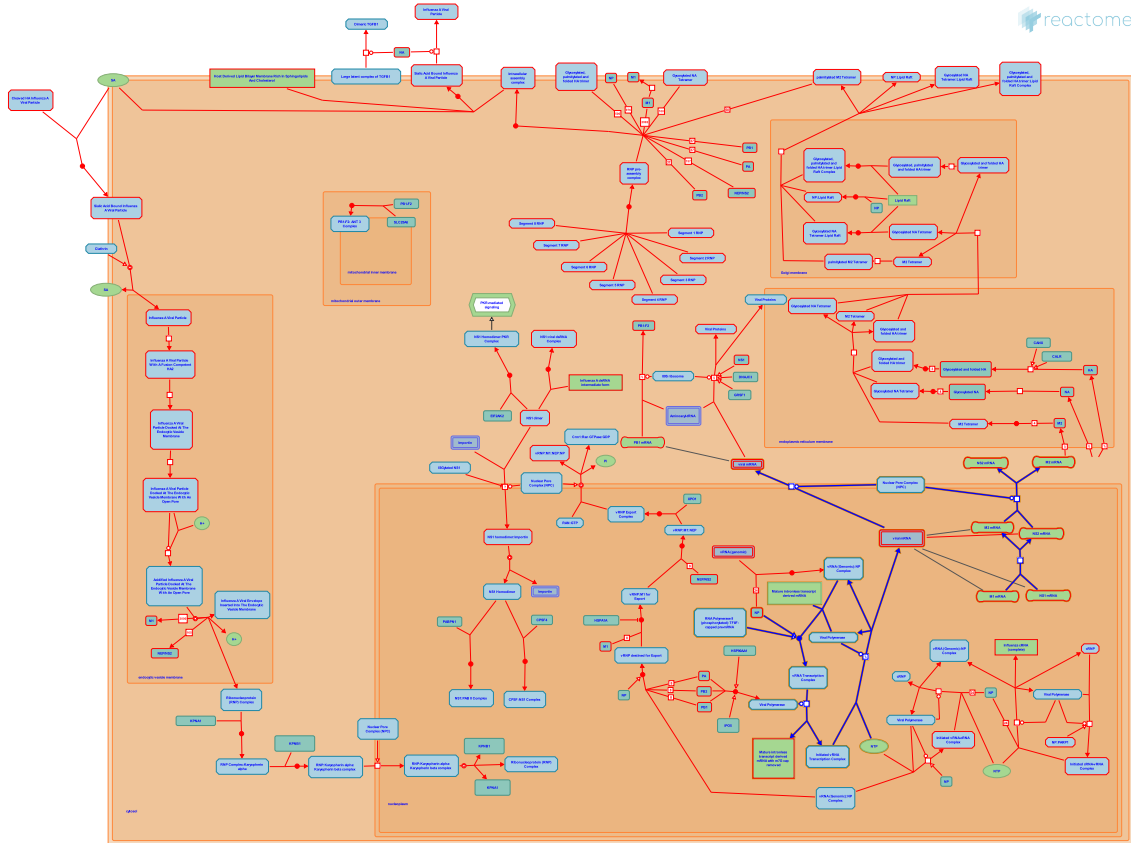


Viral Messenger RNA Synthesis



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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the [Reactome Textbook](https://reactome.org/textbook/).

03/05/2024

Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references

- Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics*, 18, 142. [↗](#)
- Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics*, 33, 3461-3467. [↗](#)
- Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res*, 46, D649-D655. [↗](#)
- Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology*, 14, e1005968. [↗](#)

Reactome database release: 88

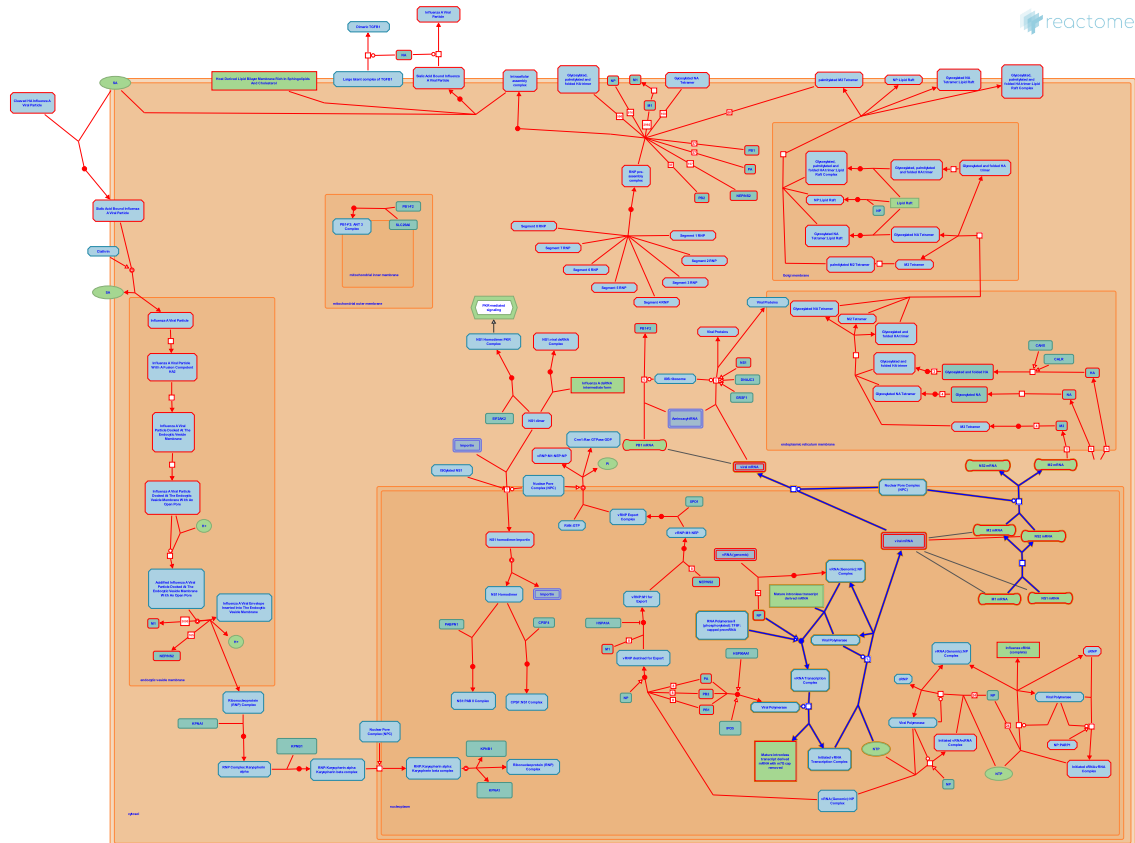
This document contains 1 pathway and 6 reactions ([see Table of Contents](#))

Viral Messenger RNA Synthesis ↗

Stable identifier: R-HSA-168325

Compartments: nucleoplasm

Diseases: influenza



Like the mRNAs of the host cell, influenza virus mRNAs are capped and polyadenylated (reviewed in Neumann, 2004). The methylated caps, however, are scavenged from host cell mRNAs and serve as primers for viral RNA synthesis, a process termed 'cap-snatching' (Krug, 1981; Hagen, 1994). The PB2 polymerase protein binds the cap, activating endonucleolytic cleavage of the host mRNA by PB1. The 3' poly-A tracts on viral messages are generated by polymerase stuttering on poly-U tracts near the 5' end of the template vRNA (Robertson, 1981; Zheng, 1999). The second process allows polyadenylation of viral mRNAs when the host cell polyadenylation process has been inhibited (Engelhardt, 2006; Amorim, 2006). Notably, early transcripts (including NP and NS1) accumulate in the cytoplasm before late transcripts (M1, HA, and NS2), and in varying abundances, suggesting additional control mechanisms regulating viral gene expression (Shapiro, 1987; Hatada, 1989; Amorim, 2006).

Literature references

- Fodor, E., Neumann, G., Brownlee, GG., Kawaoka, Y. (2004). Orthomyxovirus replication, transcription, and polyadenylation. *Curr Top Microbiol Immunol*, 283, 121-43. ↗
- Amorim, MJ., Digard, P. (2006). Influenza A virus and the cell nucleus. *Vaccine*, 24, 6651-5. ↗
- Fodor, E., Engelhardt, OG. (2006). Functional association between viral and cellular transcription during influenza virus infection. *Rev Med Virol*, 16, 329-45. ↗

Editions

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Assembly of an Active Transcription Complex ↗

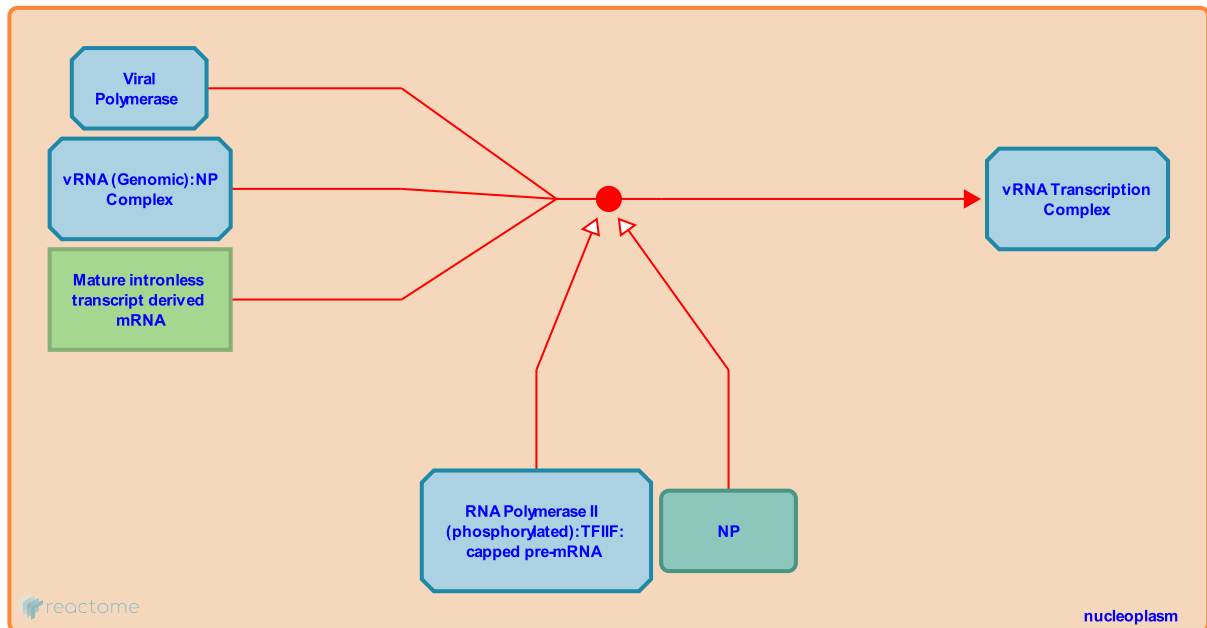
Location: [Viral Messenger RNA Synthesis](#)

Stable identifier: R-HSA-168326

Type: binding

Compartments: nucleoplasm

Diseases: influenza



The 5' end of the vRNA associates with a binding site on the PB1 subunit of the viral RNA polymerase, distinct from the 3' vRNA binding site, which is subsequently bound forming a loop. These binding events set off allosteric conformational changes in the trimeric polymerase complex that induce PB2 binding of the methylated cap on a host pre-mRNA (Plotch, 1981; Cianci, 1995; Li, 1998; Brownlee, 2002; Kolpashchikov, 2004). PB2 amino acids 242-282 and 538-577 are involved in cap binding (Honda, 1999). Direct or indirect interaction with active, transcribing host RNA polymerase II is thought to supply host mRNA for the caps (Bouloy, 1978; Engelhardt, 2005).

Followed by: [Priming and Initiation of Transcription](#)

Literature references

- Krystal, M., Tiley, L., Cianci, C. (1995). Differential activation of the influenza virus polymerase via template RNA binding. *J Virol*, 69, 3995-9. ↗
- Honda, A., Mizumoto, K., Ishihama, A. (1999). Two separate sequences of PB2 subunit constitute the RNA cap-binding site of influenza virus RNA polymerase. *Genes Cells*, 4, 475-85. ↗
- Sharps, JL., Brownlee, GG. (2002). The RNA polymerase of influenza a virus is stabilized by interaction with its viral RNA promoter. *J Virol*, 76, 7103-13. ↗
- Krug, RM., Ramirez, BC., Li, ML. (1998). RNA-dependent activation of primer RNA production by influenza virus polymerase: different regions of the same protein subunit constitute the two required RNA-binding sites. *EMBO J*, 17, 5844-52. ↗
- Plotch, SJ., Krug, RM., Bouloy, M., Ulmanen, I. (1981). A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell*, 23, 847-58. ↗

Editions

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Priming and Initiation of Transcription ↗

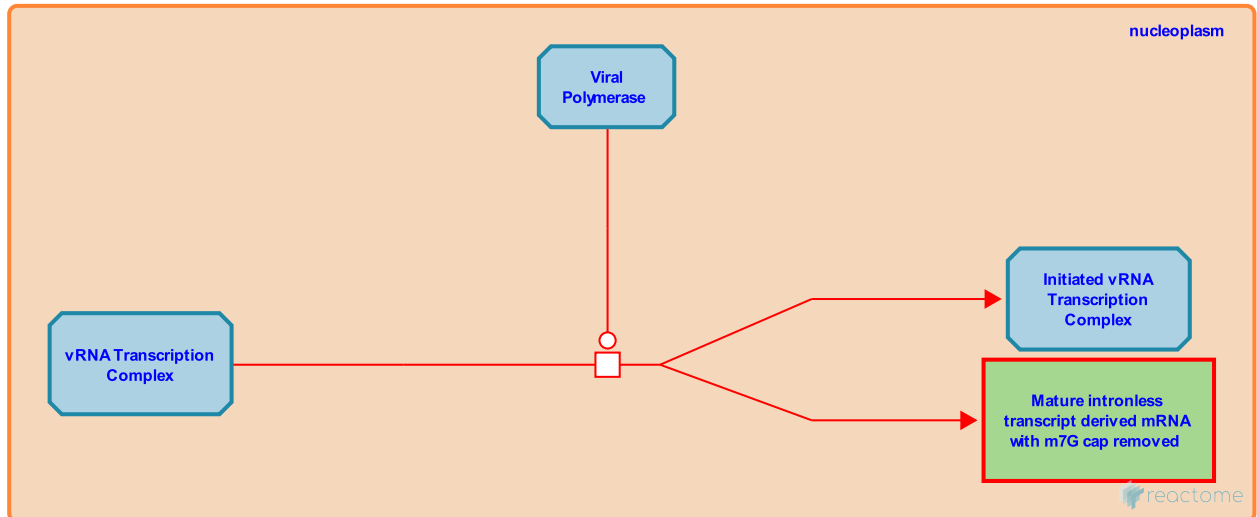
Location: [Viral Messenger RNA Synthesis](#)

Stable identifier: R-HSA-168280

Type: transition

Compartments: nucleoplasm

Diseases: influenza



The host cell mRNA bound to viral RNA polymerase PB2 subunit is cleaved by the viral RNA polymerase PB1 subunit's endonuclease activity, and the capped 5' end plus 10-13 nucleotides of the host mRNA remains bound to the polymerase complex (Plotch, 1981; Krug, 1981; Hagen, 1994; Cianci, 1995; Li, 1998; Li, 2001). Viral mRNA may be protected against cap-snatching by the polymerase complex itself, which tightly binds capped viral mRNA (Shih, 1996). A guanine residue, complementary to a cytosine in the vRNA, is added to the host-derived cap, catalyzed by the RNA polymerase activity of the PB1 viral RNA polymerase subunit (Beaton, 1981; Toyoda, 1986).

Preceded by: [Assembly of an Active Transcription Complex](#)

Followed by: [Elongation, Polyadenylation and Termination](#)

Literature references

- Krug, RM., Rao, P., Li, ML. (2001). The active sites of the influenza cap-dependent endonuclease are on different polymerase subunits. *EMBO J*, 20, 2078-86. ↗
- Chung, TD., Krystal, M., Butcher, JA., Hagen, M. (1994). Recombinant influenza virus polymerase: requirement of both 5' and 3' viral ends for endonuclease activity. *J Virol*, 68, 1509-15. ↗
- Krug, RM. (1981). Priming of influenza viral RNA transcription by capped heterologous RNAs. *Curr Top Microbiol Immunol*, 93, 125-49. ↗
- Plotch, SJ., Krug, RM., Bouloy, M., Ulmanen, I. (1981). A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell*, 23, 847-58. ↗

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Elongation, Polyadenylation and Termination ↗

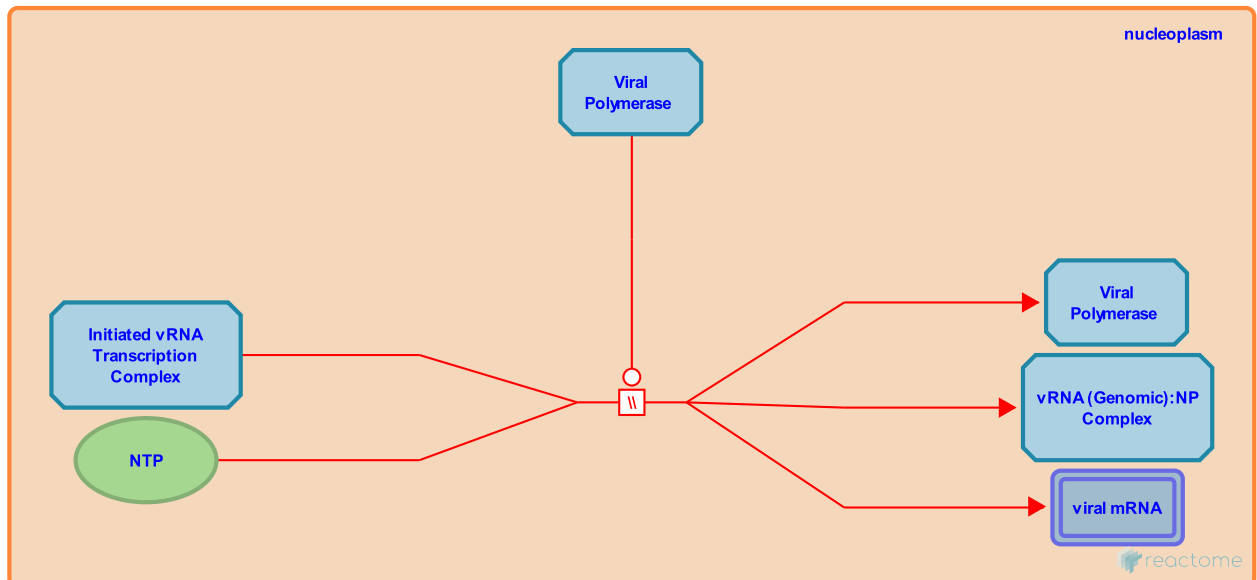
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Stable identifier: R-HSA-168301

Type: omitted

Compartments: nucleoplasm

Diseases: influenza



Catalyzed by the RNA polymerase activity of the viral PB1 subunit, an mRNA complementary to the bound vRNA is synthesized (Plotch, 1977). PA and PB2 move down the growing mRNA in complex with PB1, with PB2 possibly dissociating from the cap (Braam, 1983). However, the 5' end of the vRNA may remain bound during elongation as the template is threaded through in a 3' to 5' direction until a polyadenylation signal is encountered (Poon, 1998; Zheng, 1999).

A poly-uridine sequence motif, consisting in most cases of 5-7 U residues, abuts the "panhandle" duplex structure in the vRNA; this sequence is approximately 16 nucleotides from the 5' end of this RNA duplex structure within the vRNA promoter. Encountering this signal, the viral RNA polymerase stutters, leading to the synthesis of a poly-A tail on the viral mRNA (Robertson, 1981; Luo, 1991; Li, 1994; Poon, 1998; Zheng et al. 1999).

Preceded by: [Priming and Initiation of Transcription](#)

Followed by: [Viral mRNA Splicing \(M, NS segments\)](#), [Viral mRNA Export](#)

Literature references

Braam, J., Krug, RM., Ulmanen, I. (1983). Molecular model of a eucaryotic transcription complex: functions and movements of influenza P proteins during capped RNA-primed transcription. *Cell*, 34, 609-18. ↗

Garcia-Sastre, A., Palese, P., Lee, HA., Zheng, H. (1999). Influenza A virus RNA polymerase has the ability to stutter at the polyadenylation site of a viral RNA template during RNA replication. *J Virol*, 73, 5240-3. ↗

Plotch, SJ., Krug, RM. (1977). Influenza virion transcriptase: synthesis in vitro of large, polyadenylic acid-containing complementary RNA. *J Virol*, 21, 24-34. ↗

Poon, LL., Pritlove, DC., Brownlee, GG., Sharps, J. (1998). The RNA polymerase of influenza virus, bound to the 5' end of virion RNA, acts in cis to polyadenylate mRNA. *J Virol*, 72, 8214-9. ↗

Palese, P., Luytjes, W., Luo, GX., Enami, M. (1991). The polyadenylation signal of influenza virus RNA involves a stretch of uridines followed by the RNA duplex of the panhandle structure. *J Virol*, 65, 2861-7. ↗

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Viral mRNA Splicing (M, NS segments) ↗

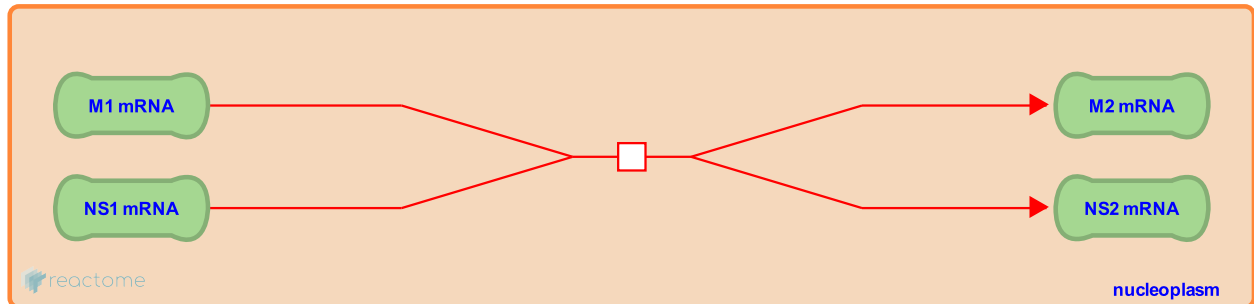
Location: [Viral Messenger RNA Synthesis](#)

Stable identifier: R-HSA-192781

Type: transition

Compartments: nucleoplasm

Diseases: influenza



The viral polymerase complex produces positive-sense viral mRNA with host-cell derived 5' methyl caps. Alternately spliced mRNA transcribed from M and NS vRNA segments 7 and 8, producing the spliced mRNA for M2 and NEP/NS2, respectively, are thought to be coupled to the cellular splicing and export mechanisms (Lamb, 1980; Lamb, 1981; Chen, 2000; Li, 2001). As segments 7 and 8 each encode two proteins, splicing must be regulated allowing for alternative mRNAs, with the spliced products in the minority (approximately 10%). M1 splicing may be regulated by the viral polymerase and the cellular SR splicing protein SF2/ASF (Shih, 1995; Shih, 1996); while NS1 splicing appears to be regulated by the viral mRNA intrinsically (Alonso-Caplen, 1991; Valcarel, 1991).

Preceded by: [Elongation, Polyadenylation and Termination](#)

Followed by: [Export of Spliced Viral mRNA](#)

Literature references

- Baker, CC., Krug, RM., Chen, ZY., Li, Y., Wang, W. (2001). The 3'-end-processing factor CPSF is required for the splicing of single-intron pre-mRNAs in vivo. *RNA*, 7, 920-31. ↗
- Lai, CJ., Lamb, RA., Choppin, PW., Chanock, RM. (1980). Mapping of the two overlapping genes for polypeptides NS1 and NS2 on RNA segment 8 of influenza virus genome. *Proc Natl Acad Sci U S A*, 77, 1857-61. ↗
- Chen, Z., Krug, RM. (2000). Selective nuclear export of viral mRNAs in influenza-virus-infected cells. *Trends Microbiol*, 8, 376-83. ↗
- Lai, CJ., Lamb, RA., Choppin, PW. (1981). Sequences of mRNAs derived from genome RNA segment 7 of influenza virus: colinear and interrupted mRNAs code for overlapping proteins. *Proc Natl Acad Sci U S A*, 78, 4170-4. ↗

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Export of Spliced Viral mRNA ↗

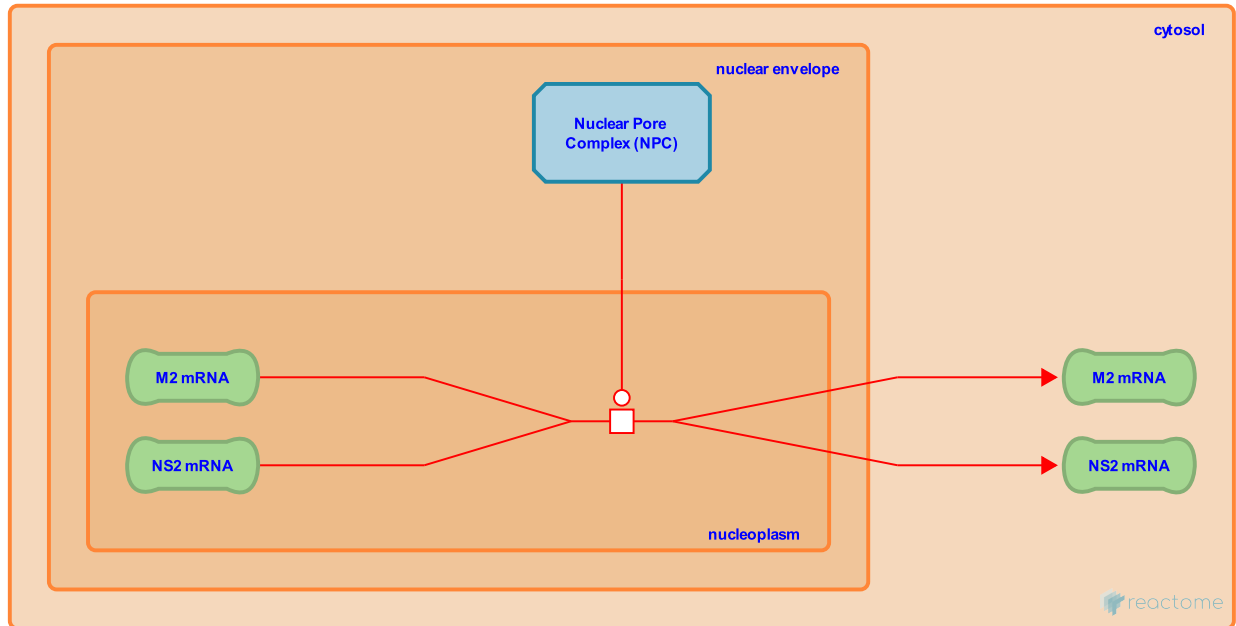
Location: [Viral Messenger RNA Synthesis](#)

Stable identifier: R-HSA-192925

Type: transition

Compartments: nucleoplasm

Diseases: influenza



In the cases of spliced, polyadenylated mRNA transcribed from M (segment 7) and NS (segment 8) vRNA templates (producing the spliced mRNA for M2 and NS2/NEP, respectively), export may be coupled to aspects of the cellular splicing and export mechanisms (Chen, 2000; Alonso-Caplan et al, 1992; Amorim, 2006). Simultaneously, the export of cellular mRNA appear to be inhibited by the viral NS1 protein, which binds to the cellular cleavage and polyadenylation specificity factor (CPSF), preventing polyadenylation and completion of pre-mRNA processing (Nemerof et al., 1998; Fortes, 1994; Lu, 1994; Li, 2001).

Preceded by: [Viral mRNA Splicing \(M, NS segments\)](#)

Literature references

Qiu, Y., Krug, RM., Alonso-Caplan, FV., Nemeroff, ME. (1992). Nucleocytoplasmic transport: the influenza virus NS1 protein regulates the transport of spliced NS2 mRNA and its precursor NS1 mRNA. *Genes Dev*, 6, 255-67. ↗

Chen, Z., Krug, RM. (2000). Selective nuclear export of viral mRNAs in influenza-virus-infected cells. *Trends Microbiol*, 8, 376-83. ↗

Editions

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Viral mRNA Export ↗

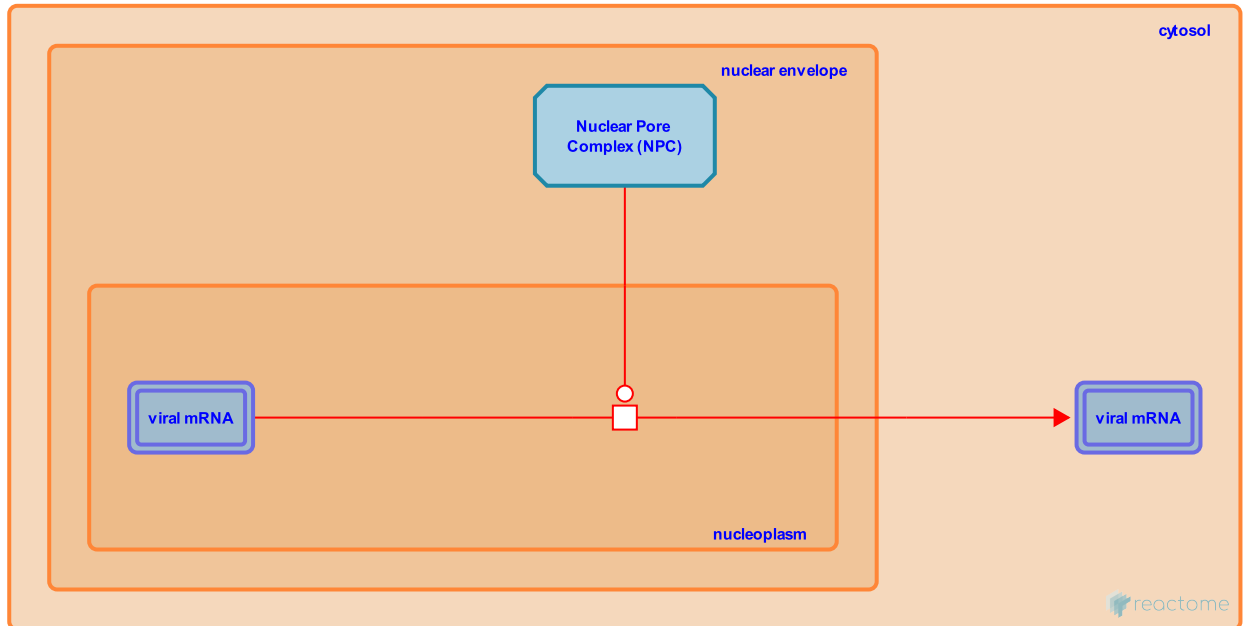
Location: [Viral Messenger RNA Synthesis](#)

Stable identifier: R-HSA-192627

Type: transition

Compartments: nucleoplasm

Diseases: influenza



The viral polymerase complex produces positive-sense viral mRNA with host-cell derived 5' methyl caps. Capped viral mRNAs are selectively exported from the host cell nucleus through a currently unclear mechanism that may rely on components of the host cell mRNA export machinery (Chen, 2000; Engelhardt, 2006). Polyadenylation of viral mRNA appears to be required for influenza mRNA export (Poon, 2000). A coupling of viral mRNA export with cellular pre-mRNA processing complexes, recruited by phosphorylation of host RNA polymerase II C-terminal domain which interacts with the viral polymerase (Engelhardt, 2005), has been proposed as controlling the export of a subset (M1, HA, and NS1, but not NP) of viral mRNA from the nucleus (Amorim, 2007).

Preceded by: [Elongation, Polyadenylation and Termination](#)

Literature references

- Amorim, MJ., Digard, P., Medcalf, L., Dalton, RM., Read, EK. (2007). Nuclear Export of Influenza A Virus mRNAs Requires Ongoing RNA Polymerase II Activity. *Traffic*, 8, 1-11. ↗
- Fodor, E., Engelhardt, OG. (2006). Functional association between viral and cellular transcription during influenza virus infection. *Rev Med Virol*, 16, 329-45. ↗
- Chen, Z., Krug, RM. (2000). Selective nuclear export of viral mRNAs in influenza-virus-infected cells. *Trends Microbiol*, 8, 376-83. ↗

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