CLEAVAGE OF pm7G FROM mRNA 5' TERMINAL CAP STRUCTURES BY PYROPHOSPHATASE ACTIVITY IN EMBRYONIC CHICK LENS CELLS

GENE C. LAVERS
Department of Biochemistry, New York University Dental Center, New York, N. Y. 10010, U.S.A.

(Received 16 July, 1977)

Abstract. The presence of pyrophosphatase activity in embryonic lens cells which cleaves pm7G and ppGm from m7G(5')pppGm was demonstrated. It was also found that m7G(5')pppG, but not G(5')pppG, was hydrolyzed, and conversion of m7GpppG to m7G*pppG, in which the 5-membered ring of the m7G moiety is open, abolished its hydrolysis. For the caps hydrolyzed, pm7G was released only in the presence of lens cellular fraction; pm7G inhibited cap hydrolysis.

I. INTRODUCTION

Most eukaryotic cellular and viral mRNAs contain a 5'terminal structure of the general forms G(5')pppN, m7G(5')pppNm, and m7G(5')pppNm pNm [1]. The presence of the m7G moiety in the 'cap' structure seems to increase the association of mRNA with ribosomes leading to formation of functional initiation complexes [2–5]. Cap structures such as m7GpppGm and pm7G (i.e., 5'-mGMP, its component) inhibit the translation of capped mRNA [6–8]. mRNA lacking m7G in the 5'terminus (i.e., pppG-) [9] or mRNA from which the m7G has been removed chemically from the cap [2, 10], shows decreased translational function. Moreover, RNAs with a 5'terminal cap structure have greater stability [11] and infectivity [12] compared to corresponding uncapped RNAs.

Recently, a pyrophosphatase has been detected [13] and characterized [14] from HeLa cells; it selectively hydrolyzes pm7G from m7GpppN(m) caps, has decreased activity on capped oligonucleotides [7–10] residues long, but does not hydrolyze pm7G from capped mRNA. Two nucleases from tobacco [12, 15] and potato [16] also hydrolyze cap structures.

Two methyltransferase activities have been found in the 15 day embryonic chick lens which utilize S-adenosyl-methionine (AdoMet) to modify G(5')pppG to m7GpppGm [17]. During that study, in addition to the appearance of m7GpppGm in the reaction mixture, pm7G and ppGm were found; addition of GMP, GDP or GTP to reaction mixtures did not lead to the appearance of pm7G or ppGm.

This communication shows that a pyrophosphatase activity is present in embryonic lens
cells which specifically hydrolyzes the pyrophosphate bond of 5′terminal mRNA cap structures closest to the m'G moiety. The products of the reaction catalyzed by the pyrophosphatase activity on m'GpppG(m) are pm'G and ppG(m).

II. MATERIALS AND METHODS

Bacterial alkaline phosphatase (BAP) from *E. coli* (grade BAPF) and *Crotalus atrox* venom nucleotide pyrophosphatase type III (E.C. 3.6.1.9) were purchased from Worthington and Sigma, respectively. GpppG, m'GpppGm, and component markers were purchased from P-L Laboratories, S-adenosyl-methionine (AdoMet) and S-adenosyl-homocysteine (AdoHcy) from Sigma and [3H-CH₃]-AdoMet (9.7 Ci mmol⁻¹) from New England Nuclear.

A cytoplasmic fraction, free of polyribosomes and messenger ribonucleoprotein (mRNP) complex particles, was prepared from 15 day embryonic chick lens cells as previously described [17]. A lens homogenate, prepared with 0.34 M sucrose containing 50 mM Tris-HCl 25 mM KCl, 8 mM MgCl₂ and 8 mM 2-mercaptoethanol, was freed of nuclei and mitochondria by centrifugation, adjusted to 0.5% triton X-100, then polyribosomes and mRNP complex particles were removed by centrifugation. The conditions for the methylating modification of GpppG with [3H-CH₃]-AdoMet and lens cellular fraction have been described [17]. The complete cap hydrolysis reaction mixture (25 µl) contained 10 mM Tris-HCl (pH 7.5), the appropriate cap structure, and 5 µl of lens fraction. In some instances 160 µM AdoHcy was present to inhibit endogenous methyltransferases [17]. After incubation at 37 °C, reactions were stopped by mixing with one volume of phenol:chloroform (1:1, v/v) containing 0.1% 8-hydroxyquinoline [18]. The reaction products in the aqueous phase were freed of phenol:chloroform by ether extraction and then the ether was blown off by a stream of nitrogen. Procedures used for enzymatic digestion with BAP and nucleotide pyrophosphatase, for high voltage paper electrophoresis, for elution of samples from analytical papers, for paper chromatography, and for isotope counting have been described [17]. Samples to be chromatographed on DEAE-cellulose were loaded onto columns in 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA and 7 M urea, and eluted with a 0.0–0.5 M NaCl gradient in the same buffer. Charge markers, prepared by RNase A digestion of yeast tRNA, were co-chromatographed with the sample. Messenger RNA and corresponding 5′terminal cap structures from reovirus [13] particles were prepared as described and generously provided by Y. Furuuchi, M. Morgan, and A. J. Shatkin of the Roche Institute of Molecular Biology, Nutley, New Jersey, U.S.A.

III. RESULTS AND DISCUSSION

An examination of the embryonic chick lens has revealed the presence of enzymes which modify GpppG, a mRNA 5′terminal cap structure, to m'GpppGm by transfer of methyl groups from S-adenosyl-methionine [17]. Figure 1 shows a typical analysis by high voltage paper electrophoresis of an incubated reaction mixture in which GpppG is modified to m'GpppGm. The [3H-CH₃]-labeled material in the m'GpppGm region was shown to be [3H-