

Class: SARS-CoV RNA-dependent RNA polymerase

Attributes: (1, 2)

Accession #: NP_828869

Synonyms: nsp12, RdRp

Molecular weight:

Number of amino acids: 931

Structure:

1) Homology: (2)

- the 5' RDRP fragment diverged from other Coronavirus taxa prior to divergences between and within groups 1-3
- the 3' RDRP fragment diverged from other coronavirus homologs more recently, after divergences between and within groups 1-3
 - the potential horizontal transmissions of s2m to SARS and the 3' region of RDRP are correlated
 - perhaps from an ancestor of IBV, the only coronavirus with s2m

2) Domains: (1)

- N-term domain (aa 1-375)
- C-term domain (aa 376-932)
 - polymerase catalytic site
 - catalytic domain consist of fingers, palm and thumb subdomains that form an encircled nucleic acid-binding tunnel

Fingers subdomain

- composed of residues 376-584 and 626 to 679
- contains an N-term portion (aa 405-444) that forms a long loop emanating from the fingertip that bridges the fingers and thumb subdomains
 - suggested to be involved in recognition of nucleotide substrate, protein-protein interactions and oligomerization of the polymerase
- composed of two polypeptide segments
 - N-term segment
 - segment spanning motifs A and B of the palm subdomain
- the base of the fingers is mainly alpha helical
- the tip of the fingers is primarily beta-strands and random coils
- there are two conserved sequence motifs F and G that play important roles in polymerization

Motif F

- proposed to consist of three submotifs F1, F2 and F3 (SARS-CoV has no F2)
- forms part of a 'beta-strand, loop, beta-strand' structure that extends from the fingers to interact with the thumb
- contains several highly conserved basic residues (Lys545{submotif F1} and Lys551 and Arg553 {submotif F3})
- residues are predicted to form part of the rNTP-binding pocket and help position the template overhang

Motif G

- consist of conserved SXGXP possibly followed by a conserved basic residue
- motif corresponds to Ser501, Gly503, Pro505 and Lys511
- forms a 'loop and alpha-helix' structure
- predicted to be involved in positioning of the 5' template strand

Palm subdomain

- residues 585-625 and 680-807
- forms the catalytic core of the polymerase
- primarily comprised of central three-stranded beta-sheet flanked by two alpha helices on one side and a beta sheet and an alpha helix on the other
- residues forming the catalytic active site are found within motifs A and C
- contains four highly conserved sequence motifs and a unique fifth motif

Motif A

- contains two highly conserved residues separated by four residues (Asp618 and Asp623)
 - Asp618 near the end of the beta strand with Asp760 and Asp761 in motif C form the catalytic center
 - may be involved in binding divalent metal ions required for catalysis
 - Asp623 located in short alpha helix
 - expected to be involved in sugar selection
- composed of a beta-strand and short alpha helix structure
- the beta strands of motif A and C form the central beta sheet

Motif B

- forms a loop and alpha helix structure
- the alpha helix of motif B together with the alpha helix of motif D packs beneath the central beta sheet
- contains several conserved residues Ser682, Gly683, Thr687 and Asn691
 - participate in recognition of the correct nucleic acid and selection of the correct substrate
 - Ser682, Gly683, Thr687 appear to interact with the nucleotide that base-pairs with the incoming rNTP
 - Asn691 is proposed to contribute to the specificity of polymerase for rNTP versus dNTPs

-via a hydrogen-bonding interaction with Asp623 of motif A which in turn hydrogen-bonds to the 2'OH of rNTP

Motif C

- forms a beta strand, turn, beta strand hairpin structure
- contains the highly conserved XSDD motif (Leu758-Ser759-Asp760-Asp761) at the polymerase active site
 - conserved Asp are located at the turn
 - Ser759 appears to help position the 3' primer terminus and / or priming nucleotide
 - together with Asp618 of motif A, the two conserved aspartates of motif C form the polymerase active site
 - Asp760 is coordinated with the metal ions during catalysis
 - Asp761 may help position the side chains of the other two aspartates and the 3' primer terminus by interacting with the 3' terminal phosphate of the primer strand

Motif D

- forms an alpha helix, turn and short beta strand
- alpha helix flanks the central beta sheet
- the c-term beta strand forms an antiparallel beta sheet the beta strand of motif A
- contains a hydrophilic residue in the middle of the alpha helix (Lys783) and a polar residue at the C-term of the helix (Tyr787) and an aromatic residue at the turn (Tyr788)
- motif likely involved in stabilizing the core structure of the catalytic domain and in helping position motif A

Motif E

- has a beta strand, turn and beta strand structure that is part of a three stranded antiparallel beta sheet
- corresponds to aa 810-820
- residues at the turn (Cys813 and Ser814) help position the primer strand at the polymerase active site
 - likely contribute to the fidelity of processive polymerization

Thumb subdomain

- C-term portion (aa 808-932)
- likely assumes an alpha helical structure
- thumb subdomain has great flexibility
 - essential for nucleic acid binding
 - essential for polymerization
 - appears to function as part of a translocation track during polymerization
- predicted to have a relatively unobstructed nucleic acid binding cleft that can accommodate dsRNA

3) Substrate Interaction: (1)

- the 2'-OH group of a docked canonical rNTP interacts with Asp623 of motif A and Asn691 of motif B
- the 3'-OH of the rNTP also forms a hydrogen bond with Asp623

4) Inhibitor Design: (1)

- analog inhibitors should contain groups at the 2' and 3' positions capable of making hydrogen bonding interaction with the neighboring Asp623 and Asn691
- potential nucleoside inhibitors should have the C3' endo sugar pucker conformation to make hydrogen bond at 3' position and avoid steric conflicts with 2' position

Processing:

- Released from gene one by 3C_lpro at Ser4370 and Gln5301|Ala5302

Location: unknown

Functions: (1)

- may be involved in interactions with the leader or intergenic sequences during transcription of nested mRNAs or
- in protein-protein interact w/ helicase or
- host and/or viral proteins involved in coronavirus replication

Abundance: unknown

Responsibilities:	Collaborators:
Oligomerization	polymerase
Create new viral RNAs	mRNAs
Functional consequence unknown	helicase
Functional consequence unknown	caveolin-1 (3)

- 1) Xu, X., et al., (2003) Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design, *Nucleic Acids Res*, **31(24)**, 7117-30.
- 2) Rest, J. and Mindell, D., (2003) SARS associated coronavirus has a recombinant polymerase and coronaviruses have a history of host-shifting., *Infect Genet Evol*, **3(3)**, 219-25.
- 3) Cai, Q.C., et al., (2003) Putative caveolin-binding sites in SARS-CoV proteins, *Acta Pharmacol Sin*, **24(10)**, 1051-9.