SUNTEXT REVIEWS

SunText Review of Virology
ISSN: 2766-5003

Open Access
Review Article
Volume 2:1

New Biotechnological Exploration on COVID-19 Proteins: Functions, Mutational Profiles and Molecular Targets for Drug Design

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Received date: 08 February 2021; **Accepted date:** 20 February 2021; **Published date:** 27 February 2021

Citation Chakraborty AK, Chanda A (2021). New Biotechnological Exploration on COVID-19 Proteins: Functions, Mutational Profiles and Molecular Targets for Drug Design. SunText Rev Virol 2(1): 115.

DOI: https://doi.org/10.51737/2766-5003.2021.015

Abstract

Corona virus spread is gaining momentum worldwide and every country is losing lives and lock down has crippled normal life, education and industry with huge GDP loss. Recently, corona virus research has accelerated with discovery of many new targets and at least 20 vaccines have been under clinical trials and three vaccines already have started to inoculate million people worldwide. However, corona virus is gathering mutations in its genome at a rate 1-2 nucleotide per month fostering a chance of vaccine failure. Thus, up-gradation of knowledge is necessary and this review will search recent development on biochemistry and molecular biology of COVID-19 and related corona virus like SARS and MERS. A great deal of research has appeared in reputed journals on sixteen non-structural proteins (nsp1-nsp16). Specifically, drug discovery against nsp3 protease and nsp12 RNA-dependent RNA polymerase was phenomenal. Similarly, knowledge of spike protein interaction with ACE-2 receptor of human cells have advanced with development of effective vaccine as well as other therapeutics. Surely, we will focus on recent bioinformatics research on non-structural proteins, nsp2, nsp13 and nsp16 those have implicated as RNA Topoisomerase, RNA helicase-Capping Guanine methyl transferase and 2'-O rRNA Uridine methyl transferase respectively. We urge to develop phyto-drugs based on ancient Hindu and Chinese civilization as most corona virus proteins have been expressed and purified.

Keywords: Non-structural proteins; Corona virus; RNA topoisomerase; D614G spike protein mutation; P4715L RdRP mutation; I300F nsp2 mutation

Introduction

Invisible SARS-CoV-2 29.9kb single (+)-stranded RNA virus (120nm) has created a panic in this Earth by killing 3 million and affecting 600 million [1-3]. Previously, six different corona viruses had been found to infect humans, including CoV-229E, CoV-HKU1, CoV-OC43, CoV-NL63, SARS-CoV and Middle East respiratory syndrome corona virus (MERS-CoV) [4]. COVID-19 virus is more deadly than its related SARS and MERS type's corona viruses. Severe COVID-19 is more common in adults aged ~70 years with co-morbidities such as diabetes, cardiovascular disease, and chronic respiratory disease. Corona virus has structural proteins (S, M, E, and N) and two large orf1ab (7096aa) and orf1a (4405aa) poly-proteins in same reading frame (Figure 1).

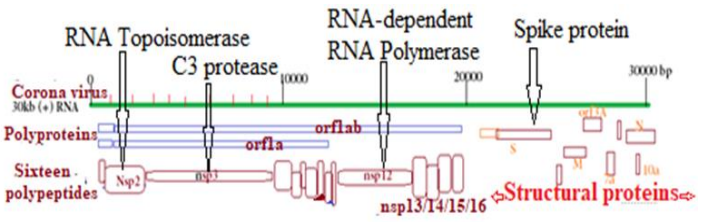

Figure 1: *Structure of corona virus and its proteins.*

Orf1ab degraded into sixteen non-structural proteins (Nsp1-Nsp16) [5]. Mutation in the corona virus genome may occur at a rate 1-2 mutation per year. SARS-CoV-2 mutations were reported

thus by many workers worldwide and very recently NCBI Virus portal analysed and reported such mutations in the database. As for example, showed in the phylogram, two distinct clades as cluster 1 and cluster 2. Cluster 1 contained the mutations 1397G > A, 11,083G > T, 28,688 T > C, and 29,742G > T, while the second cluster had 23,403 A > G, 3037 C > T, and 14,408 C > T as the dominant mutations in the Corona virus isolates from Turkey [6]. P4715L mutation in the RNA-dependent RNA polymerase also has reported in many countries possibly favouring mutation rate of corona virus and I120F mutation in the nsp2 RNA topoisomerase has reported [7] (Figure 1).

Exploration on the polyprotein (orf1ab) associated non-structural proteins (NSP)

The major function of the sixteen ORF1ab associated corona virus proteins was demonstrated in Table-1. Nsp1 is 180aa long and about 19.8KD protein which has 23% minimal similarity to porcine epidemic diarrhea virus. It has similar α/β fold structure (PDB: 2HSX) related to SARS and MERS corona viruses with higher negatively charged amino acid residues (Asp, Glu). D75E mutation was shown as frequent mutation in different corona virus isolates and V56I similar amino acid substitution also has reported [8]. It has been reported that SARS-CoV nsp1 binds to the 40S ribosomal subunit and inhibits host mRNA translation using a two-pronged strategy [9,10]. It may induce cellular mRNA cleavage at the 5'-UTR site but not viral RNA [11]. It may also be an immune modulator and functions through activation of NFκβ transcription factor. Further, a role of nsp1 protein in the regulation of Calcineurin/NFAT signalling pathways has suggested where peptide inhibitor CspA that binds cyclophillins, may be an important drug against corona virus (Gordon et al. 2020). We identified a R24C mutation in the nsp1 protein of COVID-19 from US origin (Figure 2).

Figure 2: *Detection of Nsp1 protein R24C mutation (protein id. QQJ95198) in US isolates of corona virus.*

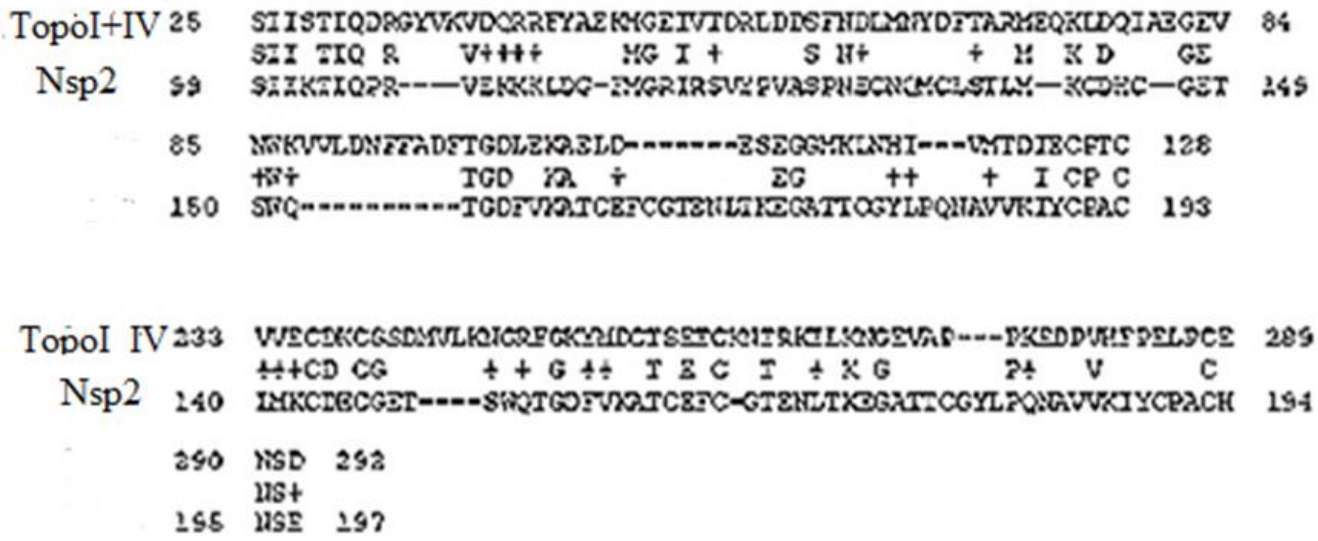

Figure 3: *Some similarities between Vibrio haemolytica topoisomerase I+IV and nsp2 protein of COVID-19 (Chakraborty, 2020).*

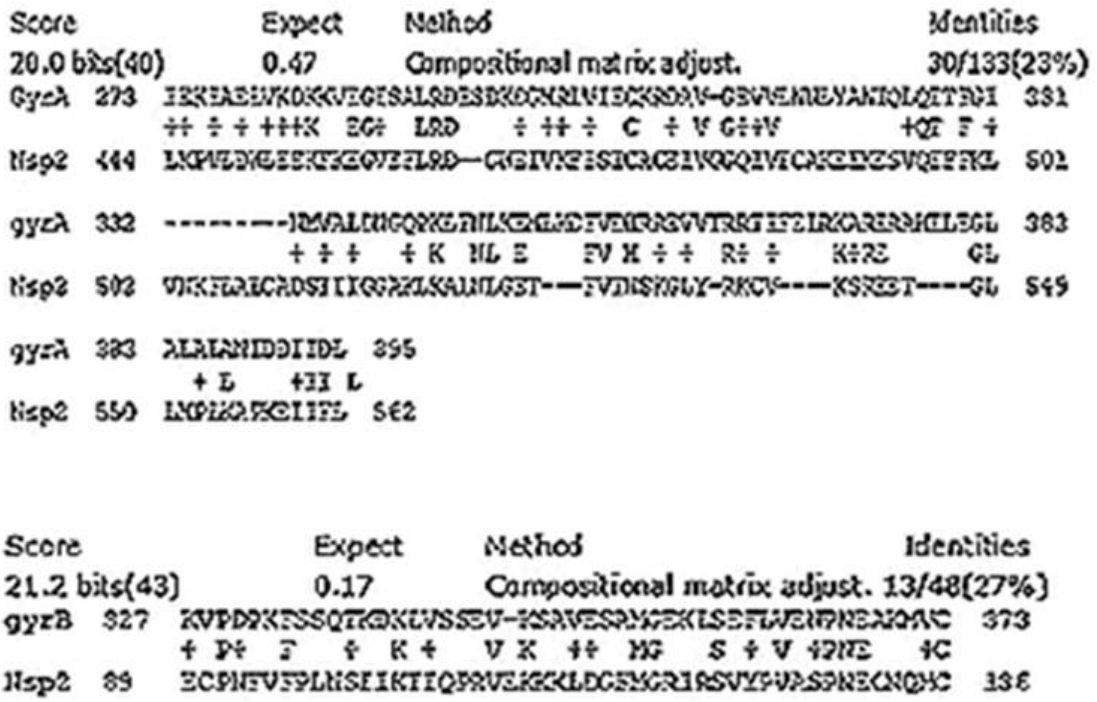

Figure 4: *Similarities of nsp2 protein with DNA gyrase subunit A and subunit B of Bacteria (Chakraborty, 2020a).*

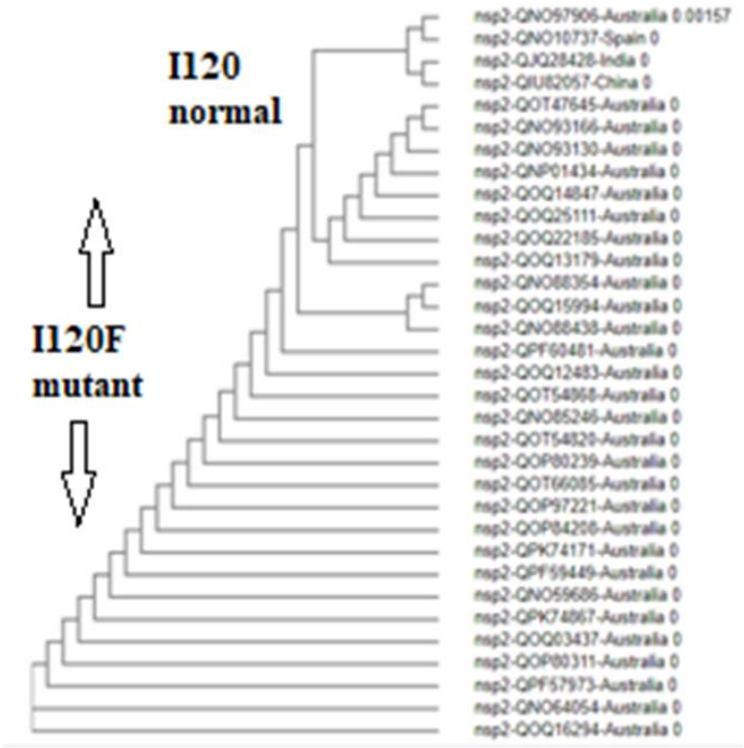

Figure 5: *Spread of I300F nsp2 mutant of COVID-19 into Australia (orf1a protein ids were shown). (Chakraborty, 2021; Journal of Antivirals and Antiretrovirals, in press).*

Figure 6: *Detection of T265I nsp2 mutation in US isolates. Note that in Australian isolates I300F mutation was prominent.*

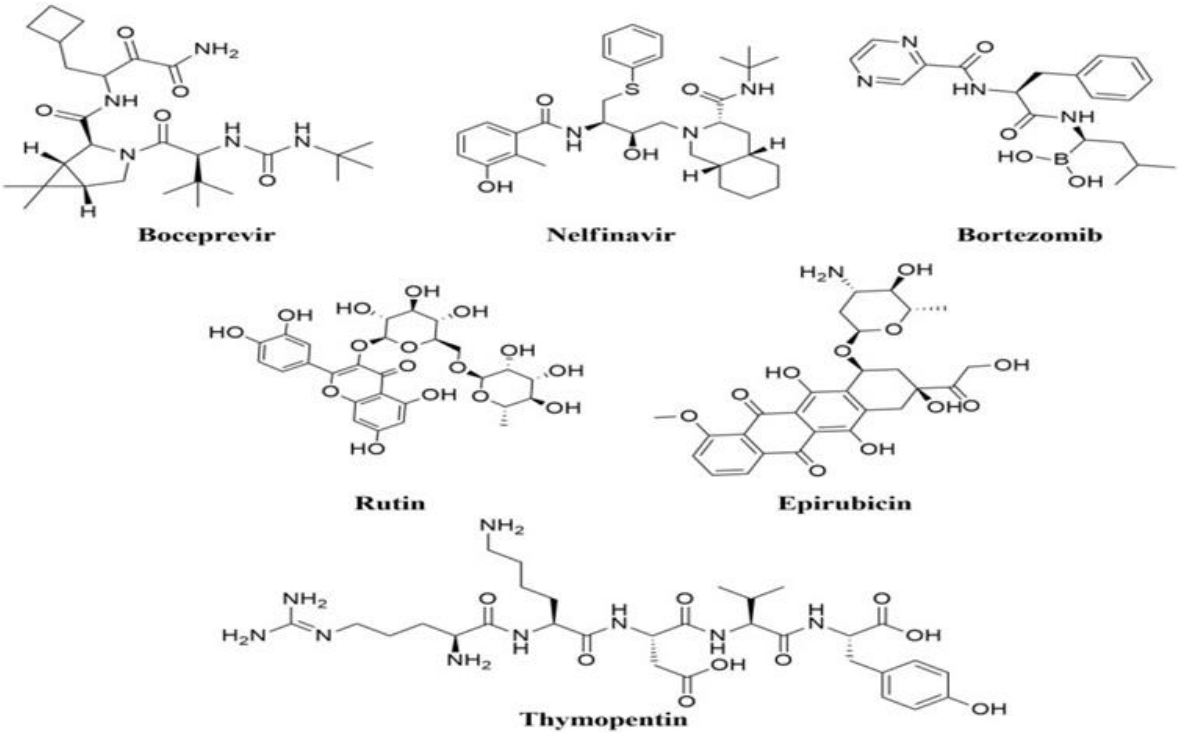

Figure 7: *Chemical structures of few corona virus Nsp3 C3 protease inhibitors (Mandour et al., 2020; Fu et al. 2020).*

Figure 8: *Detection of two important mutations (S2215A and S2874L) in the nsp4 proteins of COVID-19. China January, 2020 isolate orf1ab sequence was taken as standard amino acid sequence (protein id. QIU82056).*

Figure 9: *Detection of nsp6 mutations of COVID-19 in the Brazilian isolates.*

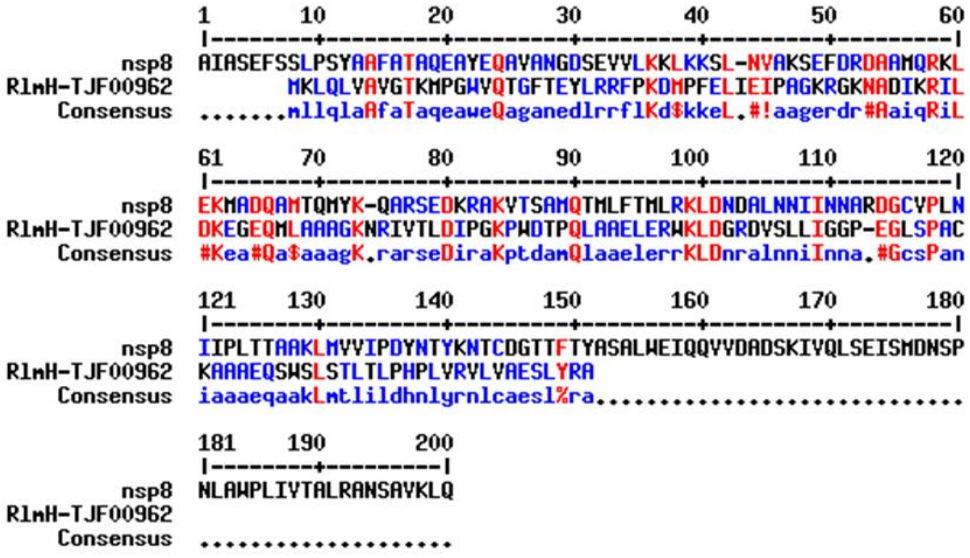

Figure 10: *A weak similarity between nsp8 and RlmH rRNA methyl transferase of Escherichia coli.*

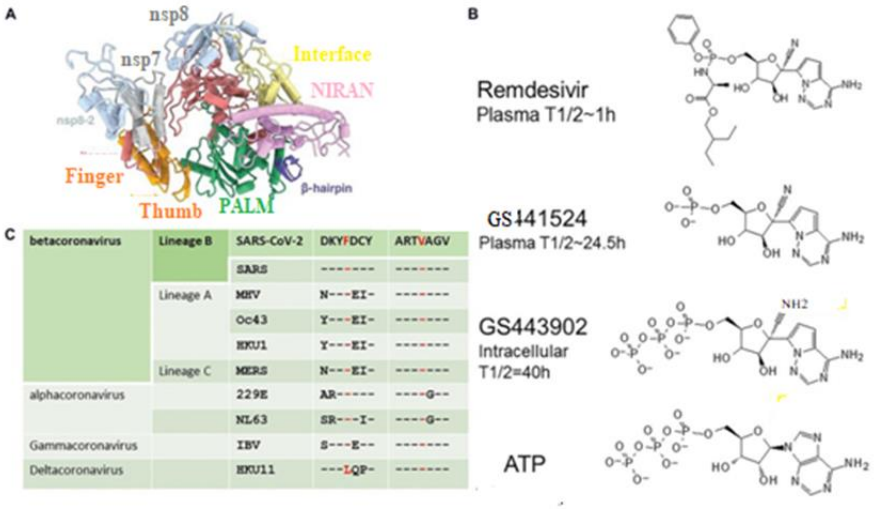

Figure 11: *Structural feature of Nsp12 RdRP of corona viruses and competitive inhibitors used as drugs (Chan, 2020; Peng et al. 2020).*

Figure 12: *Detection of RNA-dependent RNA polymerase P4715L mutation that increased transmission efficiency of corona virus.*

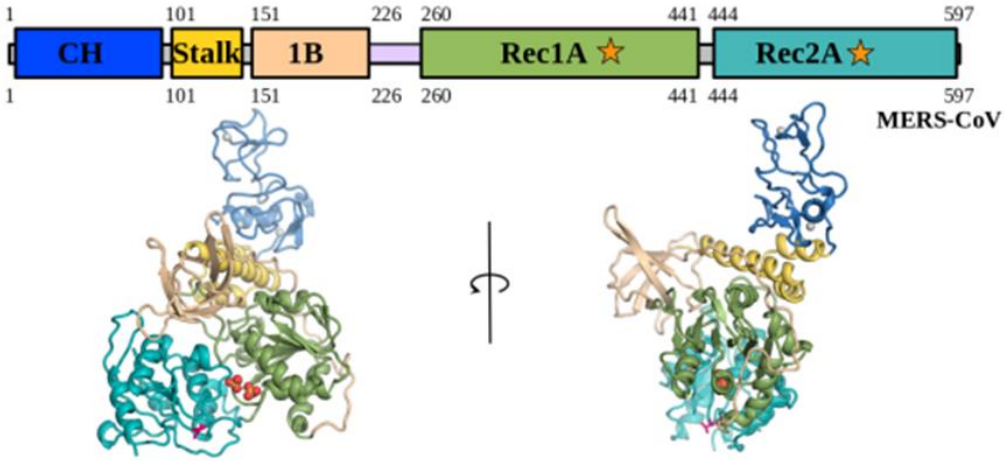

Figure 13: *MERS-CoV crystal structure of Nsp13 RNA helicase (White et al. BioRxiv 2020).*

Figure 14: *Detection of S5792L nsp13 mutation of corona virus isolates from USA.*

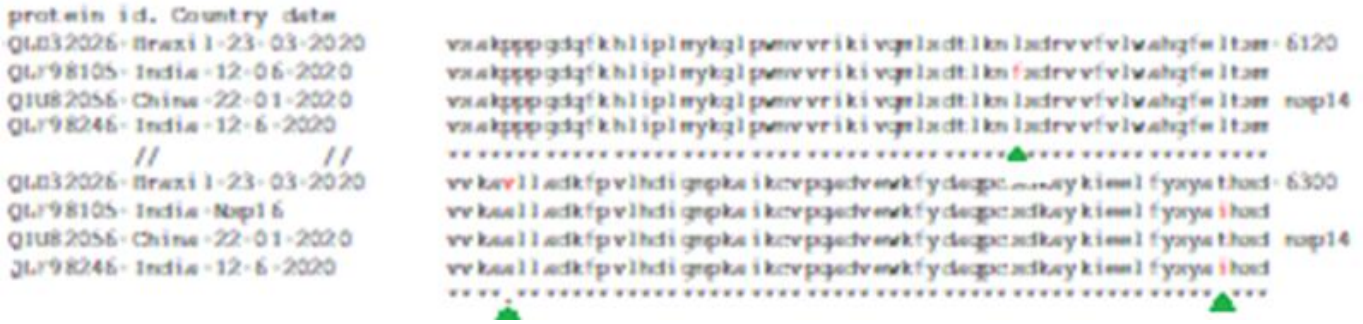

Figure 15: *Detection of L6102F, A6245V and T6297I mutations in the nsp14 protein of corona virus (SARS-CoV-2).*

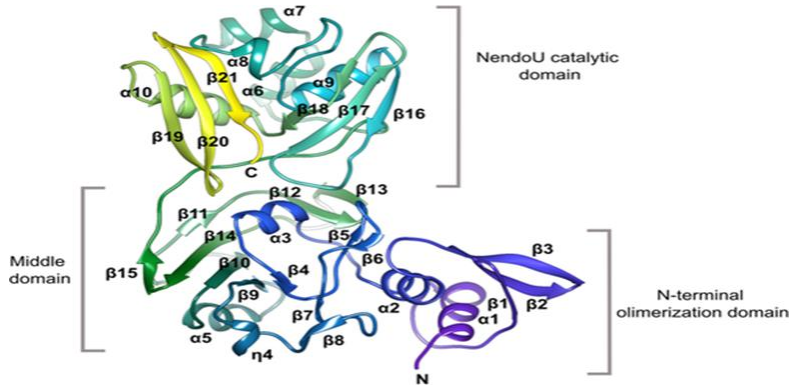

Figure 16: *Crystal structure of COVID-19 nsp15 endoribonuclease (Kim et al. 2020).*

Figure 17: *Recent S6613I mutation in the nsp15 endoribonuclease of COVID-19.*

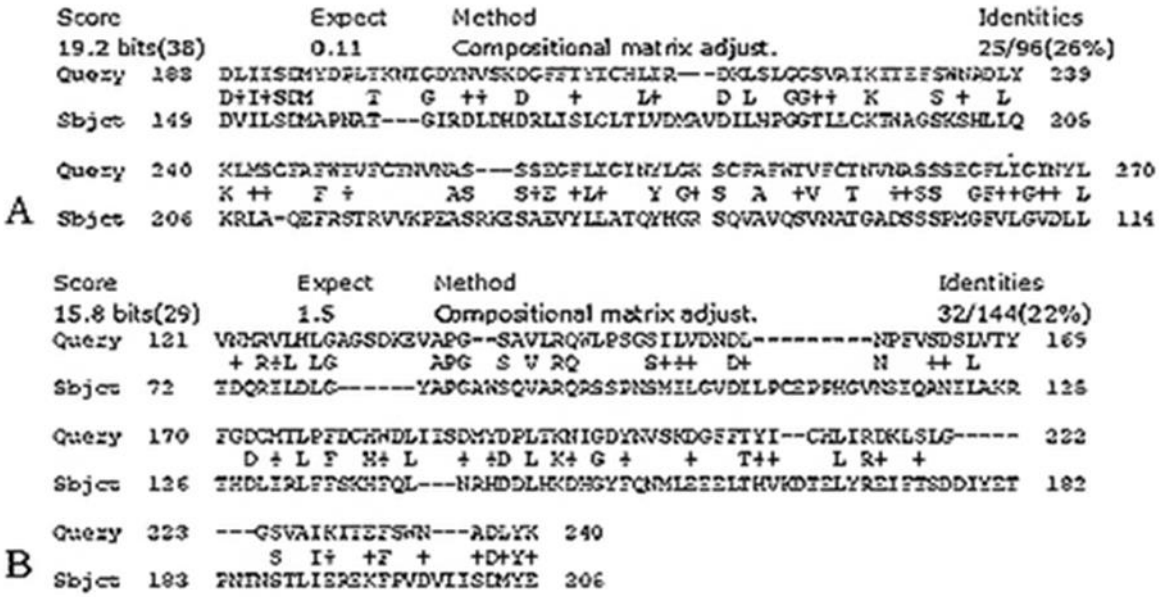

Figure 18: *Similarities between Nsp16 2'O-methyltransferase of COVID-19 and other eukaryotic 2'O-Uridine methyl transferase (A) Rat RlmE-like protein (accession-NP_001100595), (B) Yeast Mrm2 MTase (accession-QHB08519) that methylates U2791 on 21S mitochondrial rRNA.*

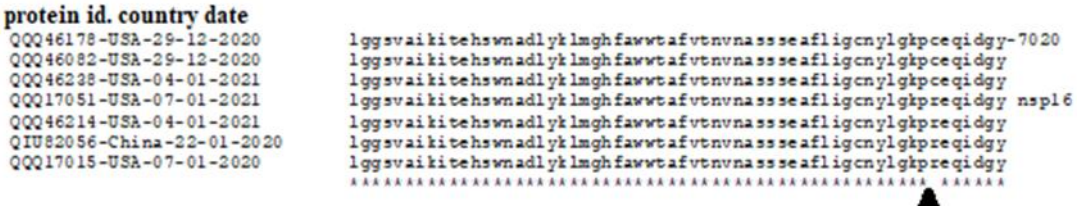

Figure 19: *Recent R7014C mutation in the nsp16 2'-O Uridine methyl transferase of COVID-19 US isolates. China January, 2020 clone was used as standard.*

Figure 20: *Detection of huge D614G mutation in Indian corona virus isolates.*

Figure 21: *Detection of a hyper-variable region (S194L/A; P199L; T205L; P207L) of nucleocapsid protein of COVID-19 in recent isolates of USA.*

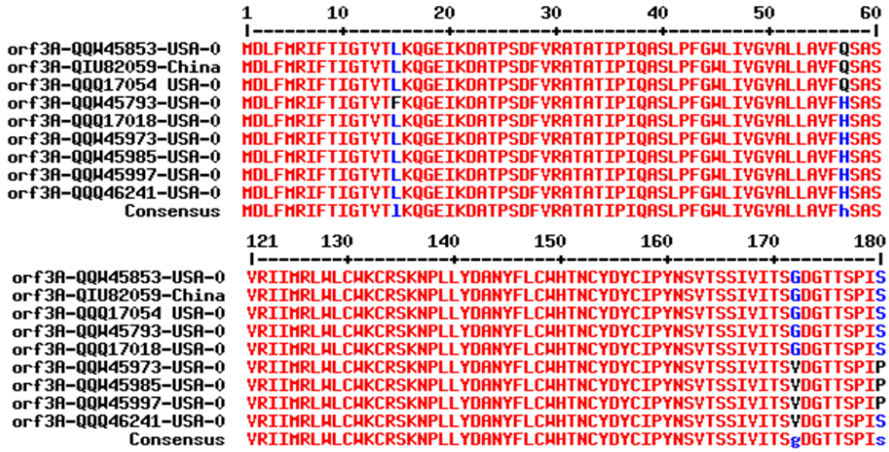

Figure 22: *Detection of new mutations (L15F, V172G, S180P) of ORF3A regulatory protein of COVID-19 in recent isolates from USA.*

Nsp2 (638aa) was suggested as RNA topoisomerase being 25% similarity stretches with *Vibrio haemolytica* topoisomerase I and topoisomerase IV [12,13]. TopoI and TopoIV chima protein had best homology to nsp2 protein of COVID-19 (figure-3). DNA

Gyrase of Escherichia coli had also some similarities and that helped to find ATPase domain of nsp2 protein (Figure 4). It was reported that nsp2 protein played an important role in the host cell survival pathway via its interaction with prohibitin (PHB) and prohibitin 2 (PHB2). The A206T, R207C and T265I mutations were predicted to cause structural alterations within the nsp2 domain. We showed recently that I120F mutation (I330F in orf1a protein) in the nsp2 protein favoured its high transmission in Australia and its spread had occurred into Bangladesh (Figure 5). We detected T265I mutation in the many isolates (Figure 6). Recently, nsp7, nsp8, nsp13 and nsp14 were assembled into replication-transcription complex with nsp12 RNA-dependent RNA polymerase for its faithful function. But without the nsp2 RNA topoisomerase such assembly may be incomplete as only topoisomerase can resolve the RNA knots and torsional stress created during transcription [14] (Figure 3-6).

Table 1: *Important functions of corona virus non-structural proteins nsp1 to nsp16.*

Name	AA length	Function of the protein	Reference
Nsp1	180	Transcription factor Translational inhibitor	[116] [8]
Nsp2	638	RNA topoisomerase Proinhibition1/2 binding & signalling Replication	[12] [81] [102]
Nsp3	1945	Papain-like Protease Polyprotein cleavage	[124] [16]
Nsp4	500	DMV formatiom	[93]
Nsp5	306	3C-lke Protease	[104]
Nsp6	290	Autophagosome and DMV formation	[105]
Nsp7	83	Cofators of Nsp12	[106]
Nsp8	198	DNA primase in RNA polymerase	[109]
Nsp9	113	RNA binding	[110]
Nsp10	139	Scaffold for Nsp14 and Nsp16	[112] [113]
Nsp11	13	Unknown cleavage product	?
Nsp12	919	RNA-dependent RNA polymerase	[65] [82]
Nsp13	601	RNA helicase, 5'-triphosphatase Capping 2'-O Guanosine Methyl Transferase	[80] [105] [5]
Nsp14	527	Exoribonuclease, Guanine-N7-methyl transferase	[117] [46] [48]
Nsp15	346	Endoribonuclease	[107] [108] [49]

Nsp16	298	RlmE rRNA Methyl Transferase	[121] [14]

Nsp3 large protein (1945aa) is a C3 Protease that cleaves corona virus polyproteins (orf1ab=7096aa and orf1a=4405aa) into functional sixteen enzymes. One of the best-characterized drug targets among corona viruses is the main protease that cleaves 11 cleavage sites on orf1ab polyprotein with a cut site is Leu-Gln↓(Ser, Ala, Gly). Along with the papain-like protease, this enzyme is essential for processing the polyproteins (790KD) that are translated from the viral RNA [15]. Peptidomimetic α-ketoamide compounds were selectively target MERS C3-protease. There is huge possibility of drug design because cleavage specificity was unique to corona protease [16]. Loninavir, nelfinavir, lopinavir, darunavir, beceprevir, ritonavir were reported good antiviral drugs targeting corona virus proteases but ritonavir inhibited Cytochrome P4503A4 enzyme and thus low dose must be used [17]. Few inhibitor structures were presented in figure-7 as described previously (Figure 7).

Nsp4 is 500aa length and is an accessory protein of C3 protease. SARS-CoV nsp4 H120 and F121 residues in the lumenal loop are essential for binding to nsp3. Thus, H120 and F121 substitutions in nsp4 cause defect in membrane rearrangement functions abolishing viral replication and propagation. Showed that SARS-CoV C3 protease was inhibited by a screened lead compound GRL0617 (IC50=20μM) controlling corona virus replication in Vero cells [18]. A complex dipeptide (compound 5h) with Ki 0.006μM was significant that inhibit SARS-CoV C3 protease [19]. We detected two abundant mutations in the nsp4 protein (S2215A and S2874L) in COVID-19 isolates of USA. Halomethyl ketone compounds inhibited 3CL pro protease of Corona virus [20] (Figure 8).

Nsp5 (~306aa) was a papain-like protease. The corona virus nsp5 protease is a conserved and indispensable virus-encoded enzyme which remains a key target for therapeutic design. However, past attempts to target the active site of nsp5 with inhibitors have failed stressing the need to identify new conserved non-active-site targets for therapeutic development. Recent study describes the discovery of a novel conserved structural region of the nsp5 protease of related mouse hepatitis virus (MHV) which may provide a rational basis for new drug development [21,22]. The nsp6 is ~290aa length membrane protein. It was reported that avian corona virus (infectious bronchitis virus, IBV) nsp6 protein helped to generate autophagosomes from the endoplasmic reticulum preventing viral components for degradation in lysosomes [23]. However, function of the protein remains elusive. However, we detected few mutations (T3586I and L3606F) in the COVID-19 isolates from Brazil (Figure 9).

Nsp7 (183aa) and Nsp8 (189aa) were small proteins and were regulatory subunits to Nsp12 RNA-dependent RNA polymerase and involved in the RNA synthesis complex [24]. However, nsp8 protein was proposed as vital methylating enzyme with some similarity to rRNA RlmH type methyl transferase and such RNA binding protein had also similarity with S30 transposase. Nsp9 (113aa) and Nsp10 (139aa) are also small proteins and may involve in the assembly of nsp14 ribonuclease [25]. We have reported that COVID-19 nsp9 and nsp10 proteins have homologies to S8 and S10 ribosomal proteins as well as RlmG and ErmD rRNA methyl transferases and may inhibit host mitoribosome assembly and protein synthesis in human [26]. NSP11 is made of thirteen amino acids and the first nine amino acids (sadaqsfln) are identical to the first nine in NSP12. No function of nsp11 was predicted yet (Figure 10).

The RdRp of SARS-CoV-2 is composed of a catalytic subunit of Nsp12 polymerase which divided into fingers, palm and thump domains and associated with Nsp7 and Nsp8 small proteins [27]. The Nsp12 subunit contains an N-terminal nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain, an interface domain and a C-terminal RdRp domain [28]. Remdesivir nucleoside analogue was a most valuable drug against Corona virus targeting Nsp12 RNA-dependent RNA polymerase [29]. RdRP inhibitors like remdesivir, ribavirin, lopinavir and favipiravir inhibited SARS-CoV-2 replication in vitro and remdesivir was a good drug against COVID-19 disease. However, presently dexamethasone, chloroquine and other non-targeted drugs were shown to relieve corona virus pathogenesis [30]. In figure-11 we showed the crystal structure of nsp12 protein as well its conserved motifs and its inhibitors. We proposed that P4715L mutation in the nsp12 involved in increased pathogenesis and COVID-19 transmission (Figure 11,12).

Nsp13 is a RNA helicase with 5'-3' NTPase activity in presence of 5mM Mg+2 ions and 8mM ATP and both activities are inhibited by bismuth salts [31-37]. However, we predicted Nsp13 as capping 2'-O Guanine methyl transferase as demonstrated its similarity to mouse capping methyl transferase [38]. The crystal structure was evaluated for MERS-CoV nsp13 RNA helicase and few mutations were detected by multiple alignments of COVID-19 orf1ab proteins [39] (Figure 13,14).

Nsp14 is a riboexonuclease with N7-Guanine methylase activity and maybe related to Rlm rRNA methyltransferase [40-45]. Critical residues of nsp14 essential for the methyl-transferase activity on GTP likely for the fidelity of RdRp were identified which were included F73, R84, W86, R310, D331, G333, P335, Y368, C414, and C416 residues [46]. Nsp10 may be required for Nsp14 structure and functions in vivo (Oganda et al. 2020). The

ExoN activity was located at the amino-terminal part of nsp14 and ExoN activity residues was divided into three motifs: I (DE), II (E), and III (D) where nsp14 was similar to DEDD exonuclease superfamily, catalyzing DNA proofreading. The N7-MTase domain of nsp14 protein exhibits a non-canonical MTase fold with a rare β-sheet insertion and a peripheral zinc finger. Thus, nsp14 required for replication and transcription of SARS-CoV [47,48]. We detected few mutations (L6102F, A6245V and T6297I) in the nsp14 protein of COVID-19 (Figure 15).

Nsp15 is a ~346aa length Mg++ ions dependent endoribonuclease producing 2'-3'-cyclic phosphodiester and 5'-OH ends acting on ss-RNA and ds-RNA [49]. Nsp15 of SARS, MERS and COVID-19 have good structural similarities and an important drug target site. NSP15 is reported to be involved in RNA replication and processing of subgenomic RNAs but the function is still not clearly understood (Deng et al. 2017). We suggested that COVID-19 nsp15 protein might be related to Dcm methyl transferase with RecA recombinase fold with some similarity to ribosomal protein S22 [50]. Nsp15 interaction with retinoblastoma tumour suppressor protein was shown and binding of pRb to nsp15 activated its endonuclease activity as also reported for mouse hepatitis virus. The structural studies of SARS-CoV, MERS-CoV and SARS-CoV-2 nsp15 showed that the protein forms hexamers made of dimers of trimers. The nsp15 was 39 kDa and folded into three domains: N-terminal, middle domain, and C-terminal catalytic NendoU domain [51]. The active site was located in a shallow groove between the two β-sheets with active residues His235, His250, Lys290, Thr341, Tyr343, and Ser294. Ser294 together with Tyr343 gave urine specificity, resembling the roles of Phe120 and Thr45 in RNaseA enzyme. Recombinant nsp15 was shown to have Mn2+-dependent endoribonuclease activity that cut dsRNA substrates with specificity towards uridylate in unpaired regions. Nsp15 suppressed interferon response in macrophages enhancing viral replication and transmission as evidenced in related porcine epidemic diarrhea corona virus [52,53]. The crystal structure of the protein was evaluated and a S6613I mutation for COVID-19 nsp15 protein was detected by GenBank database search (figure 16,17).

Nsp16 was a 2'-O rRNA Uridine methyl transferase [54,55]. It has similarity to yeast and rat rRNA uridine methyl transferase and very conserved among related corona viruses [56]. Recently, Robson et al has given evidences that Nsp13 and Nsp14 might be also involved in the minus strand RNA synthesis involving RpRp (Nsp12) as well as small accessory proteins nsp7 and nsp8 [57]. Corona virus's nsp16 are much conserved. COVID-19 nsp16 has similarities to RlmE Uridine methyltransferases of many eukaryotes (Figure 18,19).

Exploration on the Structural Proteins of COVID-19

Spike protein of COVID-19 is 1273aa long. The S-protein is the key membrane protein of corona virus that binds to the ACE receptor of human and animal cells and is the site for antiviral therapy [58,59]. It has 60% homology to bat corona viruses and may form trimer for biological activity [60,61]. It was importantly discovered that cellular protease furin cleaved the spike protein at the S1/S2 site and that cleavage was an essential step for S-protein-mediated cell-cell fusion and entry into human lung cells [62]. Samyuktha & Kumar explored the role of D614G mutation in India and such spike protein mutation have now spread worldwide [63]. Among the synonymous SNPs in the S protein, the C23929T (Y789Y) mutation was observed in Turkish isolate with high prevalence in Indian strains (>39%) and in US strains. We detected huge D614G mutation in the corona virus spike protein of Indian as well as isolates from other countries (Figure 20).

N-protein of COVID-19 is 419aa long. Experiments revealed that N-protein bound to leader RNA of replicating and transcribing genome of corona virus [64]. N-protein was shown to regulate host–pathogen interactions, such as actin reorganization, cell cycle progression, and apoptosis. The N-protein is also a highly immunogenic and abundantly expressed protein during infection, capable of inducing protective immune responses against SARS-CoV-2 [65-68]. N-protein interaction in corona virus life cycle also was a good target for novel drug design [69,70]. We detected few mutations (S194L, S194A, P199L, T205L and P207L) in the N-protein of corona virus isolated in the United States of America. We have also detected Q9H (protein id. QQW45860), P67S (QQQ45180/92; QQQ46004/188) and Q389L (QQW45980/92, QQW46004) mutations during another multi-alignment analysis (Figure 21).

Membrane protein (M-protein) of COVID-19 is 222aa long. M-protein is the most abundant surface protein that sorts viral components to be incorporated into SARS virions [71,72]. M-protein transmembrane domain forms lattice of M proteins at ERGIC membranes whereas S and E membrane proteins were integrated into the lattice through interactions with M protein. Virus-like particles were obtained in animal cells co-expressed with M-protein as well as N-protein. Further, M-protein was accumulated in the golgi-complex and might be interacted with other protein during corona virus pathogenesis [73]. The SARS-CoV-2 E-protein is a small 75aa long integral membrane protein which forms pentameric bundle in the membrane [74]. It has 10 amino acid hydrophobic NH2-terminus followed by large transmembrane domain of about 25aa and then a short hydrophilic carboxy end which needed for golgi complex localization. E-protein may be involved in apoptosis in transfected animal cells [75,76]. The hydrophobic region of the TMD contains predicted amphipathic α-helix that oligomerizes to form an ion-conductive pore in membranes. The SARS-CoV E

protein has recently been found to contain a binding motif known as the postsynaptic density protein 95 (PSD95), Drosophila disc large tumour suppressor (Dlg1) and zonula occludens-1 protein (PDZ)-binding motif. E-protein is localized mainly to the ER and Golgi-complex where it participates in the assembly, budding, and intracellular trafficking of infectious virions and C-terminus is important for such localization [77-79]. SARS-CoV-2 E-protein V25A mutation in the transmembrane domain affects the homopentameric conformation of E-protein and also a triple cysteine motif harbouring mutations (L39M, A41S/V, C43F/R/S, C44Y, and N45R) [80-83].

Exploration on the Accessory Regulatory Proteins of COVID-19

Orf3a protein of COVID-19 is 275aa long. It is conserved protein among the Corona viruses and is required for virus replication and release. The five most frequent mutations are V13L, Q57H, Q57H+A99V, G196V and G252V. Ten of the seventeen mutant sites occur within the transmembrane (TM) domain of ORF3a and are in contact with the central pore or side tunnels [84]. Others described induction of apoptosis by orf3a protein of SARS and COVID-19 in Vero E6 and other different animal cells [85-87]. The orf3a sequence has a predicted signal sequence at a 1–16 and three transmembrane domains at amino acids 34–56, 77–99 and 103–125. Furthermore, the C-terminal region of the orf3a protein shares 53% (aa 209–264) and 40% (aa 152–254) similarity with the Plasmodium calcium pump and the Shewanella outer-membrane porin. We also showed new mutations of (L15F, V172G, S180P) ORF3A regulatory protein of COVID-19 in recent isolates from USA (Figure 22).

The orf7a protein of COVID-19 is 121aa long. The orf7a protein has shown to induce apoptosis in cells [88]. ORF7a has a fifteen amino-acids NH2-terminal signal peptide, an eighty amino acids luminal domain, a twenty one amino acids transmembrane domain, and a five amino acids cytoplasmic tail [89]. SARS coronavirus orf7a protein restricts cell cycle progression at G0/G1 phase via the cyclin D3/pRb pathway [90]. Further, in association of bone marrow stromal antigen, orf7a restrict corona virus release at a greater way likely interfering glycosylation [91]. The ORF7a protein has a structural homology with ICAM-1 which binds to the T lymphocyte integrin receptor LFA-1 and SARS-CoV2 would have a similar structural interaction with LFA-1and related leukocyte integrin Mac-1 found in macrophages [92].

Exploration of Vaccine Production against COVID-19

Presently, at least ten vaccine candidates are in stage-III clinical trials on 20-40 thousand human worldwide and few vaccines were inoculated in the 16million public of UK, USA, India, Russia and China [93]. Mostly Spike protein DNA vaccine has got momentum as well as attenuated corona virus vaccine, spike mRNA vaccine and peptide vaccines. Vaccine usually is a protein or synthetic peptides from Corona virus that can elicits humoral antibody (IgG) as well as T-cell mediated ability to destroy virus and spike protein was targeted for this purpose [94]. Attenuated or killed Corona virus (Covaxin, Bharat Biotech, India) was used like Pox vaccination. As genetic information in cells processed from DNA to RNA to protein, scientists exploited DNA vaccine using adenoviral vector as well as mRNA vaccine for the protection of Corona virus. Indian Serum Institute used protein as well as killed virus where as Russia used mRNA vaccine (Sputnik V). Oxford + Astra-Zeneca used Spike protein DNA vaccine using adenovirus vector [95]. United States (Moderna+Pfizer) and Germany's BioNTech engineered mRNA vaccine of spike protein which needs -40oC for storage. The spike protein (S-protein), the receptor protein of corona virus that bound to ACE-2 receptor of lung cells of human and animal was mostly targeted for vaccine preparation [96]. But we thought RNA topoisomerase and methyl transferees may be good vaccine candidates. Oxford and AstraZeneca expect to produce up to three billion doses of the vaccine in 2021. Modern supplied 20 million doses in 2020 and will deliver 500 million doses in 2021. Vaccination already started worldwide and 10 million people were inoculated with first dose and second dose would be mandatory after 1-3 months. It was reported that Ad-5 spike protein DNA vaccine was more effective to elicit antibody quickly (Table 1).

Future Implications on the Corona Virus Research

Worldwide devastation spread of corona virus is shocking. Recently many doctors have reported the multi-drug resistant bacterial infection during corona virus pathogenesis [97]. Thus, although vaccination may reduce the corona spread but antibiotics void is a serious threat to human and animal where new drug development must be accelerated for pan drug resistant bacteria [98]. Recently, we have shown that Cassia fistula bark CU1 phyto-chemical actively inhibited many multi-drug resistant bacteria targeting RNA polymerase enzyme. We hope direct antimicrobials are also necessary for corona virus as numerous mutations have reported in S-protein [99-105]. Gene therapy using antisense, ribozyme and CRISPR-Cas6 technologies with toxic drug delivery applying nanotechnology application may be welcome. Nsp2 RNA topoisomerase may be a good target for antiviral therapy as topoisomerases of many microorganisms have targeted [105-110]. Evaluated the potential antiviral activity of the PIKfyve kinase inhibitor APY0201 against COVID-19 and HIV protease inhibitor nelfinavir mesylate hydrate and the antagonist of the serotonin receptors 5-HT1B and 5-HT1D, GR 127935 hydrochloride hydrate [111-120].

Conclusion

We have reviewed the recent research on biochemistry and molecular biology of corona virus proteins. Molecular targets were highlighted and many mutations in the important corona virus genes were detected worldwide. The crystal structures of many proteins were presented and new functions of nsp2, nsp13 and nsp16 proteins were elaborated. Huge corona virus spread was seen with D614G spike protein mutation worldwide as well as I300F RNA topoisomerase mutation in Australia and P4715L RdRP mutation was also prominent everywhere [121-124].

Acknowledgement

We thank Dr Jayashree Laha, Principal of Raja N L Khan Women's College for his interest in Corona virus research and drug design. Dr. Angsuman Chanda is Head of the post graduate department of Zoology and AKC is visiting professor.

Funding

No funding received from external sources

Compete of Interest and Ethical Issues

The authors have no competing interest. No human sample used in this study. It is bioinformatics work using NCBI and PubMed databases.

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