The biosynthesis and functional roles of methylated nucleosides in eukaryotic mRNA

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Abstract

Modified nucleosides are present in mRNA of all eukaryotes, albeit at much lower levels than in other RNA moieties such as rRNA, tRNA, and snRNA. Modification by methylation occurs on the terminal guanosine of the cap (N^7-methylguanosine), and the first two encoded nucleosides (2'-O-methylnuculeosides) in most higher eukaryotes. Additional modifications of cap nucleosides occur in special cases where the cap is derived by trans-splicing in nematodes and kinetoplastids. Modification by methylation also occurs at internal adenosine residues in many species (N^6-methyladenosine). Modification by deamination occurs at specific adenosine residues (forming inosine) and cytidine residues (forming uridine) in very specific cases leading to post-transcriptional editing. Numerous studies have shown the importance of the cap N^7-methylguanosine in translation, splicing, transport, and mRNA stability. The role of the 2'-O-methylnucleosides is not as well understood, but there is evidence that these modifications play some role in translation efficiency. The role of internal N^6-methyladenosine residues is least known, and is the focus of this review. The formation of N^6-methyladenosine is catalyzed by a complex enzyme containing a subunit (MT-A70) that co-localizes with nuclear speckles and appears to be widely expressed in all higher eukaryotes. Loss of this enzyme leads to a sporulation defect in yeast and to apoptosis in mammalian cells, although the exact mechanism by which the effects occur remains obscure.

1 Introduction

Post-transcriptional modifications of eukaryotic mRNA include the methylation of a small subset nucleosides, both within the 5'-terminal cap structure and within the body of the mRNA. The 5'-terminal cap guanine residue, first and second transcribed nucleosides, and specific internal residues can be methylated at positions either on the base or on the ribose moiety. These methylations lead to the formation of N^7-guanine (m^7G), 2'-O-methylnucleosides (N_m), and N^6-methyladenosine (m^6A). These methylated nucleosides represent nearly the entire repertoire of modified nucleosides in eukaryotic mRNA, a much more limited set than is found in other eukaryotic RNA species. (The only others are inosine and

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uridine, which will only be briefly considered in this review but are covered in detail in this volume in separate chapters by Hoopengardner et al. and Smith et al.). As is the case for many nucleoside modifications in rRNA, tRNA, and snRNA, the functions of these methylated nucleosides are not completely understood. This chapter will briefly review the biogenesis, the structure, and the function of the methylated cap, and will then focus in detail on internal m^6A residues, the enzyme complex that catalyzes the sequence-specific methylation of internal adenosine residues, and their function. Interesting features of mRNA N^6-methyladenosine methyltransferase include its multicomponent nature, the co-localization of this enzyme with splicing factors in nuclear speckles, and the prediction that it is a prototypical member of a conserved group of RNA methyltransferases, which most closely resemble the β class of methyltransferases, which previously had been known to include only prokaryotic DNA:m^6A and DNA:m^4C methyltransferases. This methyltransferase activity appears to be essential for important gene regulatory functions as it is necessary for induction of sporulation in yeast, and its absence leads to apoptosis in a human cell line.

2 Methylated nucleosides present in eukaryotic mRNA

2.1 The 5'-terminal cap structure

The general structure of the mRNA cap is $m^7GpppN_{1(m)}pN_{2(m)}pN...$, where the terminal guanine is invariably methylated at the N^7-position on the purine ring. N_1, which can be any of the four nucleosides, is generally methylated on the 2'-O-positon of the ribose ring, and N_2 can also be methylated in the same fashion (Rottman et al. 1974; Wei et al. 1975; Shatkin 1976; Banerjee 1980; and reviewed by Reddy et al. 1992). Cap structures containing only 7-methylguanine are designated Cap 0, those containing a 2'-O-methylnucleoside at position N_1 are designated Cap 1, and if both N_1 and N_2 are 2'-O-methylnucleosides the structure is designated Cap 2. If N_1 is 2'-O-methyladenosine, it can also be further methylated at the N^6-position of the purine ring. Both Cap 1 and Cap 2 structures predominate in mRNAs in higher eukaryotes, with Cap 1 being found with five times the frequency of Cap 2 in mammalian mRNA (Perry and Kelley 1976).

An exception to this generality is found for eukaryotic organisms whose mRNA 5'-termini are formed by trans-splicing of a spliced leader from the 5- end of a SL RNA. In the kinetoplastids, *Trypanosoma brucei* and *Crithidia fasciculate*, trans-splicing of a 39-41 nucleotide spliced leader yields a Cap 4 structure $m^7G(5')ppp(5')m_2{}^6A_mpA_mpC_mpm^3U_mp$ (Bangs et al. 1992). This unique cap has been shown to be synthesized co-transcriptionally on the 140 nucleotide SL RNA prior to trans-splicing to the mRNA (Mair et al. 2000). In nematodes (e.g. *C. elegans* and *A. lumbricoides*), a 2,2,7-trimethylgaunosine capped spliced leader of 22 nucleotides is added to some mRNAs via trans splicing from an ~100 nucleotide Sm snRNP (Liou and Blumenthal 1990; van Doren and Hirsh 1990; Maroney et al. 1995). Collectively, these cap methylation events account for the presence of