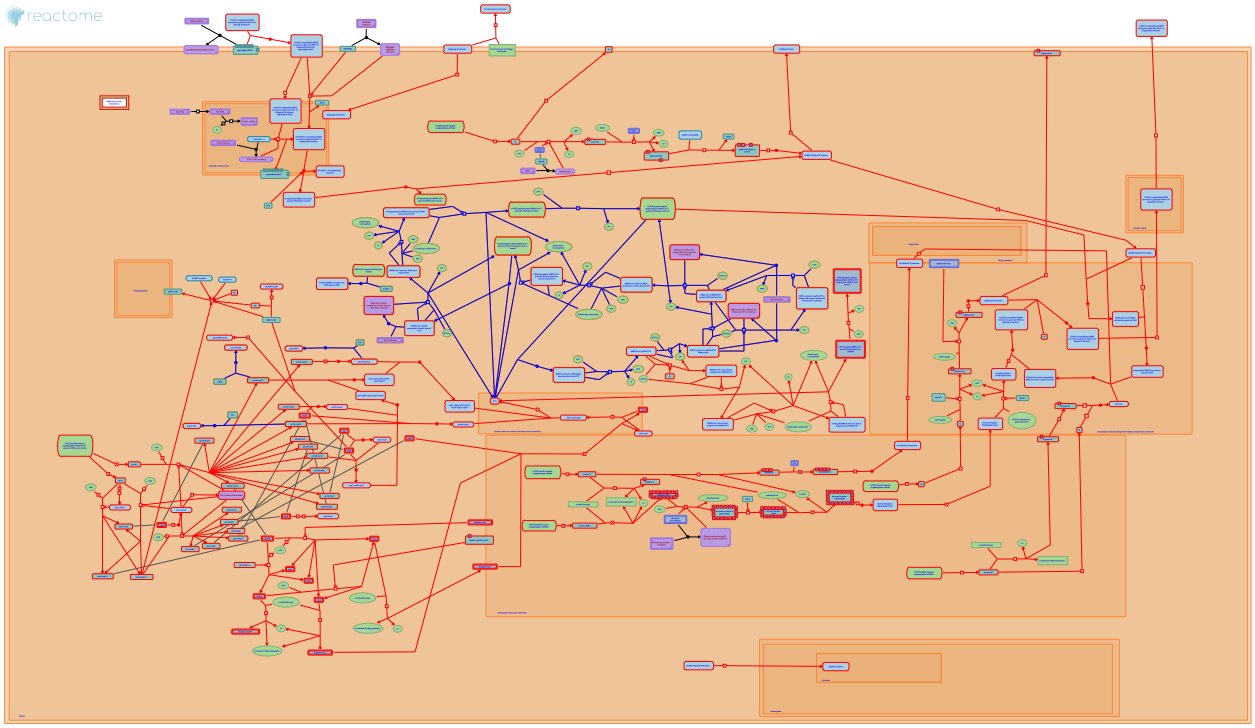
Replication of the SARS-CoV-1 genome



Acencio, ML., Jassal, B., Mazein, A., Orlic-Milacic, M., Shoichet, BK.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

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.
The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

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. ↗

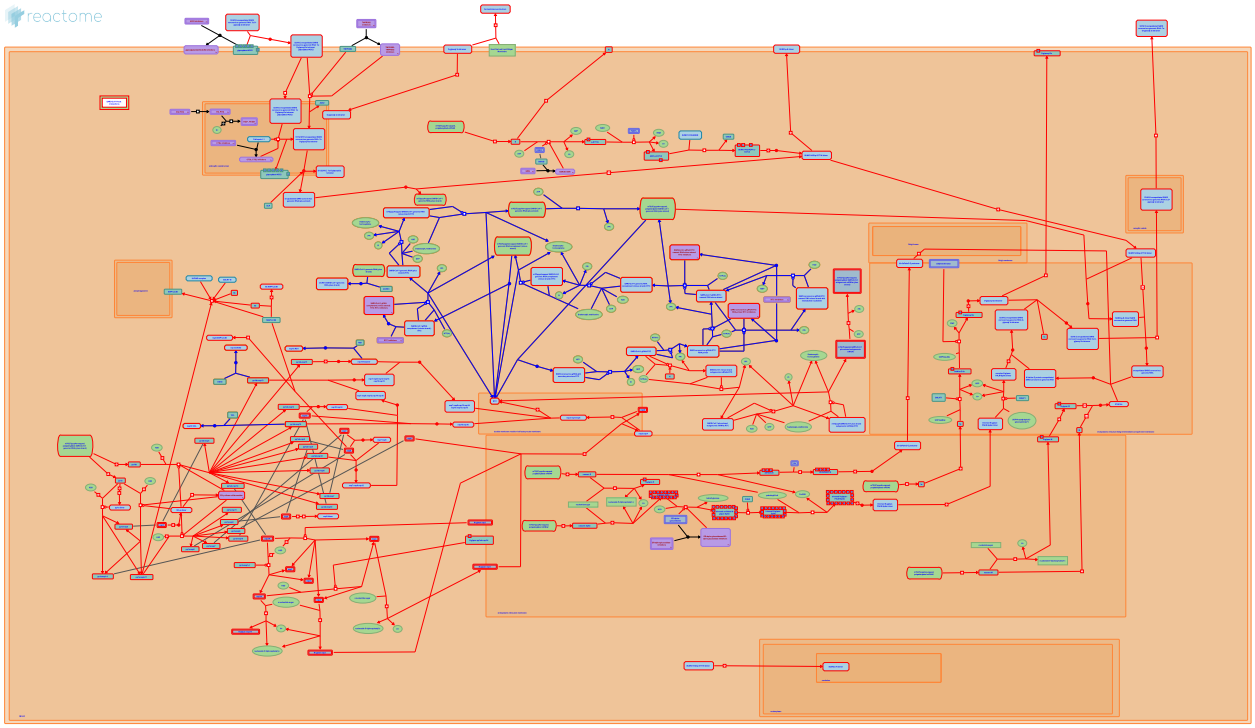
Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

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. ↗

Replication of the SARS-CoV-1 genome ↗

Stable identifier: R-HSA-9682706

Diseases: severe acute respiratory syndrome



The plus strand RNA genome of the human SARS coronavirus 1 (SARS-CoV-1) is replicated by the viral replication-transcription complex (RTC) composed of nonstructural proteins nsp3-nsp16, encoded by open reading frames ORF1a and ORF1b. Two RTC proteins, nsp8 and nsp12, possess 5'-3' RNA-dependent RNA polymerase activity. nsp12 is the main RNA polymerase, while nsp8 is thought to act as an RNA primase. nsp14 acts as a 3'-5' exonuclease, increasing the fidelity of the RTC. nsp14 also has the RNA capping activity and, in concert with nsp16, it caps viral plus strand and minus strand genomic and subgenomic RNAs, which confers stability to viral RNAs by enabling them to escape interferon-mediated innate immune responses of the host. nsp13 is an RNA helicase which is thought to melt secondary structures in the genomic RNA during replication and transcription. The plus strand genomic RNA is first used to synthesize the minus strand genomic RNA complement, which is subsequently used as a template for synthesis of plus strand viral RNA genomes that are packaged into mature virions. For review, please refer to Yang and Leibowitz 2015, Snijder et al. 2016, Fung and Liu 2019.

Literature references

Ziebuhr, J., Snijder, EJ., Decroly, E. (2016). The Nonstructural Proteins Directing Coronavirus RNA Synthesis and Processing. *Adv. Virus Res., 96*, 59-126. ↗

Fung, TS., Liu, DX. (2019). Human Coronavirus: Host-Pathogen Interaction. *Annu. Rev. Microbiol., 73*, 529-557. ↗

Leibowitz, JL., Yang, D. (2015). The structure and functions of coronavirus genomic 3' and 5' ends. *Virus Res., 206*, 120-33. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

Replication transcription complex binds SARS-CoV-1 genomic RNA ↗

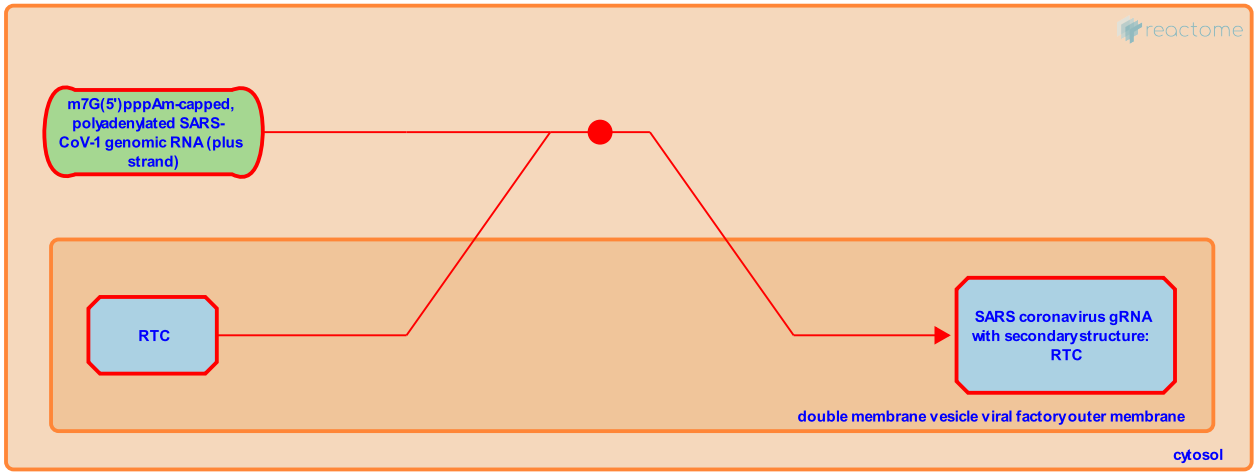
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9681314

Type: binding

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



The replication transcription complex (RTC) binds to the 3' end of the viral plus strand genomic RNA to initiate synthesis of the complementary minus strand. A 36 nucleotide sequence from the 3'-UTR of the plus strand, predicted to form a stable stem-loop structure, seems to be the minimal cis-acting RNA element required for the viral RNA-directed RNA polymerase (nsp12) to initiate RNA synthesis. The polyA tail also seems to play a role in the initiation of replication of viral genomic RNA (Ahn et al. 2012).

Followed by: nsp13 helicase melts secondary structures in SARS-CoV-1 genomic RNA template

Literature references

Ahn, DG., Oh, JW., Choi, JK., Taylor, DR. (2012). Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. *Arch. Virol., 157*, 2095-104. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp13 helicase melts secondary structures in SARS-CoV-1 genomic RNA template ↗

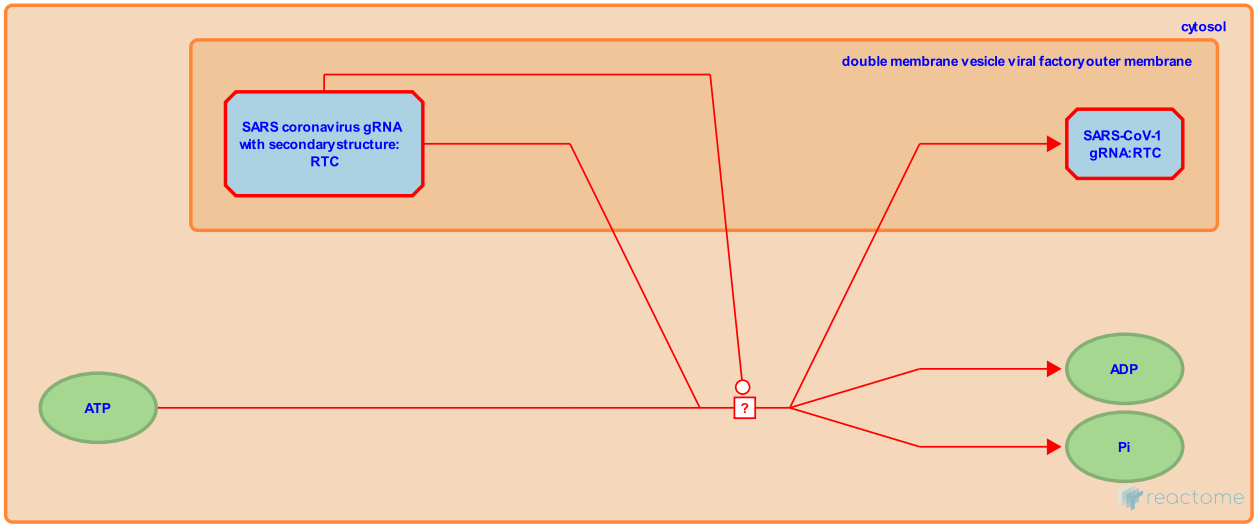
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9682695

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



nsp13 is an ATP-dependent human SARS coronavirus 1 (SARS-CoV-1) helicase that functions in the 5'-3' direction to unwind double stranded RNAs that have a 5' single strand overhang at least 20 nucleotides long. nsp13 can also act on double strand DNA in vitro, but dsRNA is thought to be its physiological substrate. The catalytic activity of nsp13 is increased in the presence of nsp12, the viral RNA-dependent RNA polymerase. nsp13 is needed for the replication of SARS-CoV-1 and is thought to act by melting secondary structures in the genomic RNA template during replication, and also to be involved in unwinding of RNA duplexes during transcription of viral genes. nsp13 is a promising target for experimental anti-SARS-CoV-1 drugs (Tanner et al. 2003, Ivanov et al. 2004, Bernini et al. 2006, Chen et al. 2009, Lee et al. 2010, Adedeji et al. 2012).

Preceded by: Replication transcription complex binds SARS-CoV-1 genomic RNA

Followed by: nsp8 generates RNA primers

Literature references

Spiga, O., Huang, J., Prischi, F., Tanner, JA., Bracci, L., Bernini, A. et al. (2006). Tertiary structure prediction of SARS coronavirus helicase. *Biochem. Biophys. Res. Commun., 343*, 1101-4. ↗

Huang, JD., Watt, RM., Tanner, JA., Chai, YB., Lu, LY., Peiris, JS. et al. (2003). The severe acute respiratory syndrome (SARS) coronavirus NTPase/helicase belongs to a distinct class of 5' to 3' viral helicases. *J. Biol. Chem., 278*, 39578-82. ↗

Ivanov, KA., Ziebuhr, J., Thiel, V., Dobbe, JC., Snijder, EJ., van der Meer, Y. (2004). Multiple enzymatic activities associated with severe acute respiratory syndrome coronavirus helicase. *J. Virol., 78*, 5619-32. ↗

Sarafianos, SG., Eoff, RL., te Velthuis, AJ., Adedeji, AO., Singh, K., Marchand, B. et al. (2012). Mechanism of nucleic acid unwinding by SARS-CoV helicase. *PLoS ONE, 7*, e36521. ↗

Lin, X., Wen, YM., Poon, KM., Chen, JY., Wang, YX., Chen, WN. et al. (2009). Interaction between SARS-CoV helicase and a multifunctional cellular protein (Ddx5) revealed by yeast and mammalian cell two-hybrid systems. *Arch. Virol., 154*, 507-12. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp8 generates RNA primers ↗

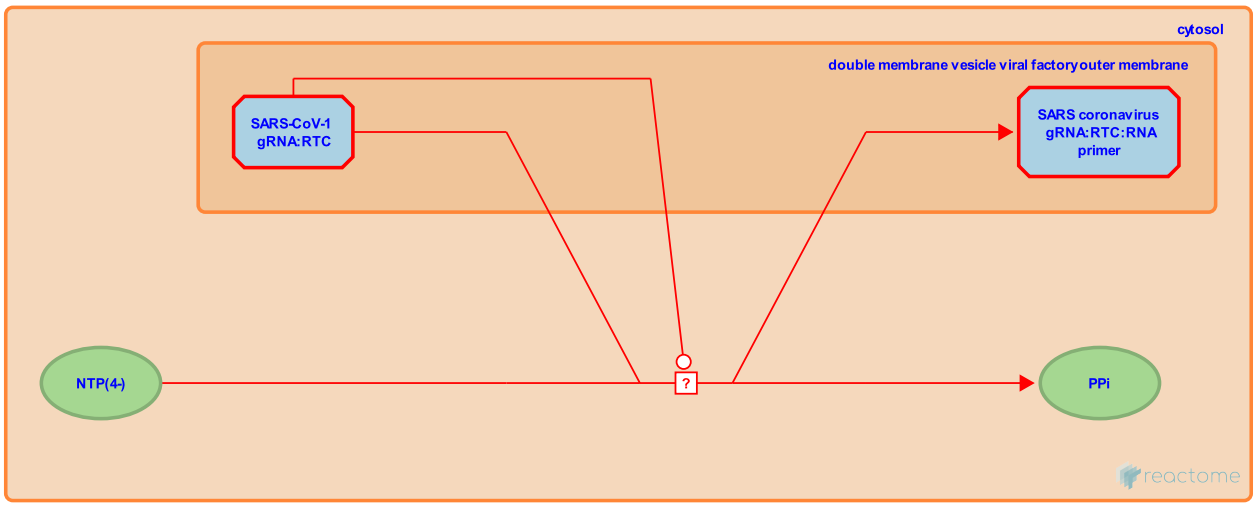
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9681651

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



nsp8 functions as an RNA-dependent RNA polymerase (RdRp) that serves as the primase for nsp12, the main RdRp of the SARS coronavirus 1 (SARS-CoV-1) (Imbert et al. 2006), as it is capable of de novo RNA synthesis (te Velthuis et al. 2011). nsp8 synthesizes short oligonucleotides (less than 6 bases long) using genomic RNA as a template. nsp8 requires at least one cytidine residue in the template sequence for its activity. Activity is dependent on manganese ions (Imbert et al. 2006). nsp8 can also extend primers but is 20-fold less efficient than nsp12 (te Velthuis et al. 2011).

Preceded by: nsp13 helicase melts secondary structures in SARS-CoV-1 genomic RNA template

Followed by: nsp12 synthesizes minus strand SARS-CoV-1 genomic RNA complement

Literature references

Ferron, F., Canard, B., Guillemot, JC., Gorbalenya, AE., Bourhis, JM., Egloff, MP. et al. (2006). A second, non-canonical RNA-dependent RNA polymerase in SARS coronavirus. *EMBO J., 25*, 4933-42. ↗

te Velthuis, AJ., van den Worm, SH., Snijder, EJ. (2012). The SARS-coronavirus nsp7+nsp8 complex is a unique multimeric RNA polymerase capable of both de novo initiation and primer extension. *Nucleic Acids Res., 40*, 1737-47. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp12 synthesizes minus strand SARS-CoV-1 genomic RNA complement ↗

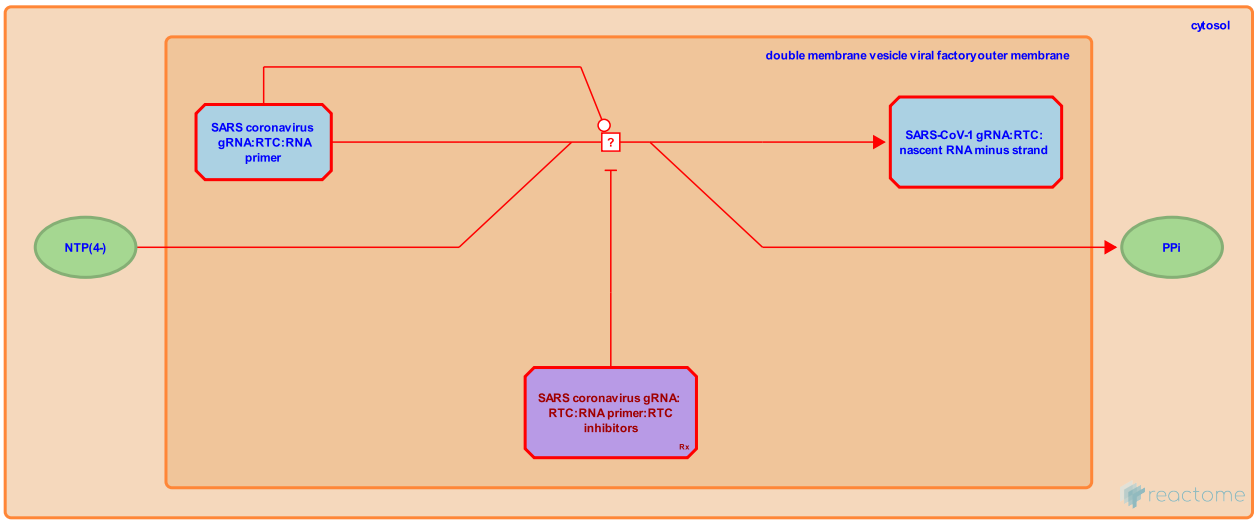
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9681674

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



Virally encoded RNA-dependent RNA polymerase (nsp12, also known as RdRP) is the key component of the replication transcription complex (RTC). As the human SARS coronavirus 1 (SARS-CoV-1) is a plus strand RNA virus, nsp12 first synthesizes the complementary minus RNA strand. The purified SARS-CoV-1 nsp12 shows both primer dependent and primer-independent RNA synthesis activities using homopolymeric RNA templates. The catalytic activity of nsp12 is strictly dependent on manganese ions (Mn2+) and primers when the template is a viral-genome-derived RNA representing part of the 3'-UTR of the plus strand with a polyA tail. A 36 nucleotide sequence from the 3'-UTR, predicted to form a stable stem-loop structure, seems to be the minimal cis-acting RNA element required for nsp12 to initiate RNA synthesis (Ahn et al. 2012). The complex of nsp7 and nsp8 confers processivity to nsp12 (Subissi et al. 2014).

Preceded by: nsp8 generates RNA primers

Followed by: nsp12 misincorporates a nucleotide in nascent RNA minus strand

Literature references

Ahn, DG., Oh, JW., Choi, JK., Taylor, DR. (2012). Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. *Arch. Virol., 157*, 2095-104. ↗

Canard, B., Collet, A., Gorbalenya, AE., Subissi, L., Decroly, E., Imbert, I. et al. (2014). One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. *Proc. Natl. Acad. Sci. U.S.A., 111*, E3900-9. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp12 misincorporates a nucleotide in nascent RNA minus strand ↗

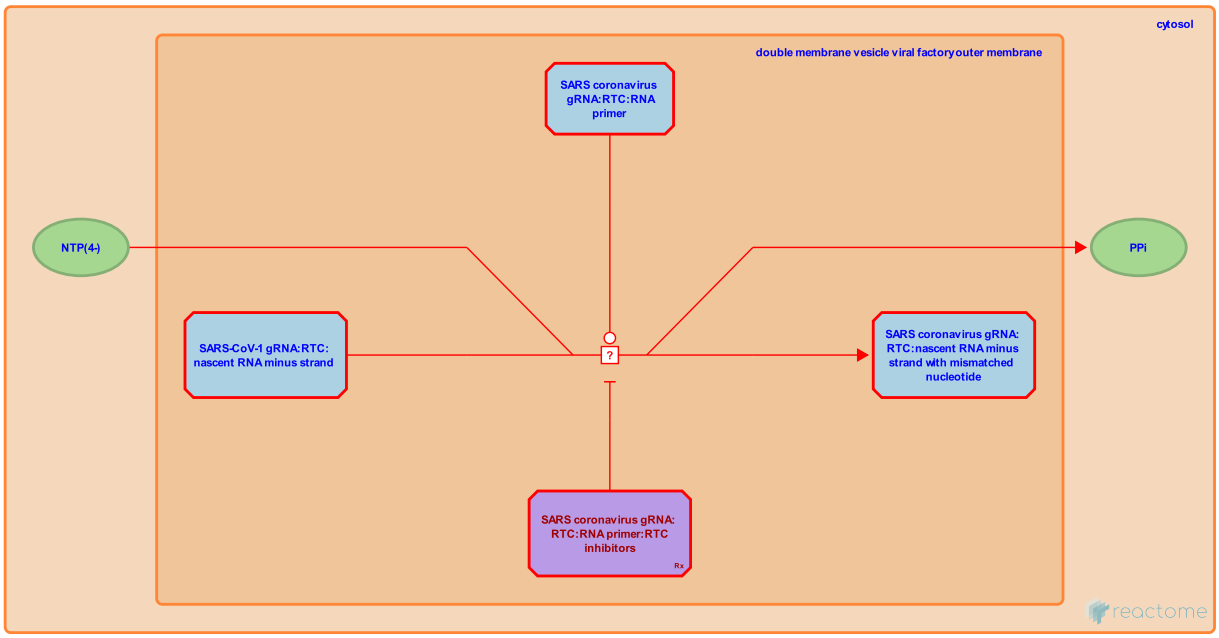
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9682563

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



In the presence of functional nsp14, which acts as a 3'-to-5' exonuclease, the mutation rate during human SARS coronavirus 1 (SARS-CoV-1) replication is 9 x 10^-7 (9E-7) per nucleotide per replication cycle or 2.2 x 10^-5 (2.2E-5) non-redundant substitutions per nucleotide, which translates into 2-3 nucleotide substitutions for each replicated SARS-CoV-1 genome. When nsp14 is defective, the mutation rate during SARS-CoV-1 replication increases to 1.2 x 10^-5 (1.2E-5) mutations per nucleotide per replication cycle or 3.34 x 10^-4 (3.34E-4) non-redundant substitutions per nucleotide, which translates into 12-23 nucleotide substitutions for each replicated SARS-CoV-1 genome (Eckerle et al. 2010). Here the process is annotated in two steps, nsp12- mediated misincorporation of a base (this reaction) and nsp14-mediated detection and removal of that base (next reaction).

Preceded by: nsp12 synthesizes minus strand SARS-CoV-1 genomic RNA complement

Followed by: nsp14 acts as a 3'-to-5' exonuclease to remove misincorporated nucleotides from nascent RNA

Literature references

Li, K., Becker, MM., Scherbakova, S., Lu, X., Denison, MR., Graham, RL. et al. (2010). Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. *PLoS Pathog.*, *6*, e1000896. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

SARS coronavirus gRNA:RTC:RNA primer binds RTC inhibitors ↗

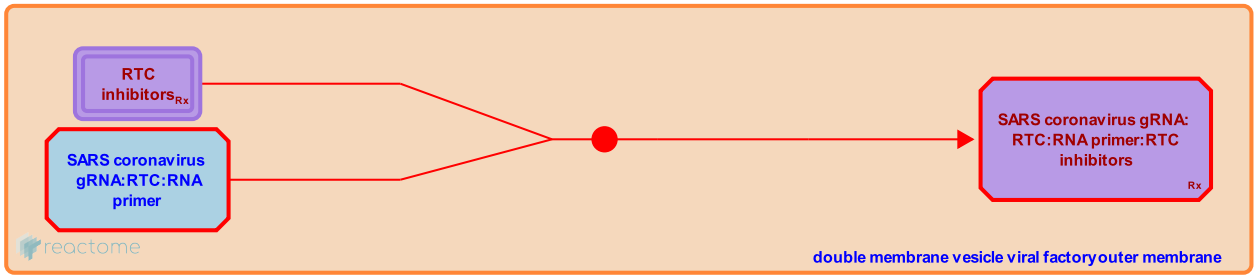
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9680262

Type: binding

Compartments: double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



Remdesivir (GS-5734) is an investigational nucleotide analogue drug that was developed for its broad spectrum antiviral potential against Ebola and Marburg virus activity (Siegel et al. 2017). It targets and inhibits viral RNA-dependent RNA polymerase (nsp12, RdRP), the key component of the replication transcription complex (RTC) (Agostini et al. 2018, Brown et al. 2019, Gordon et al. 2020). Remdesivir is being investigated for potential antiviral activity against SARS-CoV-2 by targeting viral replication (Agostini et al. 2018). Gordon et al. demonstrate remdesivir possesses broad antiviral activity against RNA viruses, including SARS-CoV, MERS-CoV and SARS-CoV-2 in-vitro (Gordon et al. 2020b). It could prevent asymptomatic, mild or moderate COVID-19 cases from progressing to severe disease (clinical trials NCT04252664, NCT04257656) but results so far in infected people have been mixed.

Molnupiravir (EIDD-2801) is an isopropylester prodrug of the ribonucleoside analogue N4-hydroxycytidine (NHC, EIDD-1931) that shows broad spectrum antiviral activity against various RNA viruses including Ebola, Influenza and CoV (Toots et al. 2019). NHC acts as a competitive alternative substrate for virally encoded RNA-dependent RNA polymerases. NHC was shown to inhibit multiple genetically-distinct Bat-CoV viruses in human primary epithelial cells without affecting cell viability. Prophylactic/therapeutic oral administration of NHC reduced lung titers and prevented acute lung failure in C57B/6 mice infected with CoV. The potency of NHC against multiple coronaviruses, its therapeutic efficacy, and oral bioavailability in vivo, all highlight its potential as an effective antiviral against SARS-CoV-2 and other future zoonotic coronaviruses (Sheahan et al. 2020). The clinical trial NCT04575584 was terminated prematurely for ethical reasons because molnupiravir reached its endpoint of effectiveness against COVID-19.

Literature references

Gotte, M., Gordon, CJ., Feng, JY., Tchesnokov, EP., Porter, DP. (2020). The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J. Biol. Chem.*. ↗

Hart, M., Kolykhalov, AA., Bluemling, GR., Sticher, ZM., Plemper, RK., Painter, GR. et al. (2019). Characterization of orally efficacious influenza drug with high resistance barrier in ferrets and human airway epithelia. *Sci Transl Med, 11*. ↗

Amirian, ES., Levy, JK. (2020). Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. *One Health, 9*, 100128. ↗

Gotte, M., Woolner, E., Gordon, CJ., Feng, JY., Perry, JK., Tchesnokov, EP. et al. (2020). Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem., 295*, 6785-6797. ↗

Harcourt, J., Schäfer, A., Saindane, M., Hill, CS., Sims, AC., Zhou, S. et al. (2020). An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci Transl Med.* ↗

Editions

2020-03-26	Authored, Edited	Jassal, B.
2020-05-14	Reviewed	Shoichet, BK.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp14 acts as a 3'-to-5' exonuclease to remove misincorporated nucleotides from nascent RNA ↗

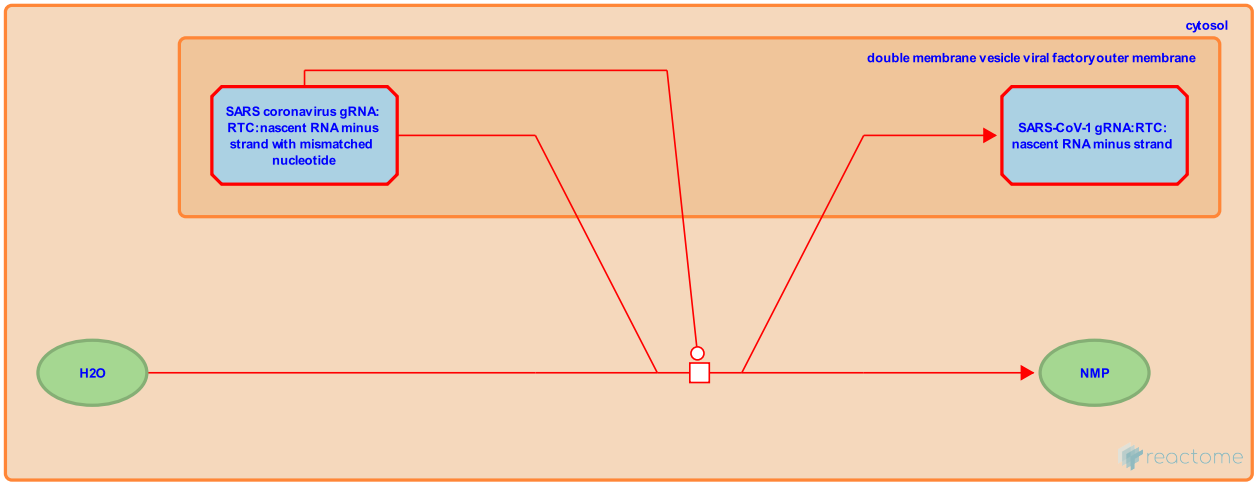
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9682603

Type: transition

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



nsp14 acts as 3'-5' exonuclease (Minskaia et al. 2006, Chen et al. 2007) that preferentially excises mismatched nucleotides from double stranded RNA (Minskaia et al. 2006, Bouvet et al. 2012). Binding to nsp10 increases the exonuclease activity of nsp14 (Bouvet et al. 2012, Subissi et al. 2014, Bouvet et al. 2014). nsp14 increases the fidelity of human SARS coronavirus 1 (SARS-CoV-1) replication by the nsp12 RNA-dependent RNA polymerase by 21-fold (Eckerle et al. 2010).

Preceded by: nsp12 misincorporates a nucleotide in nascent RNA minus strand

Followed by: RTC completes synthesis of the minus strand genomic RNA complement

Literature references

Hu, T., Guo, D., Jiang, M., Chen, P., Liu, Q., Chen, XS. (2007). Biochemical characterization of exoribonuclease encoded by SARS coronavirus. *J. Biochem. Mol. Biol., 40*, 649-55. ↗

Canard, B., Gluais, L., Subissi, L., Decroly, E., Imbert, I., Bouvet, M. (2012). RNA 3'-end mismatch excision by the severe acute respiratory syndrome coronavirus nonstructural protein nsp10/nsp14 exoribonuclease complex. *Proc. Natl. Acad. Sci. U.S.A., 109*, 9372-7. ↗

Li, K., Becker, MM., Scherbakova, S., Lu, X., Denison, MR., Graham, RL. et al. (2010). Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. *PLoS Pathog., 6*, e1000896. ↗

Campanacci, V., Canard, B., Hertzig, T., Minskaia, E., Gorbalenya, AE., Ziebuhr, J. et al. (2006). Discovery of an RNA virus 3'->5' exoribonuclease that is critically involved in coronavirus RNA synthesis. *Proc. Natl. Acad. Sci. U.S.A., 103*, 5108-13. ↗

Drosten, C., Canard, B., Betzi, S., Guillemot, JC., Lécine, P., Snijder, EJ. et al. (2014). Coronavirus Nsp10, a critical co-factor for activation of multiple replicative enzymes. *J. Biol. Chem., 289*, 25783-96. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

RTC completes synthesis of the minus strand genomic RNA complement ↗

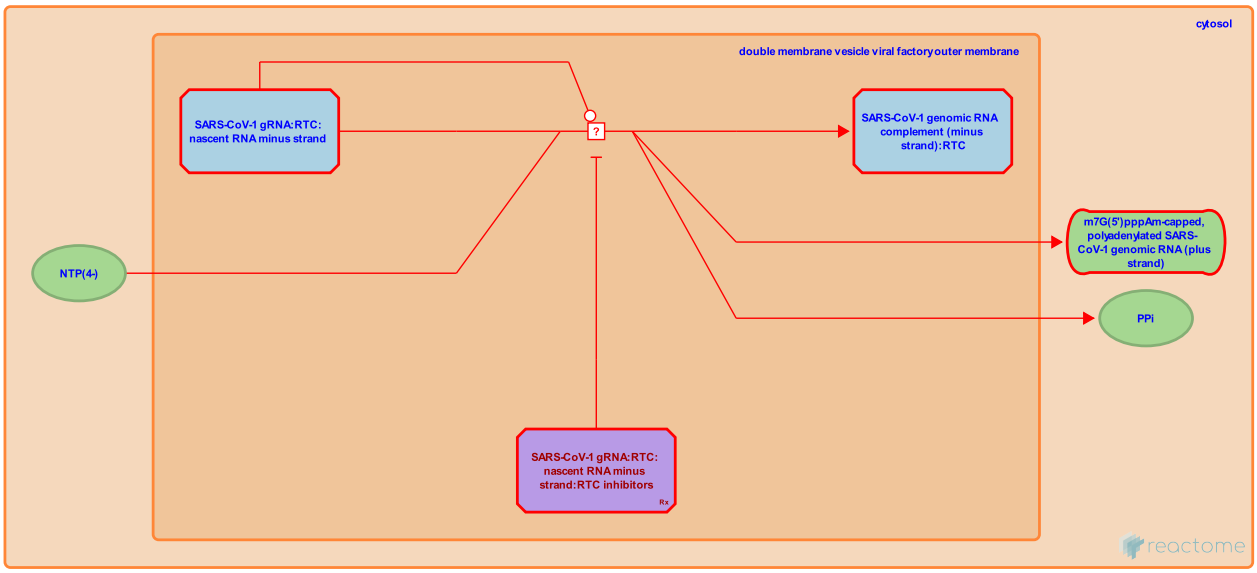
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9682465

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



The replication-transcription complex (RTC) completes synthesis of the genomic RNA complement (minus strand). The complex of nsp7 and nsp8 confers processivity to nsp12, the virally encoded RNA-dependent RNA polymerase that replicates the viral genomic RNA, enabling the RTC to complete the RNA synthesis with a very low dissociation rate. nsp7 plays a crucial role in maintaining binding of the RTC to the RNA. nsp14 subunit of the RTC does not affect the processivity (Subissi et al. 2014).

Preceded by: nsp14 acts as a 3'-to-5' exonuclease to remove misincorporated nucleotides from nascent RNA

Followed by: nsp14 acts as a cap N7 methyltransferase to modify SARS-CoV-1 gRNA complement (minus strand)

Literature references

Canard, B., Collet, A., Gorbalenya, AE., Subissi, L., Decroly, E., Imbert, I. et al. (2014). One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. *Proc. Natl. Acad. Sci. U.S.A., 111*, E3900-9. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

SARS-CoV-1 gRNA:RTC:nascent RNA minus strand binds RTC inhibitors ↗

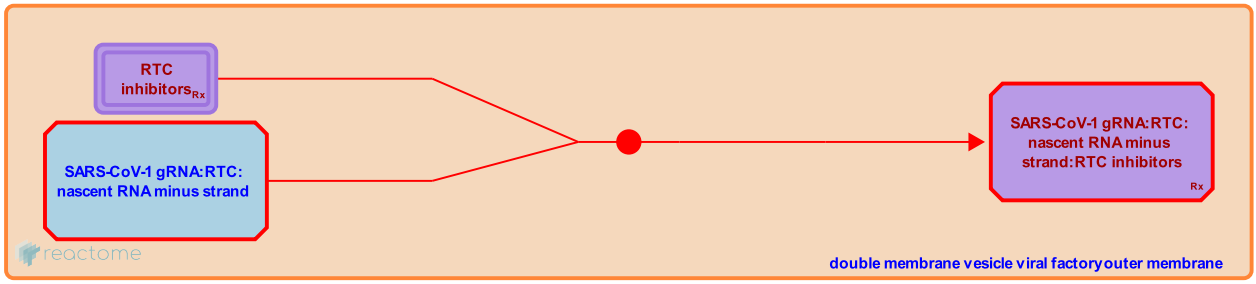
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9687388

Type: binding

Compartments: double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



Remdesivir (GS-5734) is an investigational nucleotide analogue drug that was developed for its broad spectrum antiviral potential against Ebola and Marburg virus activity (Siegel et al. 2017). It targets and inhibits viral RNA-dependent RNA polymerase (nsp12, RdRP), the key component of the replication transcription complex (RTC) (Agostini et al. 2018, Brown et al. 2019, Gordon et al. 2020). Remdesivir is being investigated for potential antiviral activity against SARS-CoV-2 by targeting viral replication (Agostini et al. 2018). Gordon et al. demonstrate remdesivir possesses broad antiviral activity against RNA viruses, including SARS-CoV, MERS-CoV and SARS-CoV-2 in-vitro (Gordon et al. 2020b). It could prevent asymptomatic, mild or moderate COVID-19 cases from progressing to severe disease (clinical trials NCT04252664, NCT04257656) but results so far in infected people have been mixed.

Molnupiravir (EIDD-2801) is an isopropylester prodrug of the ribonucleoside analogue N4-hydroxycytidine (NHC, EIDD-1931) that shows broad spectrum antiviral activity against various RNA viruses including Ebola, Influenza and CoV (Toots et al. 2019). NHC acts as a competitive alternative substrate for virally encoded RNA-dependent RNA polymerases. NHC was shown to inhibit multiple genetically-distinct Bat-CoV viruses in human primary epithelial cells without affecting cell viability. Prophylactic/therapeutic oral administration of NHC reduced lung titers and prevented acute lung failure in C57B/6 mice infected with CoV. The potency of NHC against multiple coronaviruses, its therapeutic efficacy, and oral bioavailability in vivo, all highlight its potential as an effective antiviral against SARS-CoV-2 and other future zoonotic coronaviruses (Sheahan et al. 2020). The clinical trial NCT04575584 was terminated prematurely for ethical reasons because molnupiravir reached its endpoint of effectiveness against COVID-19.

Literature references

Gotte, M., Gordon, CJ., Feng, JY., Tchesnokov, EP., Porter, DP. (2020). The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J. Biol. Chem.*. ↗

Hart, M., Kolykhalov, AA., Bluemling, GR., Sticher, ZM., Plemper, RK., Painter, GR. et al. (2019). Characterization of orally efficacious influenza drug with high resistance barrier in ferrets and human airway epithelia. *Sci Transl Med, 11*. ↗

Amirian, ES., Levy, JK. (2020). Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. *One Health, 9*, 100128. ↗

Gotte, M., Woolner, E., Gordon, CJ., Feng, JY., Perry, JK., Tchesnokov, EP. et al. (2020). Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem., 295*, 6785-6797. ↗

Harcourt, J., Schäfer, A., Saindane, M., Hill, CS., Sims, AC., Zhou, S. et al. (2020). An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci Transl Med*. ↗

Editions

2020-05-07	Authored, Edited	Jassal, B.
2020-05-14	Reviewed	Shoichet, BK.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp14 acts as a cap N7 methyltransferase to modify SARS-CoV-1 gRNA complement (minus strand)

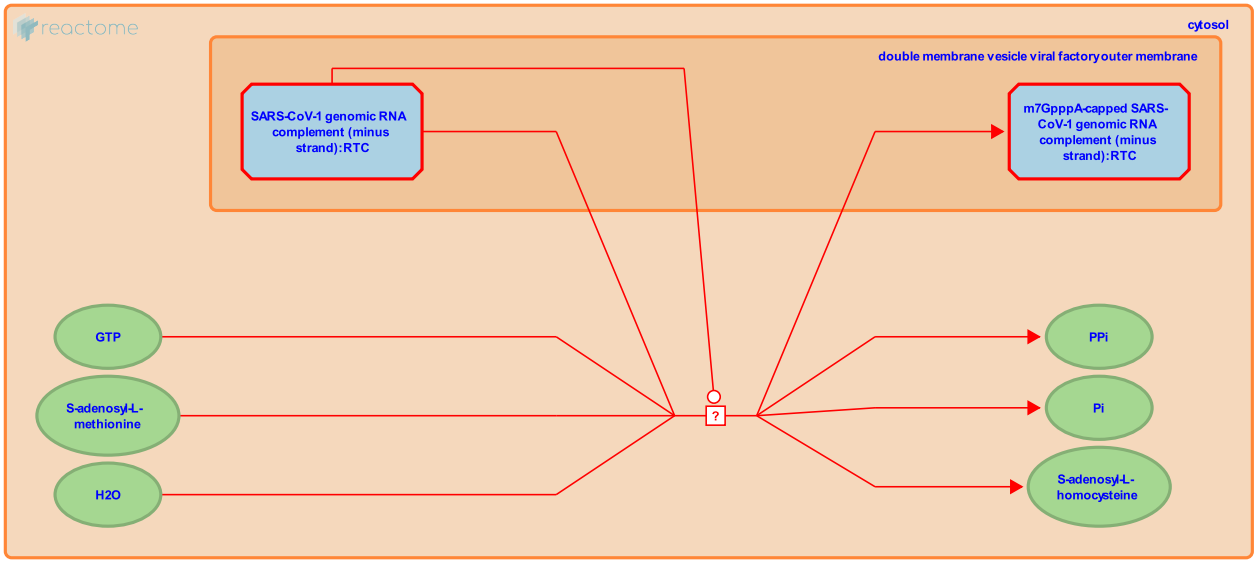
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9684018

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



The genomic and subgenomic mRNAs of SARS-CoV-1 coronavirus, including the minus strand genomic RNA complement, are presumed to be capped at their 5′ end, based on studies of the mouse hepatitis virus (MHV) (Lai and Stohlman 1981) and the equine torovirus (van Vliet et al. 2002). The non-structural protein 14 (nsp14) acts as an RNA guanine-N7-methyltransferase (N7-MTase) that completes the synthesis of the cap-0 on SARS-CoV-1 minus strand genomic RNA. The cap-0 represents N7-methyl guanosine connected to the 5′ nucleotide through a 5′ to 5′ triphosphate linkage, and is also known as m7G cap or m7Gppp cap. The N7-MTase domain maps to the carboxy-terminal part of nsp14 (Chen et al. 2009). Cap-0 formation requires three sequential reactions catalyzed by RNA triphosphatase (TPase), guanylyltransferase (GTase), and N7-MTase. There is no evidence that nsp14 possesses TPase and GTase activities, and no other SARS-CoV-1 proteins with these activities have been identified, so the identities of the enzymes that mediate these required steps remain unknown. Based on the study of the human coronavirus 229E, non-structural protein 13 (nsp13) may have a TPase activity in addition to its established helicase activity (Ivanov and Ziebuhr 2004).

Preceded by: RTC completes synthesis of the minus strand genomic RNA complement

Followed by: nsp16 acts as a cap 2'-O-methyltransferase to modify SARS-CoV-1 gRNA complement (minus strand)

Literature references

Guo, D., Cai, H., Chen, Y., Pan, J., Xiang, N., Tien, P. et al. (2009). Functional screen reveals SARS coronavirus non-structural protein nsp14 as a novel cap N7 methyltransferase. *Proc. Natl. Acad. Sci. U.S.A., 106*, 3484-9.

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp16 acts as a cap 2'-O-methyltransferase to modify SARS-CoV-1 gRNA complement (minus strand) ↗

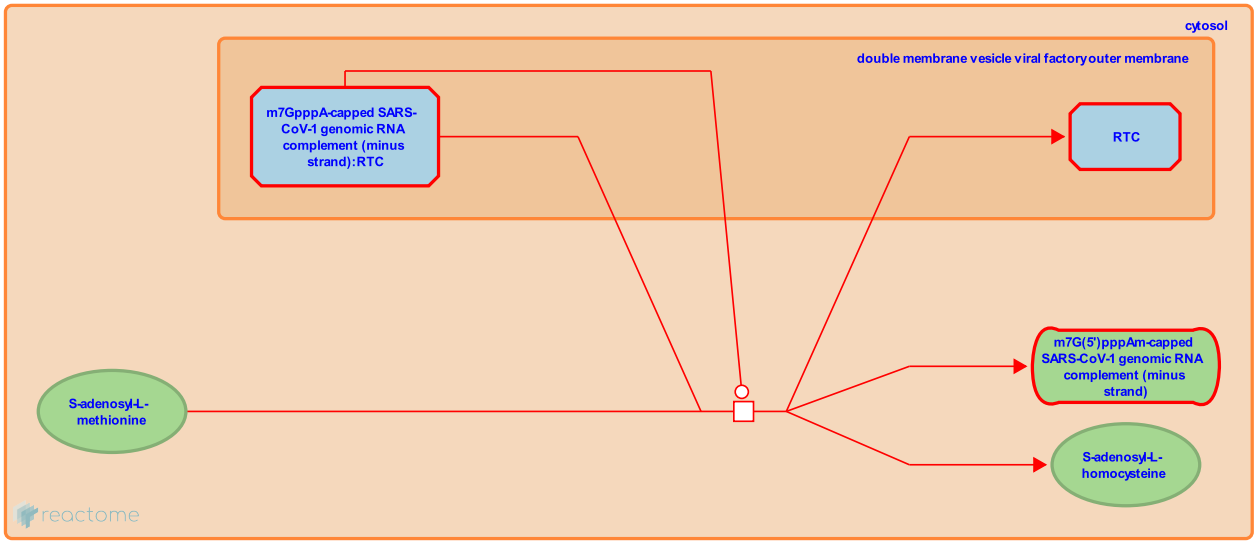
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9684030

Type: transition

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



The genomic and subgenomic mRNAs of SARS-CoV-1 coronavirus, including the minus strand genomic RNA, are presumed to be capped at their 5′ end, based on studies of the mouse hepatitis virus (MHV) (Lai and Stohlman 1981) and the equine torovirus (van Vliet et al. 2002). Non-structural protein 16 (nsp16) acts as a 2'O-methyltransferase that converts coronavirus cap-0 to cap-1, which was first demonstrated with nsp16 cloned from the feline coronavirus (FCV) (Decroly et al. 2008). Cap-0 represents N7-methyl guanosine connected to the 5′ nucleotide through a 5′ to 5′ triphosphate linkage (also known as m7G cap or m7Gppp cap). Cap-1 is generated by an additional methylation on the 2′O position of the initiating nucleotide, and is also known as m7GpppNm. Non-structural protein 10 (nsp10) acts as an activator of nsp16 and is necessary for cap-1 synthesis (Bouvet et al. 2010, Decroly et al. 2011). Coronavirus RNAs with cap-1 are protected from IFIT-mediated interferon response. IFITs are interferon-induced proteins with tetratricopeptide repeats that recognize unmethylated 2'-O RNAs and act to inhibit expression of virally encoded mRNAs (Menachery et al. 2014).

Preceded by: nsp14 acts as a cap N7 methyltransferase to modify SARS-CoV-1 gRNA complement (minus strand)

Followed by: RTC binds SARS-CoV-1 genomic RNA complement (minus strand)

Literature references

Bricogne, G., Ferron, F., Canard, B., Ortiz-Lombardia, M., Gluais, L., Papageorgiou, N. et al. (2011). Crystal structure and functional analysis of the SARS-coronavirus RNA cap 2'-O-methyltransferase nsp10/nsp16 complex. *PLoS Pathog., 7*, e1002059. ↗

Canard, B., Selisko, B., Decroly, E., Imbert, I., Snijder, EJ., Bouvet, M. et al. (2010). In vitro reconstitution of SARS-coronavirus mRNA cap methylation. *PLoS Pathog., 6*, e1000863. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

RTC binds SARS-CoV-1 genomic RNA complement (minus strand) ↗

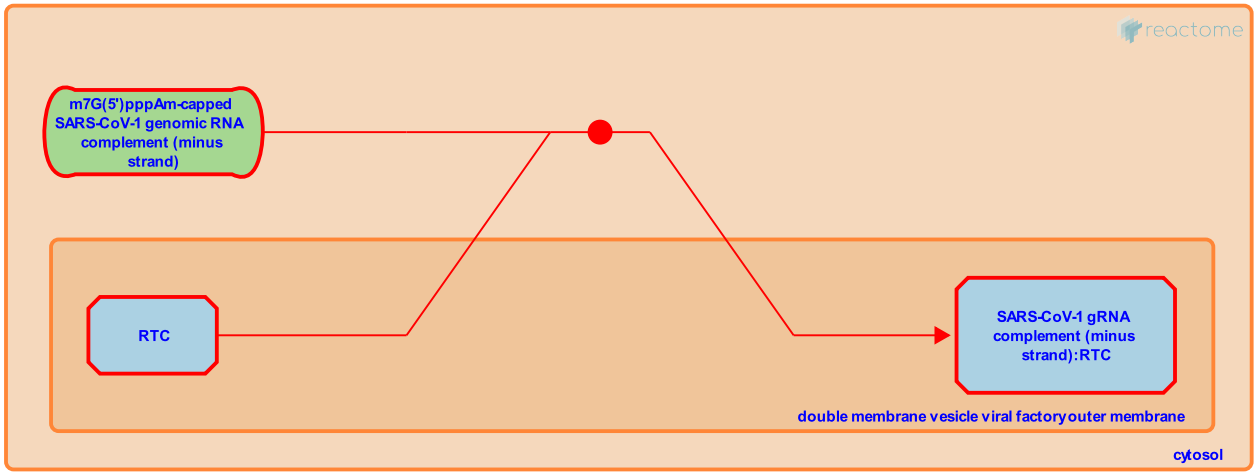
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9685597

Type: binding

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



After synthesizing the complementary minus RNA of the plus strand viral genomic RNA, SARS-CoV-1 replication-transcription complex (RTC) associates with the minus strand to initiate plus strand synthesis and to initiate transcription of subgenomic (sg) mRNAs (Ahn et al. 2012).

Preceded by: nsp16 acts as a cap 2'-O-methyltransferase to modify SARS-CoV-1 gRNA complement (minus strand)

Followed by: RTC synthesizes SARS-CoV-1 plus strand genomic RNA

Literature references

Ahn, DG., Oh, JW., Choi, JK., Taylor, DR. (2012). Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. *Arch. Virol., 157*, 2095-104. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

RTC synthesizes SARS-CoV-1 plus strand genomic RNA ↗

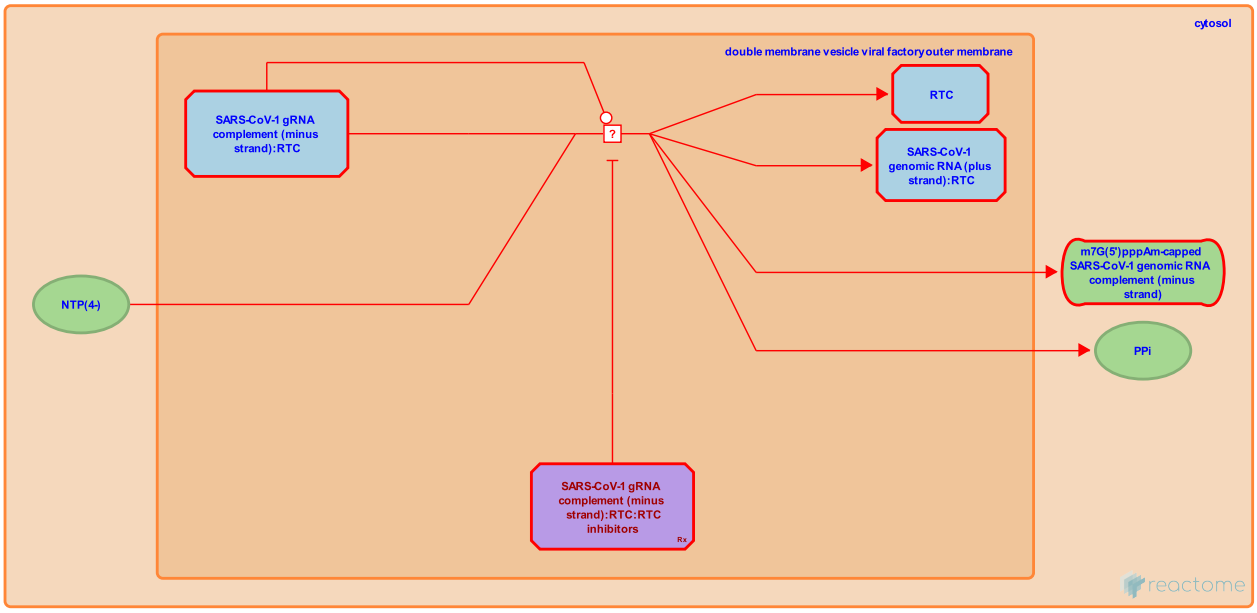
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9681840

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



After synthesizing the complementary minus RNA of the plus strand viral genomic RNA, virally encoded RNA-dependent RNA polymerase (nsp12, also known as RdRP) uses the minus strand as a template to generate viral genomic RNA that can be packaged into virions. Purified SARS-CoV-1 nsp12 shows both primer dependent and primer-independent RNA synthesis activity in vitro. nsp12 is able to initiate RNA synthesis with as little as 37 nucleotides of RNA from the 3' end of the minus strand viral RNA (complementary to the 5'-UTR of the plus strand genomic RNA - c5'-UTR). Similar to the 3'-UTR of the plus strand, the 3' end of the minus strand (c5'-UTR) is predicted to form a stable stem-loop structure and seems to be the minimal cis-acting RNA element required for nsp12 to initiate RNA synthesis using the minus strand as a template (Ahn et al. 2012). It is unclear if replication of the minus strand is primer-dependent. The complex of nsp7 and nsp8 confers processivity to nsp12 (Subissi et al. 2014).

Preceded by: RTC binds SARS-CoV-1 genomic RNA complement (minus strand)

Followed by: nsp14 acts as a cap N7 methyltransferase to modify SARS-CoV-1 gRNA (plus strand)

Literature references

Ahn, DG., Oh, JW., Choi, JK., Taylor, DR. (2012). Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. *Arch. Virol., 157*, 2095-104. ↗

Canard, B., Collet, A., Gorbalenya, AE., Subissi, L., Decroly, E., Imbert, I. et al. (2014). One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. *Proc. Natl. Acad. Sci. U.S.A., 111*, E3900-9. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

SARS-CoV-1 gRNA complement (minus strand):RTC binds RTC inhibitors ↗

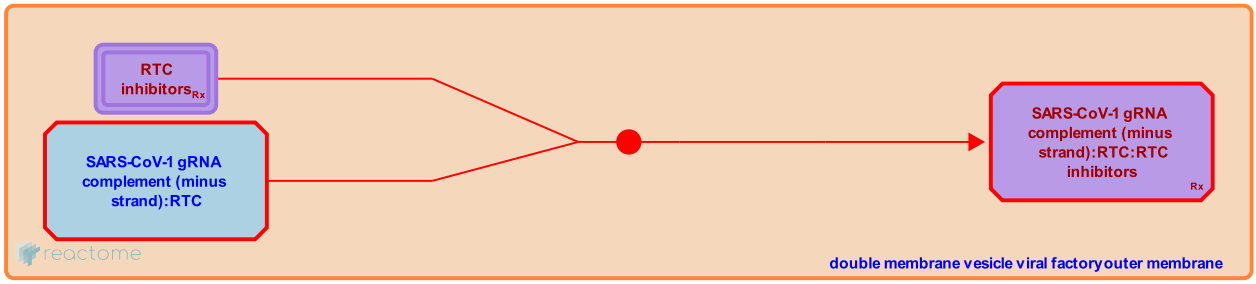
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9687384

Type: binding

Compartments: double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



Remdesivir (GS-5734) is an investigational nucleotide analogue drug that was developed for its broad spectrum antiviral potential against Ebola and Marburg virus activity (Siegel et al. 2017). It targets and inhibitis viral RNA-dependent RNA polymerase (nsp12, RdRP), the key component of the replication transcription complex (RTC) (Agostini et al. 2018, Brown et al. 2019, Gordon et al. 2020). Remdesivir is being investigated for potential antiviral activity against SARS-CoV-2 by targeting viral replication (Agostini et al. 2018). Gordon et al. demonstrate remdesivir possesses broad antiviral activity against RNA viruses, including SARS-CoV, MERS-CoV and SARS-CoV-2 in-vitro (Gordon et al. 2020b). It could prevent asymptomatic, mild or moderate COVID-19 cases from progressing to severe disease (clinical trials NCT04252664, NCT04257656) but results so far in infected people have been mixed.

EIDD-2801, is an isopropylester prodrug of the ribonucleoside analogue N4-hydroxycytidine (NHC, EIDD-1931) that shows broad spectrum antiviral activity against various RNA viruses including Ebola, Influenza and CoV (Toots et al. 2019). NHC acts as a competitive alternative substrate for virally encoded RNA-dependent RNA polymerases. NHC was shown to inhibit multiple genetically-distinct Bat-CoV viruses in human primary epithelial cells without affecting cell viability. Prophylactic/therapeutic oral administration of NHC reduced lung titers and prevented acute lung failure in C57B/6 mice infected with CoV. The potency of NHC against multiple coronaviruses, its therapeutic efficacy, and oral bioavailability in vivo, all highlight its potential as an effective antiviral against SARS-CoV-2 and other future zoonotic coronaviruses (Sheahan et al. 2020).

Literature references

Gotte, M., Gordon, CJ., Feng, JY., Tchesnokov, EP., Porter, DP. (2020). The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J. Biol. Chem.*. ↗

Hart, M., Kolykhalov, AA., Bluemling, GR., Sticher, ZM., Plemper, RK., Painter, GR. et al. (2019). Characterization of orally efficacious influenza drug with high resistance barrier in ferrets and human airway epithelia. *Sci Transl Med, 11*. ↗

Amirian, ES., Levy, JK. (2020). Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. *One Health, 9*, 100128. ↗

Gotte, M., Woolner, E., Gordon, CJ., Feng, JY., Perry, JK., Tchesnokov, EP. et al. (2020). Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem., 295*, 6785-6797. ↗

Harcourt, J., Schäfer, A., Saindane, M., Hill, CS., Sims, AC., Zhou, S. et al. (2020). An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci Transl Med.* ↗

Editions

2020-05-07	Authored, Edited	Jassal, B.
2020-05-14	Reviewed	Shoichet, BK.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp14 acts as a cap N7 methyltransferase to modify SARS-CoV-1 gRNA (plus strand) ↗

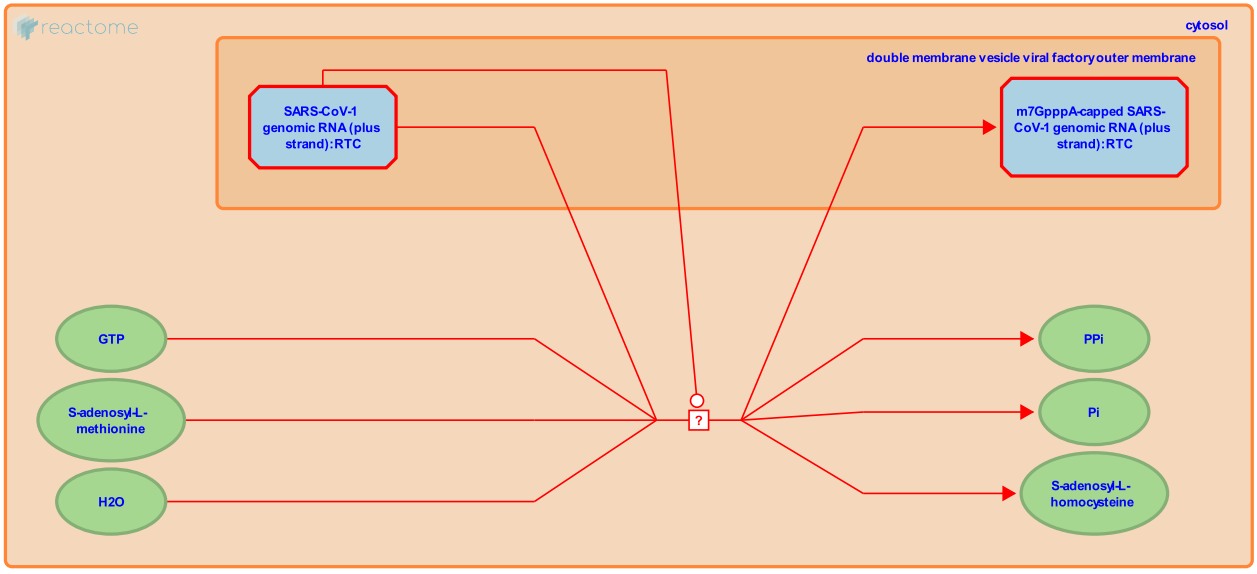
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9684017

Type: uncertain

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



The genomic and subgenomic mRNAs of SARS-CoV-1 coronavirus, including the plus strand genomic RNA, are presumed to be capped at their 5′ end, based on studies of the mouse hepatitis virus (MHV) (Lai and Stohlman 1981) and the equine torovirus (van Vliet et al. 2002). Non-structural protein 14 (nsp14) acts as an RNA guanine-N7-methyltransferase (N7-MTase) that completes the synthesis of the cap-0 on the SARS-CoV-1 plus strand genomic RNA. Cap-0 represents N7-methyl guanosine connected to the 5′ nucleotide through a 5′ to 5′ triphosphate linkage, and is also known as m7G cap or m7Gppp cap. The N7-MTase domain maps to the carboxy-terminal part of nsp14 (Chen et al. 2009). Cap-0 formation requires three sequential reactions catalyzed by RNA triphosphatase (TPase), guanylyltransferase (GTase), and N7-MTase. There is no evidence that nsp14 possesses TPase and GTase activities, and no other SARS-CoV-1 proteins with these activities have been identified, so the identities of the enzymes that mediate these required steps remain unknown. Based on the study of the human coronavirus 229E, non-structural protein 13 (nsp13) may have a TPase activity in addition to its established helicase activity (Ivanov and Ziebuhr 2004).

Preceded by: RTC synthesizes SARS-CoV-1 plus strand genomic RNA

Followed by: nsp16 acts as a cap 2'-O-methyltransferase to modify SARS-CoV-1 gRNA (plus strand)

Literature references

Guo, D., Cai, H., Chen, Y., Pan, J., Xiang, N., Tien, P. et al. (2009). Functional screen reveals SARS coronavirus non-structural protein nsp14 as a novel cap N7 methyltransferase. *Proc. Natl. Acad. Sci. U.S.A., 106*, 3484-9. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp16 acts as a cap 2'-O-methyltransferase to modify SARS-CoV-1 gRNA (plus strand)

↗

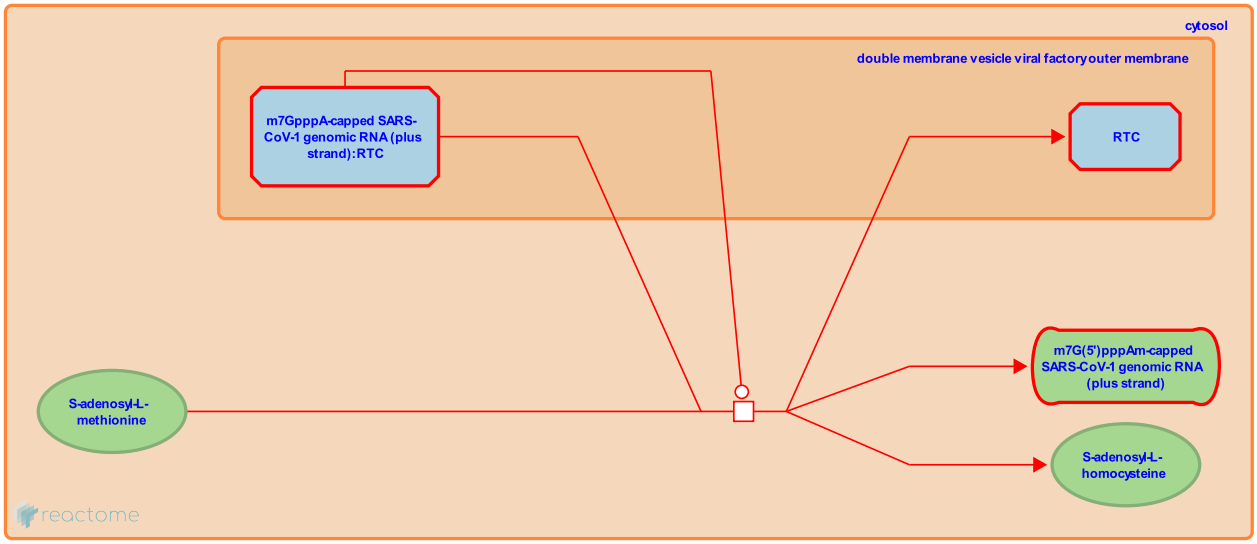
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9684032

Type: transition

Compartments: cytosol, double membrane vesicle viral factory outer membrane

Diseases: severe acute respiratory syndrome



The genomic and subgenomic mRNAs of SARS-CoV-1 coronavirus, including the plus strand genomic RNA, are presumed to be capped at their 5′ end, based on studies of the mouse hepatitis virus (MHV) (Lai and Stohlman 1981) and the equine torovirus (van Vliet et al. 2002). The non-structural protein 16 (nsp16) acts as a 2'O-methyltransferase that converts coronavirus cap-0 to cap-1, which was first demonstrated with nsp16 cloned from the feline coronavirus (FCV) (Decroly et al. 2008). Cap-0 represents N7-methyl guanosine connected to the 5′ nucleotide through a 5′ to 5′ triphosphate linkage (also known as m7G cap or m7Gppp cap). Cap-1 is generated by an additional methylation on the 2′O position of the initiating nucleotide, and is also known as m7GpppNm. Non-structural protein 10 (nsp10) acts as an activator of nsp16 and is necessary for cap-1 synthesis (Bouvet et al. 2010, Decroly et al. 2011). Coronavirus RNAs with cap-1 are protected from IFIT-mediated interferon response, as IFITs recognize unmethylated 2'-O RNAs. IFITs are interferon-induced proteins with tetratricopeptide repeats that recognize unmethylated 2'-O RNAs and act to inhibit expression of virally encoded mRNAs (Menachery et al. 2014).

Preceded by: nsp14 acts as a cap N7 methyltransferase to modify SARS-CoV-1 gRNA (plus strand)

Followed by: Polyadenylation of SARS-CoV-1 genomic RNA (plus strand)

Literature references

Bricogne, G., Ferron, F., Canard, B., Ortiz-Lombardia, M., Gluais, L., Papageorgiou, N. et al. (2011). Crystal structure and functional analysis of the SARS-coronavirus RNA cap 2'-O-methyltransferase nsp10/nsp16 complex. *PLoS Pathog., 7*, e1002059. ↗

Canard, B., Selisko, B., Decroly, E., Imbert, I., Snijder, EJ., Bouvet, M. et al. (2010). In vitro reconstitution of SARS-coronavirus mRNA cap methylation. *PLoS Pathog., 6*, e1000863. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

Polyadenylation of SARS-CoV-1 genomic RNA (plus strand) ↗

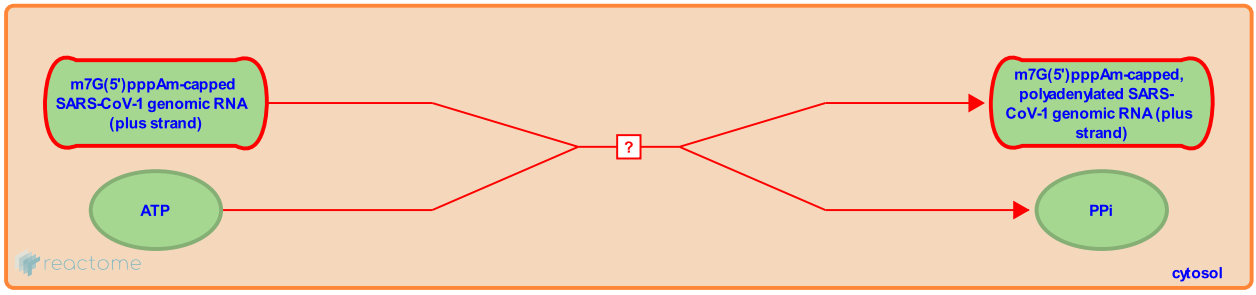
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9685519

Type: uncertain

Compartments: cytosol

Diseases: severe acute respiratory syndrome



SARS-CoV-1 plus strand genomic RNA, like genomic RNAs of other coronaviruses, possesses a polyadenylation signal in its 3'UTR and is polyadenylated by an undetermined viral RNA polymerase, possibly nsp8 or nsp12 (Spagnolo and Hogue 2000, Peng et al. 2016, Tvarogova et al. 2019).

Preceded by: nsp16 acts as a cap 2'-O-methyltransferase to modify SARS-CoV-1 gRNA (plus strand)

Literature references

Wu, HY., Tsai, TL., Lin, CN., Lin, CH., Lo, CY., Peng, YH. (2016). Characterization of the Role of Hexamer AGUAAA and Poly(A) Tail in Coronavirus Polyadenylation. *PLoS ONE, 11*, e0165077. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

ZCRB1 binds 5'UTR of SARS-CoV-1 genomic RNA ↗

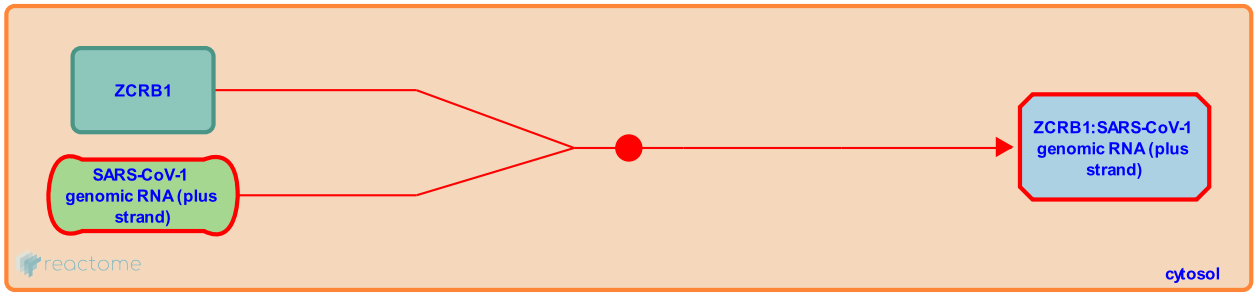
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9682009

Type: binding

Compartments: cytosol

Diseases: severe acute respiratory syndrome



Human zinc finger CCHC-type and RNA-binding motif-containing protein 1 (ZCRB1, also known as MADP1) binds to the 5'UTR of the plus strand genomic RNA of the SARS-CoV-1, as well as other coronaviruses, infectious bronchitis virus (IBV) and human coronavirus OC43. ZCRB1 normally localizes to the nucleus, where it is a component of the U12-type spliceosome. Upon infection with a coronavirus, ZCRB1 appears in the cytosol. Binding of ZCRB1 to the 5'UTR stem of coronavirus genomic RNA is thought to be necessary for efficient transcription of viral genes (Tan et al. 2012).

Literature references

Tan, YW., Hong, W., Liu, DX. (2012). Binding of the 5'-untranslated region of coronavirus RNA to zinc finger CCHC-type and RNA-binding motif 1 enhances viral replication and transcription. *Nucleic Acids Res., 40*, 5065-77. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp13 binds DDX5 ↗

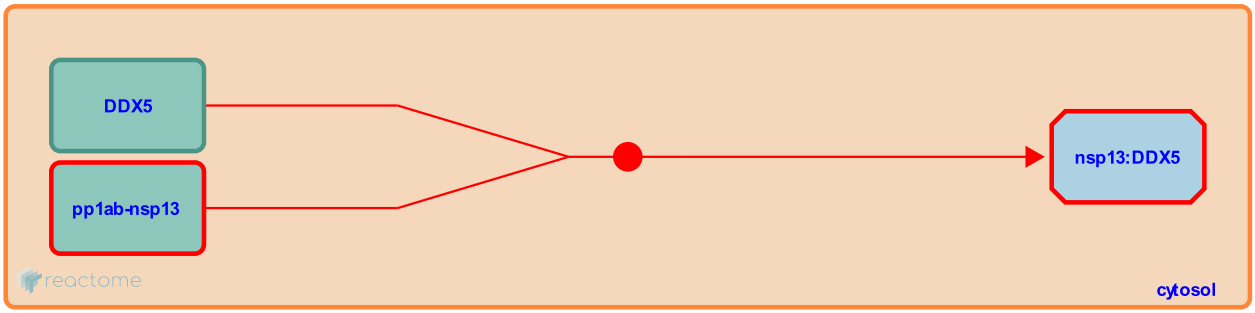
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9682631

Type: binding

Compartments: cytosol

Diseases: severe acute respiratory syndrome



nsp13, the helicase of the human SARS coronavirus 1 (SARS-CoV-1) binds to DDX5, a host protein implicated in transcription, pre-mRNA processing, RNA degradation, RNA export, ribosome assembly and translation. DDX5 knockdown inhibits viral replication (Chen et al. 2009).

Literature references

Lin, X., Wen, YM., Poon, KM., Chen, JY., Wang, YX., Chen, WN. et al. (2009). Interaction between SARS-CoV helicase and a multifunctional cellular protein (Ddx5) revealed by yeast and mammalian cell two-hybrid systems. *Arch. Virol., 154*, 507-12. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp15 binds RB1 ↗

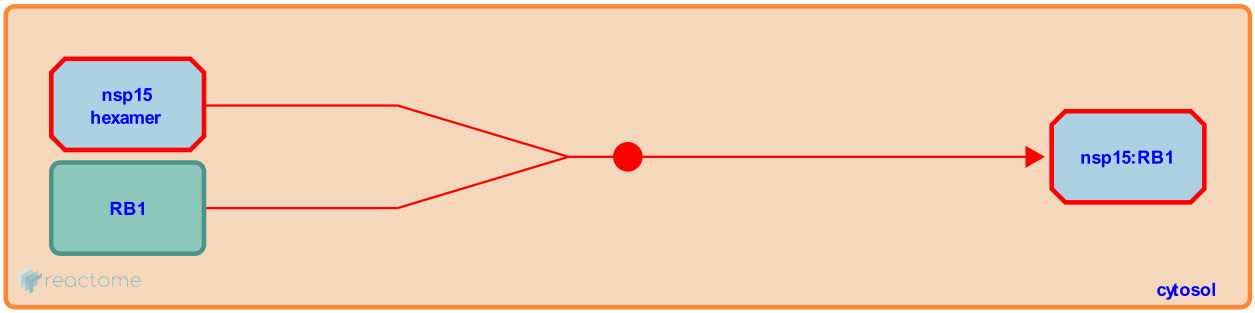
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9682712

Type: binding

Compartments: cytosol

Diseases: severe acute respiratory syndrome



Human SARS coronavirus 1 (SARS-CoV-1) non-structural protein 15 (nsp15) contains the LXCXE/D motif characteristic of proteins that bind to the retinoblastoma protein RB1. Binding to human RB1 increases the endonuclease activity of nsp15 but is not required for it. RB1 bound to nsp15 is retained in the cytosol. Interaction of nsp15 with RB1 likely affects the cell cycle of infected cells and probably modulates cytotoxicity of SARS-CoV-1 (Bhardwaj et al. 2012).

Literature references

Kao, CC., Liu, P., Bhardwaj, K., Leibowitz, JL. (2012). The coronavirus endoribonuclease Nsp15 interacts with retinoblastoma tumor suppressor protein. *J. Virol., 86*, 4294-304. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

nsp16 binds VHL ↗

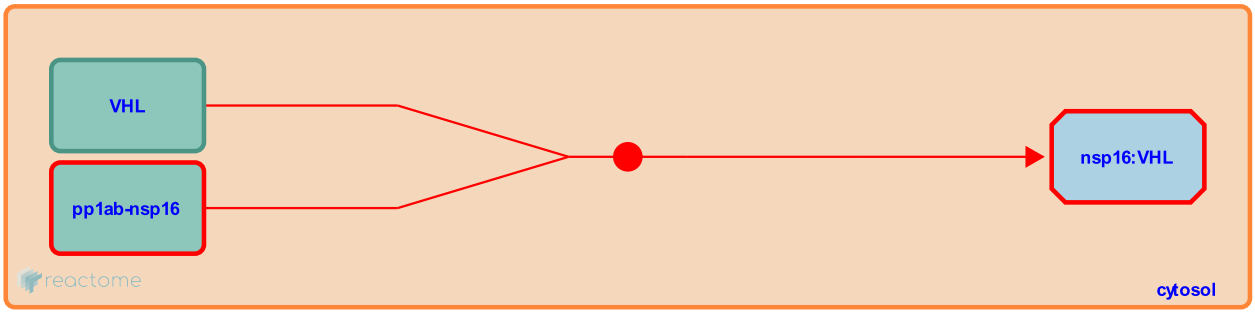
Location: Replication of the SARS-CoV-1 genome

Stable identifier: R-HSA-9683455

Type: binding

Compartments: cytosol

Diseases: severe acute respiratory syndrome



Human von Hippel Lindau (VHL) protein, a tumor suppressor that acts as a component of an E3 ubiquitin ligase complex, interacts with the non-structural protein 16 (nsp16) of the human SARS coronavirus 1 (SARS-CoV-1) and the mouse hepatitis virus, also a coronavirus. VHL negatively regulates SARS-CoV-1 replication, but the exact mechanism is not known (Yu et al. 2015).

Literature references

Hou, P., Guo, D., Chen, Y., Chen, S., Wang, M., Yu, X. (2015). VHL negatively regulates SARS coronavirus replication by modulating nsp16 ubiquitination and stability. *Biochem. Biophys. Res. Commun., 459*, 270-276. ↗

Editions

2020-04-30	Authored	Orlic-Milacic, M.
2020-05-11	Edited	Orlic-Milacic, M.
2020-05-27	Reviewed	Mazein, A., Acencio, ML.

Table of Contents