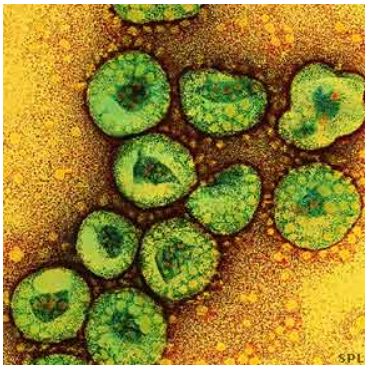
Severe Acute Respiratory Syndrome (SARS) Coronavirus



Coronaviruses

Coronaviruses are single stranded enveloped RNA viruses that have a helical geometry. Coronaviruses are the largest of RNA viruses with a genome size of up to 31kb. These viruses are grouped in the order Nidovirales. The structure common to all coronaviruses consists of spike (S), envelop (E), membrane (M), and nucleocapsid (N) proteins. There are currently three different groups of coronaviruses. Some of the notable viruses are group 2b SARS, group 1 Human coronavirus, and TGEV.

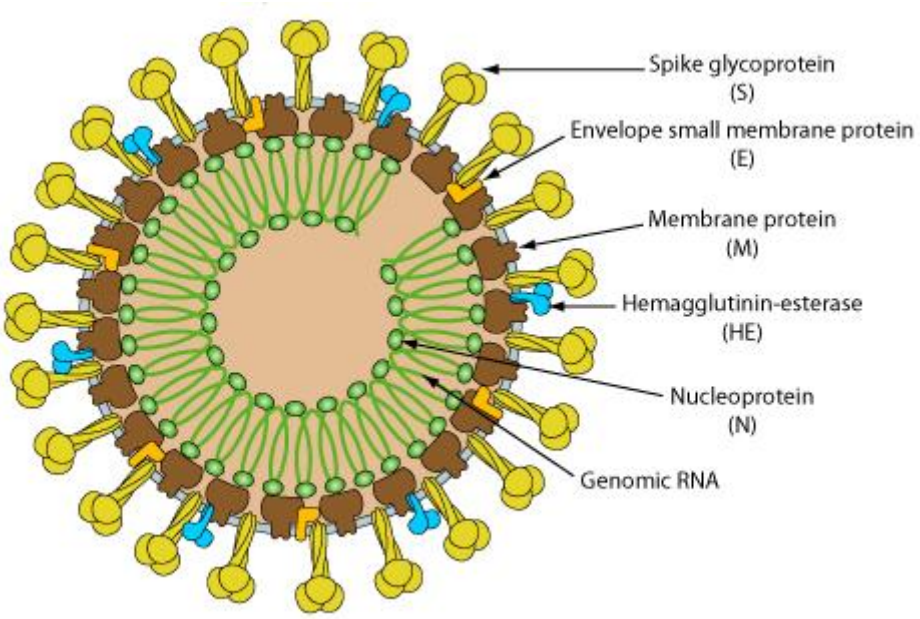
SARS coronavirus belongs to a family of coronaviruses that are enveloped, positive-stranded and single stranded RNA. Its genome is about 27 to 31.5kb, which is one of the largest among RNA viruses. SARS is like other coronaviruses in that it is polycistronic and the genome expression starts with translation of two large ORFs 1a and 1b. The SARS virus has 13 known genes and 14 known proteins. It also has 14 ORFs. There are 265bp in the 5'UTR and 342bp in the 3'UTR. Conserved proteins in all of coronaviruses are encoded by the overlapping ORFs 1a and 1b and ORFs2,4,5,6,9a. Certain protein enzymes also look like they are conserved among RNA viruses; these are: PLpro, CLpro, RdRp, and 2'-O-MT. Coronaviruses usually express pp1a (the ORF1a polyprotein) and the PP1ab polyprotein with joins ORF1a and ORF1b. The polyproteins are then processed by enzymes that are encoded by ORF1a. Product proteins from the processing includes various replicative enzymes such as RNA dependent polymerase, RNA helicase, and proteinase. The replication complex in coronavirus is also responsible for the synthesis of various mRNAs downstream of ORF 1b, which are structural and accessory proteins. Two different proteins 3CLpro and PL2pro cleave the large polyproteins into 16 smaller subunits. A unique domain in SARS is SUD (SARS-CoV unique domain), which is upstream of PL2pro and has 375 amino acids. The 3'-proximal part of the genome has five unique ORFs that are not present in other group 2 coronaviruses.

The life cycle of the SARS coronavirus starts when it enters a host cell by membrane fusion. The viral nucleic acid is then replicated, and proteins are synthesized. Assembly

of the nucelocapsid is done in the rough ER by putting together N protein and genomic RNA. The golgi apparatus swells to form smooth vesicles containing the nucleocapsides, which then bud from the golgi. The last step of forming the complete virus is the assembling of the envelops with the nucleocapsids. The smooth vesicles fuse with the cell membrane to release the viruses.

Severe Acute Respiratory Syndrome Virus Morphology

The morphology of the SARS coronavirus is characteristic of the coronavirus genus as a whole. These viruses have large pleomorphic spherical particles with bulbous surface projections that fomr a corona around particles. The envelop of the virus contains lipid and appears to consist of a distinct pair of electron dense shells. The internal component of the shell is a single-stranded helical ribonucleoprotein. There are also long surface projections that protrude from the lipid envelop. The size of these particles are about 80-90nm.

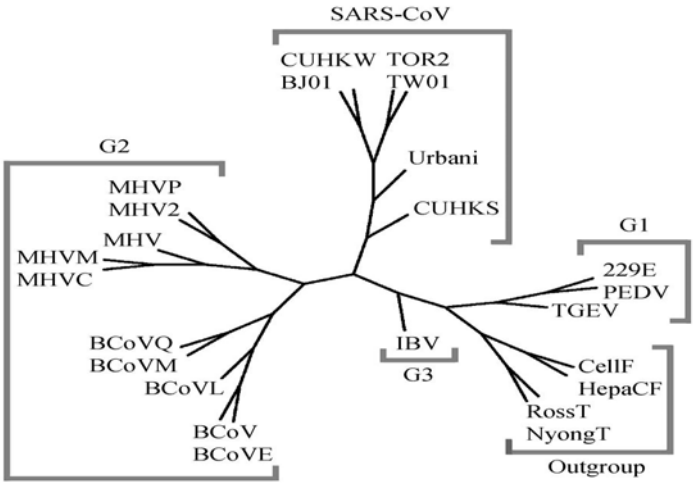


Severe Acute Respiratory Syndrome Virus Pathology

Virions first attached themselves to the surface of the host and their envelops fused with the cell membrane and the nucleocapsides enter the cell. The virus does not enter the cell via endocytosis, which is the mechanism employed by other coronaviruses. The viruses replicate their DNA inside the host cells. The SARS coronavirus infects type 2 pneumocytes, which are important for secreting pulmonary surfactants that reduce surface tension and preserves the integrity of alveolar space. SARS also infects type 1 pneumocytes, which are the primary targets early on in the infection. The spike protein also plays an important role in the pathogenesis of SARS. The S protein increases ER stress and the unfolded protein response (UPR).

Severe Acute Respiratory Syndrome Virus Evolution

SARS is most closely related to group 2 coronaviruses, but it does not segregate into any of the other three groups of coronaviruses. The closest outgroup to the coronaviruses are the toroviruses, with which it has homology in the ORF 1b replicase and the two viron proteins of S and M. SARS was determined to be an early split off from the group 2 coronaviruses based on a set of conserved domains that it shares with group 2.



Clinical/Therapeutic Considerations

Existing treatments for SARS coronavirus include the use of ribavirin, which is a nucleoside analog that has activity against numerous viruses in vitro. However, upon further study, it was shown that ribavirin did not have much activity against SARS-CoV. Another method of treatment was the use of corticosteroids, which aims to suppress cytokine-induced lung injury in phase 2 of SARS. However, this mode of therapy has the risk of complications of avascular necrosis and fungal disease. IFN-alpha was also used in combination with corticosteroids as a treatment. This drug acts to inhibit cytopathic effects of SARS-CoV, reducing viral replication. Treatment with IFN-alpha after exposure to virus yielded intermediate results. The success of clinical testing approved the treatment.

There are several other potential treatments for SARS. Glymcyrrhizin is an active component of liquorice roots. This compound can be made into a vaccine since it elicits a strong SARS-CoV specific immune response. Human monoclonal antibody (huMab) recognizes the antigen S1 protein, which recognizes the angiotensin-converting enzyme-2 receptor that leads to viral entry, in SARS-CoV. This has neutralizing activity that show huMab to be a useful inhibitor of of viral entry.

There are various proposed methods to treat and prevent SARS that are based on the discovered viral proteins, and the inhibition of their activities. A potential treatment is the inhibition of S protein cleavage by Ben-HCl. The Xa factor can cleave the S protein into S1 and S2 subunits, which is postulated to facilitate viral infection.

Another approach to tackle the virus is through vaccination. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of SARS-CoV spike protein can induce strong immune responses that provide protection against SARS.

Another proposed treatment is the targeting of the Mpro proteinase to prevent it from cleaving polyproteins in the SARS virus. The two large polyproteins that are translated undergo extensive proteolytic processing to produce functional proteins. The Mpro proteinase is particularly important since it cleaves the polyprotein at 11 conserved sites involving Leu-Gln sites. The 3D structure of the Mpro proteinase has been solved, which allows the possibility of inhibitor design. There are findings that a hexapeptyidyl inhibitor of the human rhinovirus 3C proteinase has a binding mode that can be used as a model for developing an inhibitor for the SARS Mpro proteinase.

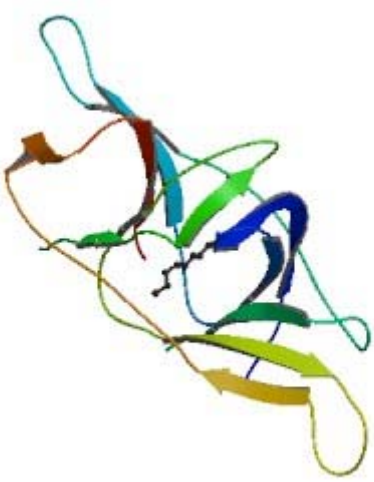
Comparison To Other Viruses

The PL2pro gene, which codes for a proteinase, is a conserved gene with group 2 Coronaviruses. The Mpro proteinase gene is highly conserved among coronaviruses. The genes that encode the Mpro proteinase is conserved among the three coronaviruses assigned, Human Coronavirus, SARS Coronavirus, and TGEV.

SARS Coronavirus is distantly related to group 2 coronaviruses, which includes the Bovine Coronavirus. It is not part of the group because it lacks genes that are present on group 2 viruses, such as PL1pro, CPD-like genes, and HE genes. A gene that is unique to SARS Coronavirus is SUD (SARS-Coronvirus unique domain). It is thought to be a proteinase.

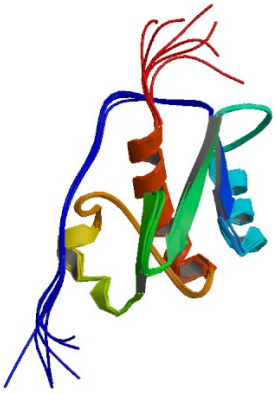
Collection of SARS PDB structures

3D crystal structures of proteins are very useful for molecular biologists since they can be used to inform the research of the location of active sites and key residues that carry out the chemistry of interest. They are also especially valuable for engineers who want to manipulate or alter the function of a particular protein. They are better informed when carrying out directed evolution or making libraries.

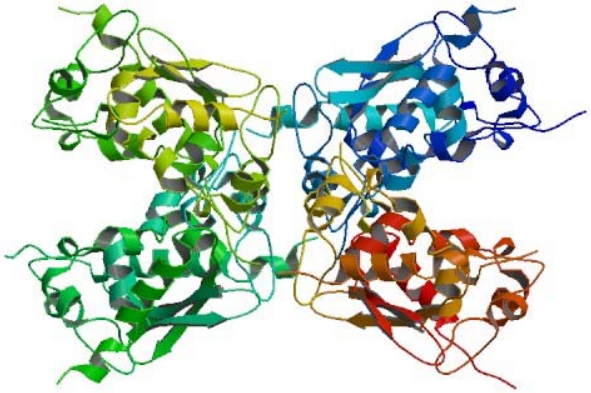


This is the protein structure of ORF-9b. The protein is produced from an alternate open reading frame within the nucleocapsid gene. The protein is likely to be involved in membrane attachment and associates with intracellular vesicles. The fold of the protein is a dimeric beta structure with amphipathic surfaces.

Meier C, Aricescu AR, Assenberg R, Aplin RT, Gilbert RJ, Grimes JM, Stuart DI. The crystal structure of ORF-9b, a lipid binding protein from the SARS coronavirus. Structure. 2006 Jul;14(7):1157-65.

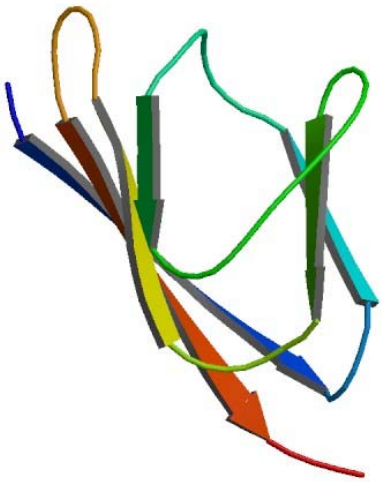


This is the protein structure of Non-structural protein 3 of the replicase polyprotein 1a.



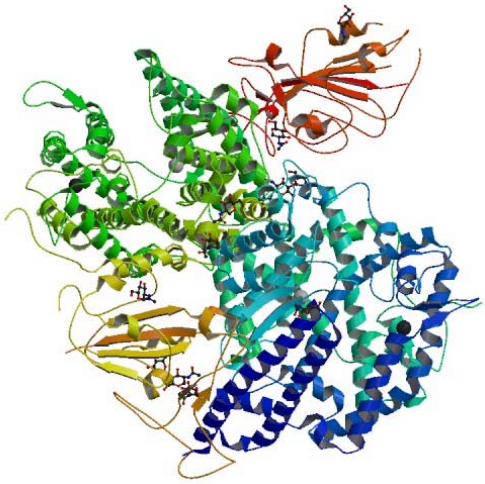
This is the protein structure of the nucleocapsid protein. The function of the protein is to form an envelop around the virus. The protein is a strong antigen and associates with various host cell motifs. This protein is of interest and a potential drug target. The interaction of this protein with the host can be more thoroughly studied since the crystal structure is available, and some means of interrupting that interaction could prove to prevent infection.

Chen CY, Chang CK, Chang YW, Sue SC, Bai HI, Riang L, Hsiao CD, Huang TH. Structure of the SARS coronavirus nucleocapsid protein RNA-binding dimerization domain suggests a mechanism for helical packaging of viral RNA. J Mol Biol. 2007 May 11;368(4):1075-86. Epub 2007 Mar 2.



This is the protein structure of ORF7A accessory protein. The function of the protein has not been currently determined. The fold of the protein, however, is similar to the Ig superfamily. It is also noted that the protein is expressed and maintained intracellularly after infection.

Nelson CA, Pekosz A, Lee CA, Diamond MS, Fremont DH. Structure and intracellular targeting of the SARS-coronavirus Orf7a accessory protein. Structure. 2005 Jan;13(1):75-85.



This is the protein structure of the tail spike. The tail spike protein attaches to the ACE2 receptor of its host. From the study of this crystal structure, vaccine treatments can be developed based on making truncated disulfide-stabilized receptor-binding domains.

Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.