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REVIEW ARTICLE

THE PROTEINS OF SARS- CoV-2 AND THEIR FUNCTIONS

Zohreh Taheri Kangarshahi [1], Shermin Lak [2], Mona Ghadam [3], Nasrin Motamed [4]✉, Sorosh Sardari [5]✉, Samin Rahimi [6]

[1] Department of Genetics, Faculty of Basic Sciences, University of Maragheh, Maragheh, Iran,
Email: azintaheri7171@yahoo.com
[2] National institute of genetic engineering and biotechnology (NIGEB) 14965/161, Tehran, Iran,
Email: Sherminlak3@gmail.com
[3] National institute of genetic engineering and biotechnology (NIGEB) 14965/161, Tehran, Iran,
Email: monaghadam@gmail.com
[4] Department of cellular and molecular biology, School of biology, University of Tehran, Tehran, Iran,
Email: motamed2@khayam.ut.ac.ir
[5] Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran,
Email: ssardari@hotmail.com
[6] Department of biology, Faculty of Basic Sciences, University of Maragheh, Maragheh, Iran,
Email: samin6686@gmail.com

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Summary

Coronavirus 2 (SARS- CoV-2) leads to Coronavirus disease 2019, is recognized as a lethal epidemic in 2020. SARS-CoV-2 is an enveloped, non-segmented, positive sense RNA virus that belongs to the beta-corona family of viruses. The genome of this virus is about 30 kb representing 16 non-structural proteins (Nsp1-16), four structural proteins (N, M, E, S) and nine accessory proteins are encoded by its genome, which are involved in survival and pathogenesis the viruses. In order to produce medicines and vaccines for SARS-CoV-2, it is essential to fully understand the genomic structure of the virus and function of its proteins. This review collects and investigates the functional properties of SARS-CoV-2 proteins that have been reported to date.

Key words: SARS- CoV-2; proteins functional; proteins structure

1. Introduction

Coronavirus (COVID-19) is the causing agent of severe acute respiratory syndrome by coronavirus 2 (SARS-CoV-2) as a lethal disease that has become an unprecedented threat to human societies since 2019 (1).

✉ Nasrin Motamed, Professor of Molecular Genetics, University of Tehran, School of biology, Department of cellular and molecular biology, Tehran, Iran
motamed2@khayam.ut.ac.ir

✉ Sorosh Sardari, Professor of Biotechnology, Pasteur Institute of Iran, Biotechnology Research Center, Department of Medical Biotechnology, Tehran, Iran
ssardari@hotmail.com

The disease was recognized by the World Health Organization (WHO) as an epidemic on March 11th, 2020 (2). Most of the COVID-19 mortality is due to respiratory failure (3) with clinical symptoms such as fever or chills, shortness of breath, muscle and body aches (4). In addition, recent studies have exhibited that patient mortality is significantly more in people over 65 years of age than those aged 18 to 65 years (5). Recent reports indicate that a significant number of patients with severe SARS-CoV-2 infection develop complications of venous and arterial thromboembolism (6). Also, one study revealed that all patients with COVID-19 had elevated blood fibrinogen levels at the time of hospitalization (7). In general, the SARS-CoV-2 is a family member of positive-sense RNA viruses (8). SARS-CoV-2 has evolved due to rapid mutation and recombination with another coronavirus in the body. These viruses can alter tissue tropism, cross from barriers, and adapt to different epidemiological conditions (9). Phylogeny based domain similarity shows that SARS-CoV-2 is a distinct breed from other coronaviruses such as Bat-SARS, which belong to the genus Beta-coronavirus (β-CoVs) (10). There are about 380 amino acid changes in different proteins of the SARS-CoV-2 genome compared to the proteins in the SARS-CoV genome that have been reported so far. Amino acid changes for positions 348, 27 and 5 have occurred in different accessory proteins, protein S and protein N (11). Accessory proteins play an important role in virus pathogenesis and regulation of interferon signaling pathways and production of pro-inflammatory cytokines (9). The structure of the SARS-CoV-2 genome includes open reading frames (ORFs) that encode accessory proteins, structural proteins, and nonstructural proteins (NSPs). Among the 29 ORFs in SARS-CoV-2, sixteen nonstructural proteins (Nsps), four structural proteins including spike glycoprotein (S), envelope protein (E), membrane protein (M) and nucleocapsid (N) protein, and eight accessory proteins including ORF3a, ORF3b (NP_828853.1, not present in SARS-CoV-2), ORF6, ORF7a, ORF7b, ORF8a, ORF8b, and ORF9b (NP_828859.1, not present in SARS-CoV-2) (12,13). In fact, two-thirds of the virus genome consists of ORF1a and ORF1ab, and the ORF1ab expression requires a ribosomal frame (-1) upstream of the ORF1a stop codon, which reduces gene expression in the ORF1ab region (14). ORF1a encodes the PP1a polyprotein, and ORF1ab causes the expression of the PP1ab polyprotein (15). Then these proteins are processed into 16 non-structural proteins (Nsp) that encode pp1a, Nsp1-11 and pp1ab, Nsp12-16. The remaining one third of the virus genome encodes structural and accessory proteins (14). Various morphologies of surface spikes cause different cell proliferation. Nsps encodes enzymes involved in replication and transcription (13,14), such as important protease (Nsp5 and Nsp3) and RNA-dependent RNA polymerase (Nsp12), helicase/triphosphatase (Nsp13), exoribonuclease (Nsp14), endonuclease (Nsp15) (2). To combat this virus, it is necessary to identify new safe and effective treatment strategies, including antiviral therapy, vaccines, and immunomodulatory drugs (17). The aim of this article is to evaluate the structure and function of SARS-CoV-2 proteins and the role of these proteins in the pathogenesis of the virus.

2. The pathogenic mechanism of SARS-CoV-2 cell entry

Two spike proteins, S1 and S2, attach to the host cell membrane, and the virus enters the host cell through endocytosis or fusion of the plasma membrane using the angiotensin-converting enzyme 2 receptor (ACE2). If entry

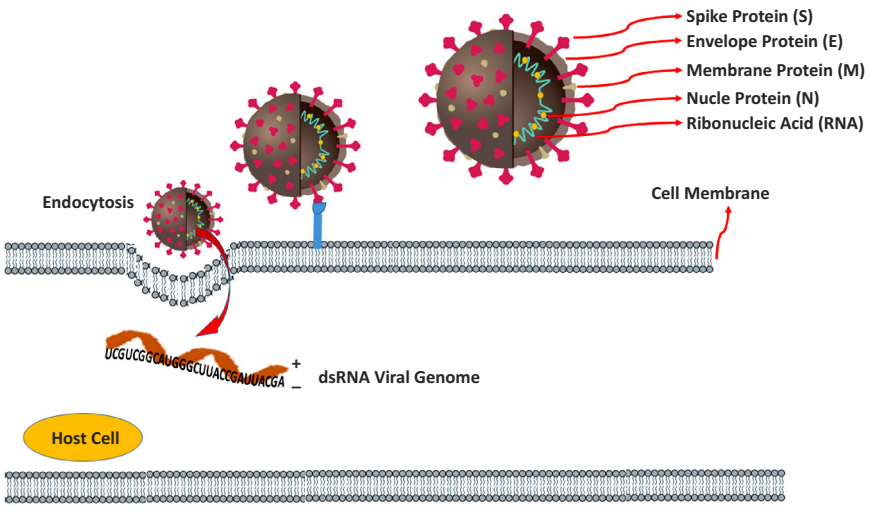

Figure 1. Schematic figure of SARS-CoV-2 and its host entry mechanism.

occurs through the endosome, cathepsin L activates spike proteins, which can also activate TMPRSS2 cellular serine by proteinase. However, the entry route through membrane fusion is much more efficient for the virus due to the reduced potential for stimulation of the intracellular signaling cascade in the host cell. Once inside the cell, viral RNA is translated and amplified. RNA-dependent RNA polymerase (RdRp) transcribes RNA related to virus structural proteins such as S, E, and M protein in the rough endoplasmic reticulum (RER) of the host cell. Translated proteins are released on the RER surface after preparation for virion generation and subgenomic transcription. Then, it accumulates in the Golgi apparatus and is collected through vesicles and fuses containing nucleocapsid (N) proteins in the form of genomic RNA and collected in the cytoplasm and sent to the cell surface. Finally, viruses made on the inner surface of the cell membrane are expelled from the host cell through exocytosis (16) (Fig 1).

3. SARS-CoV-2 proteins

SARS-CoV-2 is composed of 29 proteins with different roles in the pathogenesis process (25) (Table1 and Fig 2).

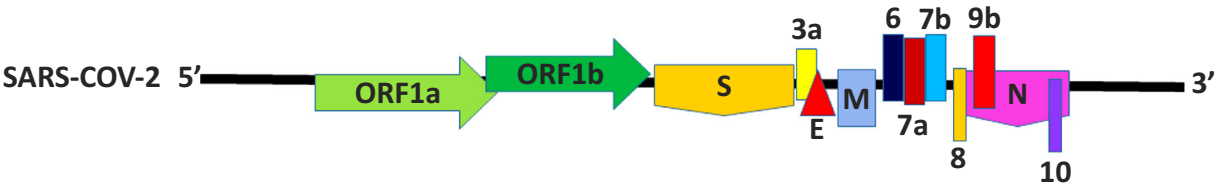

Figure 2. Representation of (+) ss RNA of SARS-CoV-2 with leader sequence (LS), poly-A tail at 5' and 3' UTR and region encoding proteins ORFs, spike (S), ORF3b, Envelope (E), membrane (M), ORF9b, ORF14, nucleocapsid (N) and NSPs.

Table 1. The proteins found in SARS-CoV-2.

Protein	Number of amino acid	Function	Role in SARS-CoV-2 life cycle of host interaction
Nsp1	180	It inhibits translation by blocking the mRNA channel entry region in the free 40S subunits of the 43S pre-primer complex and the empty and, non-translating 80S ribosome	It is possible to reduce the severity of the disease by inhibiting this protein
Nsp2	638	The association of Nsp2 with STOML2 increases mitochondrial metabolism and decreases apoptosis	It is possible to reduce the severity of the disease by inhibiting this protein
Nsp3	1945	It has important protease activity to release essential proteins for viral activity	It is possible to reduce the severity of the disease by inhibiting this protein
Nsp4	500	Nsp4 interacts with RNF5 to resist against host viral responses	It is possible to reduce the severity of the disease by inhibiting this protein
Nsp5	306	It plays a key role in post-translational processing of the replicas gene	It has an important roles in the survival and proliferation of SARS-CoV-2
Nsp6	290	It inhibits the formation of autophagosome / autolysosome vesicles of ER and plays an important role in controlling virus replication	It has an important roles in the survival and proliferation of SARS-CoV-2
Nsp7	83	It has a key role in the coronavirus RNA polymerase (RdRp) activation process	It has an important roles in the survival and proliferation of SARS-CoV-2
NSP8	198	It has a key role in the coronavirus RNA polymerase (RdRp) activation process	It has an important roles in the survival and proliferation of SARS-CoV-2

Protein	Number of amino acid	Function	Role in SARS-CoV-2 life cycle of host interaction
Nsp9	113	Nsp9 has no specific role but it is most likely responsible for RNA synthesis in virus	Unknown
Nsp10	139	A role in RNA capping Process	Unknown
Nsp11	13	Unknown	Unknown
Nsp12	932	It has a key role in the coronavirus RNA polymerase (RdRp) activation process	It has an important roles in the survival and proliferation of SARS-CoV-2
Nsp13	601	Nsp13 is a superfamily helicase 1 that possesses a variety of enzymatic properties including helicase, GTPase, and ATPase	It has an important roles in the survival and proliferation of SARS-CoV-2
Nsp14	527	A role in RNA capping Process	It helps in resisting the immune response by different ways and aid the virus escape from the host's immune system
Nsp15	346	It inhibits the production of interferons (IFNs) and interferon signaling	It helps in resisting the immune response by different ways and aid the virus escape from the host's immune system
Nsporf16	298	A role in RNA capping process	It has an important roles in the survival and proliferation of SARS-CoV-2
ORF3a	274	ORF3a protein is involved in NF-kB activation and NLRP3 inflammation	important roles in the survival and proliferation of SARS-CoV-2
ORF3b	22	It has anti-IFN-I activity	It can reduce the severity of the disease by inhibiting these proteins
ORF6	61	It inhibits the production of interferon and plays an important role in viral pathogenesis	It has an important roles in the survival and proliferation of SARS-CoV-2
ORF7a	122	It binds to SARS-CoV-2 ribosomal transport proteins HEATDR3 and MDN1	Unknown
ORF7b	44	It is not essential for virus replication	It is not essential for virus replication *in vitro*
ORF8b	121	It reduces the expression of host MHC class I proteins	It is possible to reduce the severity of the disease by inhibiting these proteins
ORF9b	97	It inhibits the host immune response *Type* I *interferons* (IFNs)	It helps in resisting the immune response by different ways and aid the virus escape from the host's immune system
ORF9c	73	It can activate immune evasion and coordinate cellular alters vital for the life cycle of SARS-CoV-2	It helps in resisting the immune response by different ways and aid the virus escape from the host's immune system
ORF10	38	Unknown	Unknown
Protein E	75	It plays a role in various stages of the virus life cycle such as envelope formation, pathogenicity, germination and assembly during the replication cycle of the virus	It has important roles in the survival and proliferation of SARS-CoV-2

Protein	Number of amino acid	Function	Role in SARS-CoV-2 life cycle of host interaction
Protein S	1273	S1 subunits bind to the ACE2 target receptor, whereas S2 subunits mediate the host and viral membrane fusion	It has important roles in the survival and proliferation of SARS-CoV-2
Protein M	222	It can mediate budding and assembling of viral particles via employment of other structural proteins into ER-Golgi-intermediate compartment	It has important roles in the survival and proliferation of SARS-CoV-2
Protein N	419	This protein is a critical factor in the *viral infections* and it is involved in the positive-strand RNA virus	It has an important roles in the survival and proliferation of SARS-CoV-2

3.1. Nsp1 (nonstructural protein 1)

The amino acid sequence identity of Nsp1 in SARS-CoV-2 is about 84% comparison with SARS-CoV, which could be an indicator of similar biological properties and functions. Nsp1 is encoded by ORF1a (19) and it is expressed after entering and infecting host cells to inhibit the expression of host proteins. It has been shown that the C-terminal domain of SARS-CoV-2 Nsp1 can inhibit translation by blocking the mRNA channel entry region in the free 40S subunits of the 43S pre-primer complex and the empty and, non-translating 80S ribosomes (18). Also Nsp1 binding to the ribosome leads to endonucleolytic cleavage and degradation of the mRNA host (12). However, host mRNAs are broken by host endonucleases because Nsp1 has no endonucleolytic activity (19).

Nsp1 also suppresses the expression of type I interferon, antiviral signaling pathways and innate immune functions of the host (18). *In vitro* tests show that mutations in the gene of this protein attenuate the virus. Natural compounds such as glycyrrhizic acid including licorice and galangan, gingeronone and shogaol from Sitharathai also interact with Nsp1. These compounds can be considered as new drug candidates against COVID-19, and it is suggested that their validation will be followed by other researchers (19) (Fig 3).

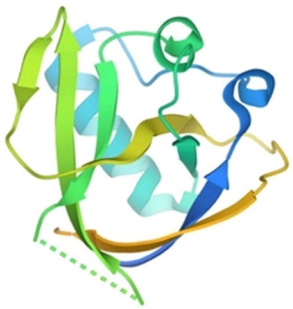

Figure 3. Crystal Structure of Nsp1 from SARS-CoV-2, Chains A, sequence length: 180. (PDB ID: 7K3N, DOI Citation: Semper, C., Watanabe, N., Chang, C., Savchenko, A., 2020-09-30, Crystal Structure of NSP1 from SARS-CoV-2, Center for Structural Genomics of Infectious Diseases (CSGID). DOI: 10.2210/pdb7K3N/pdb).

3.2. Nsp2

It is a non-structural protein with less than 70% amino acid sequence identity between SARS-CoV and SARS-CoV-2. SARS-CoV and SARS-CoV-2 interact with host cell components such as ERLIN1, ERLIN2, RNF170, VDAC2 and STOML2. ERLIN1 and ERLIN2 have been shown to have stronger interactions in SARS-CoV than in SARS-CoV-2; but the intensity of interaction with RNF170 in both is the same. SARS-CoV-2 through interactions with host cell components, tends to favorable environment in host cell for survival and proliferation. For example,

the association of Nsp2 with STOML2 increases mitochondrial metabolism and decreases apoptosis, so the virus can use these conditions to its own advantage. SARS-CoV-2 has specific interactions with FOXK1 and NR2F2, PLD3, KIN and MAZ (20).

3.3. Nsp3

Nsp3 is a multi-domain protein, which shows differences in SARS-CoV and SARS-CoV-2 (21). Mutations and changes in amino acids have been observed in this protein compared to SARS virus, which may explain SARS-CoV-2 being more contagious than SARS-CoV. Nsp3 at position 543 has a serine amino acid, while the same position of SARS virus, the amino acid glycine is observed. Also, SARS-CoV-2 Nsp3 contains proline at position 192 and due to its steric bulge and rigidity, proline can affect protein conformation, but in the same position, SARS virus contains an apolar amino acid (22)(Fig 4).

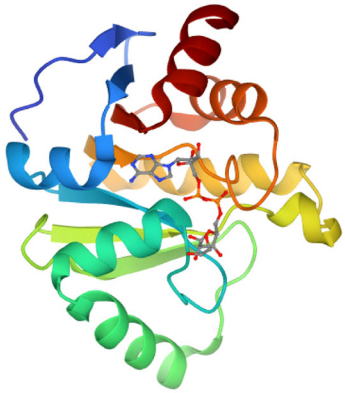

Figure 4. Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 in the complex with ADP ribose. Sequence Length: 17. (PDB ID: 6W02, DOI Citation: Michalska, K., Kim, Y., Jedrzejczak, R., Maltseva, N., Endres, M., Mececar, A., Joachimiak, A., Center for Structural Genomics of Infectious Diseases (CSGID). DOI: 10.2210/pdb6WEY/pdb).

3.4. Nsp4

Nsp4 is a non-structural protein with 80% amino acid sequence identity reported between SARS-CoV and SARS-CoV-2. SARS-CoV and SARS-CoV-2 interact with host cell components such as E3 ubiquitin ligase3 RNF5 (20). RNF5 interacts with VISA (virus-induced signaling adapter) in mitochondria and causes ubiquitin and destruction. VISA becomes an important adapter for the induction of type 1 interferon and the host response against the virus (23). As a result, Nsp4 interacts with RNF5 to resist against host viral responses. Other examples of common interactions between SARS-CoV and SARS-CoV-2 are ERLIN1 / 2, LONP1, HERPUD1, GET4, and BAG2. ERLIN1 and ERLIN2 can also interact with Nsp2. The glycosylation of Nsp4 in SARS-CoV and SARSC-COV-2 induces interaction with STT3B, MAGT1, CANX and DDOST. Specific SARS-CoV-2 interactions include RPS27A, SLC39A7, and HSPA5 (20).

3.5. Nsp5 (3C-like proteinase)

The structural gene Nsp5 is consists of 306 amino acids and two homodimers Nsp5A and Nsp5B.This protein has 11 cleavage sites Which can produces mature nonstructural proteins (Nsps) (19, 15). Nsp5 protein belongs to a class of cysteine protease is responsible for degradation of viral peptides in functional units for replication and packaging of the virus within the host cells (24). ORF pp1a sequence of SARS-CoV-2 encodes Nsp5 as two polypeptides, including the papain-like protease (PLP or Nsp3) and the chymotrypsin-like protease (3CLpro or Nsp5), which during translation are divided into Nsps Mature (15). 3CLpro, also known as Mpro and plays a key role in post-translational processing of the replicas gene (25). Targeting the Mpro enzyme inhibits virus maturation and enhances the innate immune response of the host against COVID-19 (26). Studies also show that the Nsp5 gene sequence in SARS-CoV-2 is approximately 95% similar to SARS-CoV (27) (Fig5).

Figure5. 3C-like proteinase (Nsp5), Chains A, Sequence Length: 306. (PDB ID: 6XR3, DOI citation: Anson, B., Ghosh, A.K., Mesecar, A., Center for Structural Genomics of Infectious Diseases (CSGID)X-ray Structure of SARS-CoV-2 main protease bound to GRL-024-20 at 1.45A, 2020-07-10 .DOI: 10.2210/pdb6XR3/pdb).

3.6. Nsp6

Nsp6 has molecular weight of 34 kDa and transmembrane helices (TM) structure with aC-terminus. Nsp6, with Nsp3 and Nsp4, stimulates new rearrangements in host cell membranes and is involved in the formation of replication-transcription complexes (RTCs) or replication organs (RO). These replication factors also play important role during the life cycle of the virus and viral infection and controlling virus replication. Expression of these three proteins in the SARS-CoV causes the formation of various membrane structures in host cells, including Dual Membrane Vesicles (DMVs), large viron-containing vacuoles (LVCV), cubic membrane structures (CMS), and endoplasmic reticulum (ER). Protein Nsp6 also inhibits the formation of autophagosome / autolysosome vesicles of ER. Nsp6 induces autophagy by activating the omegasome pathway (15). The autophagosomes produced by Nsp6 are larger than before infection, but they are smaller in size and may support the coronavirus infectivity by limiting the ability of autophagosomes (28). 322 Interactions between viral proteins have been identified in SARS-CoV-2-Human and its host cells, which target the innate immune signaling pathway. Also The SARS-CoV-2 Nsp6 protein interacts with the Sigma receptor, which regulates the ER stress response and blocks the ER-induced autophagosome / autolysosome vesicle, and limits virus production. Detected drugs and compounds with high potential to inhibit COVID-19, including drugs or molecules that target Sigma-1 and Sigma-2 receptors and effectively inhibit virus replication. These drugs or molecules include antipsychotics, haloperidol, melperone(which are used to treat schizophrenia), and also antihistamines such as clemastine and cloperastine, the compound PB28 (29).

3.7. Nsp7, Nsp8, Nsp12

Nsp12 alone can perform the polymerase reaction with very low efficiency, while the presence of Nsp7 and Nsp8 cofactors, which are in the form of a hexadecameric and cylindrical structure, dramatically stimulates the polymerase activity (15). It seems that the structure of the hollow cylindrical have a priming role and can accommodate dsRNA (30) but the CryoEM structure of the Nsp12-Nsp7-Nsp8 complex of SARS-CoV-2 indicates that the Nsp7-Nsp8 complex cannot place essential amino acids for primary activity near the Nsp12 catalytic center; therefore, it is not considered a primacy activity (31). However, the Nsp7-Nsp8-Nsp12 complex plays a key role in the coronavirus RNA polymerase (RdRp) activation process, which appears to be active in vitro (14). For complete transcription and replication of the viral genome, several other Nsp subunits are required to aggregate into a holoenzyme complex, including Nsp10, Nsp13, Nsp14, and Nsp16, whose exact functions in RNA synthesis are not well understood (32) (Fig6).

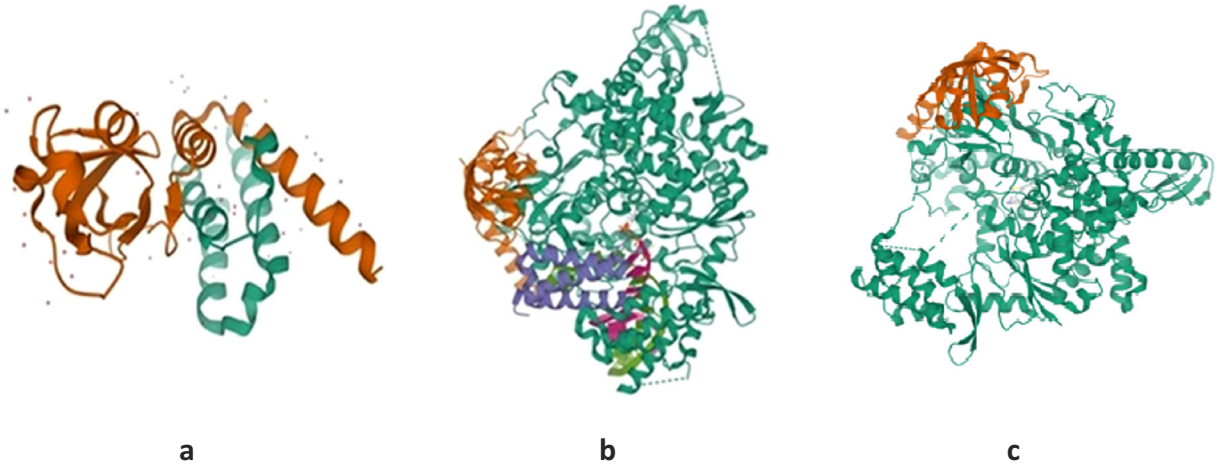

Figure 6. a. Crystal structure of the Nsp7-Nsp8 complex of SARS-CoV-2. (PDB ID: 6YHU, DOI Citation: Konkolova, E., Klima, M., Boura, E., Crystal structure of the nsp7-nsp8 complex of SARS-CoV-2, 2020-03-31. DOI: 10.2210/pdb6YHU/pdb); b. The Nsp12-Nsp7-Nsp8 complex bound to the template-primer RNA and triphosphate form of Remdesivir (RTP). (*PDB ID: 7BV2*, DOI Citation: Yin, W., Mao, C., Luan, X., Shen, D., Shen, Q., Su, H., Wang, X., Zhou, F., Zhao, W., Gao, M., Chang, S., Xie, Y.C., Tian, G., Jiang, H.W., Tao, S.C., Shen, J., Jiang, Y., Jiang, H., Xu, Y., Zhang, S., Zhang, Y., Xu, H.E. The nsp12-nsp7-nsp8 complex bound to the template-primer RNA and triphosphate form of Remdesivir(RTP), (2020) Science 368. DOI: 10.2210/pdb7BV2/pdb); c. SARS-Coronavirus NSP12 bound to NSP8 co-factor. (PDB ID: 6NUS, DOI citation: Kirchdoerfer, R.N., Ward, A.B. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. 2019-02-01, National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID). DOI: 10.2210/pdb6NUS/pdb).

3.8. Nsp9

Nsp9 has no specific role but it is most likely responsible for RNA synthesis in virus. This non-structural protein (113 amino acid in CoV-19) acts as a dimeric ssRNA-binding protein in viral replication. Nsp9 as an important factor engages other proteins in the replicas complex in virus. In fact, the product derived from Nsp9, along with Nsp10, Nsp8, and Nsp7 is located in the complex of replication. This non-structural protein can interact with nsp8 protein for its roles. Also, Nsp9 impairment results in synthesis of the damaged viral RNAs. Nsp9 protein in SARS-CoV possesses DNA/RNA binding activity, but it has been demonstrated that the interaction between Nsp9 and ssDNA/ssRNA (single-stranded nucleic acid binding proteins) is non-specific and weak(33) (Fig7).

Figure 7. Peptide-bound SARS-CoV-2 Nsp9 RNA-replicas, Sequence Length: 113. (PDB ID: 6W9Q, DOI citation: Littler, D.R., Gully, B.S., Riboldi-Tunnicliffe, A., Rossjohn, J. Peptide-bound SARS-CoV-2 Nsp9 RNA-replicase, 2020-03-23. DOI: 10.2210/pdb6W9Q/pdb).

3.9. Nsp10, 16, 14

Coronaviruses, like eukaryotic cells has RNA capping that prevent detection by the host cell and increase its stability. The proteins Nsp10, Nsp13, Nsp14 and Nsp16 are involved in RNA capping process (34, 35). Nsp14 is a nonstructural protein with two functions of exonuclease (ExoN) (in N-terminal region) and methyltransferase (in C-terminal region). The primary amino acid sequence of Nsp3 aligned between SARS-CoV (NP_828862.2) and SARS-CoV-2 (YP_009725299.1) and sequence identity and sequence similarity are 76.0% and 91.8%. Studies on The primary amino acid sequence alignment show amino acid sequence identity between SARS-CoV and SARS-CoV-2 is 95%(36). These enzymes remove nucleoside monophosphates from nucleic acids (3′ to 5 ′) by a mechanism that depends on two divalent metal ions and a water molecule. In SARS-CoV, inactivation of ExoN reduces replication. The effect of ExoN inactivation can be very different in types of viruses, even between two closely CoVs. Experiments on SARS-CoV and MHV-A59 showed that inactivation of ExoN, although it weakens the virus, it is not lethal while in SARS-CoV-2 and MERS-CoV viruses it is lethal and Unlike SARS-CoV and MHV, it affects replication, which shows ExoN's vital function in SARS-CoV-2 and MERS-CoV replication. Nsp14 with methyl-transferase activity has the action of guanine-N7-methyltransferase (N7-MTase) (34, 35, 36). It has also been shown that SARS-CoV-2 Nsp14 can inhibit interferon production and signaling (37). Nsp16 is a 2′ methyltransferase (O-Mtase -2′) that causes Cap-1. The Nsp10 also binds to Nsp16 and Nsp14 and increasing their activity. Nsp16 without Nsp10 is not actived. It is predicted that Nsp10 activates nsp16 by causing structural changes (34, 35). Nsp10 activates the exonuclease activity of Nsp14, while its effect on Nsp14 methyltransferase activity has not been observed that Nsp10 has the ability to bind to RNA and the human adapter protein complex 2. Nsp16 and Nsp14 both are dependent on SAM (S-adenosylmethionine) (34) (Fig8).

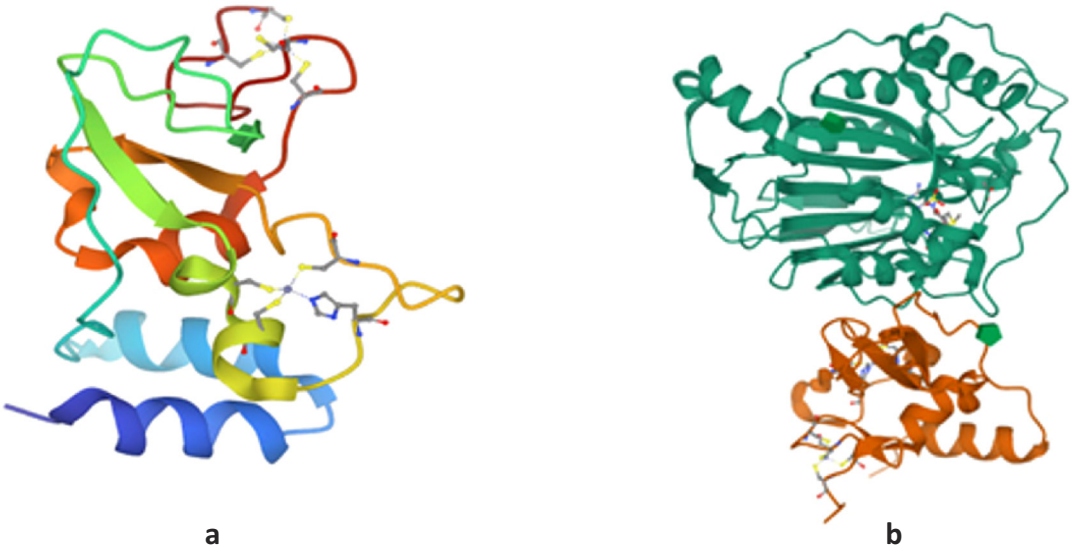

Figure 8. a. Nonstructural protein 10 (nsp10) from SARS CoV-2. (PDB ID: 6ZCT, DOI citation: Rogstam, A., Nyblom, M., Christensen, S., Sele, C., Lindvall, T., Rasmussen, A.A., Andre, I., Fisher, S.Z., Knecht, W., Kozielski, F. Nonstructural protein 10 (nsp10) from SARS CoV-2,2020-06-12. DOI: 10.2210/pdb6ZCT/pdb); b. Crystal structure of 2019-CoV Nsp16-Nsp10 complex. (PDB ID: 6W75, DOI Citation: Minasov, G., Shuvalova, L., Rosas-Lemus, M., Kiryukhina, O., Wiersum, G., Godzik, A., Jaroszewski, L., Stogios, P.J., Skarina, T., Satchell, K.J.F., 1.95 Angstrom Resolution Crystal Structure of NSP10 - NSP16 Complex from SARS-CoV-2 2020-03-25,Center for Structural Genomics of Infectious Diseases (CSGID). DOI: 10.2210/pdb6W75/pdb).

3.10. Nsp11

Nsp11 codes a short protein, which depends on the coronavirus and encompasses 13–23 residues. This protein possesses only 13 amino acids in coronavirus 2019 and is produced by cleaving the polyprotein pp1a via Mpro/3CLpro protease at the Nsp10/11 junction (38). The function of Nsp11 is unknown (15).

3.11. Nsp13

Nsp13 is a superfamily 1 helicase that possesses a variety of enzymatic properties including helicase, GTPase, and ATPase. The C-terminal is acts as a helicase domain, a 597 residue cleavage product is released from pp1ab by the 3CLpro activity, and the N terminal of this helicase is Zinc-binding domain. This protein can unwind both DNA and RNA substrates through duplex regions of 22 and 33bp, in the 5' to 3' direction respectively. The function of Nsp13 possesses most efficiently with GTP, dATP/ATP hydrolysis. Nsp13 not only is working with Nsp12 for genome replication, it is also responsible for mRNA capping in virus. Therefore, this protein plays an essential function in viral RNA replication. Transcription/replication complex consists of these viral replicas that produce the whole viral genomes. Since helicase is vital for viral proliferation and replication, thus it can be a potential target for antiviral therapies. In other words, the prohibition of these helicases can interfere with the viral metabolism without considerable side-effect in patients. Moreover, a couple of researches have been focused on the prevention of helicase activity in the treatment of hepatitis and in animal model like herpes simplex virus. The property of nonstructural protein 13 is triphosphatase function via the active site of NTPase, so this protein might also act in the capping of viral RNAs through RNA 5′-triphosphatase activity(39) (Fig 9).

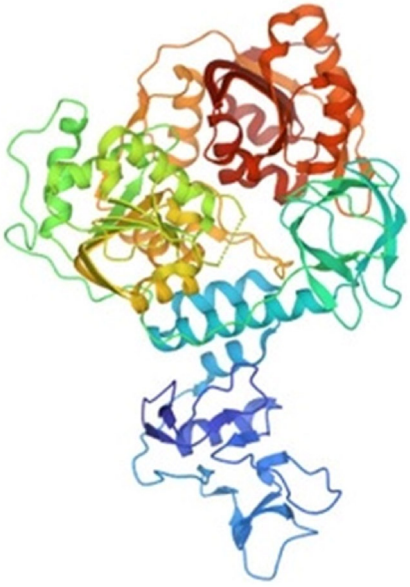

Figure 9. SARS-CoV-2 helicase Nsp13, Chains A and B, Sequence Length: 603. (PDB ID: 7NIO_1, DOI Citation: Newman, J.A., Yosaatmadja, Y., Douangamath, A., Bountra, C., Gileadi, O. 2021-02-12, SARS-CoV-2 helicase NSP13 Represented by Chain A,Crystal structure of the SARS-CoV-2 helicase APO form. DOI: 10.2210/pdb7NIO/pdb).

3.12. Nsp15 (uridylate-specific endoribonuclease (Nsp15/NendoU))

Nsp15 is an endoribonuclease designated EndoU (40). This protein inhibits the production of Interferons (IFNs) and interferon signaling (37). To create a pattern for virus synthesis, Positive-sense RNAs are replicated to negative sense RNAs, called PUNs and at the end of 5 'has a polyuridine sequence that is predicted by fold back converted to stem-loop structure that are identified as double-stranded RNA by host PRRs (pattern recognition receptors) such as MDA5 (CoV specific) and PKR and OAS and can activate interferon, but EndoU cleaves the polyuridine sequence and thus MDA5 is not activated and inhibits interferon production (40) .The amino acids His 235, His250, Lys 290 are conserved in all coronaviruses and are involved in protein ribonuclease activity (41). Glisoxepide, which is used to type 2 Diabetes Treatment, and Idarubicin, which is used to leukemia Treatment, are inhibited SARS-CoV-2 EndoU. These two drugs bind to the active site of the enzyme through intermolecular interactions with the amino acids His 235, His 250 and Lys290 and inactivate it. The effectiveness of these drugs requires further studies in vitro and in vivo (42) (Fig 10).

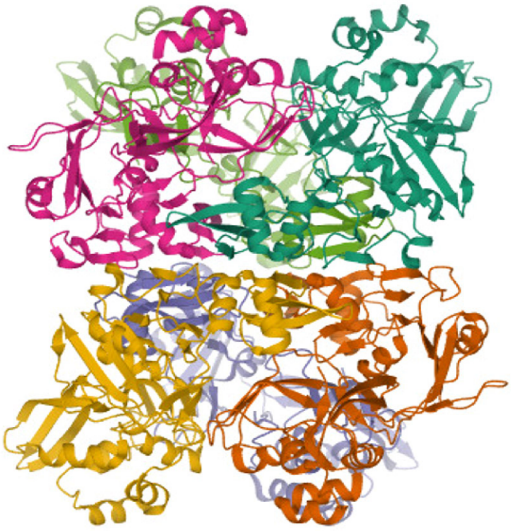

Figure 10. Cryo-EM structure of SARS-CoV-2 NSP15 NendoU at pH 6.0. (PDB ID: 7ME0, DOI citation: Godoy, A.S., Song, Y., Nakamura, A.M., Noske, G.D., Gawriljuk, V.O., Fernandes, R.S., Oliva, G. Cryo-EM structure of SARS-CoV-2 NSP15 NendoU at pH 6.0,2021-04-06, Sao Paulo Research Foundation (FAPESP). DOI: 10.2210/pdb7ME0/pdb).

3.13. ORF3a

The ORF3a gene encodes a protein called TRAF, the ion channel and binding domain kaolin (25), which has 274 amino acids and is the second largest sub genomic RNA in the SARS-CoV genome (43). Reports have been shown that if a mutation occurs in the ORF3a gene region, it activates NF-κB and NLRP3 inflammation, indicating that the ORF3a protein is involved in NF-kB activation and NLRP3 inflammation (15, 8). One of the important features of ORF3a protein in SARS-CoV genomes is the presence of cysteine-rich domain (CRD). ORF3a protein is abundantly expressed in infected and transfected cells and accumulates in intracellular membranes and plasma (9). This protein plays an essential role in the spread of viral particles and causes apoptosis and necrosis in infected cells (8). In the SARS-CoV genome, a common mutation between the ORF3a gene and the spike gene has been observed, which indicates the function of ORF3a protein is related to spike protein (44). ORF3a also interacts with TRAF3, which activates ubiquinone ASC and eventually activating the maturation of caspase-1 and IL-1β (43). Reports of amino acid sequence comparisons between ORF3a proteins in SARS-CoV and SARS-CoV-2 show that Sequence identity and sequence similarity are 72.4% and 90.2% (15) (Fig 11).

Figure 11. Cryo-EM structure of SARS-CoV-2 ORF3a. (PDB ID: 7KJR, DOI citation: Kern, D.M., Hoel, C.M., Kotecha, A., Brohawn, S.G. Cryo-EM structure of SARS-CoV-2 ORF3a, 2020-11-18. DOI: 10.2210/pdb7KJR/pdb).

3.14. ORF3b

The length of ORF3b is significant difference between SARS-CoV and SARS-CoV-2. Although ORF3 length in SARS-CoV-2 is shorter (22 amino acids), it has greater anti-IFN-I activity than SARS-CoV (on average 153 amino acids). All of the ORF3bs with higher anti-IFN I activity are located in the cytosol, while their inactive or attenuated counterparts are found in the nucleus and cytosol. Accordingly, less Anti-IFN I activity in SARS-CoV can be attributed to NLS (nuclear localization signal) in C-terminal SARS-CoV and its absence in SARS-CoV-2. This may justify further pathogenicity of SARS-CoV-2 (45). The experiments resulted in a high proportion of false negatives in early infection, but it has been seen that the combined use of ORF3b and ORF8 a highly sensitive method for detecting patients with COVID-19. As a result, this method can be useful in serological tests to overcome false negative results (46).

3.15. ORF6

Beta-coronaviruses such as SARS-CoV and MERS-CoV encode several interferon antagonists to evade host innate immune activation (37). SARS-CoV ORF6 protein is an accessory protein that plays an important role in viral pathogenesis. Using a yeast two-hybrid system, ORF6 was shown to be associated with Nsp8 (15). A report showed that the ORF6, ORF8, and N proteins in SARS-CoV-2 could inhibit the IFN-β promoter, the ISRE promoter, and the NF-κB element (47). Also, Yuen et al. reported that the expression of multiple SARS-CoV-2 protein could inhibit the production of interferon. Of all 29 SARS-CoV-2 proteins, ORF6 is the most interferon antagonists that reducing the activity of the interferon-beta (IFN-beta) promoter more than 100-fold (37).

2.16. ORF7a

ORF7a in SARS (SARS-CoV) encodes a unique type I membrane protein with 122 amino acids, consisting of 15 residue N-terminal, 81 residue luminal domain, 21 residue membrane, and a 5 residue cytoplasmic tail whose function residues unknown. ORF7a has a seven-strand structure similar to immunoglobulin (Ig) domain, and this protein is expressed in cells infected with SARS-CoV. The short cytoplasmic tail of ORF7a has positively charged residues near the membrane and contains the sequences Lys103, Arg104, and Lys105. These three [Arg/Lys] [X] [Arg/Lys] sequences are found in various Golgi proteins and appear to be needed to identify the vesicular COPII system involved in the transfer of proteins from the ER to the Golgi. It was observed that the ORF7a cytoplasmic tail was not sufficient for the accumulation of CD4 protein on the cell surface. In contrast, the presence of two transmembrane domains, ORF7a and cytoplasmic tail (26 amino acids), leads to the accumulation of the CD4 marker protein in Golgi (48). ORF7a also binds SARS-CoV-2 to the ribosomal transport proteins HEATDR3 and MDN1 (26) and inhibits cellular translation in SARS-CoV (49). A 392-nucleotide deletion starting at nt 29,424 was identified in SARS-CoV-2 ORF7a and this deletion leads to the complete elimination of ORF7b and creates a new ORF by fusion of N-terminal ORF7a with ORF8. This is reported the largest deletion in SARS-CoV-2. Also in WA-UW-5812, a 227 -nucleotide deletion was detected that leading to a new ORF by fusion of the end of N- terminal ORF7a with ORF7b (Fig 12) (49).

Figure 12. Structure of the SARS-CoV-2 ORF7A encoded accessory protein. (PDB ID: 6W37, DOI Citation: Nelson, C.A., Minasov, G., Shuvalova, L., Fremont, D.H., Center for Structural Genomics of Infectious Diseases (CSGID), STRUCTURE OF THE SARS-CoV-2 ORF7A ENCODED ACCESSORY PROTEIN. DOI: 10.2210/pdb6W37/pdb).

3.17. ORF7b

The SARS-CoV ORF7b protein is 44 amino acids and is highly hydrophobic and has no specific sequence homology to other viral or cellular proteins. Because ORF7b is highly hydrophobic, it has been hypothesized that ORF7b is a membrane protein and possibly a viral structural protein (50). Although ORF7b expression has not been recorded in virus-infected cells, specific antibodies against the ORF7b protein found in the serum of people who have improved. The ORF7b protein is an accessory proteins, and the ORF7b start codon overlaps with the ORF7a stop codon. Reports indicate that the ORF7b protein is not essential for virus replication in vitro (51).

3.18. ORF8b

ORF8b in SARS-CoV-2 contains 121 amino acids. Recent studies have shown that this protein reduces the expression of host MHC class I proteins. Overexpression of ORF8b can disrupt the host type I IFN signaling pathway. This protein rapidly forms an accessory protein that interferes with the host's immune response in several ways. Antibodies against this protein can inhibit the SARS-CoV-2 (52) (Fig 13).

Figure 13. Structure of the SARS-COV-2 ORF8 encoded accessory protein. (PDB ID: 7JX6, DOI Citation: Hall, P.D., Nelson, C.A., Fremont, D.H., Center for Structural Genomics of Infectious Diseases (CSGID), 2020-08-26 STRUCTURE OF THE SARS-CoV-2 ORF8 ENCODED ACCESSORY PROTEIN. DOI: 10.2210/pdb7JX6/pdb).

3.19. ORF9b

It is located in the mitochondrial membrane and inhibit the host immune response Type I interferons (IFNs). Overexpression of TOM70 in IFN-I signaling pathway escapes from the ORF9b inhibitory function. Recent studies suggest that targeting the ORF9B-TOM70 interaction could be a new treatment strategy for SARS-CoV-2. This protein is an ORF within the N gene (nucleocapsid) that inhibits the expression of Type I interferons and targets mitochondria (53) (Fig 14).

Figure 14. X-ray Crystallographic Structure of Orf9b from SARS-CoV-2. (PDB ID: 6Z4U, DOI Citation: Weeks, S.D., De Graef, S., Munawar, A. 2020-05-25. X-ray Crystallographic Structure of Orf9b from SARS-CoV-2. DOI: 10.2210/pdb6Z4U/pdb).

3.20. ORF9c

ORF9c codes 73 amino acid protein that disrupts antiviral immune response. ORF9c protein includes a putative trans-membrane domain, which impaired antiviral processes and interacted with membrane proteins in cellular compartments in the cell line of lung epithelial. This protein also can interact with different host proteins such as Sigma receptors, showing its involvement in the ER stress response and lipid remodeling. The product derived from ORF9c also may target the signaling pathway of NF-kB. Transcriptome, interactome, and proteomic studies combined with bioinformatics tools demonstrated that the changed expression of ORF9c can impair complement signaling, antigen presentation, and interferon signaling, while activating the signaling pathway of IL-6. The recent works showed that ORF9c can activate immune evasion and coordinate cellular alters vital for the life cycle of SARS-CoV-2 (54).

3.21. ORF10

The mRNA of ORF10 contains 117 nucleotides and 38 amino acids, but its function is unknown (55, 15). A study in India also reported that no new mutations were observed in ORF10 (55) (Fig 15).

Figure 15. Chains A, Sequence Length: 1014, Organism: Moritella marina. (PDB ID: 6RIW, DOI Citation: Santin, O., Moncalian, G. PfaC Keto synthase-Chain length factor 2019-04-25, Spanish Ministry of Science, Innovation, and Universities. DOI: 10.2210/pdb6RIW/pdb).

3.22. Protein E

Protein E is the smallest structural protein in all coronaviruses (56). This protein plays an essential role in various stages of the virus life cycle such as envelope formation, pathogenicity, germination and assembly (57) during the replication cycle of the virus, the infected cells expressed a lot of protein E. Most of the protein is located in the intracellular trafficking area such as ER, Golgi, ERGIC where it participates in the assembly and germination of the virus (58). The amino acid sequence identity of E protein in SARS-CoV and SARS-CoV-2 74.94% is similar. Protein E has a short N-terminal region and a large hydrophobic membrane domain (involved in ion channel formation) and terminates to the hydrophilic c-terminal. The results of the human SARS-CoV-2 E study showed that protein E is pentameric structure and consists of 35 alpha helix (α-helix) and 40 loops and the alpha helixes and loops in the protein move randomly, then modulates ion channel activity, which contributes to the pathogenicity of the virus. It has been shown that after binding of belachinal, macaflavanone E, vibsanol B to protein E, are prevented random protein movement and human SARS-CoV-2 E function. Hence, it may be used as a disease control drug that requires further studies *in vitro* and in *is vivo* (57) (Fig 16).

Figure 16. SARS-CoV-2 Envelope Protein Transmembrane Domain: Pentameric Structure Determined by Solid-State NMR. (PDB ID: 7K3G, DOI Citation: Mandala, V.S., Hong, M., McKay, M.J., Shcherbakov, A.S., Dregni, A.J. National Institutes of Health/National Institute of General Medical Sciences, 2020-09-11 SARS-CoV-2 Envelope Protein Transmembrane Domain: Pentameric Structure Determined by Solid-State NMR. DOI: 10.2210/pdb7K3G/pdb)

3.23. Protein S

The protein S, namely spike protein, identifies the human angiotensin-converting enzyme 2 (ACE2) on the surface of host cells, mediating the attachment of the viral particles to the target cells. The spike protein is known as a glycoprotein that its architecture has been revealed previously. The precursor of S full-length protein, 1273 amino acids in SARS-CoV-2, is cleaved into glycosylated subunits, S2 and S1. S1 subunits bind to the ACE2 target receptor, whereas S2 subunits mediate the host and viral membrane fusion. To date, multiple inhibitors like SARS-CoV spike mouse polyclonal antibodies have been established that inhibited spike protein in SARS-CoV-2 through interfering of viral entry into cell (13) (Fig 17).

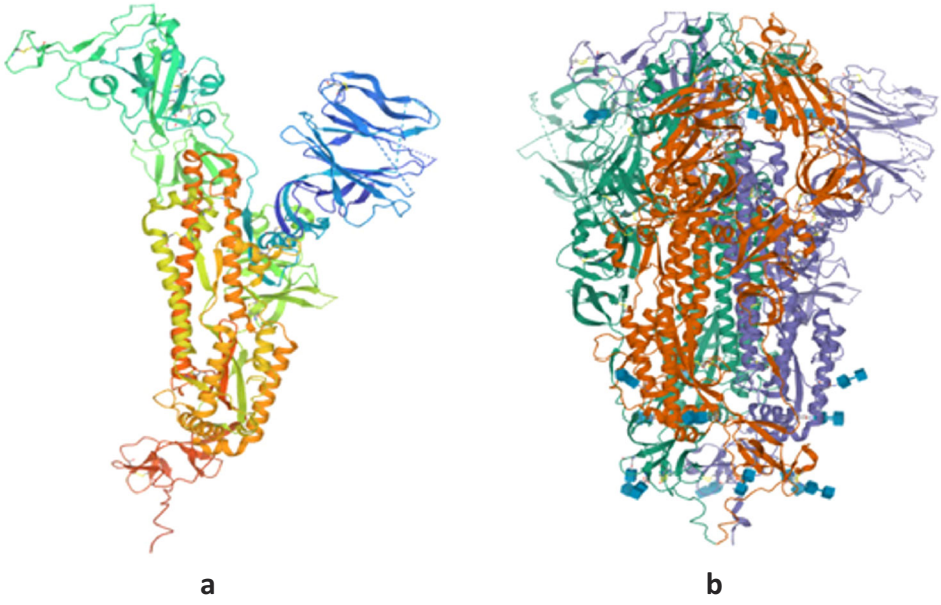

Figure 17. a. Spike glycoprotein, Chains A and B, Sequence Length: 1310. (PDB ID: 6XLU_1, DOI Citation: Ouyang, S., Hongxin, G. Structure of SARS-CoV-2 spike at pH 4.0 Represented by Chain A, 2020-03-03, National Natural Science Foundation of China. DOI: 10.2210/pdb6XLU/pdb); b. Structure of the SARS-CoV-2 spike glycoprotein (closed state). (PDB ID: 6XLU, DOI citation: Zhou, T., Tsybovsky, Y., Olia, A., Kwong, P.D. : 2020-06-29, Structure of SARS-CoV-2 spike at pH 4.0. DOI: 10.2210/pdb6XLU/pdb).

3.24. Membrane (M) (ORF5)

Membrane/matrix protein consists of 222 amino acid in COVID-19 and is much conserved. Membrane protein is concerned as the most abundant structural component of the virion that can mediate budding and assembling of viral particles via employment of other structural proteins into ER-Golgi-intermediate compartment (ERGIC) (59).

3.25. Protein N

Nucleoproteins or nucleocapsid proteins can include nucleosomes, ribosomes, and nucleocapsids. This protein is a critical factor in the viral infections and it is involved in the positive-strand RNA virus. In addition, nucleoproteins are essential for the virus accumulation. This protein is essential in increasing the replication and transcription efficiency of the virus genome. The nucleoprotein is located in cytoplasm and in all parts of the micronucleus and nucleus of infected cells. This protein is involved in several functions, including virus nucleus formation, replication, transcription, and translation. Nucleoprotein is an important structural that binds to the RNA genome of virus particle and generates the ribonucleoprotein nucleus. Nucleoprotein is the second most important protein after protein S that is targeted by the immune system. Protein N is an important virus antigen that is involved in the packaging of the virus and the release of virus particles. After infection, N protein enters the host cell to replication and packaging of viral RNA and virus release (60) (Fig 18).

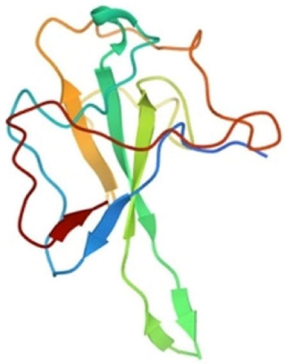

Figure 18. Crystal structure of SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain. (PDB ID: 6M3M, DOI Citation: Chen, S., Kang, S, Crystal structure of SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain 2020-03-04, National Natural Science Foundation of China (NSFC). DOI: 10.2210/pdb6M3M/pdb).

Conclusion

The SARS-CoV-2 virus has infected more than 116 million people worldwide so far and has affected almost every country. Reports indicate that infection with the SARS-CoV-2 virus causes severe inflammation, damage to the lungs, kidneys, and abnormal thrombosis. It has been reported that SARS-CoV-2 types with different Accession numbers have on average 93.62% similarity sequence with SARS-CoV types (9). Due to SARS-CoV-2 selective pressure and evolution of SARS-CoV-2 through mutation, recognizing the proteins of this pandemic virus would be an effective way to combat it. In this article, we examine all the amino acid sequences of SARS-CoV-2 proteins and their molecular mechanisms. It has been shown that M, E, S, N, Nsp5, Nsp6, Nsp7, Nsp8, Nsp12, Nsp13, Nsp16, ORF3a, ORF6, Nsp14 proteins, Because of their important roles in the survival and proliferation of SARS-CoV-2, they can be considered for pharmacological purposes and vaccine production. Also, Nsp3, Nsp1, Nsp2, Nsp4, ORF3b, ORF8b proteins make SARS-CoV-2 virus more contagious than SARS-CoV, which can reduce the severity of the disease by inhibiting these proteins. ORF3b and ORF7b in SARS-CoV-2 have no genomic sequence homology with other viral proteins. Therefore, it is suggested that the sequences of these two proteins simultaneously be detected for accurate and rapid diagnosis of SARS-CoV-2 infection from other viral infections. The ORF9 (ORF9b and ORF9c), Nsp15, Nsp14, and ORF8b proteins respond to the immune system in a variety of ways, helping the virus to escape the host immune system. Proteins ORF9 (ORF9b and ORF9c), Nsp15,

Nsp14 and ORF8b resist with the immune response by different ways and help the virus escape from the host's immune system. On the other hand, the exact function of ORF7a, ORF10, Nsp11, Nsp10 and Nsp9 is not fully understood, but it is known that ORF7a is a type I transmembrane proteins and Nsp10 activates Nsp14; therefore, identification and inhibiting these proteins can be effective in the process of improvement and treatment of the disease.

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Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

Adherence to Ethical Standards

This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

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