Cap Snatching of Yeast L-A Double-stranded RNA Virus Can Operate in Trans and Requires Viral Polymerase Actively Engaging in Transcription*†

Received for publication, November 25, 2011, and in revised form, February 15, 2012 Published, JBC Papers in Press, February 24, 2012, DOI 10.1074/jbc.M111.327676

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Background: L-A virus has a novel cap-snatching mechanism to furnish its transcripts with caps.

Results: Cap snatching can operate in trans and requires the viral polymerase active in transcription.

Conclusion: Coordination between capping and transcription ensures an efficient expression of viral proteins when the polymerase is active.

Significance: There is communication between the outer capping site and the inner polymerase across the capsid layer.

Eukaryotic mRNA bears a cap structure (m7GpppX-) at the 5′ terminus crucial for efficient translation and stability. The yeast L-A double-stranded RNA virus furnishes its mRNA with this structure by a novel cap-snatching mechanism in which the virus transfers an m7Gp moiety from host mRNA to the diphosphorylated 5′ terminus of the viral transcript, thus forming on it an authentic cap structure (referred to as cap0) in the budding yeast. This capping reaction is essential for efficient viral expression. His-154 of the capsid protein Gag is involved in the cap transfer. Here we show that the virus can utilize an externally added viral transcript as acceptor in the capping reaction. The acceptor needs to be 5′ diphosphorylated, consistent with the fact that the viral transcript bears diphosphate at the 5′ terminus. A 5′ triphosphorylated or monophosphorylated transcript does not function as acceptor. N7 methylation at the 5′ cap guanine of mRNA is essential for cap donor activity. We also demonstrate that the capping reaction requires the viral polymerase actively engaging in transcription. Because the cap-snatching site of Gag is located at the cytoplasmic surface of the virion, whereas Pol is confined inside the virion, the result indicates coordination between the cap-snatching and polymerization sites. This will allow L-A virus to efficiently produce capsid proteins to form new virions when Pol is actively engaging in transcription. The coordination may also minimize the risk of accidental capping of nonviral RNA when Pol is dormant.

The 5′ cap structure (m7GpppX-) is a hallmark of eukaryotic mRNA crucial for efficient translation and stability (1–3). In cells and for most viruses, the cap structure is installed on mRNA co-transcriptionally by three sequential catalytic reactions (4, 5): removal of the 5′ γ-phosphate by RNA triphosphatase, transfer of GMP from GTP to the diphosphorylated 5′ end by guanylyltransferase, and methylation of added GMP by methyltransferase. Guanylyltransferase forms a covalent bond with GMP through Lys before transferring it to Pol II transcripts. There are some variations from this conventional capping scheme. In vesicular stomatitis virus, L protein covalently binds the 5′ monophosphorylated pre-mRNA and transfers the bound pre-mRNA to GDP (6). Then methylation at the 5′ end follows (7). Members of the alphavirus-like superfamily methylate GTP first and then transfer the m7Gp moiety to the 5′ diphosphate end of viral transcripts (8). In addition to the de novo synthesis of the cap structure, some RNA viruses steal this structure from host mRNA and furnish their mRNA with the stolen cap (cap snatching). In influenza virus, the trimeric viral polymerase binds host mRNA, cleaves the RNA endonucleolytically 10–13 nt downstream, and utilizes the capped fragment as a primer to synthesize its transcript (9–11). Negative strand RNA viruses and ambiviruses (the Orthomixoviridae, Bunyaviridae, and Arenaviridae families) have been known to use this strategy to furnish their mRNA. Recently we have found that the yeast L-A double-stranded RNA virus synthesizes capped transcripts by a novel cap-snatching mechanism (12). The virus only transfers m7Gp from host mRNA to the diphosphorylated 5′ end of the viral transcript, thus conserving the 5′ α- and β-phosphates of the transcript in the triphosphate linkage of the final product (see Fig. 1A). Furthermore, unlike influenza virus, L-A virus utilizes the capsid protein Gag rather than Pol to catalyze the reaction.

The totivirus L-A, which infects the yeast Saccharomyces cerevisiae, has a nonsegmented double-stranded RNA genome of 4.6 kb (13). The genome contains two overlapping genes gag and pol that can be decoded into two proteins, Gag (76 kDa) and Gag-Pol (170 kDa) (14). The latter is made by a −1 ribosomal frameshifting mechanism (15). The genome is packed inside of a 39-nm icosahedral capsid consisting of 60 asymmetric Gag dimmers, in which one or two Gag molecules are replaced by Gag-Pol. The N-terminal Pol region is necessary for genome packaging, although Gag alone is sufficient to form morphologically normal capsids (16).

* This work was supported by Grant RFUE2010-15768 from the Spanish Ministry of Education and Science and by Fundación Ramón Areces.
† This article contains supplemental Fig. S1.
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2 The abbreviation used are: nt, nucleotides; BAP, bacterial alkaline phosphatase; γ-S-ATP, adenosine 5′-[γ-thio]triphosphate; β-S-ADP, adenosine 5′-[β-thio]diphosphate.
**L-A Virus Cap Snatching in trans**

Isolated L-A virions have transcriptase activity and synthesize positive strand transcripts conservatively (17). The transcripts bear diphosphate at their 5' ends (18). When transcription is primed with GMP, the 5' β-phosphate of the transcript is derived from the γ-phosphate of the ATP present in the reaction. It has been known that L-A virions bind the cap structure of host mRNA and cleave it to form an m7Gp-Gag adduct through His-154 (19, 20). Previously we speculated that the m7Gp-Gag adduct is an intermediate of the cap transfer reaction and tested this hypothesis. We demonstrated that L-A virions could transfer m7Gp from mRNA to the 5' end of the viral transcript, thus forming an authentic cap0 structure on it (12). This activity was impaired by the Arg-154 mutation of Gag. Furthermore, the synthesis of capped viral transcripts in vivo and their expression were greatly compromised by the mutation, indicating the involvement of Gag in the cap-snatching reaction (12).

Here we demonstrate that L-A virus can utilize exogenously added viral transcripts as cap acceptors in the cap-snatching reaction. A diphosphate status at the viral 5' end is essential for the acceptor activity. Furthermore, the cap-snatching activity requires the viral polymerase actively engaging in transcription, indicating coordination between the cap-snatching and RNA polymerization sites in the virion.

**EXPERIMENTAL PROCEDURES**

**Cap-snatching Reaction**—The standard cap-snatching reaction in trans (25 μl) contained 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 0.1 mM EDTA, 20 mM NaCl, 5 mM KCl, 0.5 mM ATP, 0.5 mM m7GpppG, 20% PEG 4000, bentonite (4.5 mg/ml), and [32P]-labeled 16-mer (5000–10,000 cpm). The reaction was kept at 30 °C for 20–30 min. The products were extracted with phenol, precipitated with ethanol, and separated on an 8% urea/15% acrylamide gel. The gel was run in 0.5× TBE (21) at 16 V/cm for 2 h 30 min. The products were visualized by autoradiography. The 32P-cap acceptor was labeled with either [α-32P]GTP or [32P]-UTP by L-A virions in a CTP-omitted transcription reaction (18) and purified through an acrylamide gel. The cap-snatching reaction in cis is the same as the standard reaction in trans described above, except that m7GpppG and the 32P-labeled 16-mer were replaced by GTP (or GDP) and UTP, 0.5 mM each, and a 32P-labeled 5-nt cap donor (3000–5000 cpm; 0.25–0.42 pmol). The reaction was kept at 30 °C for 1 h, and the products were analyzed as described above. The 5-nt cap donor was synthesized using the mScript mRNA production system (Epicenter) as described (12). Each experiment was carried out at least twice. The variation observed between experiments was less than 10%.

**SP6 Transcription**—5' tri-, di-, and monophosphorylated 16-mers were synthesized from pTF42 by SP6 RNA polymerase using GTP, GDP, and GMP, respectively, as primers. The reaction contained 40 mM Tris-HCl, pH 7.9, 6 mM MgCl2, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 0.5 μg of PvuII-digested pTF42, 20 units of RNasin, 20 μM [α-32P]UTP (20 μCi), 0.5 mM ATP, 0.5 mM GDP, 0.5 mM GTP, 0.5 mM m7GTP, 0.5 mM m7GDP, 0.5 mM GpppG, and 0.5 mM GpppG. The labeled 16-mer was purified from an acrylamide gel and then incubated with L-A virions in a transcription mixture to which the cap analog m7GpppG was added as cap donor. As shown in Fig. 2A, the capped product appeared in the first 5-min incubation, and cap transfer was completed within

**RESULTS**

**Cap Snatching in trans**—Previously we demonstrated that the m7Gp moiety from a cap donor was transferred to the 5' end of the L-A viral transcript to form an inverted 5'-5' triphosphate linkage (cap snatching in cis). In this work, we show that m7Gp can also be transferred to an externally added viral transcript (cap snatching in trans). B, the nucleotide sequence of L-A transcript at the 5' end. The first C appears at position 17. L-A virion synthesizes 16-nt transcript (16-mer) when CTP is omitted from the transcription reaction. The 16-mer contains a single G at the 5' end.

**Miscellaneous**—TLC was done as described (18). L-A virions were purified from yeast strain TF229 (Mata his(3,4) leu2 ski2–2 L-A-HN) as described (23). Radioactive nucleotides were obtained from PerkinElmer Life Sciences. RNA 5'-polynucleotides were from Epicenter. S1 nuclease and RNAse were from Promega, and bacterial alkaline phosphatase was from Invitrogen. Tobacco acid pyrophosphatase, γ-S-GTP, β-S-GDP, m7GTP, and m7GDP were from Sigma-Aldrich. m7GpppG was from Ambion, and GpppG was from Amersham Biosciences.
Cap Snatching in trans

The 16-nt transcript labeled with \([\alpha-32P]GTP\) was used as cap acceptor. A, time course. The incubation times are indicated below the panel. B, dose response of cap snatching to the cap donor \(m^7\text{GpppG}\). The concentrations of the donor used are indicated below the panel. C, BAP resistance of the capped product. The 16-mer after capping reaction (Capped) was treated without (--) or with BAP (+) and separated on a denaturing acrylamide gel. The 16-mer without capping reaction (Acceptor) was run in parallel. D, proof of capping. BAP-resistant capped product (Capped) was gel-purified, treated with the enzymes as indicated below the panel, and analyzed on polyethyleneimine-cellulose chromatography with 0.3 M \((\text{NH}_4\)\)_2SO_4 as solvent. In lane 1, the acceptor was incubated in the absence of L-A virions (--) as control. \(m^7\text{GpppG}\) is the smallest cap donor. The capping reaction was carried out in the presence of 0.5 mM \(m^7\text{GpppG}\) or GpppG or in their absence (--) as indicated below the panel, along with NTPs (0.5 mM each) as indicated above the panel. The 16-nt transcript labeled with \([\alpha-32P]GTP\) was used as cap acceptor. In lane 6, 10 mM EDTA was also added to the reaction. In lane 1, the acceptor was incubated in the absence of L-A virions (--) as control. In lane 6, the capped product was treated with tobacco acid pyrophosphatase (TAP) first, then extracted with phenol, and finally digested again with S1 nuclease. Noncapped 16-mer (Acceptor) was also processed in parallel as control. The mobility of nonlabeled nucleotides is indicated at the left. The deduced transfer reaction is shown on the left with \(S1\) and tobacco acid pyrophosphatase cleavage sites.

Cap Snatching Requires Viral Polymerase Actively Engaging in Transcription—We examined the effect of nucleoside triphosphates in the capping reaction and found that only ATP could promote cap snatching. The other three nucleoside triphosphates did not support the reaction (Fig. 5A). There are three possible roles of ATP for cap snatching: (i) ATP may promote the reaction as an effector without being modified, (ii) the

Requirements for cap snatching in trans

A, N7 methylation of cap is required for cap donor activity. The cap-snatching reaction was carried out in the presence of 0.5 mM \(m^7\text{GpppG}\) or GpppG or in their absence (--) as indicated below the panel, along with NTPs (0.5 mM each) as indicated above the panel. The 16-nt transcript labeled with \([\alpha-32P]GTP\) was used as cap acceptor. In lane 6, 10 mM EDTA was also added to the reaction. In lane 1, the acceptor was incubated in the absence of L-A virions (--) as control. \(m^7\text{GpppG}\) is the smallest cap donor. The capping reaction was carried out in the presence of 0.5 mM \(m^7\text{GpppG}\) (lane 3), \(m^7\text{GTP}\) (lane 4), or \(m^7\text{GpppG}\) (lane 5) as cap donor. In lane 1, 16-nt transcript (Acceptor) was incubated in the absence of L-A virions (--) as control. The arrowheads indicate capped products.

30 min. The cap-snatching reaction was saturated with the high concentration of \(m^7\text{GpppG}\) used (1 mM) (Fig. 2B). We confirmed that the product was 5'-capped (Fig. 2, C and D). The acceptor contains \(^{32}\text{P}\) at the \(\alpha\)-position in the 5'-diphosphate and thus was susceptible to BAP treatment, whereas the capped product was resistant to the enzyme (Fig. 2C). After BAP treatment, the capped product was purified from the acrylamide gel and analyzed by TLC (Fig. 2D). Nuclease S1 digested the RNA body but could not work on the triphosphate linkage; thus \(m^7\text{GpppG}\) was released from the product (Fig. 3A). In the absence of L-A virions (--), \(m^7\text{GpppG}\) or GpppG did not work as cap donor (Fig. 3B, lanes 3 and 4); thus the smallest molecule with donor activity is \(m^7\text{GpppG}\).

Cap Accepting Activity Requires 5' Diphosphate—The 16-mer made by L-A virions in the absence of CTP bears diphosphate at the 5' end. To examine the role of the phosphate status at the 5' end in the cap transfer reaction, the 16-mer was treated with RNA 5' polyphosphatase to eliminate the 5' β-phosphate (Fig. 4A). Then the 5' monophosphorylated 16-mer was incubated with L-A virions for cap transfer reaction. As shown in Fig. 4B, lane 4, the 5' monophosphorylated molecule did not function as cap acceptor. We also synthesized the 5' tri-, di-, and monophosphorylated 16-mer using SP6 RNA polymerase with GTP, GDP, and GMP, respectively, as primers. Only diphosphorylated 16-mer formed capped products, whereas tri- or monophosphorylated molecules did not function as cap acceptors (Fig. 4C). Methylation at the 5' terminal G did not significantly affect cap acceptor activity in the 5' diphosphorylated molecule (Fig. 4D, lanes 4 and 6). The 5' triphosphorylated 16-mer with the terminal G N7-methylated showed no cap acceptor activity (Fig. 4D, lane 2).
hydrolysis of ATP to ADP may be required for the reaction, and
(iii) because L-A virions can incorporate m7GpppG as a primer
into transcripts (18), the presence of ATP will allow the virion
to synthesize a short transcript (m7GpppGAAAAA-OH). This
activity may somehow promote the cap-snatching reaction.
Here we analyze these possible roles of ATP.

\[\text{H9253}\text{-S-ATP could}
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\[\text{H9253}\text{-S-ATP, however, can be used as substrate for RNA}
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\[\text{polymerization (24). In fact, L-A virions can utilize this analog}
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\[\text{as efficiently as ATP for transcription (Fig. 5, lanes 3}
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\[\text{and 4). Thus these results are consistent with the involvement of trans-
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\[\text{cription in the cap-snatching reaction. Interestingly ADP,}
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\[\text{although less active than ATP, could also promote cap snach-
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\[\text{ting (Fig. 5B, lane 5). Furthermore, ADP also supported trans-
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\[\text{cription, although it is less potent than ATP (Fig. 5C, lanes 3}
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\[\text{and 5). These results again point out the importance of trans-
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\[\text{cription in cap transfer. It has been known that L-A virions are}
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\[\text{associated with nucleoside diphosphate kinase activity (25). Be-
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\[\text{cause no nucleoside triphosphates were added to the reac-
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\[\text{tion, this kinase activity could not be accountable for ATP gen-
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\[\text{eration from ADP. The data thus suggest that L-A virions are}
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\[\text{also associated with a myokinase-like activity. \beta-S-ADP did not}
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\[\text{support either cap snatching or transcription (Fig. 5, B and}
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\[\text{C, lanes 6), presumably because this hypothetical myokinase-like}
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\[\text{activity cannot utilize \beta-S-ADP to generate ATP.}
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The data presented above are consistent with the involvement of transcription and disfavor the role of ATP hydrolysis or ATP as an effector in cap snatching. In the next experiments (Fig. 6), we tried to prove the importance of transcription in cap transfer. A nonlabeled 16-mer (cap acceptor) synthesized by L-A virions was gel-purified and incubated again with L-A virions along with a 5-nt cap donor $^{32}$P-labeled at the $\gamma$-phosphate. L-A virions cannot incorporate this 5-nt cap donor as primer into transcripts as demonstrated previously (12). Cap transfer to the acceptor molecule did not occur in the presence of ATP (or GTP) alone (Fig. 6, lanes 3 and 4). This eliminates the role of ATP hydrolysis or ATP as effector in the cap-snatching reaction. Because L-A virions required guanine nucleotides as primers for transcription, ATP alone cannot drive transcription. Therefore, the failure of ATP alone to promote cap snatching is consistent with the involvement of transcription in the reaction. In fact, that ATP could support cap transfer reaction only when GTP, an initiator of transcription, was also present (Fig. 6, lane 5), verifies this notion. The omission of the 16-nt acceptor from the reaction abolished the formation of the 17-nt capped product (Fig. 6, lane 6), confirming that the added acceptor was converted to the capped product. Therefore, these data clearly eliminate the role of ATP hydrolysis or ATP as effector and directly support the involvement of transcription in cap snatching.

**Effects of Modifications at the 5’ Terminus of Acceptor on Cap Snatching in cis**—We also examined the effects of modifications at the 5’-terminus of the 16-mer on transcription-coupled cap-snatching reaction (cap transfer in cis). The 5-nt capped fragment, $^{32}$P-labeled at the $\gamma$-phosphate, was used as cap donor. When $\beta$-S-GDP was used as primer to synthesize the 16-nt transcript, L-A virions failed to form the cap structure on it (Fig. 7A, lane 3). Because this GDP analog could initiate transcription as effectively as ADP (Fig. 7B, lanes 2 and 3), the result indicates that the modification at the $\beta$-phosphate with a thiol group specifically blocked m$^7$Gp transfer reaction. Unexpectedly, the incorporation of m$^7$GDP at the 5’ terminus of the acceptor greatly enhanced the cap transfer reaction (Fig. 7A, lane 5, and supplemental Fig. S1B). This nucleotide could initiate transcription less efficiently than GDP (Fig. 7B, lanes 2 and 5); therefore, an increase in transcription was not the cause for this enhancement. By contrast, we observed that the same N7 modification did not significantly affect the cap-snatching reaction in trans (Fig. 4D, lanes 4 and 6). In the latter experiments, the modified acceptors had been synthesized by SP6 RNA polymerase. However, this lack of stimulation was not caused by artifacts related with SP6 transcription, because m$^7$GDP-primed 16-mer synthesized by L-A virions, once isolated, did not show an enhanced acceptor activity any longer in the cap-snatching reaction in trans (supplemental Fig. S1A). Therefore, the N7 methylation at the 5’ terminal G of the transcript has differential effects on the cap-snatching reactions in cis and in trans, although the final capped products are chemically identical in both reactions. Finally, the transcripts primed with m$^7$GTP also showed an enhanced acceptor activity in cis (Fig. 7A, lane 4). Because L-A virions cannot utilize 5’ triphosphorylated transcripts as cap acceptors irrespective of N7 methylation at their terminal G (Fig. 4, C and D), this result indicates that the 5’ end of the transcript was converted to diphosphate first and then accepted m$^7$Gp from the donor to form the cap structure. Furthermore, we observed that even the 6-nt molecule (m$^7$GAAAAA-OH) acquired the cap structure (Fig. 7A,
L-A Virus Cap Snatching in trans

lane 4). Therefore, the diphosphate status of the viral transcripts is secured at a very early stage of transcription, and N7 methylation at the 5′ terminal G does not inhibit the establishment of this diphosphate status (or at least the conversion from triphosphate to diphosphate).

DISCUSSION

L-A virus synthesizes its capped mRNA by a novel cap-snatching mechanism in which the m7Gp moiety of host mRNA is transferred to the diphosphorylated 5′ end of the viral transcript to form an inverted 5′-5′ triphosphate linkage. In this work, we have found that an exogenously added viral transcript can be capped by L-A virions using the dinucleotide cap analog m7GpppG as cap donor. This allowed us to analyze the capping reaction in detail. L-A virions utilize 5′ diphosphorylated viral transcripts but not tri- or monophosphorylated ones as cap acceptors. This is consistent with the fact that L-A synthesizes its transcripts with 5′ diphosphate even when transcription is primed with GTP or GMP (18). The results also indicate that L-A cannot convert an externally added transcript with tri- or monophosphate at the 5′ end to diphosphate. Furthermore, the conversion of m7GTP-primed transcript from triphosphate at the 5′ end to diphosphate is established at the very early stage of transcription. These results suggest that the diphosphate status of viral transcripts is accomplished inside of the virion, presumably by RNA triphosphatase and RNA monophosphate kinase activities. Alternatively, L-A virion may convert GTP (or m7GTP) or GMP to GDP (or m7GDP) first and then preferentially utilizes this GDP (or m7GDP) as primer for transcription.

Interestingly, the cap-snatching reaction required the viral polymerase actively engaging in transcription. Although ATP alone could support cap transfer, it was because the cap donor m7GpppG in the reaction was also utilized as a primer for transcription, resulting in the synthesis of the short transcript m7GpppGAAAAA-OH. γ-S-ATP could drive cap transfer and transcription as efficiently as ATP. Thus the hydrolysis of ATP to ADP is not required for cap snatching. In confirmation, we used an oligonucleotide cap donor to avoid the dual roles of the dinucleotide cap analog m7GppG. The viral polymerase does not incorporate this oligonucleotide donor into transcripts as a primer. Now ATP alone could not drive cap snatching any longer. The reaction requires the presence of GTP, a priming nucleotide of transcription, in addition to ATP. These results indicate cooperation between the cap-snatching and transcription reactions.

Crystallographic studies of L-A virions have identified a trench on the outer surface of Gag that includes His-154 (26, 27). The trenches are located in the Gag asymmetric dimer close to the icosahedral 2- and 3-fold symmetric axes. L-A transcripts are made inside the virion and presumably released to the cytoplasm through one of the pores located at the 5-fold axes (26). Because the Pol domain of Gag-Pol is tethered to the inner wall of the capsid, transcription causes the motion of double-stranded RNA template inside the virion. This may temporarily activate the cap-snatching sites at unspecified locations in the virion. Alternatively, the Pol domain engaging in transcription may activate the cap-snatching site of the Gag-Pol fusion protein through the peptide bond. The passage of the transcript through the pore may also activate the cap-snatching sites in Gags surrounding the 5-fold axis. In the latter two cases, we expect that the activated sites are located near the pore from which the transcript emerges. His-154 is located at the tip of a loop (residues 144–163) that is part of the upper rim of the trench. Upon m7Gp binding, the rim moves inwardly and forms a closed conformation (27). Guanylyltransferase also contains a trench that can adopt either open or closed conformations during the mRNA capping reaction (28, 29). Guanylyltransferase forms a Gp–enzyme intermediate with GTP and transfers Gp to the diphosphorylated 5′ termini of PolII transcripts to generate a nonmethylated cap structure. The cap-snatching reaction of L-A, therefore, resembles that of guanylyltransferase.

On the analogy of guanylyltransferase (28), L-A cap snatching may proceed as follows (Fig. 8). In an open conformation, Gag binds host mRNA, and then in a closed state decapping occurs. In the next open state, the decapped mRNA is released, and m7Gp remains attached to Gag by a covalent bond through His-154. In this open conformation, Gag binds the viral transcript emerging from a pore. In the following closed conformation, the transfer of m7Gp to the 5′ end of the transcript is accomplished. Finally, the capped viral transcript is released in the next open state. The hydrolysis of anhydrous phosphate bonds does not occur in this ping-pong mechanism. Thus the reaction will be reversible and does not require ATP hydrolysis. The activation of cap snatching by transcription may be accomplished by facilitating the transition between the open and closed conformations. Alternatively, binding of Gag to host

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\text{H154-pm7G} \rightarrow \text{Gag} \rightarrow \text{L-A transcript} \rightarrow \text{Capped L-A transcript}
\]
mRNA may be activated by transcription to initiate the subsequent reactions. This ping-pong mechanism may explain why N7 methylation at the 5′ terminus of the viral transcript stimulated cap snatching in cis. The most prominent feature of cap binding proteins is the presence of two aromatic amino acids in their cap-binding pockets (30, 31). The aromatic rings of these amino acids sandwich the m7G aromatic ring of the cap structure by stacking. The delocalization of the positive charge arising from the N7 methylation of the guanine contributes to strong interactions between the π-electrons of the stacked aromatic rings (32). L-A cap snatching requires cap donors to be N7-methylated (Fig. 3A) (12). Furthermore, in the trench, Tyr-452 and Tyr-538 sandwich m7GDP by a potential base stacking (27). If the viral transcript interacts with the same cap-binding pocket for subsequent m7Gp transfer, then the N7 methylation at the 5′ terminal guanine is expected to enhance the interactions. The same methylation of a viral transcript, however, did not stimulate cap snatching in trans. This needs another explanation. It may be envisaged that in the reaction in cis an emerging transcript, still anchored at the 3′ end in the pore, interacts at the 5′ end with the cap-snatching site. Furthermore, if transcription activates the cap snatching sites near the pore as postulated earlier, then the emerging transcript finds the activated sites at propinquity. By contrast, an added transcript encounters the site in a random orientation. Moreover, the transcript encounters the trenches of all 120 Gag molecules in the virion with equal probability. In addition to these entropic disadvantages, the added transcript has to compete for capping reaction with an emerging transcript made by the virion in situ. In the reaction in trans these factors might have masked the stimulatory effect of the 5′ N7 methylation in the acceptor molecule.

The physical separation of the cap-snatching site from the transcription site may ensure that not all of transcripts are capped. This may allow L-A virus to synthesize two types of transcripts: one an unmodified transcript presumably destined for encapsidation (genomic single-stranded RNA) and the other a capped transcript for translation (mRNA). The expression of M1, a satellite RNA of the L-A virus, is compromised by the Gag mutation Arg-154, whereas the maintenance of M1 is not affected by the mutation (12, 20). Therefore, encapsidation and the two subsequent polymerization reactions inside the virion, replication and transcription, do not require the capping of viral transcript. L-A virus may actively participate in the differentiation of the transcript through coordination between transcription and cap snatching. When the virus is actively synthesizing transcripts, it will also need the capsid proteins to be produced efficiently to form new virions. Furthermore, when POL is dormant, this coordination may shut off the cap-snatching site, so that the virus minimizes the accidental capping of nonviral RNA, which may be harmful for the host. Because L-A has no extracellular transmission pathway, a profound damage to the host will be a disadvantage for its own survival. It is scarcely known how RNA with 5′ diphosphate is generated in the cytoplasm. The CTL1 gene product has RNA triphosphatase activity in vitro (33). It localizes in the cytoplasm, but its physiological role is unknown. L-A virions isolated from cell Δ cells could produce transcripts with 5′ diphosphate even when transcription was primed with GTP (18). Scavenger decapping enzyme removes m7Gp from dinucleotide or oligonucleotide capped molecules, leaving the 3′ fragment with 5′ diphosphate (34, 35). Most laboratory strains of yeast harbor, in addition to L-A, another totivirus called L-BC. Gag of L-BC virus also shares the same (or similar) amino acids crucial for decapping, including His-154, at comparative positions (12). Gag of L-BC has decapping activity (19). It is likely that L-BC virus possesses a cap-snatching system similar to L-A’s and that the transcript of L-BC also bears diphosphate at the 5′ terminus. The transcripts of L-A and L-BC share the same three nucleotides at the 5′ ends (5′-GAA . . . ). It will be interesting to see whether the L-A cap-snatching site can utilize L-BC transcript as cap acceptor or vice versa. Their cohabitation in the same host may be beneficial for both viruses. Alternatively the viruses may avoid cross-capping by distinguishing self from non-self transcripts that may lack specific cap-snatching signals or simply by preferentially performing capping in cis over capping in trans in the in vivo environment.

**REFERENCES**

1. Muthukrishnan, S., Both, G. W., Furuichi, Y., and Shatkin, A. J. (1975) 5′-Terminal 7-methylguanosine in eukaryotic mRNA is required for translation. *Nature* **255,** 33–37
2. Furuichi, Y., LaFiandra, A., and Shatkin, A. J. (1977) 5′-Terminal structure and mRNA stability. *Nature* **266,** 235–239
3. Martin, S. A., and Moss, B. (1975) Modification of RNA by RNA guanylyltransferase and mRNA (guanine-7-)methyltransferase from vaccinia virions. *J. Biol. Chem.* **250,** 9330–9335
4. Venkatesan, S., Gershowitz, A., and Moss, B. (1980) Modification of the 5′ end of mRNA. Association of RNA triphosphatase with the RNA guanylyltransferase-RNA (guanine-7-)methyltransferase complex from vaccinia virus. *J. Biol. Chem.* **255,** 903–908
5. Shuman, S. (1995) Capping enzyme in eukaryotic mRNA synthesis. *Prog. Nucleic Acid Res. Mol. Biol.* **50,** 101–129
6. Ogino, T., Yadav, S. P., and Banerjee, A. K. (2010) Histidine-mediated RNA transfer to GDP for unique mRNA capping by vesicular stomatitis virus RNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 3463–3468
7. Rahme, A. A., Li, J., Kranzusch, P. J., and Whelan, S. P. (2009) Ribose 2′-O-methylation of the vesicular stomatitis virus mRNA cap precedes and facilitates subsequent guanine-N-7 methylation by the large polymerase protein. *J. Virol.* **83,** 11043–11050
8. Huang, Y. L., Hsu, Y. H., Han, Y. T., and Meng, M. (2005) mRNA guanylation catalyzed by the S-adenosylmethylone-dependent guanylyltransferase of bamboo mosaic virus. *J. Biol. Chem.* **280,** 13153–13162
9. Plotch, S. J., Bouloy, M., Ulmanen, I., and Krug, R. M. (1981) A unique cap(mGpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell* **23,** 847–858
10. Guilligay, D., Tarendeau, F., Resa-Infante, P., Coloma, R., Crepin, T., Sehr, P., Lewis, J., Ruigrok, R. W., Ortin, J., Hart, D. J., and Cusack, S. (2008) The structural basis for cap binding by influenza virus polymerase subunit PB2. *Nat. Struct. Mol. Biol.* **15,** 500–506
11. Boivin, S., Cusack, S., Ruigrok, R. W., and Hart, D. J. (2010) Influenza A virus polymerase. Structural insights into replication and host adaptation mechanisms. *J. Biol. Chem.* **285,** 28411–28417
12. Fujimura, T., and Esteban, R. (2011) Cap-snatching mechanism in yeast L-A double-stranded RNA virus. *Proc. Natl. Acad. Sci. U.S.A.* **108,** 17667–17671
13. Wickner, R. B. (2007) in *Fields Virology* (Knipe, D. M., and Howley, P. M.) 5th Ed., pp. 737–768, Lippincott Williams and Wilkins, Philadelphia, PA
14. Icho, T., and Wickner, R. B. (1989) The double-stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. *J. Biol. Chem.* **264,** 6716–6723
15. Dinman, J. D., Icho, T., and Wickner, R. B. (1991) A 1 ribosomal frameshift in a double-stranded RNA virus of yeast forms a gag-pol fusion pro...
tein. Proc. Natl. Acad. Sci. U.S.A. 88, 174–178
16. Fujimura, T., Ribas, J. C., Makhov, A. M., and Wickner, R. B. (1992) Pol of
gag-pol fusion protein required for encapsidation of viral RNA of yeast
L-A virus. Nature 359, 746–749
17. Fujimura, T., Esteban, R., and Wickner, R. B. (1986) In vitro L-A double-
stranded RNA synthesis in virus-like particles from Saccharomyces cerevi-
siae. Proc. Natl. Acad. Sci. U.S.A. 83, 4433–4437
18. Fujimura, T., and Esteban, R. (2010) Yeast double-stranded RNA virus
L-A deliberately synthesizes RNA transcripts with 5'-diphosphate. J. Biol.
Chem. 285, 22911–22918
19. Blanc, A., Goyer, C., and Sonenberg, N. (1992) The coat protein of the
yeast double-stranded RNA virus L-A attaches covalently to the cap struc-
ture of eukaryotic mRNA. Mol. Cell. Biol. 12, 3390–3398
20. Blanc, A., Ribas, J. C., Wickner, R. B., and Sonenberg, N. (1994) His-154 is
involved in the linkage of the Saccharomyces cerevisiae L-A double-
stranded RNA virus Gag protein to the cap structure of mRNAs and is
essential for M1 satellite virus expression. Mol. Cell. Biol. 14, 2664–2674
21. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A
Laboratory Manual, pp. 156, Cold Spring Harbor Laboratory. Cold Spring
Harbor, NY
22. Esteban, R., Fujimura, T., and Wickner, R. B. (1988) Site-specific binding
of viral plus single-stranded RNA to replicase-containing open virus-like
particles of yeast. Proc. Natl. Acad. Sci. U.S.A. 85, 4411–4415
23. Fujimura, T., and Wickner, R. B. (1992) Interaction of two cis sites with the
RNA replicase of the yeast L-A virus. J. Biol. Chem. 267, 2708–2713
24. Shuman, S., Spencer, E., Furneaux, H., and Hurwitz, J. (1980) The role of
ATP in in vitro vaccinia virus RNA synthesis effects of AMP-PNP and
ATPγS. J. Biol. Chem. 255, 5396–5403
25. Georgopoulos, D. E., and Leibowitz, M. I. (1987) Nucleotide phospho-
transferase, nucleotide kinase and inorganic pyrophosphatase activities of
killer virions of yeast. Yeast. 3, 117–129
26. Naitow, H., Tang, J., Canady, M., Wickner, R. B., and Johnson, J. E. (2002)
L-A virus at 3.4 A resolution reveals particle architecture and mRNA
decapping mechanism. Nat. Struct. Biol. 9, 725–728
27. Tang, J., Naitow, H., Gardner, N. A., Kolesar, A., Tang, L., Wickner, R. B.,
and Johnson, J. E. (2005) The structural basis of recognition and removal
of cellular mRNA 7-methyl G 'caps' by a viral capsid protein. A unique
viral response to host defense. J. Mol. Recognit. 18, 158–168
28. Håkansson, K., Doherty, A. J., Shuman, S., and Wigley, D. B. (1997) X-ray
crystallography reveals a large conformational change during guanyl
transfer by mRNA capping enzymes. Cell 89, 545–553
29. Chu, C., Das, K., Tyminski, J. R., Bauman, J. D., Guan, R., Qiu, W., Mon-
telione, G. T., Arnold, E., and Shatkin, A. J. (2011) Structure of the guany-
lyltransferase domain of human mRNA capping enzyme. Proc. Natl. Acad.
Sci. U.S.A. 108, 10104–10108
30. Marcotrigiano, J., Gingras, A. C., Sonenberg, N., and Burley, S. K. (1997)
Cocrystal structure of the messenger RNA 5' cap-binding protein (eIF4E)
bound to 7-methyl-GDP. Cell 89, 951–961
31. Fechter, P., and Brownlee, G. G. (2005) Recognition of mRNA cap struc-
tures by viral and cellular proteins. J. Gen. Virol. 86, 1239–1249
32. Hu, G., Gershon, P. D., Hodel, A. E., and Quiocho, F. A. (1999) mRNA cap
recognition. Dominant role of enhanced stacking interactions between
methylated bases and protein aromatic side chains. Proc. Natl. Acad. Sci.
U.S.A. 96, 7149–7154
33. Rodriguez, C. R., Takagi, T., Cho, E. J., and Buratowski, S. (1999) A Sac-
charomyces cerevisiae RNA 5'-triphosphatase related to mRNA capping
enzyme. Nucleic Acids Res. 27, 2181–2188
34. Wang, Z., and Kiledjian, M. (2001) Functional link between the mamma-
lian exosome and mRNA decapping. Cell 107, 751–762
35. Liu, H., Rodgers, N. D., Jiao, X., and Kiledjian, M. (2002) The scavenger
mRNA decapping enzyme DcpS is a member of the HIT family of pyro-
phosphatases. EMBO J. 21, 4699–4708