Ribosomal proteins homologies and methylation of mitoribosome by Corona virus Nsp9, Nsp10 and previously described Nsp13-16 proteins suggested their roles in the inhibition of host protein synthesis: Discovery of new therapeutic targets against Corona virus infections

Asit Kumar Chakraborty, Associate Professor of Biochemistry

Department of Biotechnology and Biochemistry, Oriental Institute of science and technology, Vidyasagar University, Midnapore-721102, India. Mobile:917679154141; Email:chakraakc@gmail.com

Abstract

Multi-Alignment method coupled with phylogenetic analysis we disclosed the Nsp9 and Nsp10 non-structural proteins of Corona Virus as rRNA RlmH/K methyltransferases with similarities with bin recombinase and int-core integrase fold. Further, Nsp9 has similarities to S8 ribosomal protein and Nap10 has similarity to S10 ribosomal protein. Previously, we showed Nsp13, Nsp14, Nsp15 and Nsp16 are also different types of rRNA RlmE/N and Cfr-like methyltransferases-ribonuclease with RNA helicase domains. Two domains of Nsp13 astonishingly have similarities to ribosomal proteins L6 and L9. Taken together, Nsp9/10 and Nsp13-16 proteins could mimic host ribosome assembly and also could methylate rRNA of mitobibosome preventing mitochondrial protein synthesis and oxidative phosphorylation. Low ATP synthesis causes lowering blood pressure following coma but very ATP concentration (1-10nM) surely induces platelets aggregation through vWA, collagen and GpIIb/IIIa proteins followed by fibrin formation and blood clotting as recently have seen in the lung of many Corona virus infected patients. We have also postulated that two polyproteins itself resemble like 28S and 38S mitoribosome subunits and compete with rRNAs inhibiting the ribosome turnover and new protein synthesis due to their similarities with many ribosomal proteins. Such finding may be valuable in computer-based novel drug design against Corona virus.

Keywords

Coronavirus Nsp proteins, ribosomal proteins homology, inhibition of ribosome turn over, rRNA methyltransferase, protein synthesis inhibition, low ATP formation, blood clotting, low blood pressure and coma.

Introduction

Corona viruses (family Coronaviridae) are enveloped viruses with a largest positive sense, single-stranded RNA genome of ~30kb (Woo et al. 2009; Marra et al. 2014). On genetic and antigenic criteria, CoVs have been organised into three different groups: α -CoVs, β -CoVs, and γ -CoVs (Lau et al. 2015; Dominguez et al. 2014; Lu et al. 2015). Coronaviruses primarily infect birds, mammals and human, causing many lethal respiratory syndromes resembling the common cold, such as bronchitis, pneumonia, and even severe acute respiratory syndrome (SARS). Recently coronaviral research augmented due to pandemic severe respiratory illnesses outbreaks claiming >100000 deaths due to human Corona virus (Liu et al. 2014). COVID-19 virus enters cells through ACE2 receptor-mediated endocytosis in lungs alveolar epithelial cell as well as cells in the heart and kidney (Zhao et al. 2020).

The extremely large gene 1 covers 2/3 of the 30kb genome (separated into ORF1a and orf1b) encoding polyproteins which proteolytically degraded into sixteen non-structural proteins involved in mRNA synthesis and replication of virus through (-) strand RNA synthesis by RNA-dependent RNA polymerase. The rest 1/3 of the 3' end of the RNA genome encodes the structural spike glycoprotein (S), small envelope protein (E), membrane glycoprotein (M), and nucleocapsid protein (N) as well as few small transcripts like ORF2b, ORF7a and ORD2a (see, accession no. DQ415908, KT779555, KY674941, AY884001, DQ415912, KY674941, DQ415914, , MH940245).

The function of Nsp2 was proposed by me as RNA topoisomerase and Nsp13 was determined as 2'-O-ribose capping Guanosine methyltransferase. By bioinformatics approach comparing 200 DNA and MTases, Ligases, RNases, DNases, Ribosomal proteins as well as some RNA virus associated non-structural proteins we wanted to determine the functions of nsp9 and Nsp10 of Corona virus (Decroly et al. 2008; Bollati et al. 2009). We proposed Nsp16 as RlmE-type 2'-O-ribose Uridine methyltransferase and Nsp14 as N7 Guanidine methyltransferase where as now we proposed Nsp9 as RlmG-like and Nsp10 as ErmD-type methyltransferase accounting total five rRNA methyltransferases in Corona virus genome.

The rRNA MTases methylate at least nine 23S rRNA nucleotides (G748, A1067, C1920, A2058, G2445, G2470, U2479, A2503, T2504 and G2535) on the large ribosomal subunit (Cheng et al. 2002). There are more than ten 16S rRNA modifying MTases (ArmA, RmtA to RmtH and NpmA) have characterized where as ArmA and RmtH are abundant. Ribosome decoding centre (nucleotides 1400–1500 of 16S rRNA) is the binding sites for aminoglycosides. Endogenous methyltransferases RsmI and RsmH methylate

C1402 where as RsmE methylates U1498, and RsmF methylates C1407 (Tscheme et al. 1999). Different Rlm methyltransferases methylate at various positions of bacterial 23S rRNA conferring multi-resistant to macrolides and ketolides. As for example, RlmA^{II} MTase has preference to N₁ of G748 of 23S rRNA (Jiang et al. 2018), RlmB MTase (protein id. BAI33654) modifies G2251 (Michel et al. 2002, Mao et al. 1995) while RlmC modifies m5U747 and RlmD is specific for m5U1939. RlmE and RlmF methylate 23S rRNA 2'-O-U2552 while methylates at N₂ of G1835 and N3 pseudo-Uridine for RlmH and RlmN methylates C₂ at A2503 (Punekar et al. 2013). RlmM (YgdE; EC:2.1.1.186) enzyme catalyzes the SAM-dependent 2' O-ribose methylation of C2498 in 23S rRNA of *Escherichia coli* (Pletnev et al. 2020). Dcm Methyltransferase (EC:2.1.1.137) causes DNA methylation at the C₅ or N₄ positions of cytosine. *E. coli* Dam methyltransferase has GATC sequence specificity and methylates at the adenine residue at N₆ regulating many genes (Peterson & Reich, 2006).

We investigated the homology profiles of many recombinases and transposases using CLUSTAL-Omega software. Several thousand IS elements were sequenced but could be classified into ~20 families (IS2, IS30, Tn10, Tn21 etc) on the basis of the sequences of their transposases and terminal inverted repeats as well as associated antibiotic resistant, drug efflux and metal resistant genes as found in integrons and transposons (Blakely et al. 1993; Calos & Miller, 1980; Jacques & Michael, 1998). Int1I, int and Rci are different integrases (Polard & Chandler, 1995). *Escherichia coli* RecA, RecB and recC have roles in homologous recombination, DNA repair, and the induction of the SOS response (Kowalczykowski & Eggleston, 1994; Taylor & Smith, 1985). Bacterial and mammalian RNases are also RNA modifying enzymes and exonucleases are also compared (Beintema et al. 1988; Gotte & Menegazzi, 2019; Bechhofer & Deutscher, 2019). We also compared sixty 30S plus 50S ribosomal proteins as they are powerful RNA binding proteins (Hammerling et al. 2020). We have compared amino acid sequences of those 100-150 proteins with Nsp9 and Nsp10 non-structural proteins related to polyprotein ORF1ab of coronavirus. BLAST search and Multi-alignment analysis are important tools to find the function of Unknown viral protein like Nsp9/10 of Coeonavirus. Previously, Nsp2 and Nsp13 functions were predicted as RNA helicase-topoisomerase but and capping Guanine 2'-O-Ribose methyltransferase respectively (Chakraborty, 2020; Belanger et al. 2010).

Materials and Methods

The BLAST search was done using web portal www.ncbi.nlm.nih.gov/blast and retrieve of [covid-19](https://www.ncbi.nlm.nih.gov/covid19/) and other Coronaviruses cDNA sequences were done using web portal www.ncbi.nlm.nih.gov/nucleotide or [protein](http://www.ncbi.nlm.nih.gov/protein). NCBI Primer Design Software was used for primer selection and Oligoanalyzer 3.2 software was used to analyze primer dimmer and hairpin structure. Multalin Software and CLUSTAL Omega Software were used to multiple align of protein sequences and NCBI BLAST seq-2 analysis portal used to analyze homology between two sequences. NCBI pubmed portal (www.ncbi.nlm.nih.gov/pubmed) used to retrieve references and papers. CLUSTAL Omega Phylogenetic tool used to determine the closer structural similarities among the proteins and Seq-2 BLAST was confirmed percentage of sequence homology between two related proteins (Chakraborty et al. 2017; Chakraborty, 2020). The structure and localization of Corona proteins were demonstrated in Figure-1.

Results

BLAST and CLUSTAL Omega softwares are very powerful to compare unknown DNA and protein molecules as vast number of genes were deposited in the GenBank and crystal structures and functional domains of protein sequences were known. Figure-2 disclosed the multialign-phylogenetic relations of 50 DNA/RNA modifying genes with Nsp9 and Nsp10 non-structural proteins of Corona virus and the functions of such proteins are still elusive. It was clearly indicated that Nsp9 share a relation with ermD and Nsp10 with exonuclease or RlmK. Figure-3 showed the multi-alignment and phylogenetic relation of Nsp9 and Nsp10 with seventy five *Escherichia coli* ribosomal proteins. It is clearly indicated that Nsp9 has close similarity to S8 and Nsp10 to S10 and L22 ribosomal proteins.

We detected about 20%-25% scattered homologies between Nsp9 and S8 ribosomal protein (figure-4A) and between Nsp10 and S10 ribosomal protein (figure-4B). In figure-5 we demonstrated the similarities among ErmD methyltransferase with Nsp9 and RlmG methyltransferase, int-core integrase and bin recombinase with Nsp10 protein of Corona virus. (A) Nsp9 vs. ErmD, (B) Nsp10 vs. RlmG, (C) was Nsp10 vs. bin-recombinase and (D) Nsp10 vs. int-core integrase. The ErmD has also similarities to RmtH and int integrase but such similarities are not great and expected for distance search between viruses vs, Bacteria. The result might be confusing but problem solved when we did homology search between

RlmG vs. bin-recombinase and RlmG vs. int-core integrase (figure-6AB) showing homology profile of those enzymes. The problem is BLAST multi-alignment hardly able to catch such similarities and only gave maximum homology at certain point of the sequence. Similarly, CLUSTAL multi-alignment search drastically made gaps and hard to interpret homology data unless we made phylogenetic conversion.

Previously, Nsp13 RNA helicase was shown having multiple domains and functions and ideal for targets for anti-viral drugs (Ivanov et al. 2004; Jang et al. 2008; Jia et al. 2019). We confirmed the sequence similarities of L6 and L9 ribosomal proteins with Nsp13 proteins as well as Nsp2 has a relation to L1 etc supporting such view. We are much confused with the data but it appears that many non-structural proteins of Corona virus have significant homologies with the Ribosomal proteins and thus they could interfere with the ribosomal assembly correctly. Other words, Corona virus ribosomal proteins could interact with the Human ribosome preferably in the mitochondria to methylate as well as topological changes of mRNA or rRNA to alter the translational profile of host mRNA for protein synthesis or enhanced translation of viral structural genes (Zust et al. 2011). Whatever the situation, we predicted that COX1 and COXII types proteins deficiency in mitochondria lead to low oxidative phosphorylation at the ETC and low ATP synthesis. This could cause many physiological abnormalities in host like sudden low blood pressure, coma and heart attack as found in many Corona patients. If ATP concentration in blood reached below 10nM that could lead to blood clotting which was again shown recently in lung and brain of the many Corona patients in the Netherlands.

Fig.1. Localization of Nsp9 and Nsp10 proteins on the Corona virus genome and polypeptides.

Fig.2. Multialignment of different DNA/RNA modifying enzymes with Nsp9/10 of Corona virus.

Fig.3. Multialignment of 30S and 50S ribosomal RNA binding proteins with Nsp9/10 of Corona virus.

Fig.4. Homology of Nsp9 and Nsp10 to ribosomal proteins. (A) Nsp9 vs ribosomal protein S8 homology an (B) Nsp10 vs ribosomal protein S10 homology.

	1102030405060
ernD-APC11317	MERNIKDSQNF LHN KKLVQKLV EE SNLSKEDTVYEIGPGKGMITEQLLARCKNVIAIELD
Nsp9-Coronavirus	NNELSPVALRQMSCAAGTTQTACTODNALAYNTTKGGRFVLALLSDLQDLKWARFP
Consensus	...NnedS qna Lr#kkcaaglt#eanl dd #alaYengpggGriteaLLarcq#liaarld
	61708090100110120
ernD-APC11317	NKLYQLLQDKFK NPK LTLINE DF MLTNLPDEKYKVF SN IPFNM TA EILTKLLTAPNPPQD
Nsp9-Coronavirus	KSDGTGTIYTELEPPCRFVTD T ---PKGPKVKYLYFIKGLNNLNRGMVLGSLAATVRLQ
Consensus	nkdgqglqdkel#Pkcr! ln #d...pngPdeKYlyFinglnN\$naeillglLaApnrIQ.
	121130140150160170180
ernD-APC11317	MYIIMQYEANLKYAGAPYYKDSLKSLMFKPMYDIKMVHEFDSADFTPKPNVSIYMHVHFHK
Nsp9-Coronavirus	
Consensus	
	181190200210220230240
ernD-APC11317	KKYCDIKKATHLEFMDFLSYVYAAPGKFFKEKTKKVFSYEQQKRLRKSINLDEDVTFSEH
Nsp9-Coronavirus	
Consensus	
	241250260270280289
ernD-APC11317	TYLQWVEIFNTYKQFVSDDKKKFVIGSYAKLLLEQSKLNRMRNRQKQY
Nsp9-Coronavirus	
Consensus	
	1102030405060
Nsp10	MSQAELNGELFTLERFPPNVEEEALQAEARADEYLLQQVNDVDGLTLIFNDGFGALACAL
R1nG-CDQ55171	
Consensus	
	61708090100110120
Nsp10	AERNPV S INDSFISELATRHNLRMNGIDEESVRFQDLSPLPAAPALVLIKVPKQLALLE
R1nG-CDQ55171	AGNA
Consensus	Agna
	121130140150160170180
Nsp10	TEVPANSTVLSFCFAFYDAAKA---YKDYLASGGQPI-TNCVKMLCTHTGTGQAITVTPE
R1nG-CDQ55171	QQLRALREVVTPETRIIAAAKARDVHNSTLALFEKILGTTTTSLAWKKARLIHCYVTAPE
Consensus	q#LrAnreVlsfcara!aAKA...hndtLAlgeqii.Tnctk\$ackharlgqa!ftaPE
	181190200210220230240
Nsp10	ANMDQESFGGASCCLYCRCHIDHPN--PKGFCDLKGKYV--QIPTTCANDPVGFTLKNTV
R1nG-CDQ55171	LADAPQTYSMKLDGTPMTIH-NHANVFARSGLDIGARFFLQHLPSDLEGEIADLGCGNGV
Consensus	aadaq#s%ggaldclpcrch.#HaN...arggcDigar%f..qiPsdcan#iadlgcNgV
	241250260270280290300
Nsp10	CTVCGM W KGYGCS CD QLRE P MLQ
R1nG-CDQ55171	IGLQALAQNPNASVMFTDESHMAVASSRLNVERNLPDDIARCEFHVNNSLSGIEPDRFTA
Consensus	cglca\$aqnpnaScdqlrEph\$a.....
	301310320330340350360
Nsp10	ILCNPPFHQQHAITDHIAMQMFNDARRSLKYGGELYVYVGNRHLDYFRKLKRAFGNCTTIA
R1nG-CDQ55171	
Consensus	
	361370377
Nsp10	TNNKFVILKATKYRKQR
R1nG-CDQ55171	
Consensus	

Nsp10-coronavirus
 bin-p-ADA80845
 Consensus

1 10 20 30 40 50 60
 |-----+-----+-----+-----+-----+-----|
 AGNATEVPANSTVLSFCFAFYDAAK
 MKIGYARVSTGLQNLNLQEDQLNQYGCEKIFSDHISGSKSRPGLDKAIEFARAGDTIVV
aGnaserPandkaieFaaaaddaak

Nsp10-coronavirus
 bin-p-ADA80845
 Consensus

61 70 80 90 100 110 120
 |-----+-----+-----+-----+-----+-----|
 AYKDYLASGGQPITNCVKML---CTHTGTGQAITVTPPEANMDQESFG-GASCCLYCRCH
 WRLDRLGRNMEDLITLVNELNNRGVYFSHLEENITMDKSSSTGQLLFHLFAAFEFERNL
 arlDrLarng#diincVneL....cshhgle#aITndkeanndQelfg.gAacae%CRnh

Nsp10-coronavirus
 bin-p-ADA80845
 Consensus

121 130 140 150 160 170 180
 |-----+-----+-----+-----+-----+-----|
 IDHPNPKG--FCDLKGKYVQIPTTCANDPVGF--TLKNT--VCTVCGMWKGYGCSQDL
 ILERSSAGRIARARGRYGGRPPEKLNKQDLKLLKTLTYONGPTIKTIAEQWKVSRITTIYR
 IdernpaG..aararGrYgqrPekcan#dlgl..TLk#n...!cT!aeqWKgsrscsdrL

Nsp10-coronavirus
 bin-p-ADA80845
 Consensus

181 190 192
 |-----+-----|
 REPMLQ
 LNRLNNQENKDK
 r#r\$#n#.....

	1	10	20	30	40	50	60
Nsp10-coronavirus	-----+-----+-----+-----+-----+-----						
int-core-p-ATB85826	MLNTRYHQSEYSMATDTPRIPEQGVATLPDEAMERARRRAEIIISPLAQSETVGHAEADMA						AGNATEVPANSTVLS
Consensus	aa#aeeVga#aad\$a						
	61	70	80	90	100	110	120
Nsp10-coronavirus	-----+-----+-----+-----+-----+-----						
int-core-p-ATB85826	FCFAVDAAKAYKDYLA-SGGQPITNCVKMLCTHTGTGQAITYTPEANMDQESFSGGASCC						
Consensus	AQALGLSRRQYYVLIRRAAQGSGLYTDLVPGQSGGGKKGRLPEPVERVIEELLQKRFLT						acAlaldaaqaYkdir.ArqGqgitndlkmgcsggGkGqarlpePearmdqEllqgafcc
	121	130	140	150	160	170	180
Nsp10-coronavirus	-----+-----+-----+-----+-----+-----						
int-core-p-ATB85826	LYCRCHID-HPNPKGFCOLKGYVQIPTTCAN-----DPVGF TLKNTVCTVCGMWKGYGC						
Consensus	KQKRS LA AFHREVTQYCKAQKLRYPARNTVALRIASLDPRKVIRREGQDAARDLQGVGG						lqcRchaa.Hr#pkqfCdaqglrVqarnTcAn.....DPrgfirrregcdaardlqGyGc
	181	190	200	210	220	230	240
Nsp10-coronavirus	-----+-----+-----+-----+-----+-----						
int-core-p-ATB85826	SCDQLREPHLQ						
Consensus	EPPAVTAPLEQYQIDHTYIDLIVYDDORDRQPIGRPYLTLAIDVFTRCYLGMVYTLEAPSA						ecdalraP\$eQ.....
	241	250	260	270	280	290	300
Nsp10-coronavirus	-----+-----+-----+-----+-----+-----						
int-core-p-ATB85826	VSVGLCLVHVACDKRPWLEGLNVEMDQMSGKPLLLYLDNAAEFKSEALRRGCEQH GIRL						
Consensus							
	301	310	320	330	340	350	360
Nsp10-coronavirus	-----+-----+-----+-----+-----+-----						
int-core-p-ATB85826	DYRPLGQPHYGGIVERIIGTAMQMIHDELP GTTF SNPDQRGDYDSENKAALTLRELERWL						
Consensus							
	361	370	380	390	400	410	420
Nsp10-coronavirus	-----+-----+-----+-----+-----+-----						
int-core-p-ATB85826	TLAVGTYHGSVHNGLLQPPAARWAEAVARYGVPAVYTRATSFLVDLPILRRTLRTGFV						
Consensus							
	421	430	434				
Nsp10-coronavirus	-----+-----						
int-core-p-ATB85826	IDHIHYADGHCKK						
Consensus							

Fig.5. Homologies of Nsp9 and Nsp10 protein to different DNA modifying enzymes. (A) Nsp9 vs. ErmD, (B) Nsp10 vs RlmG methyltransferase, (C) Nsp10 vs bin recombinase and (D) Nsp10 vs int-core integrase

bin-p-ADA80845
RlnG-CDQ55171
Consensus

1102030405060
|-----+-----+-----+-----+-----+-----|
MSQAELNGELFTLERFPPNVEEEALQAEAADEYLLQQVNDVDGLTLIFNDGFGALACAL
.....

bin-p-ADA80845
RlnG-CDQ55171
Consensus

61708090100110120
|-----+-----+-----+-----+-----+-----|
AERNPVSINDSFISELATRHNLRMNGIDEESVRFQDSLSPAPALVLIKVPKQLALLE
.....

bin-p-ADA80845
RlnG-CDQ55171
Consensus

121130140150160170180
|-----+-----+-----+-----+-----+-----|
 MKIGYARVSTGLQNLNLQEDQLNQYGCEKIFSDHISGSKSRPGLDKAI---
QQLRALREVYTPETRIIAAAKARDVHNSTLALF--EKILGTTTTSLAWKKARLIHCVFTH
.....nkigear!iaaaqar#lq#dqLaq%..EKILgdhisglasKrarLdha!...

bin-p-ADA80845
RlnG-CDQ55171
Consensus

181190200210220230240
|-----+-----+-----+-----+-----+-----|
-EFARAGDTIVVWRDLRLGRNMEDLITLVN---ELNMRGVSFHSLEENITHDKSSSTGQ
PELADAPQTY-SMKLDGTPWTIHNHANYFARSGLDIGARFFLQHLPSDLEGEIADLGCGR
..ELArAg#Tis.WrLDrIgrnie#hanlfa....#inaRgflqHlle#negedadlgcG#

bin-p-ADA80845
RlnG-CDQ55171
Consensus

241250260270280290300
|-----+-----+-----+-----+-----+-----|
LLFHLFAAFAEFERNLILERSSAGRIAARARGRYGGRPEKLNKQDLKLLKTLY---DNG
GVIGLQALAQMPNASVMFTDESHMAVASSRLNVERNLPDDIARCFMVNNSLSGIEPDRF
gligLqAaaa#f#anililereSaga!AararnrernrP#diarc#lklnsLs....Drg

bin-p-ADA80845
RlnG-CDQ55171
Consensus

301310320330340350360
|-----+-----+-----+-----+-----+-----|
TPIKTIAEQWKVSRTTIYRYLNRLNNQENKDK
TAILCNPPFHQQHAITDHIAMQMFNDARRSLKYGGELYVVGNRHLDYFRKLKRAFGNCTT
TaIlcnaeqhqqhaiTdhral#rlN#arrkdK.....

bin-p-ADA80845
RlnG-CDQ55171
Consensus

361370379
|-----+-----|
IATNNKFVILKATKYRKQR
.....

Fig.6. Homologies among recombinase, methyltransferase and integrase, RlmG vs bin recombinase (A) and RlmG vs int-core integrase (B).

Discussion

Mechanism of Corona virus pathogenesis is not known and no drug or vaccine yet approved today claiming every day >1000 peoples worldwide and within four months about 30 millions infections and >210000 deaths occurred mostly in the developed countries like USA, England, France, Italy and Spain. Asian and African countries also greatly have affected but due to early lock down technology death rate has restricted to hundreds only. Recently,

Russia, Canada and Netherland also have affected to some extent. Sadly Corona pandemic created 100 millions jobless and worldwide panic and WHO has suggested that the pandemic may cause 20-50 millions death due to lack of food and sanitation. We must act to prevent the spread of Corona virus. Thus, the present bioinformatics analysis of Nsp9 and Nsp10 unknown proteins of Corona virus is important and may through some highlights to its control measures targeting those suspected ribosome antagonist and rRNA methyltransferases (von Grotthuss et al. 2003; Albert et al. 2019; Hammerling et al. 2020). We previously showed that Nsp2 is a RNA topoisomerase, a great target for DNA topoisomerase inhibitors (Chakraborty, 2020b). As well as Nsp13-16 may be other RlmE and Cfr-like methyltransferases although previous report has suggested Nsp13 as ATP-dependent RNA helicase (Adedeji et al. 2012; supra, 2016; Hao et al. 2017; Subissi et al. 2014; Chakraborty, 2020c). Many enzyme has multiple domains and thus they participate in multiple enzymatic activities as Nsp13 and Nsp9/10 which also have recombinase and transposase types domains (Jiang et al. 2018). Corona virus RNA genome replication is an ideal target and Nsp2 RNA topoisomerase has not been explored (de Wilde et al. 2018). Alisporivir inhibited the SARS and MERS Corona viruses RNA replication and hydroxychloroquine was shown protective during Corona virus pathogenesis (de Wilde et al. 2017). Interferon- α b therapy also has been started and under clinical trial (Falzarano et al. 2013).

Conclusion

We proposed a model where Nsp9, Nsp10 as well as previously reported Nsp13-16 have similarities to the ribosomal proteins and assembled in mitoribosome to methylate 21S and 12S rRNAs inhibiting protein synthesis. Low synthesis of COXI and COXII to types ETC enzymes cause low ATP synthesis which accelerates low blood pressure and heart attach. Further, very low ATP concentration also favours activation of platelets aggregation following factor XIII/IX-mediated thrombin-stimulated fibrin formation leading to blood clot in the lung and brain of COVID-19 patients. So targeting those RlmE, ErmD and RlmG types methyltransferases with phytochemicals, antisense, ribozyme and Crispr-Case6 technologies may be gene medicine for Corona virus pathogenesis (Cao et al. 2015; Chakraborty, 2019). However, major focus for Corona-specific drug development was pinpointed to the ACE-2 receptor antagonist, RNA-dependent RNA polymerase (Nsp12), c3 protease (Nsp3) inhibitors (Hu et al. 2020). Similarly, for vaccine development major focus molecules was studied likely spike protein of Corona virus (Zhao et al. 2020). I have alternate thought like Nsp2 RNA topoisomerase and rRNA methyltransferases (Chakraborty, 2020abc;

Ferron et al. 2012; Gorbalenya et al. 1988; Smietansk et al. 2014). We have also designed peptide vaccine candidates and RT-PCR primers (Chakraborty et al. 2020). We and other have developed many methods of toxic drug nanocarriers and plant extracts may be valuable source for superbugs and Corona virus control (Chakraborty et al. 2018, Chakraborty, 2018; Kilianski et al. 2014; Ulasli et al. 2014). Never the less, we clearly predicted the functions of Nsp9/10 proteins of Corona virus.

Acknowledgement

I thank Sir Narendra Modi, prime Minister of India for stimulation through his Mon Ki Bath programme to fight against COVID-19.

Conflict of Interest

I have no conflict of interest to any company and any organization.

Ethical Issues

No patient data was used in this study. PDB Database and GenBank Database were analyzed.

Funding

This is lock down research from my home computer and no funding from any agency

References

- Adedeji AO, Lazarus H (2016) Biochemical Characterization of Middle East Respiratory Syndrome Coronavirus Helicase. *mSphere*. 1(5): pii: e00235-16. doi: 10.1128/mSphere.00235-16.
- Adedeji AO, Marchand B, Te Velthuis AJ, Snijder EJ, Weiss S, Eoff RL, Singh K, Sarafianos SG (2012) Mechanism of nucleic acid unwinding by SARS-CoV helicase. *PLoS One*. 7(5):e36521
- Albert B, Kos-Braun IC, Hemras AK et al. (2019) A ribosome assembly stress response regulates transcription to maintain proteome homeostasis *eLife* 2019;8:e45002. DOI: <https://doi.org/10.7554/eLife.45002>
- Bechhofer DH, Deutscher MP.(2019) Bacterial ribonucleases and their roles in RNA metabolism. *Crit Rev Biochem Mol Biol*. 54(3):242-300. doi: 10.1080/10409238.2019.1651816
- Beintema JJ, Schuller C, Irie M, Carsana A. (1988) Molecular evolution of the ribonuclease superfamily. *Prog Biophys Mol Biol*. (1988) 51:165–92. doi: 10.1016/0079-6107(88)90001-6.

Belanger F, Stepinski J, Darzynkiewicz E and Pelletier J. (2010) Characterization of hMTr1, a human Cap1 2'-O-ribose methyltransferase. *J Biol Chem.* 285 (43): 33037-33044.

Blakely G, May G, McCulloch R., et al. (1993). Two related recombinases are required for site-specific recombination at dif and cer in *E. coli* K12. *Cell* 75, 351–361. doi: 10.1016/0092-8674(93)80076-q.

Bollati M. et al. (2009) Recognition of RNA cap in the Wesselsbron virus NS5 methyltransferase domain: implications for RNA-capping mechanisms in Flavivirus. *J. Mol. Biol.* 385: 140–152.

Cao J, Forrest JC, Zhang X. (2015) A screen of the NIH Clinical Collection small molecule library identifies potential anti-coronavirus drugs. *Antiviral Res.* 114:1-10. doi: 10.1016/j.antiviral.2014.11.010.

Calos MP, Miller JH (1980) Transposable elements. *Cell* 20:579–59..

Chakraborty AK. (2020a) Coronavirus ORF1ab Polyprotein Associated Nsp16 Protein is a RlmE Methyltransferase and May Methylate 21S Mitochondrial rRNA of Host Cells Inhibiting Protein Synthesis. Preprints 2020, 2020040213. doi:10.20944/preprints202004.0213.v1.

Chakraborty AK (2020b) Coronavirus Nsp2 Protein Homologies to the Bacterial DNA Topoisomerase I and IV Suggest Nsp2 Protein Is a Unique RNA Topoisomerase with Novel Target for Drug and Vaccine Development. OSF Preprints. April 17, 2020. doi:10.31219/osf.io/tc9us.

Chakraborty AK. (2020c) Multi-Alignment Comparison of Coronavirus Non-Structural Proteins Nsp13-16 with Ribosomal proteins and other DNA/RNA modifying Enzymes Suggested Their Roles in the Regulation of Host Protein Synthesis. IndiaRxiv May 1. doi:10.35543/osf.io/qrx5.

Chakraborty AK, Poria K, Nandi SK. (2020) Universal Primer Design for the Detection of Diverged CTX-M Extended Spectrum β -Lactamases (ESBL) That Give Penicillin and Cephalosporin Resistance During Superbug Infections. In book “ Biotechnological Applications in Human Health” Editors: Sadhukhan & Premi, Springer-Nature Singapore Pte Ltd, Chapter 6, https://doi.org/10.1007/978-981-15-3453-9_6.

Chakraborty AK. (2019) Heterogeneous phyto-antibiotics and other future therapeutics against multi-drug resistant bacteria. *Adv Biochem.* 7(2):34-50.

Chakraborty AK. (2018) Nucleic-Acids Based Nanocarriers, in “Nanocarriers for Drug Delivery”. eds. Mahapatra et al. chapter-5; pp.155-172, Elsevier Press, Amsterdam. ISBN:978012814. Pp.640.

Chakraborty AK, Poiria K, Saha D, Halder C, Das S. (2018) Multidrug- Resistant Bacteria with activated and diversified MDR Genes in Kolkata Water: Ganga Action Plan and Heterogeneous Phyto-Antibiotics tackling superbug spread in India. *Ame J Drug Deli Ther.* 5 (1): 1-9.

Cheng Z, Muhlrads D, Lim MK, Parker R, Song H. (2007) Structural and functional insights into the human Upf1 helicase core. *EMBO J.* 2007 Jan 10; 26(1):253-264.

Ching YP, Zhou HJ, Yuan JG, Qiang BQ, Kung Hf HF, Jin DY. (2002) Identification and characterization of FTSJ2, a novel human nucleolar protein homologous to bacterial ribosomal RNA methyltransferase. *Genomics* 79 (1): 2-6.

d'Aquino AE, Azim T, Aleksashin NA, Hockenberry AJ, Krüger A, Jewett MC. (2020) Mutational characterization and mapping of the 70S ribosome active site. *Nucleic Acids Res.*, gkaa001.

de Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ. (2018) Host Factors in Coronavirus Replication. *Curr Top Microbiol Immunol.* 419:1-42. doi: 10.1007/82_2017_25.

Deutscher MP, Li Z. (2001) Exoribonucleases and their multiple roles in RNA metabolism. *Prog Nucleic Acid Res Mol Biol.* 66:67–105. doi: 10.1016/S0079-6603(00)66027-0.

Decroly E, Imbert I, Coutard B et al. (2008) Coronavirus non-structural protein 16 is a cap-O-binding enzyme possessing Nucleoside-2'-O-methyltransferase activity. *J Virol.* 82(16):8071-8084.

de Wilde AH, Falzarano D, Zevenhoven-Dobbe JC, Beugeling C, Fett C et al. (2017) Alisporivir inhibits MERS- and SARS-coronavirus replication in cell culture, but not SARS coronavirus infection in a mouse model. *Virus Res.* 228:7-13. doi: 10.1016/j.virusres.2016.11.011.

Dominguez SR, Shrivastava S, Berglund A, Qian Z, Goes LG et al. (2014) Isolation, propagation, genome analysis and epidemiology of HKU1 betacoronaviruses. *Gen Virol.* 95 (PT 4):836-848.

Falzarano D, de Wit E, Rasmussen AL, Feldmann F, Okumura A et al. (2013) Treatment with interferon-alpha2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. *Nature Med.* 19:1313–1317.

Ferron F, Decroly E, Selisko B, Canard B. (2012) The viral RNA capping machinery as a target for antiviral drugs. *Antiviral Res.* 96:21–31.

Gorbalenya AE, Koonin EV, Donchenko AP, Blinov VM. (1988) A novel superfamily of nucleoside triphosphate-binding motif containing proteins which are probably involved in duplex unwinding in DNA and RNA replication and recombination. *FEBS Lett.* 235(1-2):16-24.

Gotte G and Menegazzi M (2019) Biological Activities of Secretory RNases: Focus on Their Oligomerization to Design Antitumor Drugs. *Front. Immunol.* 10:2626. doi: 10.3389/fimmu.2019.02626.

Hammerling MJ, Fritz BR, Yoesep DJ, Kim DS, Carlson ED, Jewett MC. (2020) In vitro ribosome synthesis and evolution through ribosome display. *Nat Commun.* 11(1):1108. doi: 10.1038/s41467-020-14705-2.

Hao W, Wojdyla JA, Zhao R, Han R, Das R, Zlatev I. et al. (2017) Crystal structure of Middle East respiratory syndrome coronavirus helicase. *PLoS Pathog* 13(6): e1006474. <https://doi.org/10.1371/journal.ppat.1006474>.

Ivanov KA, Thiel V, Dobbe JC, van der Meer Y, Snijder EJ, Ziebuhr J. (2004) Multiple enzymatic activities associated with Severe acute respiratory syndrome coronavirus helicase. *J. Virol.*, 78, 5619–5632.

Jacques M and Michael C. (1998). *Insertion sequences*, Microbiology and Molecular Biology Reviews. 62 (3): 725–774.

Jang KJ, Lee NR, Yeo WS, Jeong YJ, Kim DE. (2008) Isolation of inhibitory RNA aptamers against severe acute respiratory syndrome (SARS) coronavirus NTPase/Helicase. *Biochem. Biophys. Res. Commun.*, 366, 738–744.

Jia Z, Yan L, Ren Z, Wu L, Wang J, Guo J, Zheng L, Ming Z, Zhang L, Lou Z, Rao Z (2019) Delicate structural coordination of the Severe Acute Respiratory Syndrome coronavirus Nsp13 upon ATP hydrolysis. *Nucleic Acids Res.* 47(12):6538-6550. doi: 10.1093/nar/gkz409.

Jiang Y, Yu H, Li F et al. (2018) Unveiling the structural features that determine the dual methyltransferase activities of *Streptococcus pneumoniae* RlmCD. *PLoS Pathog.* 14(11): e1007379.

Kilianski A, Baker SC. (2014) Cell-based antiviral screening against coronaviruses: Developing virus-specific and broad-spectrum inhibitors. *Antivir Res.* 101:105–112.

Kowalczykowski, S.C. and A.K. Eggleston. (1994) Homologous pairing and DNA strand-exchange proteins. *Annu Rev Biochem.* 63: 991-1043.

Lau SK, Woo PC, Li KS, Tsang AK, Fan RY, Luk HK et al. (2015) Discovery of a novel coronavirus, China Rattus coronavirus HKU24, from Norway rats supports the murine origin of Betacoronavirus 1 and has implications for the ancestor of Betacoronavirus lineage A. *J Virol.* 89 (6):3076-3092.

Liu C, Zhou Q, Li Y et al (2020) Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases. *ACS Cent Sci.* 6: 315–331.

Liu DX, Fung TS, Chong KK-L, Shukla A, Hilgenfeld R. (2014) Accessory proteins of SARS-CoV and other coronaviruses. *Antivir Res.* 109:97–109.

Lu G, Wang Q, Gao GF. (2015) Bat-to-human: Spike features determining ‘host jump’ of coronaviruses SARS-CoV, MERS-CoV, and beyond. *Trends Microbiol.* 23(8):468–478.

Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A et al (2003) The Genome sequence of the SARS-associated coronavirus. *Science*, 300, 1399–1404

Mao X, Schwer B, Shuman, S. (1995) Yeast mRNA cap methyltransferase is a 50-kilodalton protein encoded by an essential gene. *Mol Cell Biol.* 15:4167–4174.

Michel G, Sauve V, Larocque R, Li Y, Matte A, Cygler M. (2002) The structure of the RlmB 23S rRNA methyltransferase reveals a new methyltransferase fold with a unique knot. *Structure (Camb)* 10:1303–1315. doi: 10.1016/S0969-2126(02)00852-3.

Peterson SN, Reich NO. (2006) GATC flanking sequences regulate Dam activity: evidence for how Dam specificity may influence *pap* expression. *J Mol Biol.* 355: 459–472.

Pletnev P, Guseva E, Zanina A, Evfratov S, Dzama M et al. (2020) Comprehensive Functional Analysis of *Escherichia coli* Ribosomal RNA Methyltransferases. *Front. Genet.* 11:97. doi: 10.3389/fgene.2020.00097.

Polard P, Chandler M. (1995). Bacterial transposases and retroviral integrases. *Mol Microbiol.* 15:13–23.

Punekar AS, Liljeruhm J, Shepherd TR, Forster AC, Selmer M. (2013) Structural and functional insights into the molecular mechanism of rRNA m6A methyltransferase RlmJ. *Nucleic Acids Res.* 41(20):9537-48. doi: 10.1093/nar/gkt719.

Smietanski M, Werner M, Purta E et al. (2014) Structural analysis of human 2'-O ribose methyltransferases involved in mRNA cap structure formation. *Nat Commun.* 5:3004. doi: 10.1038/ncomms4004.

Subissi L, Imbert I, Ferron F, Collet A, Coutard B, Decroly E, Canard B. (2014) SARS-CoV ORF1b-encoded nonstructural proteins 12–16: replicative enzymes as antiviral targets. *Antiviral Res.*, 101, 122–130..

Taylor AF and Smith GR (1985). Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli*. *J Mol Biol.* 185: 431-443.

Ulasli M, Gurses SA, Bayraktar R, Yumrutas O, Oztuzcu S, Igci M, Igci YZ, Cakmak EA, Arslan A. (2014) The effects of *Nigella sativa* (Ns), *Anthemis hyalina* (Ah) and *Citrus sinensis* (Cs) extracts on the replication of coronavirus and the expression of TRP genes family. *Mol Biol Rep.* 41(3):1703-1711. doi: 10.1007/s11033-014-3019-7.

Woo PC, Lau SK, Huang Y, Yuen K-Y. (2009) Coronavirus diversity, phylogeny and interspecies jumping. *Exp Biol Med.* 234(10):1117–1127.

Wu C, Liu Y, Yan Y et al. (2020) Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods, *Acta Pharmaceutica Sinica B*, pp1-23. <https://doi.org/10.1016/j.apsb.2020.02.008>

von Grotthuss M, Wyrwicz LS, Rychlewski L (2003) mRNA cap-1 methyltransferase in the SARS genome. *Cell* 113:701-702

Zhao Y, Zhao Z, Wang Y, et al. (2020) Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. *BioRxiv* 2020; published online Jan 26. DOI:[https:// doi.19985](https://doi.org/10.1101/19985).

Zust R. et al. (2011) Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. *Nat. Immunol.* 12:137–143.