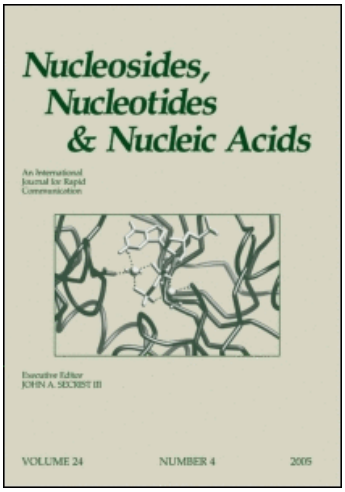

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THE CHEMISTRY OF EUKARYOTIC MESSENGER RNA

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Summary. The so-called "cap" structure of the 5'-terminus of eukaryotic messenger RNA (mRNA) is of importance for protein biosynthesis on ribosimes. We have examined the chemical synthesis and structural requirement of the 5'-terminus of mRNA.

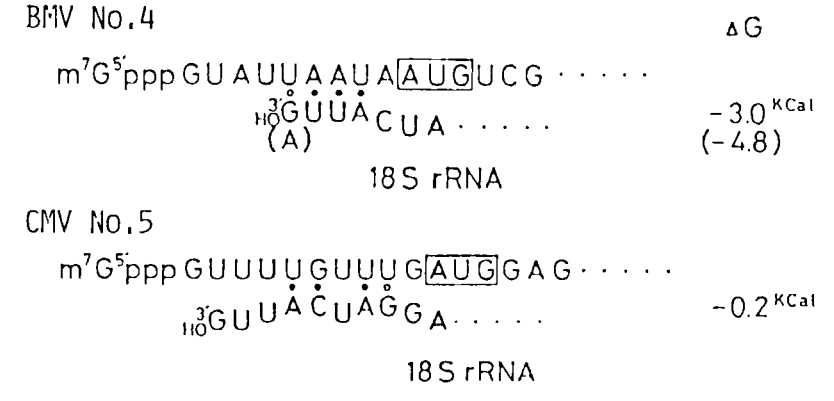
This paper describes a brief summary of the synthesis of the cap structure and the 5'-terminus of mRNA bearing the leader sequences.

A blocked structure at the 5'-terminus of mRNA of silkworm cytoplasmic polyhedrosis virus was first detected by Miura[1] and the structure was confirmed by the chemical synthesis.[2]

$$m^7G^{5'}pppAm\text{-}G\text{-}U\text{-}...$$

After that similar structures have been reported in a variety of mRNAs from eukaryotic cells and viruses, and now it is called as cap structure of eukaryotic mRNA.[3] It is known that elimination of this structure causes loss of stability of mRNA, especially against exonuclease degradation, and a decrease in ability of the initiation complex formation of mRNA for protein synthesis. In contrast to prokaryotic mRNAs, mRNAs of eukaryote is mono-cistronic whereas prokaryotic mRNAs have Shine-Dargarno sequence[4] in their up-stream of cistrons. Our question is if in eukaryotic mRNAs, S.D.-like sequences exist and participate in protein synthesis. Cucumber mosaic virus (CMV) No.5 is a small single-stranded RNA and its leader

sequence is short. The first initiation codon AUG appears at the 11th nucleotide from the 5'-terminus. The protein corded by this RNA in vitro system was characterized. The newly synthesized protein was labeled with ^{3}H leucine and ^{35}S methionine in a reticulocyte cell-free system, where CMV RNA No.5 was added as mRNA. Brome mosaic virus (BMV) RNA No.4 is a small RNA carrying the cistron of coat protein. Its leader sequence before the first initiation codon AUG is 9 nucleotides long. Analysis of the labeled peptide synthesized in the reticulocyte system was performed by gel electrophoresis with a 15% acrylamide gel slab and by fluorography. Although both CMV RNA No.5 and BMV RNA No.4 have short leader sequences with similar lengths, the latter was found to produce a protein about 100 times as efficiently as the former.[5] The difference in the leader sequence between BMV RNA No.4 and CMV RNA No.5 seems to affect affinity to ribosomal RNA. BMV RNA No.4 has the sequence UAAU, which is complementary to the 3-terminal part of 18S rRNA, whereas CMV RNA No.5 does not carry a strong continuous complementary sequence. Calculation of the affinity between the leader sequence and the rRNA by the method of Tinoco was performed.[6] Thus, BMV RNA No.4 should have a 15 times stronger affinity to ribosomes than CMV RNA No.5. The latter can form the initiation complex for protein synthesis but the efficiency is much lower than that of BMV RNA No.4. Therefore, a possible sequence complementary to rRNA ahead of the initiation codon would



enhance the first binding of mRNA to ribosomes. In onsideration of these facts, if one of the letters is replaced from U to C in the leader sequence, the affinity between BMV RNA No.4 and ribosomes may be increased. We have synthesized the one-point mutant leader sequence of BMV RNA No.4 by using new protecting groups.[7] In order to avoid side reactions on base moieties in the phosphotriester method, several protecting groups were proposed.[8] Recently, we have used the p-anisyl group for uracil moiety and isopropionyl and N,N-diphenylcarbamoyl groups for guanine residue.[9] More recently, we have tested the polymer support synthesis of oligoribonucleotide on CPG-biogel (569 Å; 100-200 mesh) was suitable for this purpose.[10]

Next, several methods for the synthesis of the cap structure were investigated.

7-Methyl guanosine is very sensitive towards alkaline conditions. Therefore, the protecting groups should be removed under acidic conditions. A stable non-methylated precapping agent, $G^{5'}$ppSPh, was prepared. It was transformed to the capping agent in by methylation with MeI in situ. It was activated by using $AgNO_3$ in the presence of an excess amount of a nucleotide pN. This silver ion catalyzed reaction required the use of an excess amount of acceptor nucleotides to obtain m^7GpppN in good yields.[11] However the silver ion mediated reaction can hardly be applied to the synthesis of capped oligoribonucleotides, because the excess use of oligomer component is undesirable. The above reaction may be rationalized by the generation of a highly active metapyrophosphate intermediate which can react further with an once-produced triphosphate so that the P-O-P bond was degraded to give a tetrapolyphosphate derivative. Consequently, it was found that coaddition of imidazole dramatically improved the silver ion catalyzed reaction. The reaction seems to proceed via a pyrophosphorimidazolidate which reacts smoothly with pN to

form the cap structure. By this method, the use of large excess amount of precapping agent was realized.

We have also been interested in the skip of the leader sequence. Therefore, m^7GpppAUG and related compounds were synthesized.[12)]

The three dimentional structure of the 5'-terminus of silkworm cytoplasmic polyhedrosis virus mRNA was classified roughly in two forms. One of them the 7-methylguanosine locates far from oligonucletide through tripolyphosphate bridge. The other is the 7-methylguanosine stacking form with the 1st letter of adenosine and tripolyphosphate bridge loops out. The CD spectrum of the confronting structure showed a chart similar to those of common oligonucleotides.[13)] Therefore, the cap structure may exist substantially in the stacking form.

The methylation reaction forming the cap structure has been examined by emplying non-methylated cap analogs. Methylation of the guanosine moiety of the cap structure with ^{3}H labbeled S-adenosylmethionine (SAM) was examined.[14)] When chemically synthesized GpppA was added as a substrate into a cytoplasmic polyhedrosis virus system, m^7GpppA was obtained. In the same system GpppG and ApppA were not methylated. On the other hand, mRNAs from reovirus were represented as m^7GpppGmpU... In vitro RNA synthesizing system of reovirus, GpppG was methylated selectively at the 7-position of one of two guanosine residues, but any methylation did not occur in the case of GpppA. These results show that the methylation enzymes in CPV and reovirus recognized strictly the structure of the confronting nucleoside residues. This supported the CD spectral results where the confronting structure showed a stacking form.

Further investigation including the biological behavior of leader sequences will be described elsewhere.

REFERENCES

1) Furuichi, Y.; Miura, K. Nature, 1975, 253, 374.
2) Yamaguchi, K.; Nakagawa, I.; Sekine, M.; Hata, T.;

Shimotohno, K.; Hiruta, M; Miura, K. Nucleic Acids Res., 1984, 12, 2939.

3) Cohn, W. E.; Volkin, E. (Ed.) 'Progress in nucleic Acid Ressearch and Molecular Biology' Academic Press, New York, 1976.

4) Shine, J.; Dalagarno, L. Proc. Natl. Acad. Sci. USA, 1974, 71, 1342.

5) Yamaguchi, K.; Hidaka, S.; Miura, K. Proc. Natl. Acad. Sci. USA, 1982, 79, 1012.

6) Tinoco, I. Jr.; Borer, P. N.; Dengler, B.; Levine, M. D.; Uhlenback, O. C.; Crothers, D. M.; Gralla, J. Nature, 1973, 246, 40.

7) Kamimura, T.; Tsuchiya, M.; Urakami, K.; Koura, K.; Sekine, M.; Shinozaki, K.; Miura, K.; Hata, T. J. Am. Chem. Soc., 1984, 106, 4552.

8) Jones, S. S.; Reese, C. B.; Shibanda, S.; Ubasawa, A. Tetrahedron Lett., 1981, 22, 4755: Gaffney, B. L.; Jones, R. A. ibid., 1982, 23, 2257: Watokins, B. E.; Rapoport, H. J. Org. Chem., 1982, 47, 4471: Watokins, B. E.; Kleyl, J. S.; Rapoport, H. J. Am. Chem. Soc., 1982, 104, 5703: Sekine, M.; Matsuzaki, J.; Satoh, M.; Hata, T. J. Org. Chem., 1982, 47, 571: Sekine, M.; Matsuzaki, J.; Hata, T. Tetrahedron Lett., 1982, 23, 5287: Kamimura, T.; Masegi, T.; Hata, T. Chem. Lett., 1982, 975; Kamimura, T.; Masegi, T.; Urakami, K.; Honda, S.; Hata, T. ibid., 1983, 1051: Trichtinger, T.; Charubara, R.; Pfleiderer, W. Tetrahedron Lett., 1983, 24, 711: Himmlesbach, F.; Pfleiderer, W. ibid., 1983, 24, 3587: Welch, C. J.; Chattopadhyaya, J. M. Acta, Chem. Scand. Ser., 1983, B 37, 147.

9) Kamimura, T.; Tsuchiya, M.; Koura, K.; Sekine, M.; Hata, T. Tetrahedron Lett., 1983, 24, 2775.

10) Tanimura, H.; Sekine, M.; Hata, T. Nucleosides & Nucleotides, in press.

11) Nakagawa, I.; Konya, S.; Hata, T. Synthesis, 1980, 556: Hata, T.; Nakawaga, I.; Shimotohno, K.; Miura, K. Chem.

Lett., 1976 987: Kamimura, T.; Osaki, Y.; Sekine, M.; Hata, T. Tetrahedron Lett., 1984, 25, 2683.

12) Sekine, M.; Nishiyama, S.; Kamimura, T.; Osaki, Y.; Hata, T. Bull. Chem. Soc. Jpn., 1985, 58, 850.

13) unpublished result.

14) Yamaguchi, K.; Nakagawa, I.; Hata, T.; Shimotohno, K.; Shimotohno, K.; Hiruta, M.; Miura, K. Nucleic Acids Res. Special Pub. No. 2, 1976, s151.