′)Ap^m··· capping group onto the RNA of satellite tobacco necrosis virus (STNV RNA) to yield intact capped STNV RNA. Studies with an in vitro system from wheat germ and limiting quantities of capped and uncapped STNV RNA show that the rates and extents of formation of initiation complexes of protein synthesis by intact capped and uncapped STNV RNA are identical, suggesting that 5′-terminal cap groups cannot function in the translation of STNV RNA. Also, the cap analogue pm^{7}G equally inhibits the initiation and the translation of limiting quantities of both capped and uncapped STNV RNA. These contrasting observations suggest that the wheat germ system contains a pm^{7}G sensitive protein and that STNV RNA has a tertiary structure that restricts the function of an added 5′-terminal capping group. This theory is supported by observations that fragmented capped STNV RNA is better at forming initiation complexes than is equally fragmented uncapped STNV RNA.

The RNA of satellite tobacco necrosis virus (STNV)[1] is a monocistronic mRNA coding only for STNV coat protein in in vitro systems from both procaryotes and eucaryotes (Klein et al., 1972; Lundquist et al., 1972; Klein & Clark, 1973; Leung et al., 1976). In vivo STNV RNA lacks a 5′-terminal capping group (Wimmer et al., 1968; Horst et al., 1971; Leung et al., 1976) and does not acquire or require a 5′-terminal capping group during its translation by an in vitro system from wheat germ (Leung et al., 1976). The purified mRNA guanylyltransferase–mRNA methyltransferases of vaccinia virions introduce a m^{7}G(5′)ppp(5′)Ap^m···5′-terminal capping group onto STNV RNA (Moss, 1977). This property allows assay of the impact of the 5′-terminal capping group upon the translation of STNV RNA. This paper reports comparisons of the mRNA potential of STNV RNA with 5′-terminally capped STNV RNA in an in vitro system from wheat germ.

Experimental Procedures

Materials. STNV RNA, STNV [^{125}I]RNA, and cell-free S-30 extracts from wheat germ were prepared as previously described (Leung et al., 1976). P_1 nuclease and various 5′-terminal fragment standards of capped mRNAs were obtained from P-L Biochemicals, Inc. [α-^{32}P]GTP, a mixture of 15 U-^{14}C-labeled L-amino acids, carrier-free Na^{125}I, and *S*-adenosyl[*methyl*-^{3}H]methionine were purchased from Amersham/Searle. Unlabeled *S*-adenosyl-L-methionine (Sigma

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[1] Abbreviations used: STNV, satellite tobacco necrosis virus; m^{7}G(5′)ppp(5′)N, m^{7}G(5′)ppp(5′)Np, m^{7}G(5′)ppp(5′)A^m, and m^{7}G(5′)ppp(5′)Ap^m, 7-methylguanosine linked by its 5′ position through three phosphate anhydrides to the 5′ position of a nucleoside, a nucleoside 3′-phosphate, a 2′-*O*-methyladenosine, or a 2′-*O*-methyladenosine 3′-phosphate, respectively; capping groups, any 5′-terminal m^{7}G(5′)ppp(5′)Np··· group on RNA; capped RNA, any RNA containing a 5′-terminal m^{7}G(5′)ppp(5′)Np··· group; pm^{7}G, 7-methylguanosine 5′-phosphate; ATP and GTP, adenosine and guanosine 5′-triphosphates; PEI-cellulose, cellulose pretreated with polyethylenimine; KMH buffer, 0.1 M KCl, 3 mM $Mg(OAc)_2$, 2 mM dithiothreitol, 20 mM Hepes–K salt, pH 7.2; KMT buffer, 0.1 M KCl, 5 mM $Mg(OAc)_2$, 10 mM Tris–OAc, pH 7.5; SAM, *S*-adenosylmethionine.

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Chemical Co.) was freed of nuclease contaminants by four phenol extractions prior to use. Fraction III mRNA guanylyltransferase–mRNA methyltransferases was generously provided by Dr. Bernard Moss. ^{125}I-Labeled standards for oligonucleotide characterization were prepared by iodination (Commerford, 1971) of known compounds.

5′-Terminal Capping of STNV RNA. STNV RNA and STNV [^{125}I]RNA were guanylated and methylated ("capped") by the procedure of Ensinger et al. (1975) using 10 μL of fraction III mRNA guanylyltransferase–mRNA methyltransferases (Martin et al., 1975) and 10 μg of RNA per 100 μL of reaction modified to contain 10 μM *S*-adenosyl-L-methionine. These reactions featured unlabeled GTP and *S*-adenosyl-L-methionine, or as required [α-^{32}P]GTP, or GTP and *S*-adenosyl[*methyl*-^{3}H]methionine as the isotopic label. All reactions were scaled up or down as needed.

Reactions to form capped STNV RNAs lacking radioisotopic label were terminated by phenol extraction. The resultant aqueous phase materials were freed of residual GTP (to allow later A_{260} assays of RNA) by partial lyophilization and later passage over a 1.5 × 40 cm column of Sephadex G-50 preequilibrated with 10 mM triethylamine–HCO_3^-, pH 7.0. The excluded A_{260} absorbing material (representing >80% of input RNA) was lyophilized to dryness and dissolved (20–40 A_{260} units/mL) in 0.3 M KOAc, pH 6.0, prior to final alcohol precipitation. "Uncapped" STNV RNA controls were incubated and treated as the capped STNV RNAs except that GTP and *S*-adenosyl-L-methionine were only added during the phenol extraction step. Concentrations of redissolved capped and uncapped STNV RNAs were determined by A_{260} measurements.

Reactions to form STNV [^{125}I]RNAs containing an unlabeled 5′-terminal capping group were terminated by phenol extraction. The resultant aqueous phase was diluted with 1/40 part of 4 M KOAc, pH 6.0, prior to alcohol precipitation of capped STNV [^{125}I]RNAs. "Uncapped" STNV [^{125}I]RNA controls were incubated and treated as the capped STNV [^{125}I]RNAs except that GTP and *S*-adenosyl-L-methionine were only added during the phenol extraction step. Residual GTP present in these capped and uncapped STNV [^{125}I]RNAs (due to omission of a gel filtration step) is inconsequential in that 1 mM GTP does not influence the translation of STNV RNA. Concentrations of redissolved capped and uncapped STNV [^{125}I]RNAs were determined from cpm assays and the specific activity of the original STNV [^{125}I]RNA.

Reactions to form STNV RNAs containing a ^{32}P-labeled 5′-terminal capping group as a result of ^{32}P uptake from [α-^{32}P]GTP were terminated by addition of 1 mL of cold 5% trichloroacetic acid, 10 mM GTP. The precipitated STNV [^{32}P]RNA was then washed by repeated solubilization in a minimum volume of 50 mM Tris-Cl, 1 mM $MgCl_2$, pH 7.6, followed by precipitation with an equal volume of 5% trichloroacetic acid, 10 mM GTP, centrifugation, and decantation until the ^{32}P levels of decanted washes reached a low level. The extent of guanylation was calculated from the cpm in the final precipitate after correction for STNV RNA loss in the washing as indicated by identical washes of a STNV [^{125}I]RNA control.

Reactions to form STNV RNAs containing ^{3}H-labeled 5′-terminal capping groups as a result of GTP dependent ^{3}H uptake from *S*-adenosyl[*methyl*-^{3}H]methionine were terminated by addition of 3 mL of cold 5% trichloroacetic acid. The precipitated STNV [^{3}H]RNA was then washed on Millipore-type HAWP filters with five 3-mL washes of cold 5% trichloroacetic acid, dried, and counted. The extent of methylation was calculated from the cpm on the filters after correction for STNV RNA loss in the washing as indicated by identical washes of a STNV [^{125}I]RNA control.

Characterization of RNA Forms. Capped and uncapped STNV RNAs and STNV [^{125}I]RNAs were characterized in the 4% acrylamide, 7 M urea denaturing gel electrophoresis system of Lockard & RajBhandary (1976). Size homogeneity of the resolved RNAs was determined from absorbance profiles of gels stained with methylene blue or from cpm profiles of serial gel slices. Fraction III mRNA guanylyltransferase–mRNA methyltransferases of Martin et al. (1975) appears free of significant nuclease contamination, but nuclease dangers still exist with the capping and reisolation procedures above. Specifically, even though we took extensive precautions against nuclease action, we had to discard approximately half of our 5′-terminally capped STNV RNA and STNV [^{125}I]-RNA preparations because of apparent degradation during our capping and reisolation procedures. The stability of resultant intact capped and uncapped STNV RNAs and STNV [^{125}I]RNAs to freezing and thawing also varied amongst preparations indicating variable low levels of residual nucleases. All the experiments reported here employ intact capped and uncapped RNAs that retain their intact character during 30-min incubation at 37 °C. All calculations that involve intact STNV RNA assume a molecular weight of 400 000.

The character of the 5′-terminal capping group added to STNV RNA by the mRNA guanylyltransferase–mRNA methyltransferases was determined using a STNV [^{32}P]RNA formed from 10 μg of STNV RNA in the presence of *S*-adenosyl-L-methionine and [α-^{32}P]GTP. The resultant STNV [^{32}P]RNA was freed of residual [α-^{32}P]GTP by passage over Sephadex G-50 as above, lyophilized to dryness, and redissolved in 5 μL of 0.01 M NaOAc, pH 6.0, containing 0.025 μg of P_1 nuclease and then incubated for 1 h at 37 °C. This hydrolyzed sample was then diluted with 5 μL of 50 mM Tris-Cl, 5 mM $MgCl_2$, pH 8.0, containing 5 μg of alkaline phosphatase and reincubated for 2 h at 37 °C prior to spotting onto thin-layer PEI-cellulose along with appropriate m^{7}G-(5′)ppp(5′)N standards. This PEI-cellulose was developed with 1 M sodium formate, pH 3.4, dried, and finally analyzed for UV absorption and ^{32}P content.

Protein Synthesis Assays. Initiation complexes were formed during 25 °C incubations in 50-μL reactions containing the KMH buffer of Leung et al. (1976) and 1 mM ATP, 20 μM GTP, 8 mM creatine phosphate, 2 μg of creatine kinase, 30 μM L-methionine, 6 μM sparsomycin, 20 μL of cell-free S-30 extract of wheat germ (Roberts & Patterson, 1973), and 0.5 μg of an intact capped or uncapped form of STNV [^{125}I]RNA. Reactions were terminated by fivefold dilution with cold KMT buffer of Leung et al. (1976) containing 60 μM aurintricarboxylic acid and then resolved and analyzed on sucrose gradients as previously described (Leung et al., 1976). The quantity of initiation complexes formed was derived from the quantity of ^{125}I label associated with resolved ribosomes within the gradient. The 0.5 μg of intact STNV [^{125}I]RNA employed in these assays was judged limiting in that the quantity of ^{125}I label bound into initiation complexes by this assay was directly proportional to the quantity of STNV [^{125}I]RNA added over a 0.1–2.0-μg range. The character of the STNV [^{125}I]RNA bound into initiation complexes was assayed by two methods. First, polyacrylamide gel electrophoretic analyses (DeWachter & Fiers, 1971) reveal that the STNV [^{125}I]RNA bound in initiation complexes after 20-min reactions is composed of approximately equal quantities of large fragments (> one-half

Table I: Quantitation of the Degree of 5′-Terminal Capping of STNV RNA

guanylation	cpm of ^{32}P incorp from [α-^{32}P]GTP: complete system	cpm of ^{32}P incorp from [α-^{32}P]GTP: STNV RNA omitted	mol of [^{32}P]guanyl[a]/mol of STNV RNA
expt 1	36 500	12 700	0.89
expt 2	4 400	1 400	0.90

methylation	cpm of ^{3}H incorp from [*methyl*-^{3}H]SAM: complete system	cpm of ^{3}H incorp from [*methyl*-^{3}H]SAM: GTP omitted	mol of [^{3}H]methyl[a]/mol of STNV RNA
expt 1	27 600	1600	1.74
expt 2	29 000	1700	1.45
expt 3	20 200	700	1.33

[a] The mole ratios of [^{32}P]guanyl or [^{3}H]methyl group incorporated per mole of STNV RNA are calculated from the specific activities of the [α-^{32}P]GTP or *S*-adenosyl[*methyl*-^{3}H]methionine employed and reflect subtraction of the minus STNV RNA or minus GTP control data. STNV [^{125}I]RNA containing ^{125}I in less than 1% of its cytidine residues is assumed to undergo similar degrees of guanylation and methylation when incubated with unlabeled GTP and *S*-adenosylmethionine in parallel experiments.

size) and intact STNV [^{125}I]RNA. Second, the ribosome-bound initiation region protected from ribonuclease A by initiation complexes formed with intact and partially fragmented STNV [^{125}I]RNA was isolated, further digested with ribonuclease A, and analyzed by two-dimensional fingerprint analysis as previously described (Leung et al., 1976). The character of each specific ^{125}I-labeled oligonucleotide resolved by a two-dimensional fingerprint procedure was determined by further digestions with specific nucleases (Woese et al., 1976), followed by electrophoretic resolution on DEAE-cellulose paper in the presence of 7% formic acid, pH 1.9, radioautographic detection of all labeled products, and comparisons of the migrations and "M" values (Brownlee, 1972) of the products relative to known standards (e.g., AA[^{125}I]C, A[^{125}I]C, and [^{125}I]C).

Protein synthesis was measured by 60 min, STNV RNA dependent incorporation of 15 U-^{14}C-labeled L-amino acids as previously described, except that all reactions contained 1.0 μg of intact capped or uncapped STNV RNA and 25 μL of cell-free S-30 extract of wheat germ.

Results

Incubation of STNV RNA with GTP, *S*-adenosyl-L-methionine, and the mRNA guanylyltransferase–mRNA methyltransferases of vaccinia virions yields STNV RNA containing a m^{7}G(5′)ppp(5′)Ap^{m}··· 5′ terminus (Moss, 1977). We confirm this observation in that our similar assays with either [α-^{32}P]GTP or GTP and *S*-adenosyl[*methyl*-^{3}H]-methionine yield STNV RNA carrying 0.9 mol of [^{32}P]guanyl group and 1.3–1.5 mol of GTP-dependent [^{3}H]methyl group per mol of STNV RNA (Table I). These preparations of labeled STNV RNA represent derivatization of the 5′ terminus of STNV RNA with the m^{7}G(5′)ppp(5′)Ap^{m}··· capping group reported by Moss (1977), because P_1 nuclease and alkaline phosphatase dependent digestion of such STNV [^{32}P]RNA (i.e., labeled with *S*-adenosyl-L-methionine and [α-^{32}P]GTP) converts all of the incorporated label to chromatographically distinct m^{7}G(5′)[^{32}P]ppp(5′)A^{m}. Most importantly, gel electrophoresis studies of STNV [^{125}I]RNA and 5′-terminally capped STNV [^{125}I]RNA under dissociating conditions in the presence of 7 M urea (Figures 1a and 1b) establish that STNV

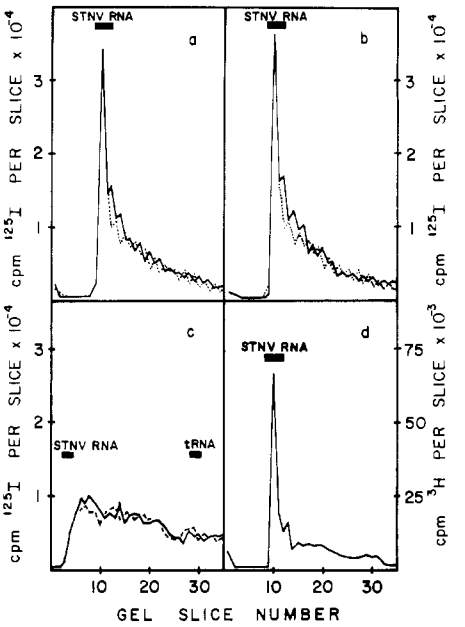

FIGURE 1: Four percent acrylamide gel electrophoretic analyses of STNV RNA forms in the presence of 7 M urea. All migrations are from left to right. (a) Five-hour assay of 5′-terminally capped STNV [^{125}I]RNA (—) and STNV [^{125}I]RNA before capping (···). (b) Five-hour assay of uncapped STNV [^{125}I]RNA (i.e., treated as 5′-terminally capped STNV [^{125}I]RNA but lacking GTP and *S*-adenosyl-L-methionine) (—) and STNV [^{125}I]RNA before capping (···). (c) Two-hour assay of 5′-terminally capped STNV [^{125}I]RNA (—) and uncapped STNV [^{125}I]RNA (···) after ten freezings, thawings, and 2-min treatments at 100 °C. (d) Five-hour assay of STNV [^{3}H]RNA capped in the presence of GTP and *S*-adenosyl[*methyl*-^{3}H]methionine.

[^{125}I]RNA can be derivatized with a m^{7}G(5′)ppp(5′)Ap^{m}··· group without destruction of the original intact character of the STNV [^{125}I]RNA. Repeated freezing, thawing, and boiling shears such STNV [^{125}I]RNAs into smaller molecules (Figure 1c). Figure 1d depicts an alternate and perhaps more sensitive nuclease assay using STNV [^{3}H]RNA (i.e., STNV RNA capped in the presence of GTP and *S*-adenosyl-[*methyl*-^{3}H]adenosine). These last data establish that no extensive nuclease action occurs near the 5′ terminus of STNV RNA during the derivatization of this RNA with a 5′-terminal capping group.

Assays of the function of 5′-terminal m^{7}G(5′)ppp(5′)Np··· groups on messenger RNAs show that these 5′-terminal capping groups function during the formation of initiation complexes upon ribosomes (Shatkin, 1976). It follows that a comparison of the rates and extents of initiation complex formation in the presence of limiting quantities of capped and uncapped STNV RNA should provide the most direct assay of the effect of a 5′-terminal capping group on the messenger properties of STNV RNA. The 5′-terminally capped and uncapped STNV [^{125}I]RNAs of Figures 1a and 1b provide ideal mRNAs for such assays because these STNV [^{125}I]-RNAs contain a high percentage of intact molecules and contain specific activities high enough to allow measurement of small quantities of STNV [^{125}I]RNA bound into density gradient resolvable initiation complexes. As seen in Figures 2 and 4, an in vitro comparison of the formation of initiation complexes by wheat germ ribosomes in the presence of equal and limiting quantities of these 5′-terminally capped and uncapped STNV [^{125}I]RNAs shows that addition of a 5′-terminal m^{7}G(5′)ppp(5′)Ap^{m}··· capping group onto intact STNV [^{125}I]RNA does not enhance either the rate or the extent of formation of initiation complexes by STNV

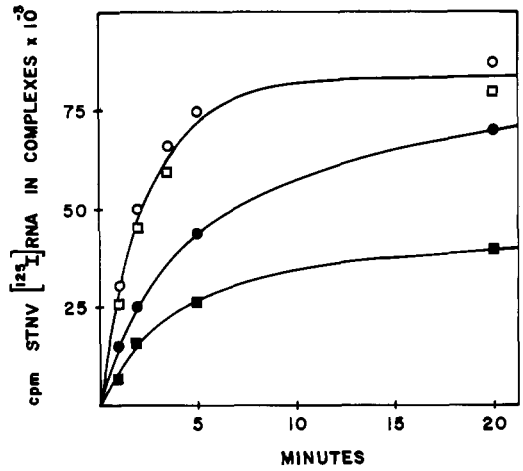

FIGURE 2: The formation of initiation complexes by 5′ terminally capped STNV [^{125}I]RNA (○), uncapped STNV [^{125}I]RNA (□), fragmented and 5′-terminally capped STNV [^{125}I]RNA (●), and fragmented uncapped STNV [^{125}I]RNA (■) of Figure 1. Approximately 5% of the intact STNV [^{125}I]RNAs added is bound into initiation complexes at 20 min under these mRNA limiting conditions.

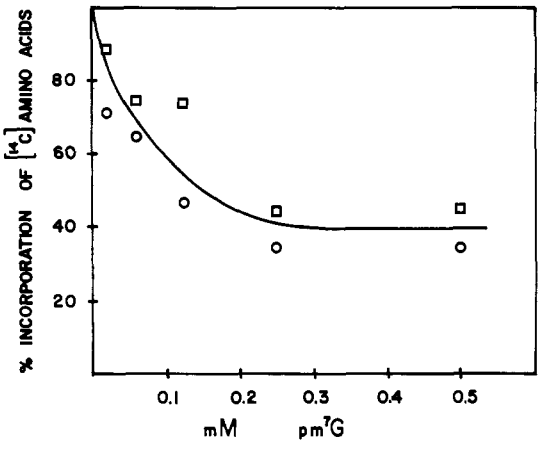

FIGURE 3: The effect of pm^{7}G upon the incorporation of 15 ^{14}C-labeled amino acids by an in vitro wheat germ system in the presence of 5′-terminally capped STNV RNA (○) or uncapped STNV RNA (□). The actual extent of ^{14}C-labeled amino acid incorporation in the absence of pm^{7}G was 18 900 cpm for 5′-terminally capped STNV RNA and 10 323 cpm for uncapped STNV RNA.

[^{125}I]RNA. Thus, a 5′-terminal capping group does not function in initiation complex formation by intact STNV RNA.

The small molecule pm^{7}G is an analogue of 5′-terminal m^{7}G(5′)ppp(5′)Np··· groups on mRNAs and inhibits the function of 5′-terminally capped mRNAs (Hickey et al., 1976; Shatkin, 1976). It follows that, if an added 5′-terminal capping group on STNV RNA is functional, then pm^{7}G should inhibit the translation of limiting quantities of 5′-terminally capped STNV RNA more than it inhibits the translation of equal quantities of uncapped STNV RNA. As seen in Figure 3, pm^{7}G demonstrates a marked yet equal inhibition of the translation of limiting quantities of both 5′-terminally capped STNV RNA and uncapped STNV RNA. This inhibition is specific for pm^{7}G for equivalent concentrations of pG do not influence the translation of these STNV RNA forms.

This inhibition of the translation of limiting quantities of capped and uncapped STNV RNA by pm^{7}G (Figure 3) can be due to the inhibition of the formation of initiation complexes or the inhibition of some subsequent event in the translation process. As seen in Figure 4, a given quantity of pm^{7}G inhibits the extent of initiation complex formation by limiting quantities

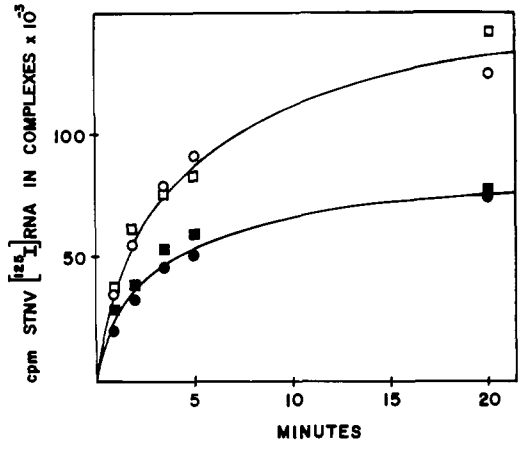

FIGURE 4: The formation of initiation complexes by 5′-terminally capped STNV [^{125}I]RNA (circles) or uncapped STNV [^{125}I]RNA (squares) in the presence (●, ■) or absence (○, □) of 0.5 mM pm^{7}G. Approximately 5% of the intact STNV [^{125}I]RNAs added is bound into initiation complexes at 20 min in the absence of added pm^{7}G.

of capped and uncapped STNV [^{125}I]RNA approximately to the same extent that it inhibits the overall translation of these STNV RNA forms (Figure 3). It follows that the inhibition of translation of capped and uncapped STNV RNA by pm^{7}G represents the pm^{7}G-dependent inhibition of the formation of initiation complexes with these STNV RNA forms. Further, the observation that pm^{7}G inhibits the formation of initiation complexes by limiting quantities of *both* 5′-terminally capped and uncapped STNV RNA suggests that the wheat germ system contains a cap specific protein that is sensitive to the cap analogue, pm^{7}G.

One additional feature of Figures 2 and 4 deserves comment. Specifically, intact STNV RNA does not appear to be an efficient mRNA in the wheat germ system. Only about 5% of the added intact capped or uncapped STNV [^{125}I]RNA binds to wheat germ ribosomes when assayed for 20 min at mRNA limiting conditions (Figure 2). Thus, it is likely that the three-dimensional structure of STNV RNA retards efficient formation in initiation complexes in the wheat germ system. Extension of this concept predicts two things may happen upon disruption of the three-dimensional structure of STNV RNA by fragmentation. First, limited fragmentation of STNV RNA could disrupt the secondary and tertiary structure of STNA RNA sufficiently to facilitate enhanced exposure of the single translation initiation site of STNV RNA. If so, fragmented STNV RNA would be a more efficient mRNA during assays of initiation complex formation, while still forming correct initiation complexes with wheat germ ribosomes. Second, limited fragmentation of STNV RNA might also disrupt secondary or tertiary features of STNV RNA that retard the function of an added 5′-terminal capping group on STNV RNA. If this is correct, then assays of initiation complex formation would show fragmented capped STNV RNA to be a better mRNA than similarly fragmented uncapped STNV RNA.

One can test these hypotheses. Repeated freezing, thawing, and boiling of 5′-terminally capped and uncapped STNV [^{125}I]RNAs shear these samples into fragments (Figure 1c). These fragments must have altered secondary and tertiary structural features relative to intact STNV RNA forms. The nature of the label in fragmented STNV [^{125}I]RNA precludes measurement of the mRNA efficiency of fragmented STNV [^{125}I]RNA in initiation complex formation because fragmentation of STNV [^{125}I]RNA decreases the quantity of uniformly distributed ^{125}I label retained by the fragments

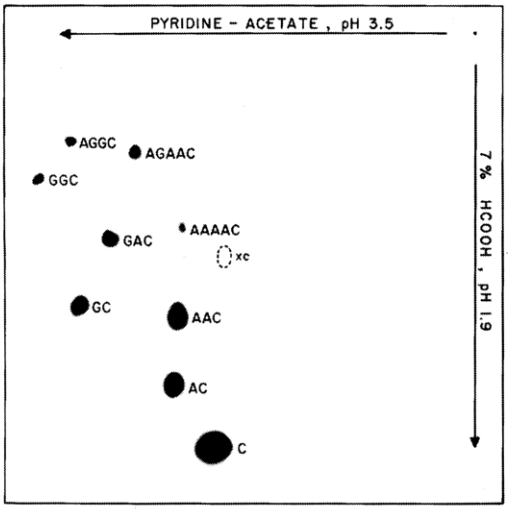

FIGURE 5: Radioautogram of a two-dimensional electrophoretic resolution of the ^{125}I-labeled products released from the ribosome-protected segment of fragmented STNV [^{125}I]RNA by exhaustive digestion with ribonuclease A. The XC marked zone indicates the migration of a xylene cyanole FF dye marker. The characterizations shown agree with those previously reported (Leung et al., 1976) except that the previously reported AAAC is now known to be AAAAC.

containing the initiation site of the STNV RNA. However, the nature of the label in the fragmented STNV [^{125}I]RNA still allows (1) assay of the correctness of initiation complexes formed by fragmented STNV [^{125}I]RNA and (2) assay of any increases in efficiency of mRNA properties of capped fragmented STNV [^{125}I]RNA relative to similarly fragmented STNV [^{125}I]RNA lacking a 5′-terminal capping group.

Figure 5 shows an analysis of the ^{125}I-labeled oligonucleotides obtained from an RNase A dependent degradation of the translation initiation fragment obtained from a ribosome protection experiment with fragmented uncapped STNV [^{125}I]RNA. The nine specific ^{125}I-labeled compounds detected and characterized are the same nine specific oligonucleotides one obtains from identical ribosome protection experiments with intact STNV [^{125}I]RNA (Leung et al., 1976). Thus fragmentation does not appear to alter the specificity of correct initiation complex formation by STNV [^{125}I]RNA. Further, studies of the rate and extent of initiation complex formation by equal and limiting quantities of fragmented capped and uncapped STNV [^{125}I]RNA (Figure 2) show that fragmented, 5′-terminally capped STNV [^{125}I]RNA is better at forming initiation complexes than is fragmented, uncapped STNV [^{125}I]RNA. Thus fragmentation of STNV RNA allows an added 5′-terminal capping group to increase the mRNA efficiency of the fragmented STNV RNA preparation. Fragmented STNV [^{125}I]RNA forms correct initiation complexes (Figure 5). The increased mRNA efficiency of fragmented capped STNV [^{125}I]RNA probably therefore represents increased formation of correct initiation complexes.

Discussion

The data reported here establish that a 5′-terminal capping group does not enhance the translation of intact STNV RNA. We had previously reported (Smith et al., 1977) that limiting quantities of intact 5′-terminally capped STNV [^{125}I]RNA and uncapped STNV [^{125}I]RNA form identical quantities of initiation complexes with wheat germ ribosomes upon long-term incubation. Thus 5′-terminally capped STNV RNA and uncapped STNV RNA compete for the same site(s) on ribosomes. The data of Figure 2 provide a more thorough time course analysis of this point and establish that addition of a 5′-terminal capping group to intact STNV RNA does not enhance the rate or the extent of initiation complex formation by intact STNV RNA. Thus STNV RNA does not contain a 5′-terminal m^{7}G(5′)ppp(5′)Np··· group in vivo (Wimmer et al., 1968; Horst et al., 1971; Leung et al., 1976); STNV RNA does not add such a 5′-terminal capping group prior to in vitro translation (Roman et al., 1976; Leung et al., 1976; Kemper & Stolarsky, 1977), and intact STNV RNA does not serve as a better mRNA in the initiation of protein synthesis when derivatized with a 5′-terminal m^{7}G(5′)ppp(5′)Ap^7··· group.

Our finding that addition of a 5′-terminal capping group does not enhance the mRNA properties of STNV RNA contrasts with Brooker & Marcus (1977) who report that addition of a 5′-terminal capping group onto STNV RNA more than doubles the rate and extent of initiation complex formation by STNV RNA. However, Brooker & Marcus (1977) are unable to introduce a 5′-terminal capping group onto STNV RNAs without "considerable cleavage" of the RNA. We show (Figure 2) that fragmentation of STNV RNA greatly alters the potential of an added 5′-terminal capping group to enhance the rate and extent of initiation complex formation by STNV RNA. The nature of the ^{125}I label employed in our studies precludes a quantitative interpretation of the efficiency of correct initiation complex formation by fragmented STNV [^{125}I]RNA preparations. However, two lines of evidence suggest that fragmentation of a 5′-terminal capped preparation of STNV RNA facilitates the formation of unnaturally high levels of initiation complexes. First, our time course studies of Figure 2 show that fragmented STNV [^{125}I]RNA containing one m^{7}G(5′)ppp(5′)Ap^m··· group per original STNV [^{125}I]RNA molecule yields, on long-term incubation, a quantity of ^{125}I label in initiation complexes equal to, or, on projection, in excess of, the quantity of ^{125}I label bound in initiation complexes formed by an equal quantity of intact STNV [^{125}I]RNA. Fragmented STNV [^{125}I]RNA must contain fewer ^{125}I cpm in the translation initiation site segment(s) of the STNV [^{125}I]RNA than that found in intact molecules of STNV [^{125}I]RNA. Figure 2 therefore dictates that fragmented capped STNV [^{125}I]RNA binds more efficiently than intact STNV [^{125}I]RNA. Second, Brooker & Marcus (1977), working with fragmented STNV RNA containing label in its added 5′-terminal capping group, report 20% and 41% of the input label of fragmented STNV RNA bound into initiation complexes during various initiation complex formations. The translation initiation site of STNV RNA is near the 5′ terminus of STNV RNA (Leung et al., 1979). Also, intact STNV RNA is an inefficient mRNA (Figures 2 and 4). The high percentages of initiation complex formation of Brooker & Marcus (1977) must therefore largely reflect the fragmented character of their capped STNV RNA.

The data of Figures 3 and 4 confirm the findings of Brooker & Marcus (1977) and Kemper & Stolarsky (1977) that increasing concentrations of pm^{7}G cause a partial inhibition of either initiation complex formation by, or in vitro translation of, intact STNV RNA. However, our results with intact capped and uncapped STNV [^{125}I]RNAs (Figure 3) show that pm^{7}G inhibits the translation of these two forms approximately equally. In contrast, Brooker & Marcus (1977) show that pm^{7}G inhibits initiation complex formation by 5′-terminally capped, yet fragmented, STNV RNA much more than it inhibits the formation of initiation complexes by intact uncapped STNV RNA. This added potential for pm^{7}G to inhibit initiation complex formation in their assays probably also reflects the fragmentation of their 5′-terminally capped STNV RNA.

Lastly, the S-30 extracts employed in these studies do contain low levels of nuclease which degrade the input intact STNV [^{125}I]RNA during long-term incubations. 5′-Terminal cap structures will protect mRNAs from certain nuclease degradations (Furuichi et al., 1977). The observed higher extent of 15 U-^{14}C-labeled amino acid incorporation (i.e., long-term translation) by limiting quantities of capped STNV RNA (Figure 3) and the slightly greater pm^{7}G dependent inhibition of long-term translation of capped STNV RNA (Figure 3) probably reflect these endogenous nuclease problems and cap dependent long-term stabilization of STNV RNA.

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H1 Histone Subfractions of Mammalian Testes. 1. Organ Specificity in the Rat†

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ABSTRACT: When H1 histones are extracted from chromatin by 5% (w/v) aqueous trichloroacetic acid, the population obtained from rat testis differs from that found in somatic organs in several ways. A readily observable difference is the presence in testicular extracts of a very prominent component that migrates more slowly than other H1 subfractions during electrophoresis in acetic acid/urea–polyacrylamide gels. We present several lines of evidence that indicate that this H1 species is not a unique product of germinal tissue. Rather, it corresponds to a minor H1 component of many other organs, namely, H1a, the first subfraction to elute during chromatography on Bio-Rex 70. Thus, H1a fractions isolated from rat testis and thymus cannot be distinguished by Bio-Rex 70 chromatography or by electrophoresis under two different conditions in which H1a is resolved from all other subfractions: (i) in the presence of acetic acid/urea; and (ii) in the presence of sodium dodecyl sulfate. While H1a evidently plays a special role during male gamete development, and constitutes about 36% of the total H1 histone present in the adult rat testis, it is present in many other organs and represents about 12% of the H1 population in rat thymus. A further distinctive feature of the rat testis H1 population is the lack of readily detectable H1b, the second subfraction to elute from Bio-Rex 70. The functional requirement(s) underlying these changes in the H1 population is not understood.

H1 histones are known to display microheterogeneity due to differences in amino acid sequence (Kinkade & Cole, 1966; Bustin & Cole, 1968; Rall & Cole, 1971), and at least six subfractions have been identified in various rat organs (Kinkade, 1969; Payim & Chalkley, 1969a,b). The distribution of these subfractions among various organs is not uniform, and this fact suggests that one or more of them may have distinctive functional roles.

For some time it has been thought that one such role might relate to meiosis. Sheridan & Stern (1967) reported over a decade ago that the process of meiosis in the lilly and the tulip is associated with a special H1-like histone that is absent or present in but trace amounts in somatic tissues. This observation has been confirmed (Strokov et al., 1973), and these authors suggested that the meiotic histone might be a com-

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