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5'-Terminal Cap Structure in Eucaryotic Messenger Ribonucleic Acids

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INTRODUCTION

Over the past 10 years the study of the structure, function, and biosynthesis of messenger ribonucleic acid (mRNA) in eucaryotic cells has been an area of intense biochemical research in various laboratories. Gratifyingly, these studies have produced fascinating discoveries which have enabled us to approach an understanding of the regulation of eucaryotic gene expression. Unlike procaryotic mRNA's, eucaryotic mRNA's are synthesized in the nucleus of a cell as part of a long transcriptional precursor unit (commonly referred to as heterogeneous nuclear RNA [hnRNA]). These precursors are apparently subjected to various post-transcriptional modifications and tailoring before mature mRNA's are finally transported to the cytoplasm (48). These post-transcriptional processes include cleavage, 5'-terminal capping, methylation, 3'-terminal polyadenylation, and splicing. Although the mRNA's in the cytoplasm carrying the hallmarks of these modifications can be studied easily, the precise molecular events which occur within the nucleus and lead to these modifications are not yet clearly understood. The main stumbling blocks to such investigations have been the difficulties in radiolabeling RNAs within nuclei in satisfactory amounts, characterizing the enzymes involved in the modification processes, and finally developing a well-defined isolated nuclear system for the in vitro study of transcription and RNA modification. Recently, great progress has been made in this direction because viruses have been used as tools in the study of cellular mRNA biosynthesis. Many vi-

ruses contain virion-associated RNA polymerases which synthesize mRNA's in vitro that carry modifications identical to those of cellular mRNA's (26). In addition, many deoxyribonucleic acid (DNA)-containing viruses replicate in cell nuclei, and in transformed cells their genomes become part of the cellular material by integration (57). Virus-specific mRNA's are synthesized in the nucleus, processed, modified, and transported to the cytoplasm, presumably by the same mechanism as cellular mRNA's. Thus, the recent understanding of mRNA structure and function has been facilitated by the continuing search into the processes involved in viral mRNA biosynthesis.

This review deals primarily with only one of the important modifications of eucaryotic mRNA's, namely 5'-terminal capping (211). The majority of eucaryotic mRNA's have been found to contain a novel 5'-terminal structure. In this 5'-cap a guanosine residue blocks the 5'-terminal penultimate base of the mRNA through a unique 5'-5' linkage containing three phosphate groups (Fig. 1). The blocking guanosine residue universally contains a methyl group at the N-7 position, and the 5'-terminal penultimate base and its adjoining residue are also sometimes methylated in the ribose moiety or when the penultimate residue is an adenosine in the N-6 position as well. The unique 5'-terminal cap structure of eucaryotic mRNA's clearly differentiates them from the polycistronic procaryotic mRNA's, which contain unblocked triphosphate ends (7). These observations also indicate that the mode of mRNA biosynthesis in the eucaryotic system is more complex than in the procaryotic system; this is commensurate with the increased complexity of the genetic organization of eucaryotic species.

Since the discovery of the cap structure in 1974, a great deal of work has been done to elucidate its structure, synthesis, and functional role. I have attempted to review as much as possible from both the published and the unpublished data available to me, highlighting the significant findings rather than describing in detail all of the papers published to date. Clearly, there are omissions, for which the responsibility is mine. The excellent reviews by Shatkin (224) and Filipowicz (70) have dealt specifically with the cap structures, and the reviews by Rottman (210), Adams (7), and Busch et al. (34) have dealt with these structures in their summaries on eucaryotic mRNA structure and function.

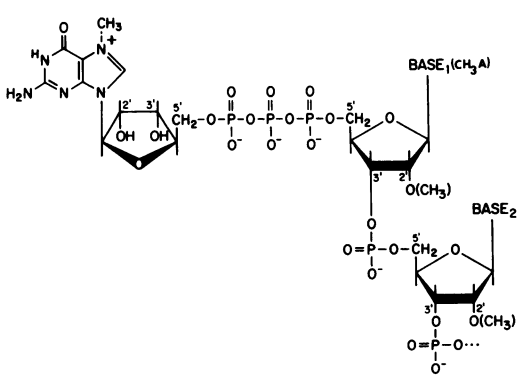

FIG. 1. *Structure of 5' cap. When base1 is adenosine, it is methylated at the N-6 position.*

5'-TERMINAL CAP STRUCTURE IN MESSENGER RIBONUCLEIC ACID (mRNA)

Discovery

The existence of a 5'-terminal blocked structure at the 5' terminus of an RNA molecule was first shown by Reddy et al. (196). These researchers studied the low-molecular-weight nuclear RNAs (4 to 8S) derived from Novikoff hepatoma cells. The primary sequence analysis of one of the RNA species revealed a blocked 5' terminus with a hypermethylated guanosine residue (2,2,7-trimethylguanosine) linked to a 2'-*O*-methylated adenosine residue through a 5'-5' pyrophosphate (PP_i) linkage. Since the precise functions of the small nuclear RNA species were not clear, the importance of the cap structure in these RNAs was not immediately evident. The 5'-terminal structures of eucaryotic mRNA's were believed to be different from those of procaryotic mRNA's since they were resistant to phosphorylation by polynucleotide kinase after prior treatment with alkaline phosphatase (211), a property shared with the low-molecular-weight nuclear RNAs. The discovery of the cap structure in mRNA's was achieved through a series of original observations made by various researchers. Perry and Kelly (186) reported the existence of methylated mRNA's in mouse L-cells. They reported the presence of approximately 2.2 methyl groups per 1,000 nucleosides in polyadenylic acid [poly(A)]-containing L-cell mRNA. In addition, a portion of the methyl label was found to be present in alkali-resistant oligonucleotides, suggesting that some of the methyl groups may be located in the ribose moieties in adjacent nucleotides or at the 5' termini of the mRNA molecules. Similarly, the existence of methylated mRNA's in Novikoff hepatoma cells was reported by Desrosiers et al. (52). Miura et al. (161, 162) observed that the 5'

termini of the double-stranded genome RNAs of cytoplasmic polyhedrosis virus (CPV) and reovirus were scarcely labeled at their 5' termini with [γ-^{32}P]adenosine triphosphate (ATP) by the polynucleotide kinase procedure. However, after sequential oxidation, β-elimination, and phosphomonoesterase digestion, both of the 5' ends of CPV and reovirus were successfully labeled with ^{32}P. These authors also showed that one of the strands of each of the 10 double-stranded genome RNAs of reovirus appeared to contain blocked 5' termini containing 2'-*O*-methylated guanosine (G^m). Furuichi (75), also working with CPV, showed that the virion-associated RNA polymerase requires *S*-adenosylmethionine (AdoMet) for transcription of the genome RNA in vitro. Moreover, all of the mRNA species synthesized in vitro were methylated as part of the initiation step of transcription, indicating that the purified virions of CPV contain a methyltransferase activity that transfers the methyl group of AdoMet to mRNA probably at or near the 5' end of nascent transcripts. Similar methyltransferase activities were later shown to be present in purified reovirus (68, 223), vaccinia virus (263), and vesicular stomatitis virus (VSV) (198). The actual identification of the site of methylation in mRNA and the eventual elucidation of the 5'-terminal methylated capped structure shown in Fig. 1 were reported independently by Furuichi et al. (79), Wei and Moss (264), and Furuichi and Miura (78), who used reovirus, vaccinia virus, and CPV, respectively. These results were followed quickly by a series of reports which demonstrated the presence of the cap structure in viral and eucaryotic mRNA's; these included reports on vaccinia virus by Urushibara et al. (256), VSV by Abraham et al. (2), mouse L-cells by Perry et al. (188, 189), rat hepatoma cells by Desrosiers et al. (53), HeLa cells by Furuichi et al. (81) and Wei et al. (261, 262), and mouse myeloma cells by Adams and Cory (8).

Isolation and Characterization of the Cap Structures

Viral mRNA's. The detailed method of isolation and characterization of the cap structures in mRNA's was established through studies of methylated mRNA's synthesized in vitro by the virion-associated polymerases of reovirus, vaccinia virus, CPV, and VSV (2, 78, 79, 264). The essential steps involved in the isolation and subsequent characterization of the cap structure, as exemplified for reovirus mRNA's (79), are described below.

(i) Phosphatase-resistant 5'-terminal phosphates in methylated mRNA's. Under normal in vitro transcription conditions (i.e., in the absence of AdoMet) in the presence of [β-γ-^{32}P]guanosine triphosphate (GTP), the reovirus mRNA's terminate with 5' $\overset{*}{p}$pGpC (83) (an asterisk denotes a labeled phosphate). However, in the presence of AdoMet, it was observed that 54% of the 5'-terminal labeled phosphate from the initiating nucleotide GTP was resistant to removal from the RNA by treatment with alkaline phosphatase. These results indicated that a population of the mRNA molecules synthesized in the presence of AdoMet must contain 5'-terminal $\overset{*}{p}$pGpC in a blocked structure.

(ii) Structure of the blocked 5' termini. To study the nature of the phosphate-protecting group, mRNA synthesized in vitro in the presence of [β-γ-^{32}P]GTP and [*methyl*-^{3}H]AdoMet was digested with nuclease P1, an enzyme that cleaves phosphodiester linkages in nucleic acids to yield 5'-mononucleotides (74). All of the *methyl*-^{3}H- and ^{32}P-labeled material migrated together between pA and pG markers on paper electrophoresis. The molar ratio of ^{32}P to *methyl*-^{3}H was 1:2. The electrophoretic migration and the net negative charge (−3) of this material were not altered after digestion with alkaline phosphatase, indicating that the phosphate moiety was resistant to the enzyme. However, when the phosphatase-resistant structure was treated with a combination of alkaline phosphatase and nucleotide pyrophosphatase, all of the ^{32}P was converted to inorganic orthophosphate (P_i) and the ^{3}H radioactivity was divided equally between material that migrated with the authentic markers 7-methylguanosine (^{7}mG) and G^m. From these results, together with other confirmatory data, it appeared that the 5'-terminal blocked structure of reovirus mRNA's in vitro was ^{7}mG$\overset{*}{p}\overset{*}{p}$pG^{m}p.... The sequence of the reactions which are described above and led to the elucidation of the structure is as follows:

$$\overset{*}{p}\overset{*}{p}\text{pG} + \text{CTP} + \text{UTP} + \text{ATP} + [\textit{methyl}\text{-}^3\text{H}]\text{AdoMet} \rightarrow {}^7\text{mG}\overset{*}{p}\overset{*}{p}\text{pG}^m\text{p}\ldots + \overset{*}{P}P_i + \text{AdoHcy} \quad (1)$$

$$^7\text{mG}\overset{*}{p}\overset{*}{p}\text{pG}^m\text{pCpUpXp} + \text{nuclease P1} \rightarrow {}^7\text{mG}\overset{*}{p}\overset{*}{p}\text{pG}^m + nP_i + \text{pC} + \text{pU} + \text{pX} \quad (2)$$

$$^7\text{mG}\overset{*}{p}\overset{*}{p}\text{pG}^m + \text{nucleotide pyrophosphatase} \rightarrow {}^7\text{mGp} + \text{pG}^m + \overset{*}{P}_i \quad (3)$$

$$^7\text{mG}\overset{*}{p}\overset{*}{p}\text{pG}^m + \text{nucleotide pyrophosphatase} + \text{alkaline phosphatase} \rightarrow {}^7\text{mG} + G^m + \overset{*}{P}_i \quad (4)$$

where CTP is cytidine triphosphate, UTP is uridine triphosphate, and AdoHcy is *S*-adenosyl-L-homocysteine.

(iii) 5′-5′ Inverted linkage of the terminal nucleotides. It is apparent from reaction 3, in which the products of nucleotide pyrophosphatase treatment of the cap structure are 5′ ^{7}mGp and pG, that a 5′-5′ inverted linkage exists between the blocking guanosine base and the penultimate residue in the RNA. A nucleotide linkage of this type contains a 2′,3′-*cis*-diol similar to the 3′-terminal nucleotides of RNA.

Cellular mRNA's. Isolation and characterization of the cap structures of cellular mRNA's have been carried out essentially by the following two methods. (i) The RNAs of cells were labeled uniformly in vivo with $^{32}P_i$ and purified from the cytoplasmic fraction. The poly(A)-containing mRNA's were purified by repeated chromatography on oligodeoxythymidylic acid-cellulose columns (186). The purified mRNA's were then digested with ribonuclease (RNase) T_2, and the products were fractionated by electrophoresis on cellulose acetate at pH 3.5 and then on diethylaminoethyl paper at the same pH (8). A series of capped and methylated oligomers were obtained; these were further characterized chemically and enzymatically as described above. Using this method, Cory and Adams (45) identified a minimum of 27 oligonucleotides containing a capped structure in mRNA's isolated from mouse myeloma cells. (ii) The cap structures in mRNA's were labeled with *methyl*-^{3}H by exposing exponentially growing cells to [methyl-^{3}H]-methionine in medium containing a reduced concentration of methionine and supplemented with sodium formate, adenosine, and guanosine to prevent incorporation of methionine methyl groups via the one-carbon pool (186). The poly(A)-containing mRNA's were analyzed for cap structures by various enzymatic procedures, as described above. A similar approach was taken to analyze cap structures in mRNA's isolated from virus-infected cells (172). mRNA's were purified by chromatography on oligo-deoxythymidylic acid-cellulose or, in certain cases, by hybridization to viral genome RNA or deoxyribonucleic acid (DNA) (138).

The cap structures isolated from eucaryotic mRNA's and subsequently characterized have the general structure ^{7}mG(5′)ppp(5′)$N_1{}^{m}pN_2{}^{m}$-pN_3p..., where N is a purine or a pyrimidine. ^{7}mG is universally present as the capping base, and the 5′-penultimate nucleoside (N_1) and its adjacent nucleoside (N_2) can also be methylated. The methylation of the latter two nucleosides may vary, generating the following types of structures: ^{7}mG(5′)ppp(5′)N_1pN_2p... (cap 0), ^{7}mG(5′)ppp(5′)$N_1{}^{m}pNp$... (cap 1), and ^{7}mG(5′)-ppp(5′)$N_1{}^{m}pN_2{}^{m}p$...(cap 2). Various RNases have different modes of action on the cap-containing regions of the mRNA's. RNase T_2, a nonspecific RNase, does not cleave the phosphodiester bond adjacent to a 2′-*O*-methylated nucleoside. Thus, after RNase T_2 digestion, mRNA's containing cap 0, cap 1, and cap 2 yield ^{7}mGpppN$_1$p, ^{7}mGpppN$_1{}^{m}$pN$_2$p, and ^{7}mGppp-N$_1{}^{m}$pN$_2{}^{m}$pN$_3$p, respectively, in addition to the internal mononucleotides. Similar products are also generated by alkaline hydrolysis of mRNA's containing the corresponding cap species. Nuclease P1, on the other hand, generates the structures ^{7}mG(5′)ppp(5)N$_1$ (referred to as the cap core) from cap 0-containing mRNA's and ^{7}mG(5′)ppp(5′)N$_1{}^{m}$ from both cap 1- and cap 2-containing mRNA's. RNase A and RNase T_1 digestions of mRNA's generate cap-containing oligonucleotides terminated with 3′-pyrimidine and guanosine, respectively. Thus, by using various RNases, different cap-containing oligonucleotides of mRNA's can be isolated (for example, by binding of the oligonucleotides to dihydroxyborate cellulose [85a, 197]), and they can be further characterized by electrophoresis (45, 79), chromatography on diethylaminoethyl-cellulose (2, 264), or high-pressure liquid chromatography (52).

Properties of Cap Structures

The cap structures have some interesting, characteristic features that impart special properties to the 5′-terminal structures and thus to the mRNA's. These are described below.

(i) The capping base is universally ^{7}mG, except for Sindbis virus mRNA, which contains a low level of ^{2,2,7}m$_3$G at the 5′ terminus (see below). So far, no other terminal base besides ^{7}mG has been found in the cap structure of eucaryotic mRNA's. Although ^{7}mG is present internally in transfer RNA, it is absent in ribosomal RNA, hnRNA, and cytoplasmic mRNA. In the latter two RNA species ^{7}mG is present only as part of the 5′-terminal cap. It is interesting to note that the N-7 atom of guanine is more susceptible to the action of alkylating compounds, and the conversion of guanine to 7-alkylguanine in nucleic acid is considered to be an important event underlying the mutagenic, cytotoxic, carcinogenic, and carcinostatic activities of biological alkylating agents (139). Alkylation of the N-7 atom of guanine in a nucleic acid also introduces a positive charge in the imidazole ring, which has been implicated in erroneous base pairing

with thymine in DNA (140). Moreover, alkylation of the N-7 atom facilitates rapid exchange of the H-8 atom with water, and the imidazole ring undergoes fission when treated with alkali (107). The 9-monosubstituted guanine derivatives, however, are resistant even when treated with hot strong alkali (107). Thus, alkylating of the N-7 atom in guanine imparts some special chemical properties to the capped 5' termini of mRNA's, but the precise functions of these are not clear at the present time.

(ii) The unique 5'-5' phosphate linkage in the cap structure results in the retention of a 2',3'-*cis*-diol on the 5'-terminal ^{7}mG. In this respect, the 3' and 5' ends of the mRNA appear to be identical. The presence of the *cis*-diol at the 5' end permits special chemical reactions usually restricted to the 3' end, such as periodate oxidation followed by labeling with [^{3}H]borohydride (173). The capping base may also be selectively removed by β-elimination by treating the oxidized nucleoside with aniline (173). The reactive dialdelyde may be reduced with cyanoborohydride in the presence of protein, yielding covalently linked protein-RNA conjugates (234). It is also possible to isolate 5'-terminal fragments of mRNA by using affinity chromatography on resins containing covalently bound borate, which were originally developed for selecting 3'-terminal fragments of RNA (208a).

(iii) Nuclear magnetic resonance studies (54, 126, 127) of the capped structure ^{7}mGpppA^{m}pAp have revealed that the three bases form a closely stacked array, resulting in increased stability. It appears that ^{7}mG5'p has enough flexibility so that it can intercalate between the two adenine residues. The 2'-*O*-methyl group present in the penultimate nucleoside induces sufficient conformational strain that the backbone of the A^{m}pA segment assumes an extended spatial configuration, enabling the ^{7}mG5'p residue to intercalate between the adenine bases (111). The presence of a long triphosphate bridge would allow ^{7}mG5'p to bend and intercalate between the adenines. Thus, it appears that the overall spatial configuration of the mRNA terminus ^{7}mG5'ppp5'A^{m}pA is a self-intercalating one which imparts a unique feature to the terminus. These features may affect stability, allow interaction with specialized proteins in ribosomes, etc.

Distribution of Capped mRNA's in Nature

After the discovery of cap structures in reovirus, vaccinia virus, CPV, and VSV, a search was made for such structures in a variety of other viral and cellular mRNA's. As Tables 1 and 2 show, cap structures are present almost universally in eucaryotic mRNA's, with only a few exceptions. Capped mRNA's are present in yeasts, molds, plants, insects, and higher organisms. The only reported exceptions among cellular mRNA's are the poly(A)-containing mRNA's isolated from HeLa cell mitochondria (90, 248). These mRNA's may contain ppA at the 5' termini. The 5' termini of maternal mRNA's obtained from developing oocytes of the tobacco hornworm (*Manduca sexta*) apparently contain a cap structure but lack methylation of the capping guanosine (117). In plant and animal viruses almost all mRNA's of both the DNA- and RNA-containing viruses contain a cap structure, except picornaviruses (108, 142) and three plant viruses, satellite tobacco necrosis virus (144, 269) and its helper virus (143) and cowpea mosaic virus (CMV) (50, 241). In the case of picornaviruses, including poliovirus and encephalomyocarditis virus, it has been shown that a protein (molecular weight, 12,000) is connected covalently to the 5'-terminal uracil of the viral genome through a phosphodiester linkage to a tyrosine residue in the protein (88, 108). A similar protein-linked genome has also been described for CMV (50, 241). Satellite tobacco necrosis virus, however, contains a di- or triphosphate (p)ppA at the 5' end (144), which is similar to bacterial and bacteriophage mRNA's (144, 269).

In a survey of cap structures present in eucaryotic mRNA's (Tables 1 and 2), an interesting pattern emerges with regard to the cap composition of various types of mRNA's. These features can be summarized as follows.

Viral mRNA's. All mRNA's contain ^{7}mG as the capping base except the few viral mRNA's that do not contain a cap (Table 1). Only the mRNA strand (i.e., the positive strand) is capped, whereas the antisense strand, such as the genome of VSV and influenza virus and one strand of the duplex genome RNA of reovirus and CPV, usually has a di- or triphosphate 5' end rather than a cap. This indicates that capping is probably restricted to mRNA's. The exceptions are the low-molecular-weight nuclear RNAs (46, 196, 202) which are capped but do not act as messengers in in vitro protein synthesizing systems (195). The 5'-penultimate base of viral mRNA's is always a purine, which may or may not be methylated. This may indicate that in viruses initiation of RNA synthesis or processing of RNA may be purine specific. When the 5'-penultimate base is adenosine, it exists in the majority of cases in a dimethylated form, N^{6}-methyl-2'-*O*-methyladenosine (N^{6}mA^{m}) (260). Plant viruses usually contain a cap 0 structure,

TABLE 1. *5′-Terminal cap structures in viral mRNA's*

Host and virus[a]	Type of genome[b]	Site of replication	5′-Terminal structure			Internal methylation	Reference(s)
			Virion RNA	In vitro mRNA	Cytoplasmic mRNA		
Mammal							
Poliovirus	ssRNA(+)	Cytoplasm	Protein-pUp		pUp	None	108, 142
EMC	ssRNA(+)	Cytoplasm	Protein-pNp		pNp	None	88
FMDV	ssRNA(+)	Cytoplasm	Protein-pNp		pNp	None	216
Sindbis virus	ssRNA(+)	Cytoplasm	^{7}mGpppApUpYpGp		^{7}mGpppApUpG[c]	m^{5}C	59–62, 103, 112
Calcivirus	ssRNA(+)	Cytoplasm	Protein-linked		No cap	ND[d]	27, 33, 64
Dengue virus	ssRNA(+)	Cytoplasm	^{7}mGpppA^{m}pNp		^{7}mGpppm^{6}A^{m}p	None	42
VSV	ssRNA(−)	Cytoplasm	(p)ppA	^{7}mGpppA^{m}p-	^{7}mGpppm^{6}A^{m}pA^{m}p	None	2, 102, 171, 204, 254
Influenza virus	ssRNA(−)	Nucleus(?), Cytoplasm	pppA	No cap	^{7}mGpppm^{6}A^{m}p/G^{m}p	3 m^{6}A	134, 169, 193
Newcastle disease virus	ssRNA(−)	Cytoplasm	ND	^{7}mGpppNp-	^{7}mGpppNp-[e]	None	43, 44
Reovirus	dsRNA(±)	Cytoplasm	^{7}mGpppG^{m}(+) ppG(−)	^{7}mGpppG^{m}p	^{7}mGpppG^{m}pC^{m}p-	None	53a, 67, 79, 82
Rous sarcoma virus	ssRNA(+)	Nucleus	^{7}mGpppG^{m}pCp		ND	10–12 m^{6}A	24, 118
Avian sarcoma virus	ssRNA(+)	Nucleus	^{7}mGpppG^{m}pCp		ND	10 m^{6}A	86, 243
Moloney murine leukemia virus	ssRNA(+)	Nucleus	^{7}mGpppG^{m}p		ND	15–23 m^{6}A	205
Feline leukemia virus	ssRNA(+)	Nucleus	No cap		^{7}mGpppG^{m}pAp ^{7}mApppG^{m}pGp	10 m^{6}A	252, 253
Insect							
CPV	dsRNA(±)		^{7}mGpppA^{m}p(+) ppA(−)	^{7}mGpppA^{m}p	ND	None	78
Fish							
Spring viremia of carp	ssRNA(−)	Cytoplasm	pppA	^{7}mGpppA^{m}p	ND	ND	96, 212
Plant							
Wound tumor virus	dsRNA(+)		ND	^{7}mGpppA^{m}p	ND	ND	199
Brome mosaic virus	ssRNA(+)		^{7}mGpppGp		ND	None	49
Tobacco mosaic virus	ssRNA(+)		^{7}mGpppGp		ND	None	119, 279
Alfalfa mosaic virus	ssRNA(+)		^{7}mGpppGp		ND	None	191
Turnip yellows virus	ssRNA(+)		^{7}mGpppGp/A		ND	None	95, 128
Cucumber mosaic virus	ssRNA(+)		^{7}mGpppNp		ND	None	246
Cucumber mosaic virus-associated RNA (CARNA 5)			^{7}mGpppGpU		ND	None	200
Barley stripe mosaic virus	ssRNA(+)		^{7}mGpppN		ND	None	9
Potato virus X	ssRNA(+)		^{7}mGpppGpA		ND	None	235
Tobacco necrotic virus	ssRNA(+)		ppApG		ND	None	143
Satellite tobacco necrotic virus	ssRNA(+)		ppApG		ND	None	144, 269

TABLE 1.—*Continued*

Host and virus[a]	Type of genome[b]	Site of replication	5′-Terminal structure			Internal methylation	Reference(s)
			Virion RNA	In vitro mRNA	Cytoplasmic mRNA		
CMV	ssRNA(+)		No cap, protein-linked		ND	ND	129, 241
Fungus							
Penicillium chrysogenum	dsRNA(±)		^{7}mGpppN^{m}p(+) ppAGY(−)		ND	ND	249
Mammal							
Vaccinia virus	DNA	Cytoplasm		^{7}mGpppA^{m}p/G^{m}p	^{7}mGpppm^{6}A^{m}p/G^{m}p	None	260, 264
Adenovirus	DNA	Nucleus			^{7}mGpppm^{6}A^{m}p/A^{m}pN$_2$^{m}p[f]	m^{6}A, m^{5}C	99, 100, 170, 273
Simian virus 40	DNA	Nucleus			^{7}mGpppm^{6}A^{m}p/G^{m}p	m^{6}A	10, 36, 91, 138
Herpes simplex virus type 1	DNA	Nucleus			^{7}mGpppN$_1$^{m}pN$_2$^{m}p[g]	m^{6}A	166
Polyoma virus	DNA	Nucleus			^{7}mGpppm^{6}A^{m}p/G^{m}pN$_2$^{m}p	m^{6}A	72

[a] Abbreviations: EMC, encephalomyocarditis virus; FMDV, foot and mouth disease virus.
[b] ss, Single stranded; ds, double stranded.
[c] mRNA contains a small proportion of $m_2^{2,7}G$ and $m_3^{2,2,7}G$ at the 5′ terminus (112).
[d] ND, Not determined.
[e] H. O. Stone, personal communication.
[f] Some of the early mRNA's contain cap 1 with N_1 = methyluridine (see reference 100).
[g] N_1 = m^{6}A^{m}, methyladenosine, or methylguanosine.

TABLE 2. *5'-Terminal cap structures in cellular mRNA's*

Cells	Cap structure[a]			Internal methylation	Reference(s)
	Cap 0	Cap 1	Cap 2		
Human HeLa cells	None	$N_1 = Pu^m, Py^m, m^6A^m$	$N_2 = Pu^m, Py^m$	m^6A	81, 261, 262, 266
HeLa cell histone	None	$N_1 = A^m, m^6A^m, G^m$	$N_2 = Pu^m, Py^m$	None	167, 242
Mouse myeloma	None	$N_1 = Pu^m, Py^m, m^6A^m$	$N_2 = Pu^m, Py^m$	m^6A	8, 45
Mouse erythroid	None	$N_1 = Pu^m, Py^m, m^6A^m$	$N_2 = Pu^m, Py^m$	m^6A	101
Mouse L-cells	None	$N_1 = Pu^m, Py^m, m^6A^m$	$N_2 = Pu^m, Py^m$	m^6A	188
Mouse kidney	None	$N_1 = N^m$	$N_2 = N^m$	Present	182, 183
Rat hepatoma	None	$N_1 = Pu^m, Py^m, m^6A^m$	$N_2 = Pu^m, Py^m$	m^6A	52, 53
Hamster kidney (BHK-21)	None	$N_1 = Pu^m, Py^m, m^6A^m$	$N_2 = Pu^m, Py^m$	m^6A, m^5C	61, 172
Monkey kidney (BSC-1)	None	$N_1 = Pu^m, Py^m, m^6A^m$	$N_2 = Pu^m, Py^m$	m^6A	138
Mouse immunoglobulin	None	$N_1 = G^m, m^6A^m$	$N_2 = A^m$	m^6A	47, 156
Human globin	None	$N_1 = m^6A^m, A^m$	$N_2 = C^m$	None	40
Mouse globin	None	$N_1 = m^6A^m, A^m$	$N_2 = C^m$	None	39
Rabbit globin	None	$N_1 = m^6A^m$	$N_2 = C^m$	None	47, 148, 150
Duck globin	None	$N_1 = N^m$	ND[b]	None	190
Ovalbumin	$N_1 = A$	$N_1 = m^6A^m, A^m$	$N_2 = Py^m$	ND	154
Protamine		Contains cap			86
Drosophila	$N_1 = C$	$N_1 = Py^m > Pu^m$	$N_2 = Pu^m, Py^m$	ND	147
Silk fibroin	None	$N_1 = A^m$	$N_2 = U^m$	m^6A	274
Aedes albopictus	None	$N_1 = Pu^m, Py^m$	$N_2 = Pu^m, Py^m$	m^6A	113
Tobacco hornworm oocyte	GpppN				117
Brine shrimp	N_1	ND	ND	ND	174
Sea urchin embryo	None	$N_1 = Pu^m > Py^m$	None	m^6A	69, 244
Slime mold	N = A > G	$N_1 = A^m$ (10%)	None	None	58
Neurospora	N = A > G	None	None	None	220
Yeast	N = A > G	None	None	None	51, 152, 240
Wheat embryo	N_1	None	None	None	98, 125, 213
Maize	N_1	None	None	None	178
Soybean seeds	N_1	$N_1 = N^m$	None	None	247

[a] Cap 0 denotes $^7mGpppN_1pN_2p\ldots$; cap 1 denotes $^7mGpppN_1{}^mpN_2p\ldots$; and cap 2 denotes $^7mGpppN_1{}^mpN_2{}^mp\ldots$. N_1 and N_2 are purine (Pu) or pyrimidine (Py) ribonucleosides.

[b] ND, Not determined.

but among animal viruses cap 0 structures are rare, with the exception of Sindbis virus genome RNA (103) and Newcastle disease virus mRNA synthesized in vitro (44). The majority of viral mRNA's contain a cap 1 (in vitro mRNA's) or cap 2 (in vivo mRNA's) structure. In addition to methyl groups in the cap structure at the 5' terminus of an mRNA, N^6-methyladenosine (N^6mA) is often present internally in a variety of mRNA's. Viruses that replicate in the cytoplasm of infected cells (such as VSV, reovirus, and vaccinia virus) do not contain internal N^6mA residues, whereas those replicating in the nucleus (simian virus 40, adenovirus, etc.) contain N^6mA located internally. The exception is Sindbis virus, in which N^6mA is present in in vivo mRNA's but not in genome RNA. These results may indicate that the methylation of internal adenosines in mRNA is a nuclear event. 5-Methylcytidine (m^5C) also has been shown to be present internally in adenovirus (233) and Sindbis virus (59) mRNA's. In the latter case, m^5C represents the only internal methylated base. Interestingly, a fraction of the Sindbis virus mRNA (26S) molecules from infected cells contain, in addition to the usual ^7mG, the hypermodified congeners $m_2{}^{2,7}G$, and $m_3{}^{2,2,7}G$ (112), similar to the low-molecular-weight nuclear RNAs (46, 202).

Cellular mRNA's. A variety of 5'-terminal capped sequences were found in higher eucaryotic mRNA's, including those of HeLa cells, myeloma cells, baby hamster kidney cells, etc. In the well-studied mouse myeloma cells (MPC-11-66) (45), a minimum of 27 5'-terminal sequences were reported. Unlike viral mRNA's, the 5'-penultimate base can be any of the four standard bases or N^6mA^m. The second base can also be any one of the standard four. mRNA's containing $^7mG(5')ppp(5')C^mpUp$ appear to be present in relatively high abundance (19%), compared with other sequences, which occur at levels of less than 1%. It is not known whether the different termini are associated with specific classes of mRNA or have any functional significance. In this respect, cellular mRNA's are more complex than viral mRNA's. The presence of pyrimidines in the 5'-penultimate position in

cellular mRNA's suggests the possibility that they may not be primary transcripts, assuming that all RNA chains are initiated by purines (153). On the other hand, it is quite possible that initiation may occur by pyrimidines (209). A better understanding of the precise nature of initiation in eucaryotic systems will help to clarify these observations.

The degrees of methylation in the cap structures are also different in cellular and viral mRNA's (Tables 1 and 2). Unlike viral mRNA's, cap 1 and cap 2 structures are present in all cellular mRNA's, although the cap 1 structure is about five times more abundant than the cap 2 structure (187). As in viral mRNA's, 5'-penultimate adenosine residues are predominantly N^6mA^m. The only exception is in *Drosophila melanogaster* mRNA's (147), which contain cap 0 structures in addition to cap 1 and cap 2 structures. Moreover, these mRNA's do not contain N^6mA^m. Like higher mammal mRNA's, *Drosophila* cell mRNA's contain purines and pyrimidines at the N_1 bases of the cap 1 structures. However, the majority of poly(A)-containing cytoplasmic RNA molecules from heat-shocked *Drosophila* cells contain purine almost exclusively as the N_1 base of cap 1. These results are consistent with other results, indicating that a special set of mRNA's is turned on during heat shock. It was also observed in *Drosophila* mRNA's that two RNase T_2-resistant dinucleotides were present in the fingerprint (147). These dinucleotides are not capped and may or may not be methylated; their precise compositions are not known. Interestingly, similar spots in the fingerprints of mouse myeloma cell mRNA's were also found (45), but they were not detected in sea urchin or *Dictyostelium* mRNA's (58).

As Table 2 shows, the diversity of cap structures is restricted to certain eucaryotes. A comparison of the 5'-terminal sequences of mammalian mRNA's and those of lower eucaryote mRNA's reveals phylogenetic trends in the cap structures. For example, only cap 0 is found in yeast mRNA and predominates in the mRNA of the unicellular slime mold *Dictyostelium discoideum*. Moreover, the N_1 base is a purine in both of these organisms. Studies of sea urchin mRNA have identified only cap 1 structures, with a purine in more than 80% of the N_1 bases. In metazoan cell mRNA (*Drosophila*) three varieties of caps are found, with a random frequency of purines and pyrimidines but no N^6mA^m in the N_1 base. In the fibroin mRNA of the silkworm and in higher organisms, cap 1 and cap 2 structures predominate, and N^6mA^m appears in the N_1 position. These observations indicate that the cap structure and its adjacent bases increase in complexity in the more developed genome structures of higher eucaryotes. The precise function of these modified structures in relation to gene regulation is not known.

The internal N^6-methylation of eucaryotic cellular mRNA's also has an interesting pattern. The mRNA's isolated from higher eucaryotic organisms, such as HeLa cells (human), L-cells (mouse), and BSC cells (monkey), invariably contain N^6mA internally. The striking exceptions are the globin and histone mRNA's, which do not contain this methylated base internally (167, 190). In contrast to the findings described above, the mRNA's isolated from lower eucaryotic organisms, such as slime molds, yeasts, etc., do not contain internal N^6mA. Although the reasons for these differences are not known, they may reflect some basic differences among eucaryotic organisms that may directly relate to the mode of mRNA biosynthesis. The methylation of internal adenosine appears to occur at specific sites in RNA. In HeLa cells and L-cells two methylated internal sequences were detected, namely, Gpm^6ApC and Apm^6ApC (217, 266). Interestingly, in avian sarcoma virus genome (55), Rous sarcoma virus (24), and simian virus 40 late mRNA's (36) identical sequences were found. Like some animal viruses, cultured hamster kidney cells and Novikoff mRNA's have been shown to contain internal m^5C at levels of 20 and 3%, respectively, of the total, the remainder being N^6mA.

MECHANISM OF THE CAPPING REACTION

Viral mRNA's

Elucidation of the various steps involved in the formation of the cap structure was made possible through studies of animal viruses that carry virion-associated RNA polymerase. Under appropriate reaction conditions, mRNA's synthesized in vitro by virion-associated RNA polymerase may contain 5'-terminal uncapped structures, capped but not methylated structures, cap 0 structures, or cap 1 structures, demonstrating that these viruses contain all of the enzymes necessary for the synthesis of cap. The mechanisms of capping that have been well elucidated by studying reovirus (83), vaccinia virus (168), CPV (76), VSV (17), influenza virus (133), etc., are summarized below.

Reovirus. Virion-associated RNA polymerase transcribes in vitro the 10 double-stranded genome RNAs into 10 mRNA species containing predominantly the 5'-terminal sequence ppGpC... (224). In the presence of the methyl donor AdoMet, the predominant 5'-terminal structure is $^7mGpppG^mpC$... (79). If methylation

is prevented by AdoHcy, 5′-terminal ppGpC... predominates (79). Reovirus cores incubated in the presence of GTP, cytidine triphosphate, and AdoMet synthesize the cap structure ^{7}mGpppG^{m}pC (83). The dinucleotide ppGpC functions as a substrate for a core-associated guanylyltransferase and is converted to GpppGpC by the addition of pG from GTP. For optimal conversion, both a diphosphate terminus and a phosphodiester bond are required in the acceptor. pGpC is not a substrate, but pppGpC is utilized after removal of the γ-phosphate by a core-associated nucleotide phosphohydrolase. GpppGpC is hydrolyzed by the cores to ppGpC in the presence of PP_i; however, this hydrolysis is markedly decreased by methylation at N-7 of guanosine. It was further shown that the β-γ-imido analog of GTP, GMPPNHP, can replace GTP for transcription; under these conditions, the 5′-terminal structure is pNHppGpC (84). Thus, hydrolysis of the β-γ bond of GTP in the acceptor RNA molecule is needed for cap formation. Based on these observations, the following reaction series was proposed for the synthesis of reovirus mRNA caps:

$$\text{pppG} + \text{pppC} \xrightarrow[\text{polymerase}]{\text{RNA}} \text{pppGpC} + PP_i$$

$$\text{pppGpC} \xrightarrow[\text{phosphohydrolase}]{\text{Nucleotide}} \text{ppGpC} + P_i$$

$$\text{ppGpC} + \text{pppG} \xleftarrow{\text{Guanylyltransferase}} \text{GpppGpC} + PP_i$$

$$\text{GpppGpC} + \text{AdoMet} \xrightarrow{\text{Methyltransferase I}} {}^7\text{mGpppGpC} + \text{AdoHcy}$$

$$^7\text{mGpppGpC} + \text{AdoMet} \xrightarrow{\text{Methyltransferase II}} {}^7\text{mGpppG}^m\text{pC} + \text{AdoHcy}$$

Thus, it appears that in reovirus mRNA's the α- and β-phosphates of GTP and the α-phosphate of the capping GTP are retained in the cap structure and that capping and initiation of RNA synthesis occur concurrently.

Vaccinia virus. mRNA's synthesized in vitro by vaccinia virus in the presence of AdoMet contain two 5′-terminal structures, ^{7}mGpppG^{m}p... and ^{7}mGpppA^{m}p... (264). The virion contains guanylyltransferase and methyltransferase activities, which modify the 5′ ends of mRNA's. The mechanism of the capping reaction is similar to the reovirus system, and the following scheme has been established (168):

$$\underset{\text{pppG}}{\overset{\gamma\beta\alpha}{}} + \underset{\text{ppN-}}{\overset{\beta'\alpha'}{}} \rightleftharpoons \underset{\text{GpppN-}}{\overset{\alpha\beta'\alpha'}{}} + \underset{PP_i}{\overset{\beta\gamma}{}}$$

$$\text{GpppN-} + \text{AdoMet} \rightarrow {}^7\text{mGpppN-} + \text{AdoHcy}$$

$$^7\text{mGpppN-} + \text{AdoMet} \rightarrow {}^7\text{mGpppN}^m\text{-} + \text{AdoHcy}$$

At limited concentrations of AdoMet, methylation occurs at the N-7 G position, whereas at higher concentrations ribose methylation of the penultimate N_1 base occurs. (See below for a detailed description of the properties of the vaccinia virus guanylyltransferase and methyltransferases which have been solubilized and purified from the virion in an active form.) Although the properties of the purified enzymes have been well characterized, it is still not clear whether the specificities of the enzymes within the virus core are the same as those of the highly purified enzymes.

Cytoplasmic polyhedrosis virus. Like reovirus, the RNA polymerase of CPV transcribes 10 genome segments in vitro (78). In contrast to reovirus and vaccinia virus, the synthesis of mRNA in vitro is stimulated considerably by the addition of AdoMet; the resulting mRNA contains the 5′-terminal capped structure ^{7}mGpppA^{m}pGp... (78). Moreover, the competitive inhibitor of methylation AdoHcy also effectively stimulates mRNA synthesis in vitro, resulting in the synthesis of viral mRNA containing 5′-terminal GpppA and ppA. Thus, it appears that CPV may have an additional regulatory mechanism(s) by which AdoMet and AdoHcy "switch on" mRNA synthesis, probably as allosteric effectors that influence the RNA polymerase and capping enzymes (85) (see below). It was further shown that the β,γ-imido analog of ATP (AMPPNHP) failed to support methylation and RNA synthesis, whereas similar analogs of GTP, cytidine triphosphate, and uridine triphosphate were effective substrates for RNA synthesis and methylation (76). These results were interpreted as due to the inability of AMPPNHP to replace 5′-ATP because it cannot be processed by phosphohydrolase, an enzyme involved in cap formation (see above). It was shown that during partial reactions in vitro with ATP and GTP as substrates, GpppA was produced and subsequently methylated to ^{7}mGpppA^{m}p in the presence of AdoMet (227). However, neither the 5′-capped compounds nor other dinucleotides, such as pppApG and ppApG, stimulated mRNA synthesis in vitro in the absence of AdoMet or AdoHcy (Y. Furuichi, personal communication). These results may imply that CPV RNA polymerase, for its full function, may require that some functional barrier be removed by an unknown process mediated by the presence of AdoMet or AdoHcy (see below).

Vesicular stomatitis virus. The virion-associated RNA polymerase of VSV synthesizes in vitro five mRNA species that are capped and have the 5' sequence GpppApApCpApG (3, 197). These 5' ends are quantitatively methylated to ^{7}mGpppA^{m}pApCpApGp... in the presence of AdoMet (2). The interesting features of the cap structures of VSV mRNA that distinguish them from the other viral mRNA cap structures described above are that (i) the α-phosphate of ATP and the α- and β-phosphates of GTP are incorporated into the cap structure (2), (ii) exogenously added ppA-containing mRNA's or GpppA cannot be capped or methylated, respectively, in the in vitro system (1), and (iii) the methyltransferase reaction takes place as follows (250):

$$\text{GpppA- + AdoMet } (<0.1\ \mu\text{M}) \xrightarrow{\text{methyltransferase}} \cdot\text{GpppA}^m\text{p-}$$

$$\text{GpppA}^m\text{p- + AdoMet } (>5\ \mu\text{M}) \xrightarrow{\text{methyltransferase}} {}^7\text{mGpppA}^m\text{p-}$$

These reactions are different from those in the reovirus and vaccinia virus systems, in which 7-methylation precedes 2'-*O*-methylation even at low AdoMet concentrations. It appears that capping and methylation in the VSV system are tightly coupled to transcription (1). The formation of the three-phosphate bridge in the VSV mRNA's, as $G^{\alpha\beta}_{pp}/{}^{\alpha}_{p}A$, indicated that the mRNA's may have been synthesized by a processing mechanism in which capping involved $G^{\alpha\beta\gamma}_{ppp}$ and 5'-$^{\alpha}_{p}$A ends from newly processed mRNA. Alternatively, the mRNA species may indeed be initiated RNA molecules with pppA ends, and capping with GTP may proceed by removal of the β,γ-phosphates of adenosine and the γ-phosphate of guanosine. This reaction, if operative, is clearly different from the reactions in the reovirus, vaccinia virus, and CPV systems described above, which have such capped structures as $G^{\alpha}_{p}/{}^{\beta\alpha}_{pp}N$. Recently, 5'-ppA-ended VSV mRNA fragments have been detected in vitro (D. Testa, P. K. Chanda, and A. K. Banerjee, unpublished data), suggesting that in the VSV system removal of the β,γ-phosphates of adenosine and the γ-phosphate of guanosine may occur at least in vitro. It is interesting to note that unlike reovirus and vaccinia virus, the capping of VSV mRNA in vitro is not inhibited by PP_i and P_i (17). Moreover, the mRNA synthesized in the presence of GMPPNHP (replacing GTP) contains the normal cap, $G^{\alpha\beta}_{pp}/{}^{\alpha}_{p}A$ (18). It was later confirmed that purified VSV contains a phosphohydrolase that can convert GTP to guanosine diphosphate (GDP) and that the latter can be converted to GTP by a virion-associated nucleoside diphosphate kinase (251). Recently, by using a fish rhabdovirus it was shown that, unlike VSV, in the presence of GMPPNHP the cap structure was $G^{\gamma}_{p}/{}^{\beta\alpha}_{pp}A$, indicating that an alternate capping reaction may be operative in this system (96). Like CPV, AMPPNHP is not used in place of ATP in the in vitro reaction for RNA synthesis. This is probably because initiation of VSV mRNA involves the synthesis of a leader RNA which is not capped and contains the 5'ppACG... sequence. This step may require ATP containing hydrolyzable β,γ-phosphates (251).

Influenza virus. The mechanism by which a 5'-terminal cap is added to influenza virus mRNA appears to be unique. It was observed that the mRNA's synthesized in vitro by the virion-associated RNA polymerase in the presence of AdoMet with or without primers, such as ApG (193), did not contain cap structures. In contrast, the viral mRNA's isolated from infected cells were capped and methylated and contained the sequences ^{7}mGpppA^{m}p..., ^{7}mGpppN^{6}mA^{m}p..., and ^{7}mGpppG^{m}p... (134). The predominant species of caps contained methylated adenosine. These results suggested that complementary RNA synthesis in infected cells initiates preferentially with adenosine and that the RNA polymerase in purified virions, unlike the RNA polymerases in other RNA viruses, did not associate with the enzymes for cap structure formation. This apparent paradox was resolved when it was found totally unexpectedly that the virion-associated RNA polymerase reaction is stimulated by exogenously added globin, reovirus, and other capped mRNA's (31, 32) and that a portion of the 5'-terminal region of the mRNA (approximately 10 to 15 nucleotides) is donated to the influenza virus mRNA in vitro. Thus, it appeared that the purified virion of influenza virus contains enzymes that can incorporate a specific 5'-terminal portion of capped and methylated mRNA's into the nascent transcripts (192) either by splicing onto the influenza virus-specific mRNA synthesized in vitro or by cleavage and extension, the added mRNA acting as a primer for RNA synthesis. This unique enzyme system can recognize only capped mRNA's containing ^{7}mG since chemically decapped globin mRNA (32) and blocked but unmethylated reovirus mRNA's do not act as

primers for in vitro mRNA synthesis. Recently, it has been shown that a similar mechanism probably also operates in vivo (133). Influenza virus-specific complementary RNA from infected cells contains a nonviral stretch of 10 to 15 nucleotides cannabilized from cellular mRNA's. These results suggest that host cell mRNA's serve as primers for viral RNA transcription in infected cells, accounting for the previously observed requirement of functional host nuclear RNA polymerase II for influenza virus replication (133).

It is clear from the studies on the viral systems described above that the presence of a virion-associated RNA polymerase greatly facilitated elucidation of the capping reaction. In those viruses that do not contain a virion polymerase, such as adenovirus, simian virus 40, polyoma virus, herpesvirus, etc., the mechanism of the capping reaction has been studied by nucleotide sequence determination of complementary RNAs pulse-labeled in vivo. By direct sequence determinations of the late promoter regions and the 5'-terminal portions of the nuclear and cytoplasmic transcription products formed during late times after adenovirus infection, Ziff and Evans (278) precisely mapped the transcripts on the promoter region. It was concluded that the capped termini of nuclear and cytoplasmic RNAs and the late promoter map together, suggesting that the initiating residues of the primary transcript are precursors of the capped terminus. From similar studies with simian virus 40 (37) and polyoma virus (72) late mRNA's, the viral transcripts also appear to be initiated molecules that are capped subsequently. In contrast to the adenovirus system, the mRNA's of these two viruses contain highly heterogeneous 5'-terminal sequences (37, 72, 89, 97). These results indicate that the RNA polymerase may initiate transcription over a range of nucleotides on the promoter region. The precise reasons for this heterogeneity in the 5'-terminal cap sequence are not known. Recently, similar heterogeneity in cap sequences has been shown in the early mRNA's of adenovirus-infected cells (99). Interestingly, one of the six early promoter sequences contains $^{7}mGpppU^{m}pN_2$ as the cap structure, indicating that a pyrimidine can be present in the N_1 position in viral mRNA's (100).

Cellular mRNA's

As described above, cellular mRNA's contain the cap structure $^{7}mGpppN_1^{m}$, where N_1 is G^{m}, $N^{6}mA^{m}$, A^{m}, methylcytidine, or methyluridine. It has been generally assumed that purines in the N_1 position indicate that caps are put on initiated RNA chains and that when the N_1 base is a pyrimidine, caps are formed on cleaved molecules (218). This assumption is mainly derived from the observation that in procaryotic systems, the RNA polymerase initiates chains predominantly with purines (153). In fact, all bases are used for initiation, but purines are favored 5 to 10 to 1 over pyrimidines, depending on the DNA template (153). Thus, RNA polymerase II of eucaryotic cells would also presumably initiate mainly or exclusively with purines. This assumption may no longer be tenable since initiation with pyrimidines has now been shown to occur in at least in one bacteriophage operon (209) and at a second promoter for ribosomal RNA synthesis in *Escherichia coli* (275). Nevertheless, it has not been demonstrated conclusively in eucaryotes whether RNA chains may indeed initiate with pyrimidines. A detailed analysis of the 5'-terminal structure of the hnRNA from mouse L-cells (the presumed precursor of mRNA) revealed the presence of purine tri- and diphosphates, such as pppG, pppA, ppG, and ppA (219), as well as the four varieties of cap structures described above. These results indicate that the 5' portions of some of the primary transcripts (those starting with purines) are directly converted to caps. Similar uncapped 5' termini have also been found in pre-mRNA molecules of Ehrlich carcinoma cells (15). The RNA chains containing pyrimidines may be generated by either (i) an initiation process or (ii) cleavage at specific sites on hnRNA. In the former case, capping and initiation may be concurrent, and in the latter capping may occur on RNAs containing monophosphates at the 5' termini. In an earlier report, Schibler and Perry (218) found large amounts of pUp in addition to ppUp and ppCp at the 5' termini of the hnRNA. It was proposed that the mRNA segments derived by cleavage could be converted by a cellular kinase action to diphosphate-terminated pyrimidines, which would then condense with GTP to form a pyrimidine-containing cap structure. Later it was found that no pyrimidine diphosphates were present in the hnRNA, thus negating this model (219). Although the biosynthesis of the pyrimidine-containing cap structure remains unclear, the existence of enzyme activities in mammalian cells which form caps by condensation of GTP with diphosphate-terminated RNA lends credence to the idea that probably all capped RNAs (including those with pyrimidine-containing caps) are initiated, primary transcripts (see below).

Recent experiments (214) have shown that in cells labeled by brief exposure to [*methyl*-^{3}H]-AdoMet the majority of the labeled 5'-terminal cap 1 structures were in hnRNA molecules hav-

ing a chain length of about 750 nucleotides or less. After longer labeling times, the proportion of cap 1 structures in hnRNA molecules longer than mRNA molecules was increased to more than 50% of the total. The cap structures in both long and short hnRNA chains contained all four 2'-*O*-methylated nucleotides in the N_1 position in about the same proportion as in mRNA. These results may indicate that hnRNA chains can indeed initiate with pyrimidines and that capping occurs very close to or at the start of chain formation.

The mechanism of capping in cellular and viral systems has also been approached by using isolated nuclei to study transcription and capping in vitro. Groner and Hurwitz (94) reported that the RNA synthesized in vitro in the presence of AdoMet by HeLa cell nuclei contained a variety of capped and methylated 5'-terminal structures, such as $^7mGppN^mp$, $^7mGppN_1{}^mpN_2{}^mp\ldots$, $^7mGpppN_2{}^mp\ldots$, and $^7mGpppN_1pN_2{}^mp$. The relative amounts of $N_1{}^m$ were as follows: guanosine, 45%; adenosine, 25%; uridine, 20%; and cytosine, 10%. Thus, in the in vitro system, in addition to the cap 1 structures, diphosphate-containing capped structures and cap 2 structures were also found. In contrast, in the hnRNA isolated from the nucleus, only cap 1 structures were found, and cap 2 structures were obtained only in the mRNA's isolated from the cytoplasm (73, 187, 215). Clearly, in contrast to intracellular nuclei, in isolated nuclei methyltransferases involved in methylating N_1 and N_2 bases are present and active. Using a subcellular system isolated from mouse L-cell nuclei, Winicov and Perry (270, 271) have also shown that cap 1 and cap 2 structures were present in the in vitro transcripts. It was further shown that cap formation in large nuclear RNA species synthesized in vitro was closely associated with transcription, as indicated by α-amanitin sensitivity and a requirement for the presence of all four ribonucleoside triphosphates. Recently, Groner et al. (92) showed that [β-^{32}P]ATP can be incorporated into the 5'-terminal capped structure of RNA synthesized in vitro by HeLa cell nuclear homogenates. Approximately 10% of the RNA chains were initiated in vitro with [β-^{32}P]ATP, and this value decreased considerably when α-amanitin was present in the reaction mixture. These results indicate that, at least for those RNA chains containing adenosine in the N_1 position, initiation and capping occur by condensation of pG from GTP on either a diphosphate or triphosphate 5' end. It was further observed that in contrast to triphosphate caps, in which the N_1 base is any one of the four 2'-*O*-methylated derivatives, the diphosphate caps ($^7mGppN^mp$) contain exclusively G^m at the N_1 position. It is not clear whether these cap-containing RNA species are artifacts of the in vitro reaction or whether capping enzymes present in the nuclei may carry out reactions which have not been observed in vivo.

Using isolated nuclei from adenovirus-infected cells, Manly et al. (155) demonstrated that adenovirus-specific RNA was synthesized in vitro and that the RNA species originated at a map position expected for late mRNA in vivo promoters (i.e., map position 16.5). Moreover, it was observed that the 5' termini of the RNAs were capped with ^7mG and that the amount of radioactivity in 7-methylguanosine monophosphate was equivalent to the amount of radioactivity in any other single nucleotide in the 5'-terminal RNase T_1 fragment. Since a large amount of the transcription observed arises from elongation of nascent chains initiated in vivo, the possibility existed that [α-^{32}P]GTP was incorporated into the 5' termini of the RNA chains already initiated in vivo. Under these conditions, a greater than equimolar amount of radioactivity in 7-methylguanosine monophosphate would have been obtained; thus, the results indicate that post-transcriptional capping does not occur at detectable levels and that the capping and transcription initiation processes are tightly linked.

ENZYMES INVOLVED IN THE CAPPING REACTIONS

From the structure of the 5' cap and the mechanism of its formation, it became apparent that multienzyme systems are involved in the formation of fully methylated capped 5' ends. The enzymes involved in the cap formation are as follows. (i) mRNA guanylyltransferase, involved in the first step of the capping reaction, catalyzes the formation of the cap structure by using GTP that is linked through a triphosphate bridge to the 5' end of the RNA. To carry out this reaction, two other enzymatic activities appear to be involved; these are (a) an enzyme that converts 5'-phosphate-terminated RNA to 5'-di- and triphosphate-terminated RNAs, which in turn can be used for capping with GTP (239) and (b) an RNA triphosphatase activity that converts triphosphate-terminated RNA to diphosphate-terminated RNA (the latter is then used as a substrate for capping) (257). (ii) mRNA (guanine-7-)methyltransferase catalyzes the transfer of a methyl group from AdoMet to the position 7 of the capping guanosine residue. (iii) 2'-*O*-Methyltransferase activity methylates the 2'-*O*-ribose moieties of the N_1 nucleotides of the mRNA's. (iv) A methyltransferase activity spe-

cifically catalyzes the transfer of a methyl group from AdoMet to the N^6 position of a 2'-*O*-methyladenosine residue at the N_1 position of the capped 5' end of mRNA.

All of the above-described enzymes have been purified from vaccinia virions by Moss and his co-workers (19, 20, 65, 157–159) with the exception of the last enzyme, which was purified by them from HeLa cells (120). RNA (guanine-7-)methyltransferase was also purified from HeLa cells (66). Purifications of some of these enzymes have also been carried out in the laboratory of Hurwitz (164, 165). Recently, both guanylyltransferase and methyltransferase activities were purified from rat liver nuclei (163). The properties and specificities of these enzymes are described below.

mRNA Guanylyltransferase and mRNA (Guanine-7-)Methyltransferase

Martin et al. (159) first reported the isolation and purification of these enzymes from vaccinia virions. They took advantage of the selective binding of these activities to homopolyribonucleotides to achieve a 200-fold increase in specific activity. Both activities remain together during affinity or ion-exchange chromatography. The purified enzyme has a molecular weight of 127,000 and contains two polypeptides of molecular weights 95,000 and 31,400 in a molar ratio close to 1:1, indicating that both activities are components of an enzyme system. This system specifically catalyzes modification of the diphosphate-containing 5' termini of mRNA, poly(A), or polyguanidylic acid (157, 158):

$$\text{ppN-} + \text{GTP} \rightleftharpoons \text{GpppN} + \text{PP}_i$$

$$\text{GpppN} + \text{AdoMet} \rightarrow {}^7\text{mGpppN} + \text{AdoHcy}$$

The enzyme prefers Mg^{2+} over Mn^{2+}, and the optimum pH is 7.8. Studies on the donor and substrate specificities of this enzyme complex revealed that diphosphate-terminated polyribonucleotides (such as $pp[N]_n$, where N can be any base) are preferred and that monophosphate termini are not used. Although mononucleotides (ppN) are capped, trinucleotides (ppNpNpN) are better substrates, and slightly longer polyribonucleotides might be optimal (157). GTP, inosine triphosphate, and deoxyguanosine triphosphate, but not ^{7}mGTP, can be used as donors.

Monroy et al. (164) reported an approximately 10,000-fold purification of the same activities from purified vaccinia virus. Slightly different properties were observed. The molecular weight of the native protein is 120,000; a major band of molecular weight 95,000 is released in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, along with minor bands having molecular weights of 59,000 and 28,000. However, when the native guanylyltransferase is purified by nondenaturing polyacrylamide gel electrophoresis, recovered, iodinated, and electrophoresed on a polyacrylamide gel containing sodium dodecyl sulfate, a major polypeptide band of molecular weight 59,000 is observed. This enzyme specifically uses 5'-triphosphate-ended RNA chains [ppp(A)n] (165). The apparent K_m for termini of ppp(A)n is 0.2 μM, indicating a high affinity of the enzyme for the substrate. During guanylylation of ppp(A)n, P_i and PP_i are both released in a 1:1 stoichiometry with cap formation. The reason for the apparent discrepancy between these results and those described by Moss and his colleagues (157, 158) are presently unclear. However, Venkatesan and Moss (257) have recently detected an additional enzyme activity (RNA triphosphatase) in purified vaccinia virions. This activity also appears to be a component of the guanylyltransferase enzyme complex. In addition, this enzyme activity is involved in the removal of γ-phosphate from triphosphate-ended RNA chains, converting the 5' end to a diphosphate, which in turn is used by the enzyme as the principal substrate. A similar mechanism has been proposed previously for the capping of reovirus mRNA's in vitro (83).

Spencer et al. (239) have reported isolation of a 5'-phosphate-phosphate polyribonucleotide kinase activity from vaccinia virus cores; this activity catalyzes the conversion of 5'-phosphate and 5'-diphosphate termini of RNAs to 5'-triphosphate forms. 5'-Hydroxyl RNA or DNA and 5'-phosphorylated DNA are not phosphorylated by this enzyme. In a system that coupled 5'-phosphate polyribonucleotide kinase and guanylyltransferase, p(A)n and pp(A)n were capped. The guanylyltransferase by itself, however, can only cap ppp(A)n. The precise role of the 5'-polyribonucleotide kinase activity in the capping process is not known, but this enzyme may be involved in the capping of mRNA's that are formed by cleavage of pre-mRNA (219) to form 5'-phosphate termini, which are then converted to capped molecules.

2'-*O*-Methyltransferase

This activity has been purified from vaccinia virions approximately 350-fold. It catalyzes the transfer of a methyl group from AdoMet to the 2'-OH of the penultimate nucleoside of the capped mRNA, such as conversion of ^{7}mGpppG in brome mosaic virus RNA to ^{7}mGpppG^m (19). The molecular weight of the enzyme is 38,000. RNAs ending on pN, ppN, or GpppN are ineffective substrates for this enzyme (20). poly(A)

and polyinosinic acid ending in ^{7}mGpppN are excellent substrates, whereas capped polyguanosinic acid, polyuridylic acid, and polycytidylic acid are poor substrates for the enzyme. The low ability of the enzyme to use dinucleotides as substrates (for example, ^{7}mGpppG) suggests that a longer polyribonucleotide chain is required. This activity is extremely sensitive to AdoHcy. With only 1 μM AdoHcy in the presence of 2.5 μM AdoMet, the activity is virtually abolished. This is in contrast to the VSV system, in which 2'-*O*-methyltransferase activity is more resistant to AdoHcy than (guanine-7-)methyltransferase (250).

Guanylyltransferases and Methyltransferases from Eucaryotic Cells

Like the enzymes in vaccinia virus, the enzymes involved in the capping reaction in eucaryotic cells have also been detected and partially purified. A soluble extract prepared from HeLa cell nuclei has been shown to catalyze the 5'-terminal modification of RNA and synthetic polyribonucleotides to form ^{7}mGpppA and ^{7}mGpppG structures (66, 265). The substrate RNA molecule appears to require the presence of at least two terminal phosphates. A polyribonucleotide with only a single 5'-terminal phosphate is not capped unless a second phosphate is added. Thus, it appears that the first step of the capping reaction in eucaryotic cells may be similar to that in the vaccinia virus and reovirus systems. More recently, Venkatesan et al. (S. Venkatesan, A. Gershowitz, and B. Moss, personal communication) have purified this enzyme 1,000-fold from HeLa cell nuclei. It has a molecular weight of 48,500 and shows optimal activity at pH 7.5 in the presence of Mn^{2+} or Mg^{2+}. Using this purified enzyme, Venkatesan and Moss have shown that RNA with ppC at the 5' end (a transcription product of phage λc17 DNA [209]) is also capped by purified mRNA guanylyltransferase, indicating that pyrimidine-ended as well as purine-ended RNAs can serve as acceptors. This result is consistent with the presence of a pyrimidine in the N_1 base of the cap in eucaryotic mRNA's and also suggests that pyrimidine-containing caps may also be part of initiated RNA molecules. Recently, an mRNA guanylyltransferase purified from wheat germ has also been shown to use only diphosphate-ended RNA (J. Keith, A. Gershowitz, S. Venkatesan, and B. Moss, personal communication).

Unlike vaccinia virus, the HeLa cell RNA (guanine-7-)methyltransferase is clearly separable from the guanylyltransferase activity (66). This enzyme specifically methylates the 5'-terminal guanosine of RNA chains ending in GpppG or GpppA. It differs from the vaccinia virus enzyme in the following respects. (i) The HeLa cell enzyme exhibits exclusively methyltransferase activity, whereas the vaccinia virus is part of an enzyme complex that also includes guanylyltransferase activity (see above). (ii) GTP and GDP are not substrates for HeLa cell enzymes, whereas these compounds can be methylated to a small extent by the viral enzyme. (iii) The HeLa cell enzyme has a molecular weight of 56,000, whereas the molecular weight of the viral enzyme is 127,000.

As discussed above, in viral and cellular mRNA's synthesized in vivo (in contrast to in vitro mRNA in the case of viruses) adenosine residues in cap structures exist largely as the unusual dimethylated nucleoside N^6 mA^m (Table 1). Consistent with this observation, an enzyme activity (molecular weight, 65,000) was observed in the postribosomal supernatant of HeLa cells; this activity specifically methylates the N^6 position of 2'-*O*-methylated adenosine in the cap structure (120). Less activity was found with RNA ending in ^{7}mGpppA, and barely detectable activity was found with RNAs ending in GpppA and ppA- or oligonucleotides of the type ^{7}mGpppA^mpN or ^{7}mGpppApN. The latter results indicate that some extension of RNA is required before the methylation of the N-6 position of adenosine may occur. The failure of this enzyme to methylate internal RNA segments makes it likely that a separate methyltransferase, not isolated so far, catalyzes the formation of internal m^6A in eucaryotic mRNA's (Table 1).

Recently, separate guanylyltransferase and RNA (guanine-7-)methyltransferase activities have been purified from rat liver nuclei (163). They have molecular weights of approximately 65,000 and 130,000, respectively. For the guanylylation reaction, dithiothreitol is essential, and Mn^{2+} (2 mM) is twice as effective as Mg^{2+} (8 mM). The capping reaction appears to involve condensation of guanosine monophosphate to a diphosphate-terminated ribopolymer [such as pp(A)n, ppGpCpC, etc.]. The triphosphate-terminated ribopolymers are capped at much lower rates than their diphosphate-terminated counterparts. In this respect, the mode of action of this enzyme is similar to the vaccinia virus enzyme reported by Martin and Moss (157, 158). Recently, Bajszar and co-workers (16) have reported that 30S ribonucleoprotein particles from rat liver nuclei contain (guanine-7-)methyltransferase and 2'-*O*-methyltransferase in addition to guanylyltransferase.

RNA (guanine-7-)methyltransferase has also been partially purified from *Neurospora crassa*

(87). It catalyzes the transfer of a methyl group from AdoMet to an RNA containing a 5′-terminal unmethylated capped structure. *N. crassa* poly(A)$^+$ RNA and reovirus mRNA are equally good as substrates. No 2′-*O*-methyltransferase activity is associated with this enzyme, which is consistent with the absence of 2′-*O*-methylation in *N. crassa* mRNA (220). Although not purified, a similar methyltransferase activity has been demonstrated in the extracts of wheat germ (173, 184; J. Keith and B. Moss, personal communication), *Artemia salina* (174), calf thymus (141), and embryonic chicken lens (136).

Recently, two separate mRNA (nucleoside-2′-*O*-)-methyltransferases from HeLa cells have been purified; one of these completes formation of cap 1 structures and the other yields cap 2 structures with suitable acceptors (S. Langberg and B. Moss, personal communication).

Enzymes That Cleave the Cap Structure

Enzymes that cleave the cap structure into ^{7}mGp and pN^m-containing mRNA have been isolated and purified free from RNase from tobacco tissue culture cells (228) and potato (276). The nucleotide pyrophosphatase purified from potato has been used to remove the caps from reovirus, rabbit globin, and *A. salina* mRNA's for studies on the effect of caps on mRNA translation (276). The corresponding enzyme from tobacco cells was used similarly to decap CPV mRNA (226, 229). Moreover, after removal of the cap from tobacco mosaic virus RNA, it was shown that the ability of the RNA to reassemble with the coat protein was unchanged, but infectivity was abolished (181). By using tobacco pyrophosphatase, the blocking nucleotide of rabbit globin mRNA was removed, and the mRNA was subsequently sequenced by the polynucleotide kinase method, using γ-ATP (63).

An enzyme activity was also detected in HeLa cell extracts; this activity specifically cleaved the dinucleotide ^{7}mGpppG^m to ^{7}mGp and ppG^m (180). It did not hydrolyze the corresponding unmethylated structure or ring-opened cap derivative. However, this enzyme failed to cleave the cap structure present in mRNA. It appears to require for activity capped oligonucleotides shorter than 10 bases (179). The low-molecular-weight nuclear hypermethylated capped RNAs were not substrates for the enzyme. The function of this enzyme in eucaryotic cells is not known. One of the proposed functions is as a scavenger by which accumulated cap structures are destroyed during degradation of mRNA's, thus avoiding inhibitory effects of caps on ribosome binding and eventually facilitating translation of capped mRNA (179). A similar nucleotide pyrophosphatase activity has also been detected in embryonic chicken lens (135). It releases ^{7}mGp from the cap structure ^{7}mGpppN (where N is adenosine, A^m, G^{m}pCp, A^{m}pApC, or A^{m}pApCpApG) (137). Phage T4-induced polynucleotide kinase has also been reported to cleave the PP$_i$ bond from a cap structure, but this has not been confirmed (4).

BIOLOGICAL ROLE OF THE CAP STRUCTURE

The presence of the unconventional cap structure in eucaryotic mRNA's prompted investigations to determine its role in mRNA function. It was quickly recognized that the cap structure carrying the polar ^{7}mG moiety may play a significant role in the interaction of mRNA with ribosomal RNA or its protein components during initiation of protein synthesis. Moreover, the 5′-terminal cap structure of mRNA may be resistant to the action of 5′-exonuclease action, thus imparting stability to the mRNA's within the cell. Finally, the capping process may be directly involved in the biosynthesis of mRNA. These areas covering the possible roles of the cap structure have been investigated in great detail, and the results strongly indicate that cap structures may indeed play an important role in conferring higher translational ability and stability to mRNA's and in addition may also be directly related to the transcription process.

Protein Synthesis

Both et al. (28) were the first to demonstrate that the cap structure in mRNA was required for efficient translation of VSV and reovirus mRNA's in a wheat germ cell-free protein-synthesizing system. The corresponding uncapped (reovirus) and capped and unmethylated (VSV) mRNA's were virtually untranslated in the same extract. Similar results were obtained when the translational abilities of capped reovirus, VSV, and globin mRNA's were compared with the corresponding mRNA's after chemical removal of the cap structures from their 5′ ends (173). Subsequently, Both et al. (29) observed that the cap structure facilitates efficient binding of reovirus mRNA's to 40S ribosomal subunits during initiation of protein synthesis. After these discoveries, a great deal of data have been accumulated over the years; the role of the cap structure in translation has been studied extensively by using a number of different mRNA's from viral and eucaryotic sources and a variety of cell-free protein-synthesizing systems. The recent reviews by Filipowicz (70) and Kozak (130) have dealt comprehensively with the functions of the cap structure in protein synthesis

and its possible involvement in the capacity of ribosomes to select the initiation regions in mRNA's. To avoid repetition, I have summarized below the observations already reviewed and added some new interesting findings regarding the role of mRNA cap structures in eucaryotic protein synthesis.

From a vast body of reports published over the years concerning the function of the cap structure in mRNA translation, some distinct patterns emerged.

Uncapped or unmethylated mRNA's are poor templates for protein synthesis in vitro. This was shown either by using standard in vitro synthesized mRNA's that lacked the cap structure or by removing the cap structure from the mRNA by chemical or enzymatic methods. The translational abilities of these mRNA's and their abilities to bind to initiation complexes were then tested in a variety of cell-free protein-synthesizing systems. The mRNA's tested were from reovirus (104, 146, 173, 175, 276), vaccinia virus (176, 185, 259), VSV (151, 175, 206, 254), CPV (226), tobacco mosaic virus (177, 226, 272), brome mosaic virus (225), globin (104, 149, 173, 226, 272, 276), protamine (86), bovine parathyroid (123), hen ovalbumin (222), and *A. salina* (174). The results indicated that virtually all mRNA's that lacked ^{7}mG were ineffective templates for protein synthesis. The observed translational deficiency of mRNA lacking ^{7}mG was more pronounced in wheat germ extract compared with reticulocyte lysates and ascite extracts. It was further observed that wheat germ extracts and reticulocyte lysates responded differently to changes in salt concentrations when translating uncapped mRNA's (25, 41, 124, 185, 272). It is not clear whether there is an intrinsic difference in the components used for chain initiation or whether the extracts differ only in the concentrations or activities of some common initiation components. Although other conditions, such as mRNA concentration (110, 146, 225, 272), initiation factors (104, 116), and temperature (259), favorably affect the translation of uncapped mRNA's under optimum conditions, the importance of the cap for translation was evident.

Recently, the role of the cap structure in translation of procaryotic mRNA's in eucaryotic cell-free systems has been studied. Various procaryotic mRNA's have been translated with accuracy in both mammalian and wheat germ cell-free systems to produce functional protein products (11), but the efficiency of translation was significantly higher in the procaryotic extract. Using vaccinia virus capping enzyme, Paterson and Rosenberg (184) were able to cap the ir vitro mRNA's coding for λ *cro* gene. The capped mRNA's were translated at relatively high efficiencies in wheat germ extract (compared with capped rabbit globin mRNA), and this efficient translation was absolutely dependent on the modification of the λ transcripts by addition of the cap structure. Like other capped mRNA's, *cro* protein translation was inhibited by the cap analog ^{7}mGpppA. These results suggest that the procaryotic mRNA's contain all of the information necessary for efficient recognition and initiation by eucaryotic translation components, except for the cap structure.

7-Methylguanosine facilitates binding of mRNA's to ribosomes. Although the translation of capped and uncapped mRNA's in vitro depended on various experimental parameters, the ribosome binding studies were unambiguous. In experiments with capped polynucleotides, such as ^{7}GpppG^{m}C(U)$_n$ and ^{7}mGpppG^{m}C(A,C)$_n$, it was shown that these polymers were able to bind more efficiently to 40S ribosomal subunits than the corresponding uncapped ribopolymers (30). Capped mRNA's were also more efficient than their uncapped counterparts in binding to ribosomes to form stable initiation complexes (151, 175, 176, 206, 259). The addition of a cap structure to uncapped mRNA by vaccinia virus enzyme increased the rate and extent of binding of vaccinia virus mRNA to wheat germ ribosomes (176). Moreover, in a mixture of mRNA's containing capped and uncapped mRNA's, capped mRNA was found to be rapidly and selectively associated with ribosomes. (175, 176). The notable exceptions are the mRNA's that normally lack the cap (e.g., poliovirus, satellite tobacco necrosis virus, cow pea mosaic virus) but nevertheless bind to ribosomes and are translated efficiently in in vitro systems. Uncapped satellite tobacco necrosis virus RNA was apparently translated equally efficiently when it was enzymatically capped (232). The latter examples indicate that in these systems cellular factors play a primary role in the translation process (see below).

From experimental data relating binding of the 40S and 80S initiation complexes to the 5′ ends of a number of mRNA's, Kozak and Shatkin (130, 132) postulated a "scanning model" in which the 40S ribosomal subunit first attaches to the 5′ end of a message, irrespective of sequence, and subsequently advances until it encounters the first AUG triplet, at which point the 40S ribosome stops and the 60S subunit joins. The presence of ^{7}mG probably facilitates the initial binding with 40S ribosome. This translation process in eucaryotic systems is clearly different from the polycistronic procar-

yotic mRNA's, in which the ribosomes can attach to multiple AUG codons in the mRNA. Eucaryotic mRNA's, on the other hand, presumably contain one functional AUG codon to account for their monocistronic nature. This model may account for the inability of the ribosome to attach to an internal AUG codon and is also compatible with the observed variability in the location of the AUG codon within the 5'-proximal regions of different messages with varying neighboring sequences. The nucleotide sequences of the 5'-proximal regions of eucaryotic mRNA's (130) indicate that the initiator codon AUG is located at variable distances, ranging from 10 to 100 bases from the 5'-terminal cap. The interaction of the 5' terminus of the message with the 40S subunit appears to be more important than the position or location of the AUG codon in the mRNA. In support of this model, Kozak (131) has recently demonstrated that eucaryotic ribosomes failed to attach to the AUG codon when present in a circular mRNA. Circularization of pUpG$(pA)_{40-80}$ or of poly(A,U,G) by T_4 RNA ligase virtually abolished the ability of the template to bind to wheat germ or reticulocyte ribosomes. In control experiments, linear forms of each polymer were able to form initiation complexes in wheat germ and reticulocyte extracts, and the circular molecules that failed to bind to wheat germ or reticulocyte ribosomes were able to bind to *E. coli* ribosomes. These results are compatible with the proposed mechanism by which eucaryotic ribosomes enter at the open 5' terminus of a message by a threading mechanism, with translation beginning at the first AUG codon encountered by the ribosome. The precise function of the cap structure in this process is not clear since, as described above, uncapped mRNA's also attach to ribosomes and are translated, although at lesser efficiency.

The role of ^{7}mG in translation has also been tested by using a polycistronic procaryotic mRNA. Rosenberg and Paterson (209) used a high-molecular-weight polycistronic in vitro λ DNA transcript containing the 5'-terminal *cro* gene in the RNA. When a cap structure was added to the 5' end of the RNA before translation in wheat germ extract, efficient translation of only the *cro* gene occurred. The inability to detect protein synthesis from any of the cistrons located internally in the transcript suggests that the eucaryotic ribosome interacts with the cap structure, which subsequently allows recognition of the first AUG located close to the 5' end. Using a similar translation system, these investigators have also shown that in the *gal* operon-derived polycistronic message only the cap-proximal cistron (*galE*) was translated in vitro. Future experiments in this area should give interesting insights into the nature of the interactions of the cap structures in procaryotic mRNA with eucaryotic ribosomes that allow the 5'-proximal AUG to be recognized in a polycistronic message.

Chemical analogs of cap structures, such as p^{7}mG, ^{7}mGDP, and ^{7}mGpppN^m, inhibit translation and ribosome binding of capped mRNA. Under appropriate ionic conditions these cap analogs inhibited translation of a variety of capped mRNA's in various cell-free extracts (13, 38, 71, 93, 110, 203, 221, 245, 258, 268). These studies indicate that some special structural features of the cap play a significant role in the recognition process during the formation of the initiation complex of mRNA and ribosomes. In a survey of the inhibitory effects of different methylated nucleosides and nucleotides in translation and ribosome binding, some interesting structural requirements for cap recognition were observed. ^{7}mG, GMP, 7-methylguanosine 2',3'-cyclic phosphate, and GpppN were not inhibitory to translation, whereas p^{7}mG, ^{7}mGDP, ^{7}mGTP, and ^{7}mGpppN^m were potent inhibitors. These results indicate that some interaction between the polar group of ^{7}mG and the negatively charged phosphate group(s) of these inhibitors is involved in altering the binding of capped mRNA to ribosomes (71, 111). Replacement of the methyl group by ethyl (7-ethyl)GDP or benzyl (7-benzyl)GDP did not decrease the inhibitory capacity of ^{7}mGDP (6), indicating that the maintenance of the positive charge (but not necessarily a methyl group at the N7 position) is essential for the inhibitory effect. This is also borne out by the observation that 7-ethylguanosine-containing capped mRNA's (mRNA's synthesized in vitro by reovirus-associated enzymes in the presence of *s*-adenosyl-*l*-ethionine) were essentially equally active in translation and binding to ribosomes compared with their methylated counterparts (80).

Cap-binding protein. Since ribosomal subunits from wheat germ or reticulocyte extracts bound efficiently to the 5'-terminal region of many mRNA's and protected cap-containing sequences against RNase digestion (132), it seemed possible that initiation complexes included proteins capable of binding mRNA (cap-binding protein [CBP]), thus protecting the cap from nuclease action. Activity of this type was detected in an *A. salina* ribosomal high-salt wash by assaying the binding of *methyl*-^{3}H-labeled ^{7}mGpppG^{m}C to membrane filters (71). By the same technique it was found that preparations of the purified rabbit reticulocyte initiation factors (for nomenclature, see Anderson et al. [12]) eIF-2, -4B, and -5 bound to mRNA (21, 22,

221). The binding of eIF-4B to capped mRNA, but not to encephalomyocarditis virus RNA, which is uncapped, was inhibited by the cap analog p^{7}mG. On this basis, it was suggested that eIF-4B recognizes and binds to the cap structure (221). By using the same experimental approach, it was shown that eIF-2 also recognizes the cap structure in mRNA (116). These researchers used the filter binding technique, which may not permit unequivocal identification of polypeptides with cap-binding activity, especially if the cap-binding component is a contaminant with a strong affinity for caps. To detect protein with cap-binding activity, Sonenberg and Shatkin (237) used a cross-linking method by which proteins in the proximity of the 5' end of mRNA in initiation complexes can be detected. Using this method and polyacrylamide gel electrophoresis, Sonenberg et al. (234) detected a 24,000-dalton polypeptide in initiation factors prepared by high-salt washes of rabbit reticulocyte, mouse ascites, and human HeLa cell ribosomes. The CBP can be specifically cross-linked to *methyl*-^{3}H-labeled capped viral mRNA as defined by ^{7}mGDP inhibition. The 24,000-dalton CBP was detected only in purified eIF-3 and -4B among the many factors tested. The strong cap interaction of the CBP was not limited to reovirus mRNA but was also observed with reticulocyte eIF-3 cross-linked to VSV, Newcastle disease virus, and vaccinia virus capped mRNA's (235). The failure to obtain nonspecific binding and cross-linking to the CBP in the presence of ^{7}mGDP suggests that this polypeptide has a single site available for cross-linking and that this site recognizes the ^{7}mG nucleotide. Recently, the CBP from reticulocyte ribosomes has been purified to homogeneity by chromatographic passage through an affinity resin prepared by coupling the levulinic acid $O^{2',3'}$-acetal of ^{7}mGDP to AH-Sepharose 4B (236). The translation of capped mRNA's from Sindbis virus, reovirus, and rabbit globin was stimulated by purified CBP in HeLa cell extracts. The translation of uncapped mRNA's from encephalomyocarditis virus and satellite tobacco necrosis virus was not stimulated under the same conditions (236), indicating that the CBP may interact with the ^{7}mG in mRNA before the formation of protein synthesis initiation complexes.

Based on these results, Sonenberg et al. (235) proposed a model for the role of CBP in the promotion of translation of capped mRNA's. The first step in protein synthesis involves the formation of a pre-initiation complex between the 5' end of capped mRNA and the CBP. Since the CBP has a high affinity for eIF-3 (234), the next step is the interaction of the pre-initiation complex containing CBP with the 40S ribosomal subunits containing eIF-3. After initiation complex formation, the CBP is released for recycling, and its interaction with the 5' end of mRNA is replaced by eIF-3 subunits. This model predicts that eIF-3 or some of its subunits would be present in the 80S initiation complexes, either on the ribosome or on the 5' terminus of the mRNA. Further characterization of the proteins that can be cross-linked to the cap structures of the mRNA's in initiation complexes should clarify the roles of CBP and eIF-3 subunits in capped mRNA translation.

Recently, CBP has been shown to have restoring activity for capped mRNA translation in cell extracts from poliovirus-infected cells. Infection with poliovirus leads to inactivation of the cap-dependent recognition mechanism of mRNA's during protein synthesis initiation. It was observed that the translation of capped mRNA's (e.g., in VSV) was markedly decreased in extracts of poliovirus-infected HeLa cells, whereas poliovirus RNA, which is not capped, continued to function well in vitro (208). The addition of only purified eIF-4B and not other initiation factors to infected cell extracts restored capped mRNA translation without increasing poliovirus synthesis. These results were interpreted as selective inactivation of eIF-4B by poliovirus during infection and thus blocking of host cell protein synthesis (i.e., the translation of capped mRNA's). In view of the stimulatory effect of CBP in translation of capped mRNA's compared with uncapped mRNA's, it was confirmed that the observed restoring activity of eIF-4B was indeed due to the presence of contaminating CBP in the initiation factor (255). The restoring activity was purified from a high-salt wash of rabbit reticulocyte ribosomes, which corresponded to the CBP with regard to electrophoretic mobility, tryptic peptide pattern, and ability to cross-link to the 5'-terminal cap in mRNA. Thus, it appears that at least in the poliovirus system cellular protein synthesis may be inhibited by inactivation of some crucial property of the CBP.

Helentjaris and Ehrenfeld (105) have also shown that a salt wash from uninfected cells stimulated translation of both cell (endogenous translation system derived from HeLa cells) and poliovirus mRNA's, whereas a salt wash from infected cells stimulated the translation only of viral mRNA. This mRNA-discriminating activity was found to be tightly associated with eIF-3 (106). Since CBP has a high affinity for eIF-3 (234), it is possible that the mRNA discriminating activity may be similar, if not identical, to the CBP.

Interesting results were obtained when similar experiments were carried out in reovirus-in-

fected cell extracts. Skup and Millward (231) have shown that extracts from uninfected L-cells translated capped reovirus mRNA at high efficiencies and that the translation was sensitive to ^{7}mGTP. The same extracts translated uncapped reovirus mRNA at low efficiencies. In contrast, extracts from infected L-cells translated uncapped reovirus mRNA at higher efficiencies, and the translation was not sensitive to ^{7}mGTP. These results suggest that like the poliovirus system, reovirus infection renders a component(s) of the protein synthesis machinery inactive, such that translation of capped mRNA's is blocked. However, reovirus mRNA, in contrast to poliovirus mRNA, is capped, and its translation continues unabated during infection. It has been claimed that reovirus mRNA's late after infection are not capped but contain pGp at the 5′ end (277). Enzyme activity in L-cell S10 extract rapidly converted diphosphate-ended mRNA's to monophosphate-ended mRNA's. Subsequently, it was shown (230) that the capping and methyltransferase activities become masked in subviral particles isolated from cells at late times after reovirus infection. Thus, the mechanism of host cell shutoff by reovirus infection may be similar to that in the poliovirus system, except that additional controlling features may exist in the former that convert capped viral mRNA to its uncapped form during infection.

mRNA Stability

One of the features that distinguishes eucaryotic mRNA's from procaryotic mRNA's is their longer half-lives. The short-lived procaryotic mRNA's are degraded exonucleolytically from the 5′ end during translation (7). Modification at the 5′ end of the eucaryotic mRNA's possibly imparts stability by virtue of conferring resistance to the action of such nucleases. One of the reasons for the observed inefficient translation of uncapped mRNA's in in vitro cell-free extracts may thus be due to preferential degradation of uncapped compared with capped mRNA's. Several experiments indicated that the cap structure stabilizes mRNA both in vivo and in cell-free extracts. Furuichi et al. (77) injected capped reovirus mRNA's into *Xenopus laevis* oocytes and observed that capped mRNA's were more stable than their uncapped counterparts. It was also found that uncapped reovirus mRNA's were preferentially degraded in cell-free extracts isolated from wheat germ or mouse L-cells. Rabbit reticulocyte lysate contained little or no nuclease activity, which is consistent with its loss of transcriptional machinery during differentiation and possibly accounts for the increased efficiency of translation of uncapped mRNA's in this system. Similar findings confirming the importance of the cap structure in stabilizing mRNA during eucaryotic protein synthesis have been obtained with the mRNA's of tobacco mosaic virus, globin, protamine, and *A. salina* (see above). In each case, the capped mRNA species remained intact, but when they were decapped, they lost their translation capabilities (>90%) in wheat germ cell-free extracts and were degraded exonucleolytically during translation. When capped and uncapped globin mRNA's were injected into frog oocytes, more than 90% of the translatability of uncapped globin mRNA was lost over a 96-h period (149). The capped globin mRNA, on the other hand, was apparently stable since it continued to be translated. These results clearly indicate that the cap structure protects mRNA from degradation and thus increases its stability. There is one unconfirmed report (5) in which removal of ^{7}mG from globin mRNA by T4-polynucleotide kinase did not alter the translational ability of the mRNA in wheat germ extract.

In contrast to the above-described results, uncapped RNAs, such as those in poliovirus (208) and satellite tobacco necrosis virus (145), which function as messengers in infected cells, are stable and are translated in various model systems. One interpretation of this finding is that at least in the case of poliovirus, the protein linked to the 5′ end of the RNA may protect it from nuclease action. However, this argument is weakened by the finding that polysome-associated poliovirus mRNA does not contain the 5′-terminal protein. It is removed from the genome RNA by endogenous proteases present in cell-free extracts. In the case of satellite tobacco necrosis virus, the stabilizing role may be played by a secondary structure at the 5′ end of the RNA (232). Although the precise reasons for the stability of these uncapped mRNA's are not known, some special interaction of proteins with the 5′ termini may account for their resistance to degradation in infected cells.

mRNA Biosynthesis

Cap structures have been detected in early stages of hnRNA synthesis in nuclei (48). Conversion of precursor transcripts to cytoplasmic mRNA's apparently involves conservation of the cap structure during several processing steps, including 3′-terminal addition of poly(A) and splicing of intervening nucleotide sequences (48). It is still not clear whether cap formation is involved or required for the initiation of mRNA

synthesis. An answer to this question has not been obtained because of the difficulties in controlling nuclear RNA synthesis in order to isolate the primary RNA products in high yields. As discussed above, the mechanisms of cap formation have been studied in detail in viral systems, and in some cases capping and mRNA synthesis are tightly coupled. For example, VSV mRNA's synthesized in vitro are always capped, and efforts to find conditions for uncapped mRNA formation have failed (17). Thus, it appears that in the VSV system capping may be essential for the synthesis and eventual chain completion of mRNA's. Although not obligatory, influenza virus may use capped primers for mRNA synthesis in vitro (32), but in vivo (133) capping appears somehow directly related to the biosynthesis of influenza virus mRNA and thus to viral replication.

CPV is a unique system, in which the capping process appears to be directly involved in mRNA transcription. The addition of AdoMet stimulates in vitro mRNA synthesis by the virion-associated RNA polymerase. More interestingly, AdoHcy, a competitive inhibitor of AdoMet, also stimulates RNA synthesis to the same extent as AdoMet (76, 160). Methylation of the cap apparently is not required for mRNA synthesis, but some structural features of AdoMet appear to be involved in the transcription complex and cause activation of RNA polymerase at the initiation site of the genome. This hypothesis is supported by the finding that the apparent K_m for ATP for the initiation of CPV mRNA synthesis is greatly reduced by increasing concentrations of AdoMet. It has been proposed that AdoMet may interact with a methylase subunit in the CPV transcription complex, resulting in an allosteric conformational change that lowers the apparent K_m for initiating ATP and activation of the RNA polymerase (85). Recently, Wertheimer et al. (267) have studied the effects of various AdoMet analogs on CPV transcription in vitro in an effort to understand which structural features of AdoMet are important for stimulation of RNA synthesis. It appears that those analogs that generally inhibit methyltransferase also stimulate RNA synthesis. Furthermore, analogs which do not inhibit methyltransferase fail to stimulate mRNA synthesis. These findings also indicate that some interaction between AdoMet and the methyltransferase turns on CPV mRNA synthesis. Stimulatory effects of AdoMet on in vitro transcription of a fish rhabdovirus have also been reported (212). More detailed studies in these viral systems should eludicate further the role of capping in mRNA synthesis.

Inhibitors of methyltransferases have been used to understand the role of methylation in mRNA synthesis, transport, and translation in vivo. Kaehler et al. (115) have shown that in vivo *S*-tubercidinyl homocysteine effectively inhibits ribose 2'-*O*-methylation of the N_1 nucleoside of the cap and base N^6 adenosine methylation in internal positions of Novikoff hepatoma mRNA's. Methylation of 5' blocking guanosine was not affected, and the cytoplasmic mRNA's contained predominantly cap 0 structures. Since this inhibitor did not affect the viability of the cell, it was concluded that ribose methylation or internal adenosine methylation was not needed for mRNA transport from the nucleus or subsequent translation in cytoplasm. Similarly, Dimock and Stoltzfus showed that cycloleucine, a competitive inhibitor of ATP:L-methionine *S*-adenosyltransferase, blocks ribose methylation and internal methylation (but not 7-methylation of guanosine) of avian sarcoma virus genome RNA in chicken embryo cells (56). Viral replication was virtually unaffected under these conditions, indicating that ribose and internal methylation are not required for avian sarcoma virus RNA synthesis, processing, and transport. Similar results were obtained by Bachellerie et al. (14) in Chinese hamster ovary cells treated with cycloleucine. In contrast, Robert-Gero et al. (201) reported that 5'-deoxy-5'-isobutyryladenosine, an analog of AdoHcy, inhibited by more than 90% the production of the Schmidt-Ruppin strain of Rous sarcoma virus. These results suggest that 5'-deoxy-5'-isobutyryladenosine may inhibit the 7-methylguanosine methyltransferase which may be essential for mRNA synthesis. In fact, Jacquemont and Huppert (114) have shown that 5'-deoxy-5'-isobutyryladenosine inhibited ^{7}mG methylation of viral mRNA, whereas m^{6}A methylation was not affected in herpes simplex virus type 1-infected cells. Recently, the antibiotic Sinefungin has been shown to inhibit Newcastle disease virus replication and also the 7-methylguanine and 2'-*O*-methylnucleoside methyltransferases of vaccinia virus in vitro (194). Cycloleucine has also been shown to inhibit VSV replication in baby hamster kidney and Chinese hamster ovary cells (35, 243a), indicating that methylation may play an important role in viral mRNA synthesis in vivo. However, inhibition of methylation (AdoHcy) in vitro has virtually no effect on VSV mRNA transcription, although it affects the polyadenylation process. poly(A) tails 2,000 bases long (approximately 10 times the normal length) were added to VSV mRNA's when mRNA synthesis was carried out in vitro in the presence of AdoHcy (207).

CONCLUSIONS

The appearance of the cap structure in eucaryotic mRNA's is clearly an important step in the evolution of living organisms. Presumably, biosynthesis of guanylyltransferase and mRNA (7-guanine)methyltransferase and the appearance of these enzymes in the nuclei of primitive eucaryotic cells marked the onset of the first production of capped mRNA's. Ribose methylation and internal methylations of mRNA are late evolutionary events, the roles of which are still not clear. The methylation of the blocking guanosine base at position 7 is also an important process because it renders the cap structure polar, thus allowing interaction with charged macromolecules. In addition, the triphosphate bridge confers a freedom of movement of ^{7}mG for similar interactions. Moreover, the structure appears to be resistant to the 5'-exonuclease action and thus may increase the half-life of the message. The involvement of the cap in mRNA translation has been documented adequately. More extensive studies on the CBP with regard to its structure and function will certainly help to elucidate the involvement of the cap in ribosome recognition. The roles of ribose methylation, $N^{6}mA^{m}$, and internal N^{6}mA in mRNA's are not well understood. Possibly, they are involved in fine tuning during initiation complex formation (121, 175), mRNA processing, and transport (23). It is not clear whether ^{7}mG has any role in the transcription of the embryonic genome during preimplantation development of mammalian embryos. There is one report (275a) describing the appearance of ^{7}mG in the RNA of mouse one-cell embryos 3 h after fertilization. This in contrast to the echinoderms, in which maternal mRNA's are capped and their translation after fertilization does not require attachment of ^{7}mG to the 5' terminus (69, 109, 224). The mechanism of capping in the viral systems has been well elucidated, and the enzymes involved in the capping reactions have now been purified from both viral and eucaryotic sources. On the other hand, an understanding of capping mechanisms in cellular mRNA is still not clear. With the continued use of more refined in vitro nuclear systems, more light should be shed on this process in the near future. Elucidation of this reaction will be of great help in understanding the more general process of mRNA biosynthesis in eucaryotic cells in general. Future experiments will clearly be directed toward a more precise understanding of the function of the cap in eucaryotic gene expression.

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LITERATURE CITED

1. **Abraham, G., and A. K. Banerjee.** 1976. The nature of the RNA products synthesized *in vitro* by subviral components of vesicular stomatitis virus. Virology **71**:230–241.
2. **Abraham, G., D. P. Rhodes, and A. K. Banerjee.** 1975. The 5'-terminal structure of the methylated messenger RNA synthesized *in vitro* by vesicular stomatitis virus. Cell **5**:51–58.
3. **Abraham, G., D. P. Rhodes, and A. K. Banerjee.** 1975. Novel initiation of RNA synthesis *in vitro* by vesicular stomatitis virus. Nature (London) **255**:37–40.
4. **Abraham, K. A., and J. R. Lillehaug.** 1976. Enzymic cleavage of m^{7}GDP from eucaryotic mRNA. FEBS Lett. **71**:49–52.
5. **Abraham, K. A., and A. Pihl.** 1977. Translation of enzymically decapped messenger RNA. Eur. J. Biochem. **77**:589–593.
6. **Adams, B. L., M. Morgan, S. Muthukrishnan, S. M. Hecht, and A. J. Shatkin.** 1978. The effect of "cap" analogs on reovirus mRNA binding to wheat germ ribosomes. J. Biol. Chem. **253**:2589–2595.
7. **Adams, J. M.** 1977. Messenger RNA, p. 81–128. *In* P. R. Stewart, and D. S. Letham (ed.), The ribonucleic acids. Springer-Verlag, New York.
8. **Adams, J. M., and S. Cory.** 1975. Modified nucleosides and bizarre 5'-termini in mouse myeloma mRNA. Nature (London) **255**:28–33.
9. **Agranovsky, A. A., V. V. Dolja, V. K. Kagramanova, and J. G. Atabekov.** 1979. The presence of a cap structure at the 5'-end of barley stripe mosaic virus RNA. Virology **95**: 208–210.
10. **Aloni, Y.** 1975. Methylated SV-40 messenger RNA. FEBS Lett. **54**:363–367.
11. **Anderson, C. W., J. F. Atkins, and J. J. Dunn.** 1976. Bacteriophage T_3 and T_7 early RNAs are translated by eukaryotic 80S ribosomes: active phage T_3 coded S-adenosylmethionine cleaving enzyme is synthesized. Proc. Natl. Acad. Sci. U.S.A. **73**:2752–2756.
12. **Anderson, W. F., L. Bosch, W. E. Cohn, H. Lodish, W. C. Merrick, H. Weissbach, H. G. Wittman, and I. G. Wool.** 1977. International symposium on protein synthesis: meeting report. FEBS Lett. **76**:1–10.
13. **Asselbergs, F. A. M., W. Peters, W. J. Van Venrooij, and H. Bloemendal.** 1978. Diminished sensitivity of re-initiation of translation to inhibition by cap analogs in reticulocyte lysates. Eur. J. Biochem. **88**:483–488.
14. **Bachellerie, J. P., F. Amalric, and M. Caboche.** 1978. Biosynthesis and utilization of extensively undermethylated poly(A) RNA in CHO cells during a cycloleucine treatment. Nucleic Acids Res. **5**:2927–2943.
15. **Bajszar, G., O. P. Samarina, and G. P. Geor-**

giev. 1976. On the nature of 5'-termini in nuclear pre-mRNA of Ehrlich carcinoma cells. Cell 9:323-332.
16. Bajszar, G., G. Szabo, A. Simoncsits, and J. Molnar. 1978. Methylated cap formation by enzymes bound to nuclear informer particles. Mol. Biol. Rep. 4:93-96.
17. Banerjee, A. K., G. Abraham, and R. J. Colonno. 1977. Vesicular stomatitis virus: mode of transcription. J. Gen. Virol. 34:1-8.
18. Banerjee, A. K., R. J. Colonno, D. Testa, and M. T. Franze-Fernandez. 1978. Mechanism of RNA synthesis *in vitro* by vesicular stomatitis virus, p. 249-259. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press Inc., New York.
19. Barbosa, E., and B. Moss. 1978. mRNA (nucleoside-2'-)-methyltransferase from vaccinia virus: purification and physical properties. J. Biol. Chem. 253:7692-7697.
20. Barbosa, E., and B. Moss. 1978. mRNA (nucleoside-2'-)-methyltransferase from vaccinia virus: characteristics and substrate specificity. J. Biol. Chem. 253:7698-7702.
21. Barrieux, A., and M. C. Rosenfeld. 1977. Comparison of mRNA binding by met-tRNA$_f$ binding protein and mRNA-associated proteins. J. Biol. Chem. 252:392-398.
22. Barrieux, A., and M. G. Rosenfeld. 1977. Characterization of GTP-dependent met-tRNA$_f$ binding protein. J. Biol. Chem. 252:3843-3847.
23. Bartkoski, M. J., and B. Roizman. 1978. Regulation of herpesvirus macromolecular synthesis. VII. Inhibition of internal methylation of mRNA late in infection. Virology 85:146-156.
24. Beemon, K., and J. Keith. 1977. Localization of N^6-methyladenosine in the Rous sarcoma virus genome. J. Mol. Biol. 113:165-179.
25. Bergmann, J. E., and H. F. Lodish. 1979. Translation of capped and uncapped vesicular stomatitis virus and reovirus mRNAs: sensitivity to ^{7}mGpppA^m and ionic conditions. J. Biol. Chem. 254:459-468.
26. Bishop, D. H. L. 1977. Virion polymerases, p. 117-278. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 10. Plenum Press, New York.
27. Black, D. N., J. N. Burroughs, T. J. R. Harris, and F. Brown. 1979. The structure and replication of calcivirus RNA. Nature (London) 274:614-615.
28. Both, G. W., A. K. Banerjee, and A. J. Shatkin. 1975. Methylation-dependent translation of viral messenger RNAs *in vitro*. Proc. Natl. Acad. Sci. U.S.A. 72:1189-1193.
29. Both, G. W., Y. Furuichi, S. Muthukrishnan, and A. J. Shatkin. 1975. Ribosome binding to reovirus mRNA in protein synthesis requires 5'-terminal 7-methylguanosine. Cell 6:185-195.
30. Both, G. W., Y. Furuichi, S. Muthukrishnan, and A. J. Shatkin. 1976. Effect of 5'-terminal structure and base composition on polyribonucleotide binding to ribosomes. J. Mol. Biol. 104:637-658.
31. Bouloy, M., M. Morgan, A. J. Shatkin, and R. M. Krug. 1979. Cap and internal nucleotides of reovirus mRNA primers are incorporated into influenza viral complementary RNA during transcription in vitro. J. Virol. 32:895-904.
32. Bouloy, M., S. J. Plotch, and R. M. Krug. 1978. Globin mRNAs are primers for the transcription of influenza viral RNA *in vitro*. Proc. Natl. Acad. Sci. U.S.A. 75:4886-4890.
33. Burroughs, J. N., and F. Brown. 1978. Presence of a covalently linked protein on calcivirus RNA. J. Gen. Virol. 41:443-446.
34. Bush, H., F. Hirsch, K. K. Gupta, M. Rao, W. Spohn, and B. C. Wu. 1976. Structural and functional studies on the "5'-cap": a survey method for mRNA. Prog. Nucleic Acid Res. Mol. Biol. 19:39-61.
35. Caboche, M., and C. La Bonnardiere. 1979. Vesicular stomatitis virus mRNA methylation *in vivo*: effect of cycloleucine, an inhibitor of S-adenosylmethionine biosynthesis, on viral transcription and translation. Virology 93:547-557.
36. Canaani, D., C. Kahana, S. Lavi, and Y. Groner. 1979. Identification and mapping of N^6-methyladenosine containing sequences in simian virus 40 RNA. Nucleic Acids Res. 6: 2879-2899.
37. Canaani, D., C. Kahana, A. Mukamel, and Y. Groner. 1979. Sequence heterogeneity at the 5'-termini of late simian virus 40 19S and 16S mRNAs. Proc. Natl. Acad. Sci. U.S.A. 76: 3078-3082.
38. Canaani, D., M. Revel, and Y. Groner. 1976. Translational discrimination of "capped" and "non-capped" mRNAs: inhibition by a series of chemical analogs of m^{7}GpppX. FEBS Lett. 64: 326-331.
39. Cheng, T.-C., and H. H. Kazazian, Jr. 1977. The 5'-terminal structures of murine alpha globin and beta globin messenger RNA. J. Biol. Chem. 252:1758-1763.
40. Cho, S., T.-C. Cheng, and H. H. Kazazian, Jr. 1978. Characterization of 5'-terminal methylation of human alpha and beta globin mRNAs. J. Biol. Chem. 253:5558-5561.
41. Chu, L.-Y., and R. E. Rhoads. 1978. Translational recognition of the 5'-terminal 7-methylguanosine of globin messenger RNA as a function of ionic strength. Biochemistry 17:2450-2454.
42. Cleaves, G. R., and D. T. Dubin. 1978. Methylation status of Dengue virus 40S RNA. Fed. Proc. 37:173.
43. Colonno, R. J., and H. O. Stone. 1975. Methylation of messenger RNA of Newcastle disease virus *in vitro* by a virion associated enzyme. Proc. Natl. Acad. Sci. U.S.A 72:2611-2615.
44. Colonno, R. J., and H. O. Stone. 1976. Newcastle disease virus messenger RNA lacks 2'-O-methylated nucleotides. Nature (London) 261: 611-614.
45. Cory, S., and J. M. Adams. 1975. The modified 5'-terminal sequences in messenger RNA of mouse myeloma cells. J. Mol. Biol. 99:519-547.
46. Cory, S., and J. M. Adams. 1975. Modified 5'-

termini in small nuclear RNAs of mouse myeloma cells. Mol. Biol. Rep. **2**:287-294.

47. **Cory, S., C. Genin, and J. M. Adams.** 1976. Modified nucleosides and 5'-end groups in purified mouse immunoglobulin light chain mRNA and rabbit globin mRNA detected by borohydride labelling. Biochim. Biophys. Acta **454**:248-262.
48. **Darnell, J. E., Jr.** 1979. Transcription units for mRNA production in eukaryotic cells and their DNA viruses. Prog. Nucleic Acid Res. Mol. Biol. **22**:327-353.
49. **DasGupta, R., F. Harada, and P. Kaesberg.** 1976. Blocked 5' termini in brome mosaic virus RNA. J. Virol. **18**:260-267.
50. **Daubert, S. D., G. Bruening, and R. C. Najarian.** 1978. Protein bound to the genome RNAs of cowpea mosaic virus. Eur. J. Biochem. **92**: 45-51.
51. **De Kloet, S. R., and B. A. G. Andrean.** 1976. Methylated nucleosides in polyadenylate-containing yeast messenger ribonucleic acid. Biochim. Biophys. Acta **425**:401-408.
52. **Desrosiers, R., K. Friderici, and F. Rottman.** 1974. Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc. Natl. Acad. Sci. U.S.A. **71**:3971-3975.
53. **Desrosiers, R. C., K. H. Friderici, and F. M. Rottman.** 1975. Characterization of Novikoff hepatoma mRNA methylation and heterogeneity in the methylated 5'-terminus. Biochemistry **14**:4367-4374.

53a. **Desrosiers, R., G. C. Sen, and P. Lengyl.** 1976. Difference in 5'-terminal structure between the mRNA and the double-stranded virion RNA of reovirus. Biochem. Biophys. Res. Commun. **73**:32-39.

54. **Dhingra, M. M., and R. H. Sarma.** 1978. NMR studies and minimum energy conformation calculations on the 5'-terminus of mammalian mRNA. Quantum Chem. Biochem. **11**:31-39.
55. **Dimock, K., and C. M. Stoltzfus.** 1977. Sequence specificity of internal methylation in B77 avian sarcoma virus RNA subunits. Biochemistry **16**:471-478.
56. **Dimock, K., and C. M. Stoltzfus.** 1978. Cycloleucine blocks internal and 5'-terminal methylations of avian sarcoma virus genome RNA. Biochemistry **17**:3627-3632.
57. **Doerfler, W.** 1977. Animal virus-host genome interaction, p. 279-399. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology. vol. 10. Plenum Press, New York.
58. **Dottin, R. P., A. M. Weiner, and H. F. Lodish.** 1976. 5'-Terminal nucleotide sequences of the messenger RNAs of *Dictyostellium discoideum*. Cell **8**:233-244.
59. **Dubin, D. T., and V. Stollar.** 1975. Methylation of Sindbis virus 26S messenger RNA. Biochem. Biophys. Res. Commun. **66**:1373-1379.
60. **Dubin, D. T., V. Stollar, C.-C. Hsu-Chen, K. Timko, and G. M. Guild.** 1977. Sindbis virus messenger RNA: the 5'-termini and methylated residues of 26 and 42S RNA. Virology **77**: 457-470.
61. **Dubin, D. T., and R. H. Taylor.** 1975. The methylation state of poly A-containing messenger RNA from cultured hamster cells. Nucleic Acids Res. **2**:1653-1668.
62. **Dubin, D. T., K. Timko, S. Gillies, and V. Stollar.** 1979. The extreme 5'-terminal sequences of Sindbis virus 26 and 42S RNA. Virology **98**:131-141.
63. **Efstratiadis, A., J. N. Vournakis, H. Donis-Keller, G. Chaconas, D. K. Dougall, and F. C. Kafatos.** 1977. End labelling of enzymatically decapped mRNA. Nucleic Acids Res. **4**: 4165-4174.
64. **Ehresmann, D. W., and F. L. Schaffer.** 1979. Calcivirus intercellular RNA: fractionation of 18-22 S RNA and lack of typical 5'-methylated caps on 36 S and 22 S San Miguel sea lion virus RNAs. Virology **95**:251-255.
65. **Ensinger, M. J., S. A. Martin, E. Paoletti, and B. Moss.** 1975. Modification of the 5'-terminus of mRNA by soluble guanylyl and methyl transferases from vaccinia virus. Proc. Natl. Acad. Sci. U.S.A. **72**:2525-2529.
66. **Ensinger, M. J., and B. Moss.** 1976. Modification of the 5' terminus of mRNA by an RNA (guanine-7-)methyltransferase from HeLa cells. J. Biol. Chem. **251**:5283-5291.
67. **Faust, M., K. E. M. Hastings, and S. Millward.** 1975. ^{7}mG(5')ppp(5')G^{m}pCpUp at the 5'-terminus of reovirus messenger RNA. Nucleic Acids. Res. **2**:1329-1343.
68. **Faust, M., and S. Millward.** 1974. *In vitro* methylation of nascent reovirus mRNA by a virion-associated methyl transferase. Nucleic Acids Res. **1**:1739-1752.
69. **Faust, M., S. Millward, A. Duchastel, and D. Fromson.** 1976. Methylated constituents of poly(A)$^{-}$ and poly(A)$^{+}$ polyribosomal RNA of sea urchin embryos. Cell **9**:597-604.
70. **Filipowicz, W.** 1978. Function of the 5'-terminal ^{7}mG cap in eukaryotic mRNA. FEBS Lett. **96**: 1-11.
71. **Filipowicz, W., Y. Furuichi, J. M. Sierra, S. Muthukrishnan, A. J. Shatkin, and S. Ochoa.** 1976. A protein binding the methylated 5'-terminal sequence, ^{7}mGpppN, of eukaryotic messenger RNA. Proc. Natl. Acad. Sci. U.S.A. **73**:1559-1563.
72. **Flavell, A. J., A. Cowie, S. Legon, and R. Kamen.** 1979. Multiple 5'-terminal cap structures in late polyoma virus RNA. Cell **16**:357-371.
73. **Friderici, K., M. Kaehler, and F. Rottman.** 1976. Kinetics of Novikoff cytoplasmic messenger RNA methylation. Biochemistry **15**:5234-5241.
74. **Fujimoto, M., A. Kuninaka, and H. Yoshino.** 1974. Substrate specificity of nuclease P_1. Agr. Biol. Chem. **38**:1555-1561.
75. **Furuichi, Y.** 1974. Methylation-coupled transcription by virus-associated transcriptase of cytoplasmic polyhedrosis virus containing double-stranded RNA. Nucleic Acids Res. **1**:802-809.
76. **Furuichi, Y.** 1978. "Pretranscriptional capping" in the biosynthesis of cytoplasmic polyhedrosis

virus mRNA. Proc. Natl. Acad. Sci. U.S.A. **75**: 1086–1090.

77. **Furuichi, Y., A. LaFinandra, and A. J. Shatkin.** 1977. 5'-Terminal structure and mRNA stability. Nature (London) **266**:235–239.
78. **Furuichi, Y., and K.-I. Miura.** 1975. A blocked structure at the 5'-terminus of messenger RNA from cytoplasmic polyhedrosis virus. Nature (London) **253**:374–375.
79. **Furuichi, Y., M. Morgan, S. Muthukrishnan, and A. J. Shatkin.** 1975. Reovirus messenger RNA contains a methylated, blocked 5'-terminal structure, $m^7G(5')ppp(5')GpCp$. Proc. Natl. Acad. Sci. U.S.A. **72**:362–366.
80. **Furuichi, Y., M. Morgan, and A. J. Shatkin.** 1979. Synthesis and translation of mRNA containing 5'-terminal 7-ethylguanosine cap. J. Biol. Chem. **254**:6732–6738.
81. **Furuichi, Y., M. Morgan, A. J. Shatkin, W. Jelinek, M. Salditt-Georgieff, and J. E. Darnell.** 1975. Methylated, blocked 5' termini in HeLa cell mRNA. Proc. Natl. Acad. Sci. U.S.A. **72**:1904–1908.
82. **Furuichi, Y., S. Muthukrishnan, and A. J. Shatkin.** 1975. 5'-Terminal $^7mG(5')ppp(5')G^mp$ *in vivo*: identification in reovirus genome RNA. Proc. Natl. Acad. Sci. U.S.A. **72**:742–745.
83. **Furuichi, Y., S. Muthukrishnan, J. Tomasz, and A. J. Shatkin.** 1976. Mechanism of formation of reovirus mRNA 5'-terminal blocked and methylated sequence, $^7mGpppGmC$. J. Biol. Chem. **251**:5043–5053.
84. **Furuichi, Y., and A. J. Shatkin.** 1977. 5'-Termini of reovirus mRNA. Ability of viral cores to form caps post-transcriptionally. Virology **77**:566–578.
85. **Furuichi, Y., and A. J. Shatkin.** 1979. Stimulation of CPV mRNA synthesis *in vitro* by S-adenosyl methionine and 5'-capping during initiation, p. 351–360. *In* E. Usdin, R. T. Borchardt, and C. R. Creveling (ed.), Transmethylation. Elsevier/North-Holland, New York.

85a. **Furuichi, Y., A. J. Shatkin, E. Stavnezer, and J. M. Bishop.** 1975. Blocked, methylated 5'-terminal sequence in avian sarcoma virus RNA. Nature (London) **257**:618–620.

86. **Gedamu, L., and G. H. Dixon.** 1978. Effect of enzymatic "decapping" on protamine mRNA translation in wheat germ S-30. Biochem. Biophys. Res. Commun. **85**:114–124.
87. **Germershausen, J., D. Goodman, and E. W. Somberg.** 1978. 5' Cap methylation of homologous poly A(+) RNA by a RNA (guanine-7-) methyltransferase from *Neurospora crassa*. Biochem. Biophys Res. Commun. **82**:871–878.
88. **Golini, F., A. Nomoto, and E. Wimmer.** 1979. The genome-linked protein of picornaviruses. IV. Difference in the VPg's of encephalomyocarditis virus and poliovirus as evidence that the genome-linked proteins are virus-coded. Virology **89**:112–118.
89. **Gosh, P. K., V. B. Reddy, J. Swinscoe, P. Lebowitz, and S. M. Weissman.** 1978. Heterogeneity and 5'-terminal structures of the late RNA's of simian virus 40. J. Mol. Biol. **126**:813–846.
90. **Grohmann, K., F. Amalric, S. Crews, and G. Attardi.** 1978. Failure to detect "cap" structures in mitochondrial DNA-coded poly(A)-containing RNA from HeLa cells. Nucleic Acids Res. **5**:637–651.
91. **Groner, Y., P. Carmi, and Y. Aloni.** 1977. Capping structures of simian virus 40 19S and 16S mRNAs. Nucleic Acids Res. **4**:3959–3968.
92. **Groner, Y., E. Gilboa, and H. Aviv.** 1978. Methylation and capping of RNA polymerase II primary transcripts by HeLa nuclear homogenates. Biochemistry **17**:977–982.
93. **Groner, Y., H. Grosfeld, and U. Z. Littaur.** 1976. 5'-Capping structures of *Artemia salina* mRNA and the translational inhibition by cap analogs. Eur. J. Biochem. **71**:281–293.
94. **Groner, Y., and J. Hurwitz.** 1975. Synthesis of RNA containing a methylated blocked 5'-terminus by HeLa nuclear homogenates. Proc. Natl. Acad. Sci. U.S.A. **72**:2930–2934.
95. **Guilly, H., and J. P. Briand.** 1978. Nucleotide sequence of turnip yellow mosaic virus coat protein mRNA. Cell **15**:113–122.
96. **Gupta, K. C., and P. Roy.** 1980. Alternate capping mechanisms for transcription of spring viremia carp virus: evidence for independent mRNA initiation. J. Virol. **33**:292–303.
97. **Haegeman, G., and W. Fiers.** 1978. Localization of the 5'-terminus of late SV40 mRNA. Nucleic Acids Res. **5**:2359–2371.
98. **Haffner, M. H., M. B. Chin, and B. G. Lane.** 1978. Wheat embryo ribonucleates. XII. Formal characterization of terminal and penultimate nucleoside residues at the 5'-ends of "capped" RNA from imbibing wheat embryos. Can. J. Biochem. **56**:729–733.
99. **Hashimoto, S., and M. Green.** 1976. Multiple methylated cap sequences in adenovirus type 2 early mRNA. J. Virol. **20**:425–435.
100. **Hashimoto, S., and M. Green.** 1979. Methylated 5'-terminal caps of adenovirus type 2 early mRNA: evidence for at least six 5'-termini. Virology **94**:254–272.
101. **Heckle, W. L., Jr., R. G. Fenton, T. G. Wood, C. G. Merkel, and B. J. Lingrel.** 1977. Methylated nucleosides in globin mRNA from mouse nucleated erythroid cells. J. Biol. Chem. **252**:1764–1770.
102. **Hefti, E., and D. H. L. Bishop.** 1975. The 5'-nucleotide sequence of vesicular stomatitis viral RNA. J. Virol. **15**:90–96.
103. **Hefti, E., D. H. L. Bishop, D. T. Dubin, and V. Stollar.** 1976. 5'-Nucleotide sequence of Sindbis viral RNA. J. Virol. **17**:149–159.
104. **Held, W. A., K. West, and J. F. Gallagher.** 1977. Importance of initiation factor preparations in the translation of reovirus and globin messenger RNA lacking a 5' terminal 7 methylguanosine. J. Biol. Chem. **252**:8489–8497.
105. **Helentjaris, T., and E. Ehrenfeld.** 1978. Control of protein synthesis in extracts from poliovirus-infected cells. I. mRNA discrimination by crude initiation factors. J. Virol. **26**:510–521.

106. **Helentjaris, T., E. Ehrenfeld, M. Brown-Luedi, and J. W. B. Hershey.** 1979. Alterations in initiation factor activity from poliovirus-infected HeLa cells. J. Biol. Chem. **254:** 10973–10978.

107. **Hendler, S., E. Fürer, and P. R. Srinivasan.** 1970. Synthesis and chemical properties of monomers and polymers containing 7-methylguanine and an investigation of their substrate or template properties for bacterial deoxyribonucleic acid or ribonucleic acid polymerases. Biochemistry **9:**4141–4153.

108. **Hewlett, M. J., J. K. Rose, and D. Baltimore.** 1976. 5′-Terminal structure of poliovirus polyribosomal RNA is pUp. Proc. Natl. Acad. Sci. U.S.A. **73:**327–330.

109. **Hickey, E. D., C. Baglioni, and L. A. Weber.** 1976. Translation of RNA from unfertilized sea-urchin eggs does not require methylation and is inhibited by 7-methyl GMP. Nature (London) **261:**71–72.

110. **Hickey, E. D., L. A. Weber, and C. Baglioni.** 1976. Inhibition of initiation of protein synthesis by 7-methylguanosine-5′-monophosphate. Proc. Natl. Acad. Sci. U.S.A. **73:**19–23.

111. **Hickey, E. D., L. A. Weber, C. Baglioni, C. H. Kim, and R. H. Sarma.** 1977. A relation between inhibition of protein synthesis and conformation of 5′-phosphorylated 7-methylguanosine derivatives. J. Mol. Biol. **109:**173–183.

112. **Hsuchen, C.-C. H., and D. T. Dubin.** 1976. Di- and trimethylated congeners of 7-methylguanine in Sindbis virus mRNA. Nature (London) **264:**190–191.

113. **Hsuchen, C.-C. H., and D. T. Dubin.** 1977. Methylated constituents of *Aedes albopictus* poly(A)-containing messenger RNA. Nucleic Acids Res. **4:**2671–2682.

114. **Jacquemont, B., and J. Huppert.** 1977. Inhibition of viral RNA methylation in herpes simplex virus type 1-infected cells by 5′,5-isobutyladenosine. J. Virol. **22:**160–167.

115. **Kaehler, M., J. Coward, and F. Rottman.** 1977. In vivo inhibition of Novikoff cytoplasmic messenger RNA methylation by S-tubercidinyl-homocysteine. Biochemistry **16:**5770–5775.

116. **Kaempfer, R., H. Rosen, and R. Israeli.** 1978. Translational control: recognition of the methylated 5′-end and an internal sequence in eucaryotic mRNA by the initiation factor that binds methionyl-$tRNA_f^{met}$. Proc. Natl. Acad. Sci. U.S.A. **75:**650–654.

117. **Kastern, W. H., and S. J. Berry.** 1976. Nonmethylated guanosine as the 5′ terminus of capped mRNA from insect oocytes. Biochem. Biophy. Res. Commun. **71:**37–44.

118. **Keith, J., and H. Fraenkel-Conrat.** 1975. Identification of the 5′-end of Rous sarcoma virus RNA. Proc. Natl. Acad. Sci. U.S.A. **72:**3347–3350.

119. **Keith, J., and H. Fraenkel-Conrat.** 1975. Tobacco mosaic virus RNA carries 5′ terminal triphosphorylated guanosine blocked by 5′ linked 7-methylguanosine. FEBS Lett. **57:**31–33.

120. **Keith, J. M., M. J. Ensinger, and B. Moss.** 1978. HeLa cell RNA(2′-*O*-methyladenosine-N^6-)-methyltransferase specific for the capped 5′-end of messenger RNA. J. Biol. Chem. **253:** 5033–5041.

121. **Keith, J. M., S. Muthukrishman, and B. Moss.** 1978. Appendix: effect of methylation of the N6 position of the penultimate adenosine of capped mRNA on ribosome binding. J. Biol. Chem. **253:**5039–5041.

122. **Kelly, D. E., and R. P. Perry.** 1975. Methylated sequences at the 5′ termini of heterogeneous nuclear RNA and messenger RNA. J. Cell Biol. **76:**205A.

123. **Kemper, B.** 1976. Inactivation of parathyroid hormone mRNA by treatment with periodate and aniline. Nature (London) **262:**321–323.

124. **Kemper, B., and L. Stolarsky.** 1977. Dependence on potassium concentration of the inhibition of the translation of messenger ribonucleic acid by 7-methylguanosine 5′-phosphate. Biochemistry **16:**5676–5680.

125. **Kennedy, T. D., and B. G. Lane.** 1975. The probable "capping" of wheat leaf messenger ribonucleates by 7-methylguanosine. Can. J. Biochem. **53:**1346–1348.

126. **Kim, C. H., and R. H. Sarma.** 1977. Spatial configuration of mRNA 5′-terminus. Nature (London) **270:**223–227.

127. **Kim, C. H., and R. H. Sarma.** 1978. Spatial configuration of the bizarre 5′-terminus of mammalian messenger RNA. J. Am. Chem. Soc. **100:**1571–1590.

128. **Klein, C., C. Fritsch, J. P. Braind, K. E. Richards, G. Jonard, and L. Hirth.** 1976. Physical and functional heterogeneity in TYMV RNA: evidence for the existence of an independent messenger coding for coat protein. Nucleic Acids Res. **3:**3043–3061.

129. **Klootwijk, J., I. Klein, P. Zabel, and A. Van Kammen.** 1977. Cowpea mosaic virus RNAs have neither ^{7}mGpppN.. nor mono-, di-, or triphosphates at their 5′ ends. Cell **11:**73–82.

130. **Kozak, M.** 1978. How do eucaryotic ribosomes select initiation regions in messenger RNA? Cell **15:**1109–1123.

131. **Kozak, M.** 1979. Inability of circular mRNA to attach to eucaryotic ribosomes. Nature (London) **280:**82–85.

132. **Kozak, M., and A. J. Shatkin.** 1978. Migration of 40S ribosomal subunits on messenger RNA in the presence of edeine. J. Biol. Chem. **253:** 6568–6577.

133. **Krug, R. M., B. A. Broni, and M. Bouloy.** 1979. Are the 5′ ends of influenza viral mRNAs synthesized *in vivo* donated by host mRNAs? Cell **18:**329–334

134. **Krug, R. M., M. A. Morgan, and A. J. Shatkin.** 1976. Influenza viral mRNA contains internal N^6-methyladenosine and 5′-terminal 7-methylguanosine in cap structures. J. Virol. **20:**45–53.

135. **Lavers, G. C.** 1977. Cleavage of pm^{7}G from mRNA 5' terminal cap structures by pyrophosphatase activity in embryonic chick lens cells. Mol. Biol. Rep. **3**:413–420.

136. **Lavers, G. C.** 1977. Detection of methyltransferase activities which modify GpppG to m^{7}GpppGm in embryonic chick lens. Mol. Biol. Rep. **3**:275–281.

137. **Lavers, G. C.** 1979. Metabolism of m^{7}GMP-containing 5' terminal mRNA cap sequences in extracts of embryonic chick lens cells. Exp. Eye Res. **28**:299–309.

138. **Lavi, S., and A. J. Shatkin.** 1975. Methylated simian virus 40-specific RNA from nuclei and cytoplasm of infected BSC-1 cells. Proc. Natl. Acad. Sci. U.S.A. **72**:2012–2016.

139. **Lawley, P. D.** 1966. Effects of some chemical mutagens and carcinogens on nucleic acids. Prog. Nucleic Acid Res. Mol. Biol. **5**:89–131.

140. **Lawley, P. D., and P. Brookes.** 1961. Acidic dissociation of 7:9-dialkylguanines and its possible relation to mutagenic properties of alkylating agents. Nature (London) **192**:1081–1082.

141. **Laycock, D. E.** 1977. Purification of eukaryotic mRNA guanylyltransferase. Fed. Proc. **36**:770.

142. **Lee, Y. F., A. Nomoto, B. M. Detjen, and E. Wimmer.** 1977. A protein covalently linked to poliovirus genome RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:59–63.

143. **Lesnaw, J. A., and M. E. Reichmann.** 1970. Identity of the 5'-terminal RNA nucleotide sequence of the satellite tobacco necrosis virus and its helper virus: possible role of the 5'-terminus in the recognition by virus-specific RNA replicase. Proc. Natl. Acad. Sci. U.S.A. **66**:140–145.

144. **Leung, D. W., K. S. Browning, J. E. Heckman, U. L. RajBhandary, and J. M. Clark.** 1979. Nucleotide sequence of the 5'-terminus of satellite tobacco necrosis virus ribonucleic acid. Biochemistry **18**:1361–1366.

145. **Leung, D. W., C. W. Gilbert, R. E. Smith, N. L. Sasavage, and J. M. Clark, Jr.** 1974. Translation of satellite tobacco necrosis virus ribonucleic acid by an *in vitro* system from wheat germ. Biochemistry **15**:4943–4950.

146. **Levin, K. H., and C. E. Samuel.** 1977. Biosynthesis of reovirus-specified polypeptides—effect of methylation on the efficiency of reovirus genome expression *in vitro*. Virology **77**:245–259.

147. **Levis, R., and S. Penman.** 1978. 5'-Terminal structures of poly(A)$^+$ cytoplasmic messenger RNA and of poly(A)$^+$ and poly(A)$^-$ heterogenous nuclear RNA of cells of the dipteran *Drosophila melanogaster*. J. Mol. Biol. **120**: 487–516.

148. **Lockard, R. E.** 1978. Different Cap I:Cap 2 ratios in rabbit α and β globin mRNA. Nature (London) **275**:153–154.

149. **Lockard, R. E., and C. Lane.** 1978. Requirement for 7-methylguanosine in translation of globin mRNA *in vivo*. Nucleic Acids Res. **5**: 3237–3247.

150. **Lockard, R. E., and U. L. RajBhandary.** 1976. Nucleotide sequences at the 5'-termini of rabbit α and β globin mRNA. Cell **9**:747–760.

151. **Lodish, H. F., and J. K. Rose.** 1977. Relative importance of 7-methylguanosine in ribosome binding and translation of vesicular stomatitis virus messenger RNA in wheat germ and reticulocyte cell-free systems. J. Biol. Chem. **252**: 1181–1188.

152. **Mager, W. H., J. Klootwijk, and I. Klein.** 1976. Minimal methylation of yeast messenger RNA. Mol. Biol. Rep. **3**:9–17.

153. **Maitra, U., and J. Hurwitz.** 1965. The role of DNA in RNA synthesis. IX. Nucleoside triphosphate termini in RNA polymerase products. Proc. Natl. Acad. Sci. U.S.A. **54**:815–822.

154. **Malek, L. T., H.-J., Breter, G. M. Hellmann, K. H. Friderici, F. M. Rottman, and R. E. Rhoads.** 1979. 5'-Terminal structure of hen ovalbumin messenger ribonucleic acid. J. Biol. Chem. **254**:10415–10420.

155. **Manley, J. L., P. A. Sharp, and M. L. Gefter.** 1979. RNA synthesis in isolated nuclei: *in vitro* initiation of adenovirus 2 major late mRNA precursor. Proc. Natl. Acad. Sci. U.S.A. **76**: 160–164.

156. **Marcu, K. B., U. Schibler, and R. P. Perry.** 1978. The 5'-terminal sequence of immunoglobulin messenger RNAs of a mouse myeloma. J. Mol. Biol. **120**:381–400.

157. **Martin, S. A., and B. Moss.** 1975. Modification of RNA by mRNA guanylyltransferase and mRNA(guanine-7-)methyltransferase from vaccinia virions. J. Biol. Chem. **250**:9330–9335.

158. **Martin, S. A., and B. Moss.** 1976. mRNA guanyltransferase and mRNA (guanine-7-)-methyltransferase from vaccinia virions. J. Biol. Chem. **251**:7313–7321.

159. **Martin, S. A., E. Paoletti, and B. Moss.** 1975. Purification of mRNA guanylyltransferase and mRNA(guanine-7-)methyltransferase from vaccinia virions. J. Biol. Chem. **250**:9322–9329.

160. **Mertens, P. P. C., and C. C. Payne.** 1978. *S*-adenosyl-L-homocysteine as a stimulator of viral RNA synthesis by two distinct cytoplasmic polyhedrosis viruses. J. Virol. **26**:832–835.

161. **Miura, K., K. Watanabe, and M. Sugiura.** 1974. 5'-Terminal nucleotide sequences of the double-stranded RNA of silkworm cytoplasmic polyhedrosis virus. J. Mol. Biol. **86**:31–48.

162. **Miura, K.-I., K. Watanabe, M. Sugiura, and A. J. Shatkin.** 1974. The 5'-terminal nucleotide sequences of the double-stranded RNA of human reovirus. Proc. Natl. Acad. Sci. U.S.A. **71**:3979–3983.

163. **Mizumoto, K., and F. Lipmann.** 1979. Transmethylation and transguanylylation in 5'-RNA capping system isolated from rat liver nuclei. Proc. Natl. Acad. Sci. U.S.A. **76**:4961–4965.

164. **Monroy, G., E. Spencer, and J. Hurwitz.** 1978. Purification of mRNA guanylyltransferase from vaccinia virions. J. Biol. Chem. **253**:4481–4489.

165. **Monroy, G., E. Spencer, and J. Hurwitz.** 1978. Characteristics of reaction catalyzed by purified guanylyltransferase from vaccinia virus. J.

Biol. Chem. **253**:4490–4498.
166. **Moss, B., A. Gershowitz, J. R. Stringer, L. E. Holland, and E. K. Wagner.** 1977. 5′-Terminal and internal methylated nucleosides in herpes simplex virus type 1 messenger RNA. J. Virol. **23**:234–239.
167. **Moss, B., A. Gershowitz, L. A. Weber, and C. Baglioni.** 1977. Histone mRNAs contain blocked and methylated 5′ terminal sequences but lack methylated nucleosides at internal positions. Cell **10**:113–120.
168. **Moss, B., A. Gershowitz, C.-M. Wei, and R. Boone.** 1976. Formation of the guanylylated and methylated 5′-terminus of vaccinia virus mRNA. Virology **72**:341–351.
169. **Moss, B., J. M. Keith, A. Gershowitz, M. B. Ritchey, and P. Palese.** 1978. Common sequence at the 5′ ends of the segmented RNA genomes of influenza A and B viruses. J. Virol. **25**:312–318.
170. **Moss, B., and F. Koczot.** 1976. Sequence of methylated nucleotides at the 5′ terminus of adenovirus-specific RNA. J. Virol. **17**:385–392.
171. **Moyer, S. A., G. Abraham, R. Adler, and A. K. Banerjee.** 1975. Methylated and blocked 5′ termini in vesicular stomatitis virus in vivo mRNAs. Cell **5**:59–67.
172. **Moyer, S. A., and A. K. Banerjee.** 1976. In vivo methylation of vesicular stomatitis virus and its host-cell messenger RNA species. Virology **70**:339–351.
173. **Muthukrishnan, S., C. W. Both, Y. Furuichi, and A. J. Shatkin.** 1975. 5′-Terminal 7-methylguanosine in eukaryotic mRNA is required for translation. Nature (London) **255**:33–37.
174. **Muthukrishnan, S., W. Filipowicz, J. M. Sierra, G. W. Both, A. J. Shatkin, and S. Ochoa.** 1975. Messenger RNA methylation and protein synthesis in extracts from embryos of brine shrimp, *Artemia salina*. J. Biol. Chem. **250**:9336–9341.
175. **Muthukrishnan, S., M. Morgan, A. K. Banerjee, and A. J. Shatkin.** 1976. Influence of 5′ terminal ^{7}mG and 2′-*O* methylated residues on messenger ribonucleic acid binding to ribosomes. Biochemistry **15**:5761–5768.
176. **Muthukrishnan, S., B. Moss, J. A. Cooper, and E. S. Maxwell.** 1978. Influence of 5′-terminal cap structure on the initiation of translation of vaccinia virus mRNA. J. Biol. Chem. **253**:1710–1715.
177. **Muthukrishnan, S., S. Szybiak, A. B. Legocki, and W. Filipowicz.** 1978. 5′-Terminal 7-methylguanosine and mRNA function. The effect of enzymatic decapping and of cap analogs on translation of tobacco mosaic virus RNA and globin mRNA *in vitro*. Eur. J. Biochem. **92**:69–80.
178. **Nichols, J. L.** 1979. "Cap" structures in maize poly(A)-containing RNA. Biochim. Biophys. Acta **563**:490–495.
179. **Nuss, D. L., and Y. Furuichi.** 1977. Characterization of the m7G(5′)pppN-pyrophosphatase activity from HeLa cells. J. Biol. Chem. **252**: 2815–2821.
180. **Nuss, D. L., Y. Furuichi, G. Koch, and A. J. Shatkin.** 1975. Detection in HeLa cell extracts of a 7-methylguanosine specific enzyme activity that cleaves $m^{7}GpppN^{m}$. Cell **6**:21–27.
181. **Ohno, T., Y. Okada, K. Shimotohno, K. Miura, H. Shinski, M. Miwa, and T. Sugimura.** 1976. Enzymatic removal of the 5′-terminal methylated blocked structure of tobacco mosaic virus RNA and its effects on infectivity and reconstitution with coat protein. FEBS Lett. **67**:209–213.
182. **Ouellette, A. J., D. Frederick, and R. A. Malt.** 1975. Methylated messenger RNA in mouse kidney. Biochemistry **14**:4361–4367.
183. **Ouellette, A. J., S. L. Reed, and R. A. Malt.** 1976. Short-lived methylated messenger RNA in mouse kidney. Proc. Natl. Acad. Sci. U.S.A. **73**:2609–2613.
184. **Paterson, M. B., and M. Rosenberg.** 1979. Efficient translation of prokaryotic mRNAs in a eukaryotic cell-free system requires addition of a cap structure. Nature (London) **279**:692–696.
185. **Pelham, H. R. B., J. M. M. Sykes, and T. Hunt.** 1978. Characteristics of a coupled cell-free transcription and translation system directed by vaccinia cores. Eur. J. Biochem. **82**: 199–209.
186. **Perry, R. P., and D. E. Kelley.** 1974. Existence of methylated messenger RNA in mouse L cells. Cell **1**:37–42.
187. **Perry, R. P., and D. E. Kelley.** 1976. Kinetics of formation of 5′ terminal caps in mRNA. Cell **8**:433–442.
188. **Perry, R. P., D. E. Kelley, K. Frederici, and F. Rottman.** 1975. The methylated constituents of L cell messenger RNA: evidence for an unusual cluster at the 5′ terminus. Cell **4**:387–394.
189. **Perry, R. P., D. E. Kelley, K. H. Frederici, and F. M. Rottman.** 1975. Methylated constituents of heterogeneous nuclear RNA: presence in blocked 5′ terminal structures. Cell **6**: 13–19.
190. **Perry, R. P., and K. Scherrer.** 1975. Methylated constituents of globin mRNA. FEBS Lett. **57**:73–78.
191. **Pinck, L.** 1975. The 5′-end groups of alfalfa mosaic virus RNA are ^{7}mG(5′)ppp(5′)Gp. FEBS Lett. **59**:24–28.
192. **Plotch, S. J., M. Bouloy, and R. M. Krug.** 1979. Transfer of 5′-terminal cap of globin mRNA to influenza viral complementary RNA during transcription *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **76**:1618–1622.
193. **Plotch, S. J., J. Tomasz, and R. M. Krug.** 1978. Absence of detectable capping and methylating enzymes in influenza virions. J. Virol. **28**:75–83.
194. **Pugh, C. S. G., R. T. Borchardt, and H. O. Stone.** 1978. Sinefungin, a potent inhibitor of virion mRNA (guanine-7-)methyltransferase, mRNA (nucleoside-′2-)methyltransferase, and viral multiplication. J. Biol. Chem. **253**:4075–4077.
195. **Rao, M. S., M. Blackstone, and H. Busch.** 1977. Effects of U-1 nuclear RNA on transla-

tion of messenger RNA. Biochemistry **16:** 2756–2762.

196. **Reddy, R., T. S. Ro-Choi, D. Henning, and H. Busch.** 1974. Primary sequence of U-1 nuclear ribonucleic acid of Novikoff hepatoma ascites cells. J. Biol. Chem. **249:**6486–6494.
197. **Rhodes, D. P., and A. K. Banerjee.** 1976. 5'-Terminal sequence of vesicular stomatitis virus messenger RNA synthesized in vitro. J. Virol. **17:**33–42.
198. **Rhodes, D. P., S. A. Moyer, and A. K. Banerjee.** 1974. In vitro synthesis of methylated messenger RNA by the virion-associated RNA polymerase of vesicular stomatitis virus. Cell **3:** 327–333.
199. **Rhodes, D. P., D. V. R. Reddy, R. MaCleod, L. M. Black, and A. K. Banerjee.** 1977. In vitro synthesis of RNA containing ^{7}mG(5')ppp(5')A^{m} by purified wound tumor virus. Virology **76:**554–559.
200. **Richards, K. E., G. Jonard, M. Jacquemond, and H. Lot.** 1978. Nucleotide sequence of cucumber mosaic virus-associated RNA 5. Virology **89:**395–408.
201. **Robert-Géro, M., F. Lawrence, G. Farrugia, A. Berneman, P. Blanchard, P. Vigier, and E. Lederer.** 1975. Inhibition of virus-induced cell transformation by synthetic analogues of S-adenosyl homocysteine. Biochem. Biophys. Res. Commun. **65:**1242–1249.
202. **Ro-Choi, T. S., C. C. Yong, D. Henning, J. McCloskey, and H. Busch.** 1975. Nucleotide sequence of U-2 ribonucleic acid. J. Biol. Chem. **250:**3921–3928.
203. **Roman, R. J. D., S. N. Booker, and A. Marcus.** 1976. Inhibition of the transition of a 40S ribosome-Met-tRNA^{met} complex to an 80S ribosome-Met-tRNA^{met} complex by 7-methylguanosine-5'-phosphate. Nature (London) **260:** 359–360.
204. **Rose, J. K.** 1975. Heterogenous 5'-terminal structures occur on vesicular stomatitis virus mRNA's. J. Biol. Chem. **250:**8098–8104.
205. **Rose, J. K., W. A. Haseltine, and D. Baltimore.** 1976. 5' Terminus of Moloney murine leukemia virus 35S RNA is ^{7}mG(5')ppp(5')-G^{m}pCp. J. Virol. **20:**324–329.
206. **Rose, J. K., and H. F. Lodish.** 1976. Translation in vitro of vesicular stomatitis virus mRNA lacking 5'-terminal 7-methylguanosine. Nature (London) **262:**32–37.
207. **Rose, J. K., H. F. Lodish, and M. L. Brock.** 1977. Giant heterogenous polyadenylic acid on vesicular stomatitis virus mRNA synthesized in vitro in the presence of *S*-adenosylhomocysteine. J. Virol. **21:**683–693.
208. **Rose, J. K., H. Trachsel, K. Leong, and D. Baltimore.** 1978. Inhibition of translation by poliovirus: inactivation of a specific initiation factor. Proc. Natl. Acad. Sci. U.S.A. **75:**2732–2736.

208a. **Rosenberg, M.** 1974. Isolation and sequence determination of the 3'-terminal regions of isotopically labeled RNA molecules. Nucleic Acids Res. **1:**653–671.

209. **Rosenberg, M., and B. M. Paterson.** 1979. Efficient cap-dependent translation of polycistronic prokaryotic mRNAs is restricted to the first gene in the operon. Nature (London) **279:** 696–701.
210. **Rottman, F. M.** 1978. Methylation and polyadenylation of heterogeneous nuclear and messenger RNA, p. 45–73. *In* B. F. C. Clark (ed)., Biochemistry of nucleic acids, vol 17. University Park Press, Baltimore.
211. **Rottman, F., A. J. Shatkin, and R. P. Perry.** 1974. Sequences containing methylated nucleotides at the 5' termini of messenger RNA: possible implications for processing. Cell **3:** 197–199.
212. **Roy, P., and J. P. Clewley.** 1978. Spring viremia of carp virus RNA and virion-associated transcriptase activity. J. Virol. **25:**912–916.
213. **Saini, M. S., and B. G. Lane.** 1977. Wheat embryo ribonucleates. VIII. The presence of 7-methylguanosine "cap structures" in the RNA of imbibing wheat embryos. Can. J. Biochem. **55:**819–824.
214. **Salditt-Georgieff, M., M. Harpold, S. Chen-Kiang, and J. E. Darnell.** 1980. The addition of 5'-cap structures occurs early in HnRNA synthesis and prematurely terminated molecules are capped. Cell **19:**69–78.
215. **Salditt-Georgieff, M., W. Jelinek, J. E. Darnell, Y. Furuichi, M. Morgan, and A. Shatkin.** 1976. Methyl labeling of HeLa cell HnRNA: a comparison with mRNA. Cell **7:** 227–237.
216. **Sangar, D. V., D. J. Rowlands, T. J. R. Harris, and F. Brown.** 1977. Protein covalently linked to foot-and-mouth disease virus RNA. Nature (London) **268:**648–650.
217. **Schibler, U., D. E. Kelley, and R. P. Perry.** 1977. Comparison of methylated sequences in messenger RNA and heterogeneous nuclear RNA from mouse L cells. J. Mol. Biol. **115:** 695–714.
218. **Schibler, U., and R. P. Perry.** 1976. Characterization of the 5'-termini of HnRNA in mouse L cells: implications for processing and cap formation. Cell **9:**121–130.
219. **Schibler, U., and R. P. Perry.** The 5'-termini of heterogenous nuclear RNA: a comparison among molecules of different sizes and ages. Nucleic Acids Res. **4:**4133–4149.
220. **Seidel, B. L., and E. W. Somberg.** 1978. Characterization of *Neurospora crassa* polyadenylated messenger ribonucleic acid: structure of the 5'-terminus. Biochem. Biophys. Res. Commun. **187:**108–112.
221. **Shafritz, D. A., J. A. Weinstein, B. Safer, W. C. Merrick, L. A. Weber, E. D. Hickey, and C. Baglioni.** 1976. Evidence for role of ^{7}mGp group in recognition of eukaryotic mRNA by initiation factor IF-M3. Nature (London) **261:** 291–294.
222. **Sharma, O. K., D. E. Hruby, and D. N. Beezley.** 1976. Inhibition of ovalbumin mRNA translation by 7-methylguanosine-5'-phosphate. Biochem. Biophys. Res. Commun. **72:** 1392–1398.
223. **Shatkin, A. J.** 1974. Methylated messenger

RNA synthesis *in vitro* by purified reovirus. Proc. Natl. Acad. Sci. U.S.A. **71**:3204–3207.

224. **Shatkin, A. J.** 1976. Capping of eucaryotic mRNAs. Cell **9**:645–653.
225. **Shih, D. S., R. DasGupta, and P. Kaesberg.** 1976. 7-Methylguanosine and efficiency of RNA translation. J. Virol. **19**:637–642.
226. **Shimotohno, K., Y. Kodama, J. Hashimoto, and K.-I. Miura.** 1977. Importance of 5′-terminal blocking structure to stabilize mRNA in eukaryotic protein synthesis. Proc. Natl. Acad. Sci. U.S.A. **74**:2734–2738.
227. **Shimotohno, K., and K-I. Miura.** 1976. The process of formation of the 5′-terminal modified structure in messenger RNA of cytoplasmic polyhedrosis virus. FEBS Lett. **64**:204–208.
228. **Shinshi, H., M. Miwa, K. Kato, M. Noguchi, T. Matsushima, and T. Sugimura.** 1976. A novel phosphodiesterase from cultured tobacco cells. Biochemistry **15**:2185–2190.
229. **Shinshi, H., M. Miwa, T. Sugimura, K. Shimotohno, and K. Miura.** 1976. Enzyme cleaving the 5′-terminal methylated blocked structure of messenger RNA. FEBS Lett. **65**:254–257.
230. **Skup, D., and S. Millward.** 1980. mRNA capping enzymes are masked in reovirus progeny subviral particles. J. Virol. **34**:490–496.
231. **Skup, D., and S. Millward.** 1980. Reovirus induced modification of cap-dependent translation in infected L-cells. Proc. Natl. Acad. Sci. U.S.A. **77**:152–156.
232. **Smith, R. E., and J. M. Clark.** 1979. Effect of capping upon the mRNA properties of satellite tobacco necrosis virus ribonucleic acid. Biochemistry **18**:1366–1371.
233. **Sommer, S., M. Salditt-Georgieff, S. Bachenheimer, J. E. Darnell, Y. Furuichi, M. Morgan, and A. J. Shatkin.** 1976. The methylation of adenovirus-specific nuclear and cytoplasmic RNA. Nucleic Acids Res. **3**:749–765.
234. **Sonenberg, N., M. A. Morgan, W. C. Merrick, and A. J. Shatkin.** 1978. A polypeptide in eukaryotic initiation factors that crosslinks specifically to the 5′-terminal cap in mRNA. Proc. Natl. Acad. Sci. U.S.A. **75**:4843–4847.
235. **Sonenberg, N., M. A. Morgan, D. Testa, R. J. Colonno, and A. J. Shatkin.** 1979. Interaction of a limited set of proteins with different mRNAs and protection of 5′-caps against pyrophosphatase digestion in initiation complexes. Nucleic Acids Res. **7**:15–29.
236. **Sonenberg, N., K. M. Rupprecht, S. M. Hecht, and A. J. Shatkin.** 1979. Eukaryotic mRNA cap binding protein: purification by affinity chromatography on Sepharose-coupled m^7-GDP. Proc. Natl. Acad. Sci. U.S.A. **76**:4345–4349.
237. **Sonenberg, N., and A. J. Shatkin.** 1977. Reovirus mRNA can be covalently crosslinked via the 5′ cap to proteins in initiation complexes. Proc. Natl. Acad. Sci. U.S.A. **74**:4288–4292.
238. **Sonenberg, N., A. J. Shatkin, R. P. Ricciardi, M. Rubin, and R. M. Goodman.** 1978. Analysis of terminal structures of RNA from potato virus X. Nucleic Acids Res. **5**:2501–2512.
239. **Spencer, E., D. Loring, J. Hurwitz, and G. Monroy.** 1978. Enzymatic conversion of 5′-phosphate-terminated RNA to 5′-di- and triphosphate-terminated RNA. Proc. Natl. Acad. Sci. U.S.A. **75**:4793–4797.
240. **Sripati, C. E., Y. Groner, and J. R. Warner.** 1976. Methylated, blocked 5′ termini of yeast mRNA. J. Biol. Chem. **251**:2898–2904.
241. **Stanley, J., P. Rottier, J. W. Davies, P. Zabel, and A. V. Kammen.** 1978. A protein linked to the 5′ termini of both RNA components of the cowpea mosaic virus genome. Nucleic Acids Res. **5**:4505–4522.
242. **Stein, J. L., G. S. Stein, and P. M. McGuire.** 1977. Histone messenger RNA from HeLa cells: evidence for modified 5′-termini. Biochemistry **16**:2207–2213.
243. **Stoltzfus, C. M., and K. Dimock.** 1976. Evidence for methylation of B-77 avian sarcoma virus genome RNA subunits. J. Virol. **18**:586–595.

243a. **Stoltzfus, C. M., K. Dimock, and S. A. Moyer.** 1979. Effect of an *in vivo* methylation inhibitor, cycloleucine, on viral and cellular mRNA structure and function, p. 399–408. *In* E. Usdin, R. T. Borchardt, and C. R. Creveling (ed.), Transmethylation. Elsevier/North-Holland, New York.

244. **Surrey, S., and M. Nemer.** 1976. Methylated blocked 5′ terminal sequences of sea urchin embryo messenger RNA classes containing and lacking poly(A). Cell **9**:589–595.
245. **Suzuki, H.** 1977. Effect of ^{7}mGppN^m on the rabbit globin synthesis. FEBS Lett. **79**:11–14.
246. **Symons, R. H.** 1975. Cucumber mosaic virus RNA contains 7-methylguanosine at the 5′-terminus of all four RNA species. Mol. Biol. Rep. **2**:277–285.
247. **Takagi, S., and T. Mori.** 1979. The 5′-terminal structure of poly(A)-containing RNA of soybean seeds. J. Biochem. **86**:231–238.
248. **Taylor, R. H., and D. T. Dubin.** 1975. The methylation status of mammalian mitochondrial messenger RNA. J. Cell Biol. **67**:428A.
249. **Tazaki, Y., and K. I. Miura.** 1977. Terminal structure involving a single-stranded stretch in the double-stranded RNA from *Penicillium chrysogenum* virus. Virology **82**:14–24.
250. **Testa, D., and A. K. Banerjee.** 1977. Two methyltransferase activities in the purified virions of vesicular stomatitis virus. J. Virol. **24**:786–793.
251. **Testa, D., and A. K. Banerjee.** 1979. Initiation of RNA synthesis *in vitro* by vesicular stomatitis virus. J. Biol. Chem. **254**:2053–2058.
252. **Thomason, A. R., D. A. Brian, L. F. Velice, and F. M. Rottman.** 1976. Methylation of high-molecular-weight subunit RNA of feline leukemia virus. J. Virol. **20**:123–132.
253. **Thomason, A. R., K. H. Friderici, L. F. Velicer, and F. Rottman.** 1978. Presence of 5′-terminal cap structures in virus-specific RNA from feline leukemia virus-infected cells. J. Virol. **26**:226–235.
254. **Toneguzzo, F., and H. P. Ghosh.** 1976. Char-

acterization and translation of methylated and unmethylated vesicular stomatitis virus mRNA synthesized in vitro by ribonucleoprotein particles from vesicular stomatitis virus-infected L cells. J. Virol. **17**:477–491.

255. **Trachsel, H., N. Sonenberg, A. J. Shatkin, J. K. Rose, K. Leong, J. E. Bergman, J. Gordon, and D. Baltimore.** 1980. Purification of a factor that restores translation of vesicular stomatitis virus mRNA in extracts from poliovirus-infected HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **77**:770–774.
256. **Urushibara, T., Y. Furuichi, C. Nishimura, and K. Miura.** 1975. A modified structure at the 5'-terminus of mRNA of vaccinia virus. FEBS Lett. **49**:385–389.
257. **Venkatesan, S., A. Gershowitz, and B. Moss.** 1980. Modification of the 5' end of mRNA: association of RNA triphosphatase with the RNA guanyltransferase-RNA (guanine-7-)-methyltransferase complex from vaccinia virus. J. Biol. Chem., in press.
258. **Weber, L. A., E. R. Feman, E. D. Hickey, M. C. Williams, and C. Baglioni.** 1976. Inhibition of HeLa cell messenger RNA translation by 7-methylguanosine 5'-monophosphate. J. Biol. Chem. **251**:5657–5662.
259. **Weber, L. A., E. D. Hickey, D. L. Nuss, and C. Baglioni.** 1977. 5'-Terminal 7-methylguanosine and mRNA function: influence of potassium concentration on translation *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **74**:3254–3258.
260. **Wei, C. M., A. Gershowitz, and B. Moss.** 1975. N^6,2'-*O*-dimethyladenosine, a novel methylated ribonucleoside next to the 5'-terminal of animal cell and virus mRNAs. Nature (London) **257**:251–253.
261. **Wei, C. M., A. Gershowitz, and B. Moss.** 1975. Methylated nucleotides block 5' terminus of HeLa cell messenger RNA. Cell **4**:379–386.
262. **Wei, C. M., A. Gershowitz, and B. Moss.** 1976. 5'-Terminal and internal methylated nucleotide sequences in HeLa cell mRNA. Biochemistry **15**:397–401.
263. **Wei, C. M., and B. Moss.** 1974. Methylation of newly synthesized viral messenger RNA by an enzyme in vaccinia virus. Proc. Natl. Acad. Sci. U.S.A. **71**:3014–3018.
264. **Wei, C. M., and B. Moss.** 1975. Methylated nucleotides block 5'-terminus of vaccinia virus messenger RNA. Proc. Natl. Acad. Sci. U.S.A. **72**:318–322.
265. **Wei, C. M., and B. Moss.** 1977. 5'-Terminal capping of RNA by guanylyltransferase from HeLa cell nuclei. Proc. Natl. Acad. Sci. U.S.A. **74**:3758–3761.
266. **Wei, C. M., and B. Moss.** 1977. Nucleotide sequences at the N^6-methyladenosine sites of HeLa cell messenger ribonucleic acid. Biochemistry **16**:1672–1676.
267. **Wertheimer, A. M., S.-Y. Chen, R. T. Borchardt, and Y. Furuichi.** 1980. S-adenosylmethionine and its analogs: structural features correlated with synthesis and methylation of mRNAs of cytoplasmic polyhedvirus virus. J. Biol. Chem., in press.
268. **Willems, M., B. E. Wieringa, J. Mulder, A. B. Geert, and M. Gruber.** 1979. Translation of vitellogenin mRNA in the presence of 7-methylguanosine 5'-triphosphate: cap analogs compete with mRNAs on the basis of affinity for initiation-complex formation. Eur. J. Biochem. **93**:469–479.
269. **Wimmer, E., A. Y. Chang, J. M. Clark, Jr., and M. E. Reichmann.** 1968. Sequence studies of satellite tobacco necrosis virus RNA. Isolation and characterization of a 5'-terminal trinucleotide. J. Mol. Biol. **38**:59–73.
270. **Winicov, I., and R. P. Perry.** 1976. Messenger RNA and heterogeneous nuclear RNA methylation *in vitro*. J. Cell Biol. **70**:391A.
271. **Winicov, I., and R. P. Perry.** 1976. Synthesis, methylation and capping of nuclear RNA by a subcellular system. Biochemistry **15**:5039–5045.
272. **Wodnar-Filipowicz, A., E. Szczesna, M. Zan-Kowalozewska, S. Muthukrishnan, U. Szybiak, A. B. Legooki, and W. Filipowicz.** 1978. 5' Terminal 7 methylguanosine and messenger RNA function: the effect of enzymatic decapping and of cap analogs on translation of tobacco mosaic virus RNA and globin messenger RNA *in vitro*. Eur. J. Biochem. **92**:69–80.
273. **Wold, W. S. M., M. Green, and T. W. Munns.** 1976. Methylation of late adenovirus 2 nuclear and messenger RNA. Biochem. Biophys. Res. Commun. **68**:643–649.
274. **Yang, N. S., R. F. Manning, and L. P. Gage.** 1976. The blocked and methylated 5' terminal sequence of a specific cellular messenger: the mRNA for silk fibroin of *Bombyx mori*. Cell **7**: 339–347.
275. **Yong, R. A., and J. A. Steitz.** 1979. Tandem promoters direct *E. coli* ribosomal RNA synthesis. Cell **17**:225–234.
275a. **Young, R. J.** 1977. Appearance of 7-methylguanosine-5'-phosphate in the RNA of mouse 1-cell embryos three hours after fertilization. Biochem. Biophys. Res. Commun. **76**:32–39.
276. **Zan-Kowalczewska, M., M. Bretner, H. Sierakowska, E. Szczesna, W. Filipowicz, and A. J. Shatkin.** 1977. Removal of 5'-terminal m^7G from eukaryotic mRNAs by potato nucleotide pyrophosphatase and its effect on translation. Nucleic Acids Res. **4**:3065–3081.
277. **Zarbl, H., D. Skup, and S. Millward.** 1980. Reovirus progeny subviral particles synthesize uncapped mRNA. J. Virol., in press.
278. **Ziff, E. B., and R. M. Evans.** 1978. Coincidence of the promoter and capped 5'-terminus of RNA from the adenovirus 2 major late transcription unit. Cell **15**:1463–1475.
279. **Zimmern, D.** 1975. The 5' end group of tobacco mosaic virus RNA is $_m{}^7G(5')ppp(5')Gp$. Nucleic Acids Res. **2**:1189–1201.