Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Biochemical principles and inhibitors to interfere with viral capping pathways

Etienne Decroly and Bruno Canard

Messenger RNAs are decorated by a cap structure, which is essential for their translation into proteins. Many viruses have developed strategies in order to cap their mRNAs. The cap is either synthetized by a subset of viral or cellular enzymes, or stolen from capped cellular mRNAs by viral endonucleases ('cap-snatching'). Reverse genetic studies provide evidence that inhibition of viral enzymes belonging to the capping pathway leads to inhibition of virus replication. The replication defect results from reduced protein synthesis as well as from detection of incompletely capped RNAs by cellular innate immunity sensors. Thus, it is now admitted that capping enzymes are validated antiviral targets, as their inhibition will support an antiviral response in addition to the attenuation of viral mRNA translation. In this review, we describe the different viral enzymes involved in mRNA capping together with relevant inhibitors, and their biochemical features useful in inhibitor discovery.

Address
CNRS, Aix Marseille University, AFMB UMR7257, Marseille, France

Corresponding authors: Decroly, Etienne (etienne.decroly@afmb.univ-mrs.fr), Canard, Bruno (bruno.canard@afmb.univ-mrs.fr)

Current Opinion in Virology 2017, **24**:87–96

This review comes from a themed issue on **Antiviral strategies**

Edited by **Lieve Naesens** and **Fabien Zoulim**

For a complete overview see the Issue and the Editorial

Available online 18th May 2017

http://dx.doi.org/10.1016/j.coviro.2017.04.003

Introduction

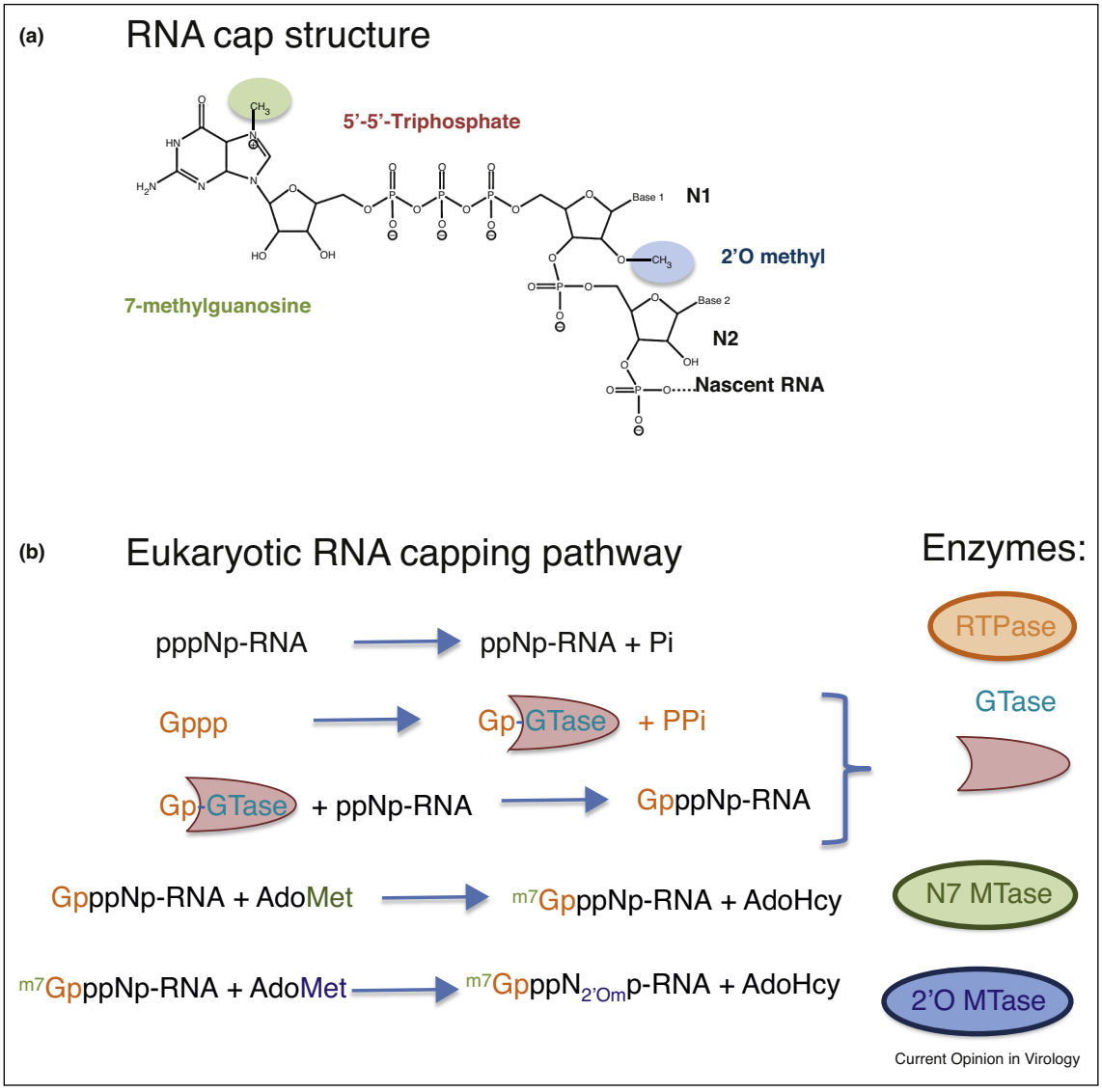
The 5′ end of nascent eukaryotic messenger RNA (mRNA) is co-transcriptionally modified by the addition of a cap structure. The cap-0 structure consists of a guanosine linked by a 5′–5′ triphosphate bridge to the RNA 5′ end (Figure 1a). This cap structure is methylated at the nitrogen in position 7 of G (cap-0 structure or ^{m7}**G**pppN). In metazoan, cap-0 is often converted into cap-1 structure by 2′-*O*-methylation of the first N_1 ribose (cap-1 structure or ^{m7}**G**ppp$N_{2'm}$) of the mRNA. This structure plays several key biological functions (reviewed in Ref. [1••]). The cap (i) increases mRNA stability by protecting mRNA from 5′ exoribonucleases; (ii) participates to pre-mRNA splicing and export to the cytoplasm; (iii) ensures the recruitment of mRNA to the ribosomes by recognizing eukaryotic translation Initiation Factor (eIF4E); and (iv) initiates the translation of mRNA into proteins. In addition, it was demonstrated that the cap structure is a marker of 'self', preventing detection by mechanisms of cellular innate immunity [2]. It was first reported that host cell sensors, such as Toll Like Receptor (TLR) and Retinoic acid-Inducible Gene (RIG)-like receptors, could detect uncapped RNAs with 5′-triphosphate ends. More recently, it was also shown that RIG-I and Melanoma Differentiation-Associated protein 5 (MDA5) recognize mis-capped RNA lacking 2′-*O*-methylation of the first transcribed nucleotide [3,4] initiating signaling cascades leading to the expression and release of cytokines and type I interferon. In turn, interferon induces an antiviral state in neighboring cells. Among the Interferon-Stimulated Genes (ISG), InterFeron-Induced protein with Tetratricopeptide repeats 1 (IFIT 1) can recognize mis-capped RNAs and inhibit their translation [5].

Within the host cell, eukaryotic mRNA is generally capped through a 'canonical' RNA capping pathway. It generally requires four sequential reactions, elucidated four decades ago, catalyzed by an RNA 5′ triphosphatase (RTPase), a guanylyltransferase (GTase), a guanine N7 methyltransferase (N7-MTase) and a 2′-*O*-MTase, respectively (Figure 1b).

In contrast, many viruses have evolved their own mRNA capping machinery in order to expedite efficient viral protein production and escape from innate immunity detection. Remarkably, pathways of viral mRNA capping are highly diverse but almost converge to the RNA cap structure common to viral and cellular mRNAs (Figure 1a) [6•]. When viruses express their own set of capping enzymes, four types of RNA capping pathways have been evidenced so far [1••,6•].

In the first one, viruses use a capping pathway similar to that observed in eukaryotic cells (Figure 1b). The phosphate at the 5′ end of the nascent viral is hydrolyzed by an RTPase activity held by an RTPase or a helicase domain. Concomitantly, a GTP is recruited by a GTPase, often forming a covalent adduct Lys-GMP before the transfer of GMP onto the RNA 5′ diphosphate end [7–11]. This occurs for DNA viruses (*e.g.*, poxviruses, mimivirus,

Figure 1



(a) Chemical structure of the eukaryotic RNA cap. **(b)** Eukaryotic 'canonical' RNA capping pathway, in which the nascent mRNA is sequentially processed by four enzymatic activities, represented as separate enzymes on the right-side of the reaction (see text for details). RTPase: RNA 5′-triphosphatase; GTase: Guanylytransferase; N7 MTase: N7-guanine RNA cap methyltransferase; 2′*O*MTase: Ribose 2′-*O* RNA methyltransferase.

baculoviruses) as well as supposedly for several positive strand RNA viruses (*e.g.*, flavivirus, coronavirus). After capping, the cap is methylated on its N7 and 2′*O* position by either one bi-functional N7/2′*O*-MTases (*e.g.*, flaviviruses [12,13]), or two separate enzymes (*e.g.*, coronaviruses [14]).

The non-segmented negative strand (NNS) viruses use a distinct RNA capping pathway (Figure 2a). The most studied NNS, VSV, codes for a large (L) protein performing both replication/transcription and capping of viral RNA [15]. The cap synthesis is ensured by a polyribonucleotidyltransferase (PRNTase), which forms a covalent link between a conserved histidine and the nascent viral mRNA. In the presence of GDP, the cap structure is formed and the MTase domain in C terminus of the L protein methylates the cap structure at the ribose 2′*O* position of the first transcribed nucleotide, followed by the cap-guanine at its N7 position.

Togaviridae also synthesize a cap structure using a non-conventional mechanism (Figure 2b). This virus family (and also bamboo mosaic virus, a plant pathogen from the related potexvirus genus) codes for an enzyme (alphavirus nsp1) that methylates the N7 of GTP and forms a covalent His-^{N7}GMP complex [16,17,18•]. The