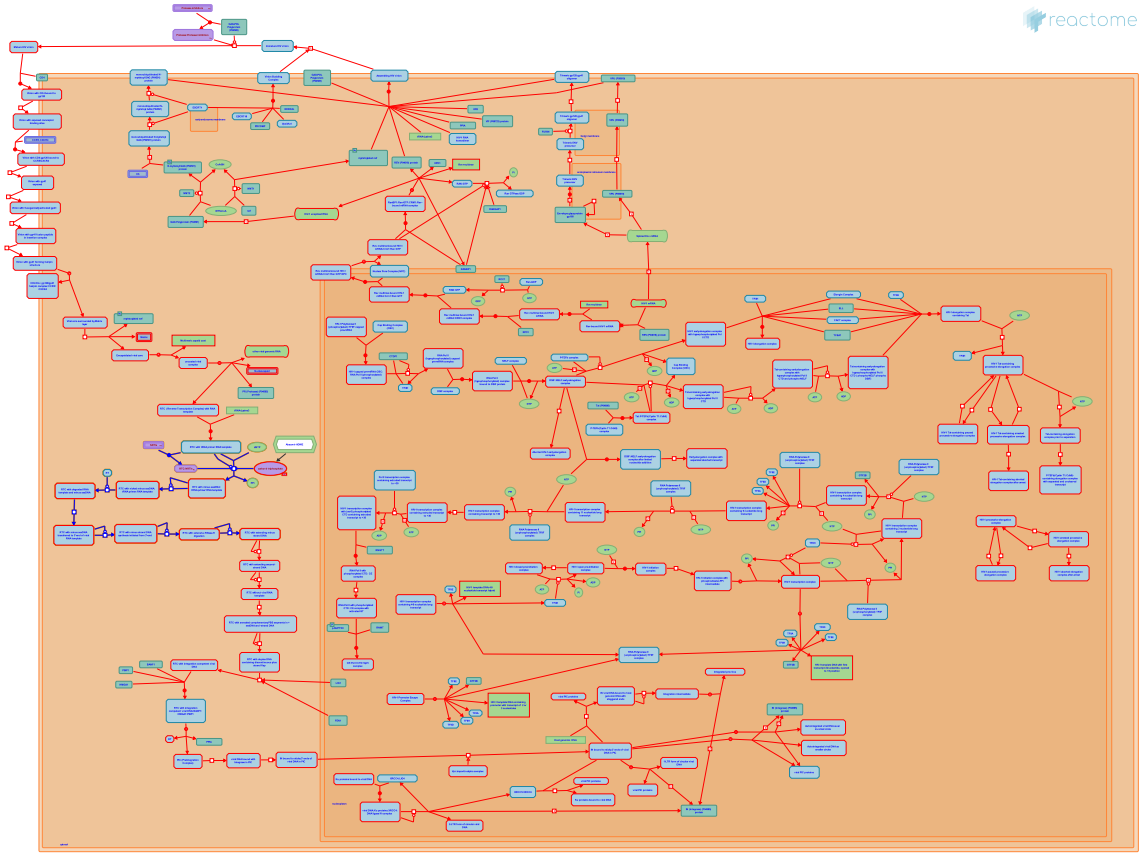
Minus-strand DNA synthesis



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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.

01/04/2024

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. ↗

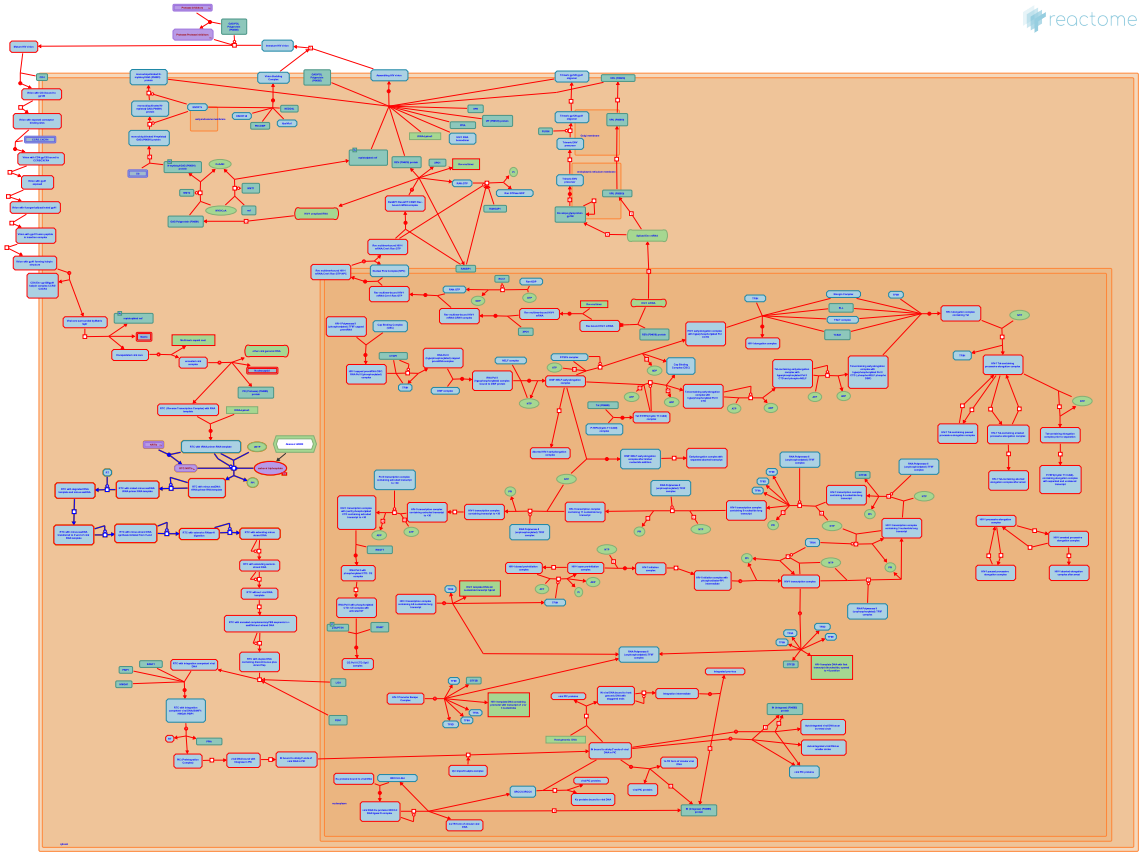
Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

Minus-strand DNA synthesis ↗

Stable identifier: R-HSA-164516

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



In the first part of reverse transcription, minus-strand synthesis, a DNA strand complementary to the HIV genomic RNA is synthesized, using the viral RNA as a template and a host cell lysine tRNA molecule as primer. The synthesis proceeds in two discrete steps, separated by a strand transfer event. As minus strand DNA is synthesized, the viral genomic RNA is degraded, also in several discrete steps. Two specific polypurine tracts (PPT sequences) in the viral RNA, one within the pol gene (central or cPPT) and one immediately preceding the U3 sequence (3' PPT) are spared from degradation and serve to prime synthesis of DNA complementary to the minus strand (plus-strand synthesis). During plus-strand synthesis, Preston and colleagues observed secondary sites of plus-strand initiation at low frequency both in the cell-free system and in cultured virus (Klarman et al., 1997). Both DNA synthesis and RNA degradation activities are catalyzed by the HIV-1 reverse transcriptase (RT) heterodimer.

Literature references

Nevinsky, GA., Litvak, S., Tarrago-Litvak, L., Andreola, ML., Sarih-Cottin, L. (1994). The reverse transcriptase of HIV-1: from enzymology to therapeutic intervention. *FASEB J, 8*, 497-503. ↗

De Clercq, E., Anne, J., Jonckheere, H. (2000). The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med Res Rev, 20*, 129-54. ↗

Whitcomb, JM., Hughes, SH. (1992). Retroviral reverse transcription and integration: progress and problems. *Annu Rev Cell Biol, 8*, 275-306. ↗

Nevinsky, GA., Litvak, S., Tarrago-Litvak, L., Bordier, B., Andreola, ML., Barr, PJ. et al. (1992). Interaction of tRNA-Lys with the p66/p66 form of HIV-1 reverse transcriptase stimulates DNA polymerase and ribonuclease H activities. *J Biol Chem, 267*, 19356-62. ↗

Varmus, HE., Hughes, SH., Coffin, JM. (1997). Reverse Transcriptase and the Generation of Retroviral DNA, Retroviruses. *Cold Spring Harbor Laboratory Press*, 121-160.

Editions

2006-05-19	Authored, Edited	Gopinathrao, G., D'Eustachio, P.
2006-10-31	Reviewed	Hughes, SH.

Synthesis of minus strand strong stop DNA (-sssDNA) ↗

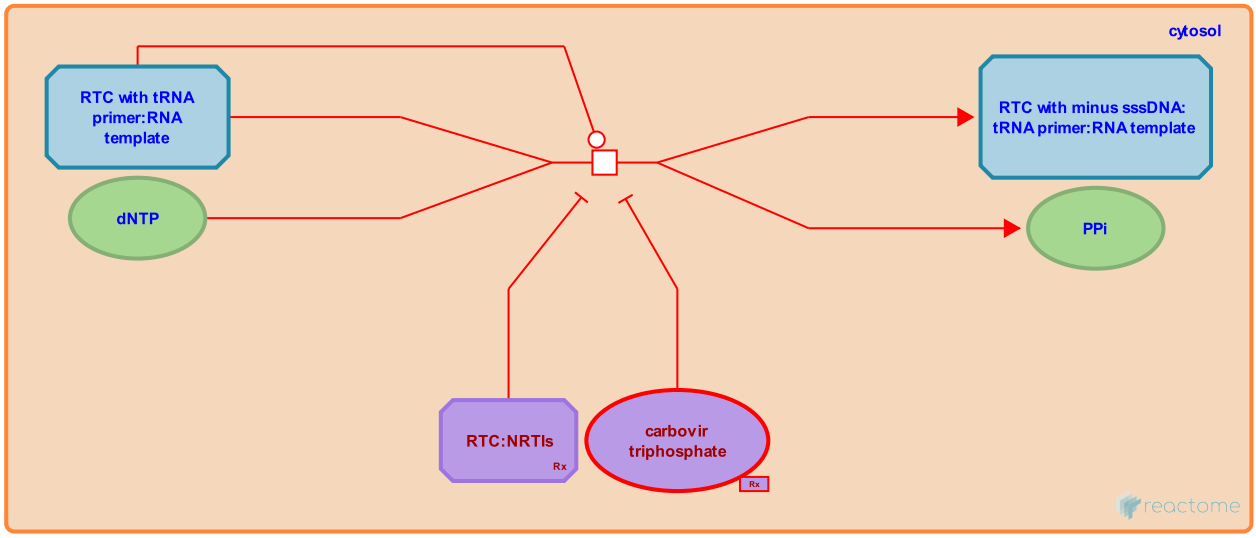
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-164504

Type: transition

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



To catalyze DNA synthesis, retroviral reverse transcriptase requires a primer strand to extend and a template strand to copy. For HIV-1, the primer is the 3'-end of a partially unwound lysine(3) tRNA annealed to the PBS (primer binding site) 179 bases from the 5' end of the retroviral genomic RNA (Isel et al. 1995). Reverse transcription of the viral genomic RNA proceeds from the bound tRNA primer to the 5' end of the viral RNA, yielding a minus-strand strong-stop DNA (-sssDNA) complementary to the R and U5 elements of the HIV-1 viral genome, as shown in the figure below (Telesnitsky and Goff 1997; Jonckheere et al. 2000). The reaction takes place in the host cell cytosol, and is catalyzed by the reverse transcriptase activity of the HIV-1 RT heterodimer.

NucleoCapsid (NC) protein prevents self-priming by generating or stabilizing a thermodynamically favored RNA-DNA heteroduplex instead of the kinetically favored TAR hairpin seen in reverse transcription experiments in vitro (Driscoll and Hughes 2000).

Followed by: RNase H-mediated cleavage of the RNA strand of the -sssDNA:RNA duplex

Literature references

De Clercq, E., Anne, J., Jonckheere, H. (2000). The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med Res Rev, 20*, 129-54. ↗

Varmus, HE., Hughes, SH., Coffin, JM. (1997). Reverse Transcriptase and the Generation of Retroviral DNA, Retroviruses. *Cold Spring Harbor Laboratory Press*, 121-160.

Editions

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RTC binds NRTIs ↗

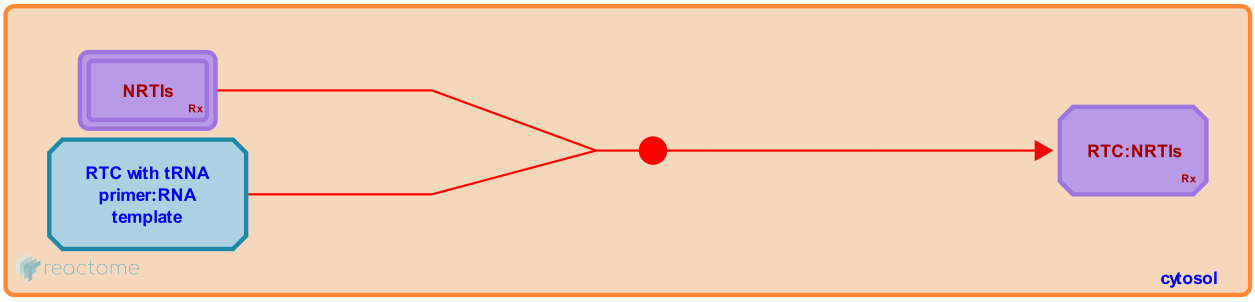
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-9697051

Type: binding

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



Antiretroviral (ARV) therapy, comprising a backbone of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) plus another ARV, has helped extend life expectancy in people living with HIV (Orkin et al. 2018). NRTIs work by inhibiting HIV reverse transcriptase (RT), preventing transcription of HIV RNA to DNA. Emtricitabine, a cytidine analogue (Feng et al. 2009, Al-Majed et al. 2020) and tenofovir, an acyclic nucleotide diester analogue of adenosine monophosphate (McConville et al. 2015, Ray et al. 2016) are compounds which inhibit RT causing chain termination and inhibition of viral protein synthesis.

Literature references

Miller, MD., Goodman, D., Myrick, F., Svarovskaia, ES., Borroto-Esoda, K., Feng, JY. et al. (2009). The triple combination of tenofovir, emtricitabine and efavirenz shows synergistic anti-HIV-1 activity in vitro: a mechanism of action study. *Retrovirology, 6*, 44. ↗

Hitchcock, MJ., Fordyce, MW., Ray, AS. (2016). Tenofovir alafenamide: A novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. *Antiviral Res., 125*, 63-70. ↗

Boyd, P., Major, I., McConville, C. (2014). Efficacy of Tenofovir 1% Vaginal Gel in Reducing the Risk of HIV-1 and HSV-2 Infection. *Clin Med Insights Womens Health, 7*, 1-8. ↗

Bakheit, AHH., Abdelhameed, AS., Al-Kahtani, HM., Al-Qahtani, BM., Al-Majed, AA. (2020). Emtricitabine. *Profiles Drug Subst Excip Relat Methodol, 45*, 55-91. ↗

Editions

2020-03-30	Authored, Edited	Jassal, B.
2020-05-14	Reviewed	Shoichet, BK.

RNase H-mediated cleavage of the RNA strand of the -sssDNA:RNA duplex ↗

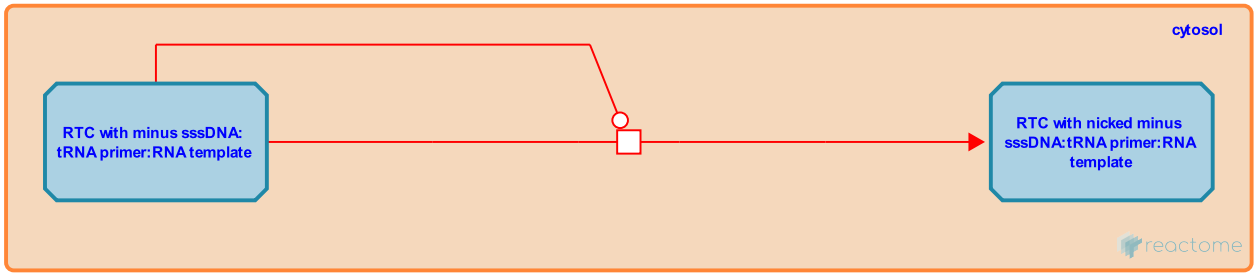
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-164519

Type: transition

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



As the reverse transcriptase activity of the HIV-1 RT heterodimer catalyzes the synthesis of minus-strand strong stop DNA (-sssDNA), the RNaseH activity of the same RT heterodimer catalyzes the degradation of the complementary viral genomic RNA sequences. Degradation of this RNA is required for the efficient transfer of the -sssDNA to the 5' end of the viral genomic RNA. The RNase H active site is positioned within the HIV-1 RT heterodimer so as to attack the RNA strand of the RNA:DNA duplex at a point 18 bases behind the site of reverse transcription (Furfine and Reardon 1991; Ghosh et al. 1995; Gopalakrishnan et al. 1992; Wohrl and Moelling 1990). The rate of RNase H cleavage is substantially lower than the rate of DNA synthesis, however (Kati et al. 1992), and may further depend on RT stalling and structural features of the viral genomic RNA template. The product of these combined DNA synthesis and RNA degradation events is a DNA strand still duplexed with extended viral genomic RNA fragments.

Preceded by: Synthesis of minus strand strong stop DNA (-sssDNA)

Followed by: RNase H-mediated degradation of the RNA strand of the -sssDNA:RNA duplex

Literature references

Le Grice, SF., Benkovic, SJ., Hughes, SH., Howard, KJ., Ghosh, M., Cameron, CE. (1995). Truncating alpha-helix E' of p66 human immunodeficiency virus reverse transcriptase modulates RNase H function and impairs DNA strand transfer. *J Biol Chem, 270*, 7068-76. ↗

De Clercq, E., Anne, J., Jonckheere, H. (2000). The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med Res Rev, 20*, 129-54. ↗

Reardon, JE., Furfine, ES. (1991). Reverse transcriptase.RNase H from the human immunodeficiency virus. Relationship of the DNA polymerase and RNA hydrolysis activities. *J Biol Chem, 266*, 406-12. ↗

Varmus, HE., Hughes, SH., Coffin, JM. (1997). Reverse Transcriptase and the Generation of Retroviral DNA, Retroviruses. *Cold Spring Harbor Laboratory Press*, 121-160.

Editions

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2006-10-31	Reviewed	Hughes, SH.

RNase H-mediated degradation of the RNA strand of the -sssDNA:RNA duplex ↗

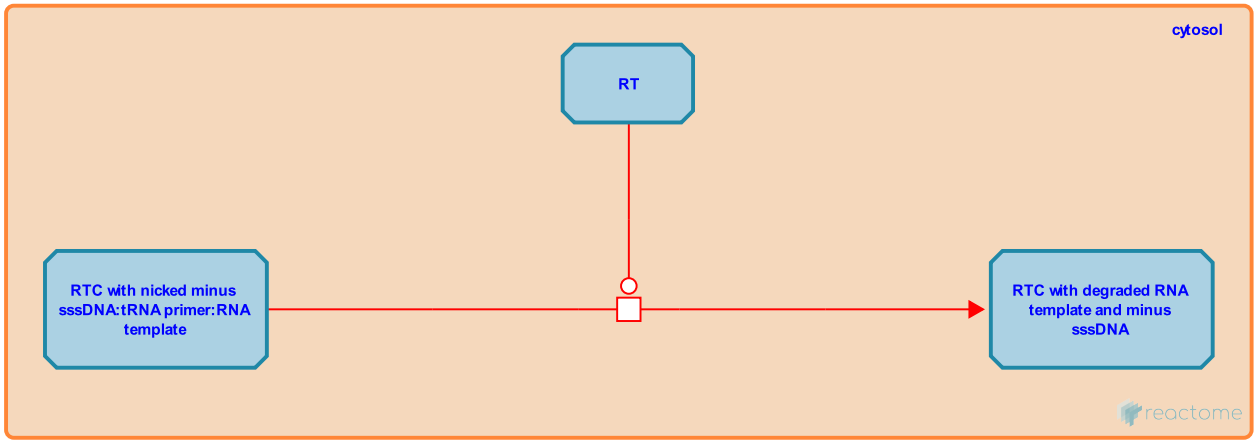
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-182859

Type: transition

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



The rate of RNase H cleavage is substantially lower than the rate of DNA synthesis (Kati et al. 1992), so the product of the combined DNA synthesis and RNA degradation events catalyzed by the RT heterodimer mediating minus-strand strong stop DNA (-sssDNA) synthesis is a DNA segment still duplexed with extended viral genomic RNA fragments. In vitro, other RT heterodimers bind the remaining RNA:DNA heteroduplexes and their RNase H domains further degrade the viral genomic RNA (Wisniewski et al. 2000a, b).

Preceded by: RNase H-mediated cleavage of the RNA strand of the -sssDNA:RNA duplex

Followed by: First strand transfer mediated by Repeated (R) sequence

Literature references

Wisniewski, M., Bambara, RA., Palaniappan, C., Balakrishnan, M., Fay, PJ. (2000). Unique progressive cleavage mechanism of HIV reverse transcriptase RNase H. *Proc Natl Acad Sci U S A, 97*, 11978-83. ↗

Wisniewski, M., Bambara, RA., Palaniappan, C., Balakrishnan, M., Fay, PJ. (2000). The sequential mechanism of HIV reverse transcriptase RNase H. *J Biol Chem, 275*, 37664-71. ↗

Kati, WM., Jerva, LF., Johnson, KA., Anderson, KS. (1992). Mechanism and fidelity of HIV reverse transcriptase. *J Biol Chem, 267*, 25988-97. ↗

Editions

2006-05-19	Authored	Gopinathrao, G., D'Eustachio, P.
2006-10-31	Reviewed	Hughes, SH.

First strand transfer mediated by Repeated (R) sequence ↗

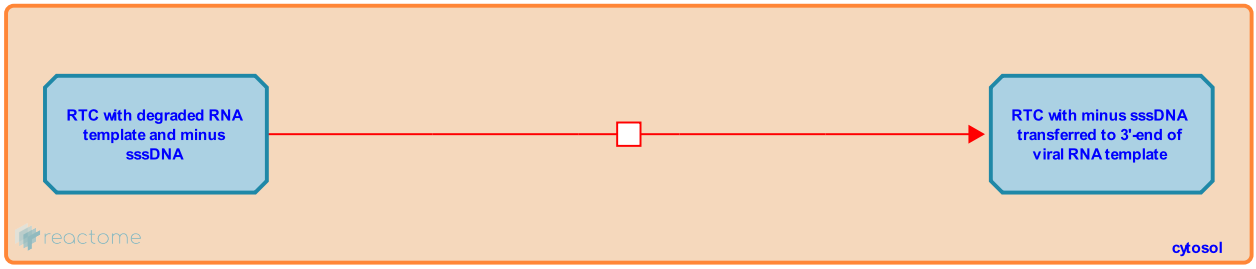
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-164503

Type: transition

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



The minus strand strong stop DNA (-sssDNA) is transferred to the 3' end of the HIV-1 genomic RNA, where the 3' end of the -sssDNA anneals to the viral genomic R sequence motif (Ghosh et al. 1995; Klaver and Berkhout 1994; Ohi and Clever 2000; Telesnitsky and Goff 1997). Viral NC (nucleocapsid) protein may play a role in this transfer (Driscoll and Hughes 2000).

Preceded by: RNase H-mediated degradation of the RNA strand of the -sssDNA:RNA duplex

Followed by: Minus strand DNA synthesis resumes

Literature references

Klaver, B., Berkhout, B. (1994). Premature strand transfer by the HIV-1 reverse transcriptase during strong-stop DNA synthesis. *Nucleic Acids Res, 22*, 137-44. ↗

Clever, JL., Ohi, Y. (2000). Sequences in the 5' and 3' R elements of human immunodeficiency virus type 1 critical for efficient reverse transcription. *J Virol, 74*, 8324-34. ↗

Editions

2006-05-19	Authored, Edited	Gopinathrao, G., D'Eustachio, P.
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Minus strand DNA synthesis resumes ↗

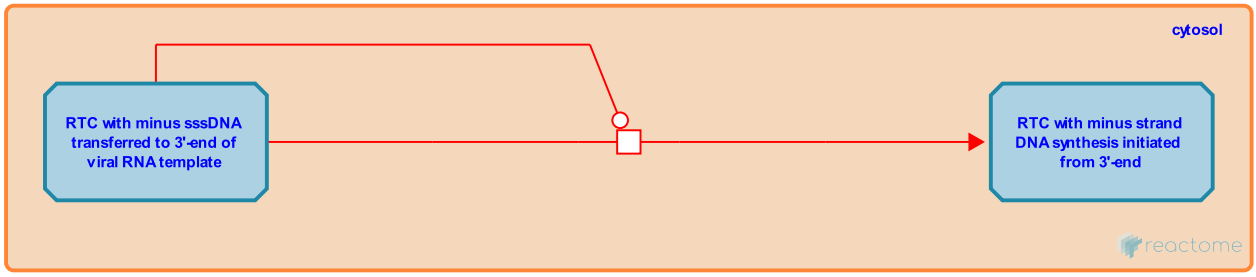
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-164520

Type: transition

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



Synthesis of minus-strand DNA proceeds toward the 5' end of the PBS motif of the template HIV genomic RNA.

Preceded by: First strand transfer mediated by Repeated (R) sequence

Followed by: RNase H-mediated cleavage of the template strand

Literature references

De Clercq, E., Anne, J., Jonckheere, H. (2000). The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med Res Rev, 20*, 129-54. ↗

Varmus, HE., Hughes, SH., Coffin, JM. (1997). Reverse Transcriptase and the Generation of Retroviral DNA, Retroviruses. *Cold Spring Harbor Laboratory Press*, 121-160.

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RNase H-mediated cleavage of the template strand ↗

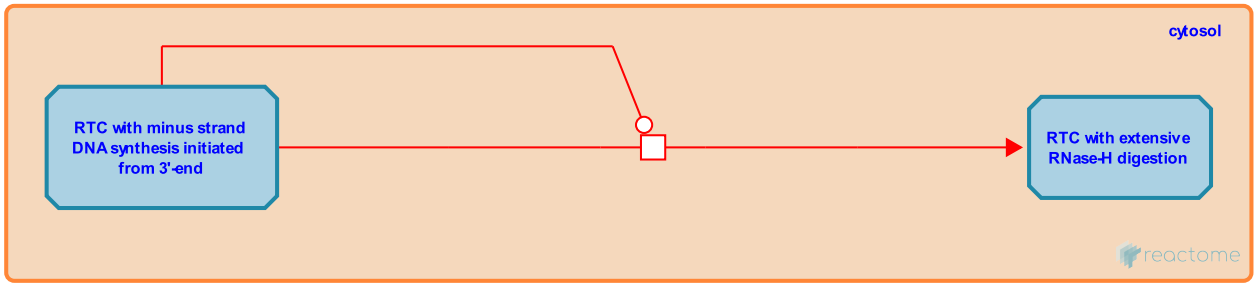
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-164528

Type: transition

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



As the reverse transcriptase activity of the HIV-1 RT heterodimer catalyzes the extension of the minus-strand DNA, the RNaseH activity catalyzes the degradation of the complementary viral genomic RNA sequences. Telesnitsky and Goff (1993) observed that two defective forms of reverse transcriptase can complement to restore retroviral infectivity. The RNase H active site is positioned within the HIV-1 RT heterodimer so as to attack the RNA strand of the RNA:DNA duplex at a point 18 bases behind the site of reverse transcription (Furfine and Reardon 1991; Ghosh et al. 1995; Gopalakrishnan et al. 1992; Wohrl and Moelling 1990). The rate of RNase H cleavage is substantially lower than the rate of DNA synthesis and the level of its activity in vivo is unclear, however (Kati et al. 1992). The product of these combined DNA synthesis and RNA degradation events is a DNA strand still duplexed with extended viral genomic RNA fragments.

Preceded by: Minus strand DNA synthesis resumes

Followed by: RNase H-mediated degradation of the template strand

Literature references

Sarafianos, SG., Alvord, WG., Julias, JG., Hughes, SH., McWilliams, MJ., Arnold, E. (2004). Effects of mutations in the G tract of the human immunodeficiency virus type 1 polypurine tract on virus replication and RNase H cleavage. *J Virol, 78*, 13315-24. ↗

De Clercq, E., Anne, J., Jonckheere, H. (2000). The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med Res Rev, 20*, 129-54. ↗

Pullen, KA., Champoux, JJ., Rattray, AJ. (1993). The sequence features important for plus strand priming by human immunodeficiency virus type 1 reverse transcriptase. *J Biol Chem, 268*, 6221-7. ↗

Varmus, HE., Hughes, SH., Coffin, JM. (1997). Reverse Transcriptase and the Generation of Retroviral DNA, Retroviruses. *Cold Spring Harbor Laboratory Press*, 121-160.

Editions

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2006-10-31	Reviewed	Hughes, SH.

RNase H-mediated degradation of the template strand ↗

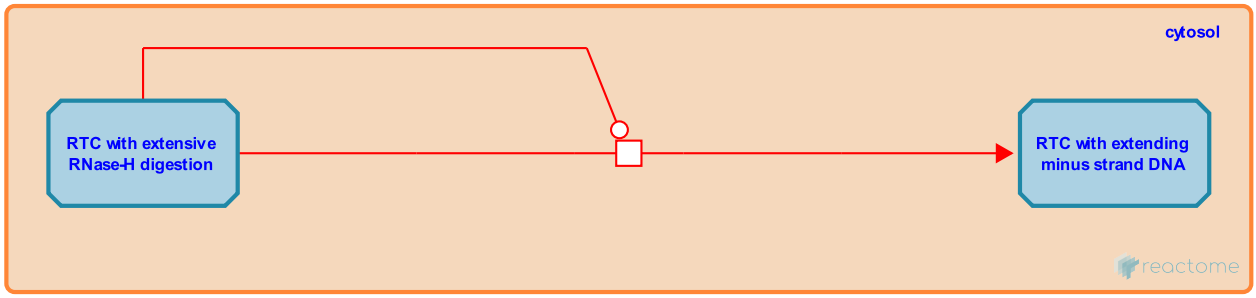
Location: Minus-strand DNA synthesis

Stable identifier: R-HSA-182795

Type: transition

Compartments: cytosol

Diseases: Human immunodeficiency virus infectious disease



The rate of RNase H cleavage is substantially lower than the rate of DNA synthesis (Kati et al. 1992), so the product of the combined DNA synthesis and RNA degradation events catalyzed by the RT heterodimer mediating minus-strand DNA synthesis is a DNA segment still duplexed with extended viral genomic RNA fragments. Other RT heterodimers bind the remaining RNA:DNA heteroduplexes and their RNase H domains further degrade the viral genomic RNA (Wisniewski et al. 2000a, b). Two PPT (polypurine tract) sequence motifs in the template, one immediately 5' to the U3 sequence and one located within the pol gene in the center of the viral genome, are spared from degradation (Charneau et al. 1992; Julias et al. 2004; Pullen et al. 1993).

Preceded by: RNase H-mediated cleavage of the template strand

Literature references

Sarafianos, SG., Alvord, WG., Julias, JG., Hughes, SH., McWilliams, MJ., Arnold, E. (2004). Effects of mutations in the G tract of the human immunodeficiency virus type 1 polypurine tract on virus replication and RNase H cleavage. *J Virol, 78*, 13315-24. ↗

Wisniewski, M., Bambara, RA., Palaniappan, C., Balakrishnan, M., Fay, PJ. (2000). Unique progressive cleavage mechanism of HIV reverse transcriptase RNase H. *Proc Natl Acad Sci U S A, 97*, 11978-83. ↗

Pullen, KA., Champoux, JJ., Rattray, AJ. (1993). The sequence features important for plus strand priming by human immunodeficiency virus type 1 reverse transcriptase. *J Biol Chem, 268*, 6221-7. ↗

Wisniewski, M., Bambara, RA., Palaniappan, C., Balakrishnan, M., Fay, PJ. (2000). The sequential mechanism of HIV reverse transcriptase RNase H. *J Biol Chem, 275*, 37664-71. ↗

Alizon, M., Clavel, F., Charneau, P. (1992). A second origin of DNA plus-strand synthesis is required for optimal Human Immunodeficiency Virus replication. *J Virol, 66*, 2814-2820. ↗

Editions

2006-05-19	Authored	Gopinathrao, G., D'Eustachio, P.
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