International Journal of
Virology & Infectious Diseases

Review Article

Structures and Functions of Coronavirus Proteins: Molecular Modeling of Viral Nucleoprotein -

Tugba Taskin Tok[1]*, Gizem Tatar[2]

[1]*Department of Chemistry, Gaziantep University, Gaziantep, Turkey*

[2]*Department of Bioinformatics and Computational Biology, Institute of Health Sciences, Gaziantep University, Gaziantep, Turkey*

***Address for Correspondence:** Taskin Tok Tugba, Department of Chemistry, Gaziantep University, Gaziantep, Turkey, 9027310, Tel: +90 342-317-1810; Fax: +90 342-360-1032; E-mail: ttaskin@gantep.edu.tr

Submitted: 01 June 2017; **Approved:** 23 June 2017; **Published:** 26 June 2017

Citation this article: Tok TT, Tatar G. Structures and Functions of Coronavirus Proteins: Molecular Modeling of Viral Nucleoprotein. Int J Virol Infect Dis. 2017;2(1): 001-001.

ABSTRACT

Background: In recent times, several life-threatening viruses have emerged and coronaviruses (CoVs) are one of these. They have infected a variety of human and animal hosts, causing illnesses that range from mostly upper respiratory tract infections in human, encephalitis and demyelination in animals, which can be fatal. They have been responsible for causing significant human and animal mortality, in addition to raising serious public health concerns worldwide. Two novel viruses Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS- CoV) were implicated to be responsible for severe acute illness in recent times. Unfortunately, no effective treatments against these viruses are available.

Results: There is an urgent need to develop new strategies to prevent or control coronavirus infections, and understanding the biology, replication, and pathogenesis of these viruses. Therefore, in this review we have described CoVs proteins structure and functions with experimental and computational studies.

Conclusion: This information may lead to a better understanding of the function of CoV proteins in the virus replication and transcription mechanism and to develop pioneering antivirals.

Keywords: Coronaviruses; SARS-CoV; MERS-CoV; CoVs protein; Molecular Modeling Methods.

INTRODUCTION

Coronaviruses (CoVs) are a diverse family of viruses. They cause a variety of diseases in mammals and birds ranging from enteritis in cows and pigs and upper respiratory disease in chickens to potentially lethal human respiratory infections. Severe acute respiratory syndrome (SARS), the first identified in 2002 and diagnosed in Southern China, occurred from a human CoV [1]. Then, exactly 10 years after the SARS-CoV emergence, a new emerging Coronavirus named Middle East Respiratory Syndrome (MERS-CoV) has infected people with a high mortality rate of nearly 50% in the Middle East. 10 March 2016 the World Health Organization (WHO) global case count for MERS was 1,651 laboratory-confirmed cases, including at least 590 deaths (case fatality rate 36%) since the first cases were reported in September 2012 [2]. The murine coronavirus, Mouse Hepatitis Virus (MHV), which is closely related to SARS and MERS-CoV, has long served as a model for study of both the molecular biology and pathogenesis of members of this viral family.

These viruses infect a variety of human and animal host cells, and also carry out their infection and replication. Also, many proteins have most important role in the replication mechanism, although that role is, as yet, poorly defined. In this case, it is necessary to know the definition of these proteins in terms of this mechanism. Therefore; this review explains the classification and genome organization of coronaviruses and then it summarizes the structure and function of these proteins in the replication and transcription mechanism from a more biophysical point of view by using molecular modeling methods. This information is expected to assist elucidation of the replication and transcription mechanism of the CoV.

Classification of coronaviruses

The classification of Coronaviruses has been based on genomic organization, similarities in genomic sequence, antigenic properties of viral proteins, replication strategies, and structural characteristics of virions, pathogenic, cytopathogenic and physicochemical properties [3]. The Coronaviruses (CoVs) are species of virus belonging to the Nidovirales order, which includes *Coronaviridae, Arteriviridae, Roniviridae* and *Mesoniviridae* families [4].The *Coronaviridae* family is the largest one of the four families, by its *genomic* sizes of *coronaviridae* range from 26 to 32 kb [5]. *Coronaviridae* virus family subdivided into two subfamilies, *coronavirinae* and *torovirinae* [6]. It is now divided into four genera, Alpha coronavirus, Beta coronavirus, Gamma coronavirus and Delta coronavirus (Figure 1).

Alpha coronaviruses type 1 species are classified feline FCoV, FECV (Feline Enteric Coronavirus) and FIPV (Feline Infectious Peritonitis Virus), the porcine TGEV (Transmissible Gastro- Enteritis Virus), Porcine PEDV (Epidemic Diarrhea Virus), PRCoV (Porcine Respiratory Coronavirus) and the canine CCoV. Alpha coronaviruses also compromise human CoVs such as HCoV-229E and HCoV-NL63, but various bat Coronaviruses. Beta coronaviruses also infect a wide range of mammalians, with species such as mice, human with SARS-CoV, HCoV-OC43, HCoV-HKU1, and MERS-CoV, Murine coronavirus (MHV) and Bovine Coronavirus (BCoV). Gamma coronaviruses are specific of birds, with one exception of a beluga whale Coronavirus. The delta coronavirus genus was created in 2012 and regroups various (HKU11, HKU12, HKU13) Coronavirus from mammals to birds [7].

Genome structure of coronaviruses

Coronaviruses encode five structural proteins in their genomes. These are the Spike (S), Membrane (M), Envelope (E) glycoproteins,

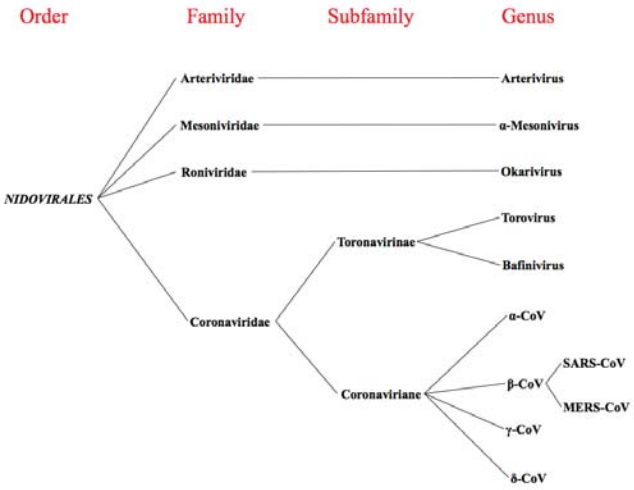

Figure 1: Classification of coronaviruses.
The first OC43 strain of the human beta coronavirus family was characterized in 1967 as agents of human respiratory diseases [8]. Until 2002, the new disease identified, Severe Acute Respiratory Syndrome (SARS), is caused by a human CoV that emerged in Southern China and Hong Kong. Following the SARS epidemic, two new human coronaviruses named HCov-HKU1 and HCov-NL63 have been identified [9-10].

Hemagglutinin Esterase (HE) and Nucleocapsid (N) protein, (Figure 2). All envelope proteins and N protein is present in all virions but HE is only present in some beta coronaviruses [11]. In addition to that, it is thought the virus particles are huddled together owing to interaction between these proteins [12,13].

S Glycoproteins: S Glycoproteins are located outside the virion and give the virion the typical shape. The S proteins form homotrimers, which allow the formation of sun-like morphologies that give the name of Coronaviruses [15-17]. S proteins bind to the virion membrane via the C-terminal transmembrane regions and they also interact with M proteins [18]. Virions can be bound to specific surface receptors in the plasma membrane of the host cell via the N-terminus of the S proteins [19].

M Glycoproteins: M Glycoproteins have three transmembrane regions. M proteins are glycosylated in the Golgi apparatus [20-22]. This modification of the M protein is crucial for the virion to fuse into the cell and to make protein antigenic [23-25]. The M protein plays a key role in regenerating virions in the cell. N protein forms a complex by binding to genomic RNA and M protein triggers the formation of interacting virions in this endoplasmic reticulum-Golgi apparatus intermediate compartment (ERGIC) with this complex [20,26,27].

E Glycoproteins: E Glycoproteins are small proteins that are composed of approximately 76 to 109 amino acids. About 30 amino acids in the N-terminus of the E proteins allow attachment to the membrane of viruses [28]. In addition, coronavirus E proteins play a critical role in the assembly and morphogenesis of virions within the cell. In one study coronavirus E and M proteins were expressed together with mammalian expression vectors to form virus-like structures within the cell [29-31]. In another study, there was a significant decrease in the ability of the recombinant mouse hepatitis virus (MHV) and SARS viruses to elicit E protein expression in the genome to support this status [32,33].

N Proteins: N proteins are phosphoproteins that are capable of binding to helix and have flexible structure of viral genomic RNA. It plays an important role in virion structure, replication and transcription of coronaviruses, because the N protein localizes in both the replication/ transcriptional region of the coronaviruses and the ERGIC region where the virus is collected [26-34].

Introduction of Virus into the Cell, Replication and Transcription

The replication of coronaviruses occurs in host cell cytoplasm. The viruses primarily bind to the receptor on the cell surface via the spike (S) protein. When S protein is bound to the receptor, a conformational structure occurs in the structure and the process of entry into the virus cell begins [35,36]. This process with endocytosis is dependant of pH through the receptor [37-40]. After entering the cytoplasm, the virus particle releases the RNA genome. This genome is a single-stranded, non-segmented RNA virus with the largest known RNA genome (gRNA), which is approximately 26-32 kb (Figure 3) [41-44]. The genome consists of seven genes. It is organized into 5' non-structural protein coding regions comprising the replicase genes (gene 1), which are two-thirds of the genome, and 3' structural and nonessential accessory protein coding regions comprising the gene 2-7[13,45].

The replicase gene 1 products are encoded two very large open reading frames ORF1a and 1b, which are translated into two large polypeptides pp1a and pp1b, which are synthesized directly from the 5' two-thirds of the genomic RNA of CoV. After synthesis of these proteins, consisting of 16 units, non-structural protein (nsp1 to nsp16) is converted with the contribution of viral proteases pp1a and pp1b [46-48]. These 16 proteins form Double-Membrane Vesicles (DMV). At the same time, this DMV is virus Replication and Transcription Complex (RTC) [49,50]. These nsp proteins, especially non-structural protein3 (nsp3), have an important role in the virion structure, the replication and transcription of CoV [51-53].

Genes 2 to 7 are translated from sub genomic mRNA. Sub genomics RNAs encode the major viral Structural proteins (S), Envelope protein (E), Membrane protein (M), Nucleocapsid protein (N), and the accessory proteins, which are essential for virus-cell-receptor binding. The newly structural synthesized proteins are released into the endoplasmic reticulum. All of these proteins, along with the N protein, are linked to the viral genomic RNA and localized in the ERGIC region [34,49,54,55] (Figure 4). Although, N protein is known to be necessary for coronavirus replication, the specific role that this protein plays in this process remains unknown. But, many studies suggest that N protein interaction with nsp3 plays a critical role in the virus replication early in infection. Therefore, the next section gives a detail information about determination of structure N and interaction mechanism of N protein and nsp3 protein.

Clarification of Structure MHV N protein and Determinants of Interaction with nsp3 Protein via the Molecular Modeling Methods

The nucleocapsid (N) protein plays an essential role in the virus structure, the replication and transcription of CoV via interactions

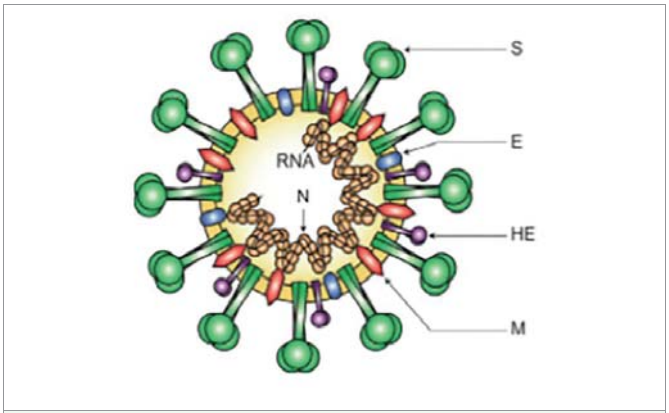

Figure 2: Coronavirus virion structure shown with structural proteins. N: Nucleocapsid protein; S: Spike protein, M: Membrane protein, HE: Hemagglutinin-Esterase and E: Envelope protein [14].

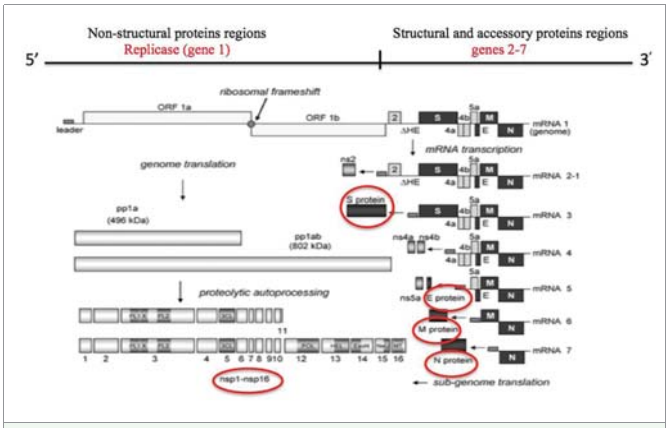

Figure 3: Mouse Hepatitis Virus (MHV) genome organization [56].

with the large positive-strand RNA viral genome. It is also shown that it plays a significant role in the pathogenesis of mouse hepatitis virus (MHV) and human CoV-induced diseases (SARS-CoV, MERS-CoV) which is a major determinant of virulence [34,57-59]. Therefore, it is necessary to determinate structure of N to understand the replication and transcription mechanism. Thus, this section gives information about the structure and function of MHV N protein.

The MHV N protein contains two structurally independent RNA binding domains which are called as N-terminal domain (NTD) and C-terminal (CTD) dimerization domain linked by a charged linker region which is rich in Ser/Arg (SR-rich linker) (Figure 5). The NTD domain (amino acids range 60-194) and SR-rich region (amino acids range 195-230) are known as they play an important role in CoV, both in viral replication and transcription [60,61]. For example; NMR chemical shift perturbation studies [62] reveal that the regions of the SR-rich and NTD in MHV N protein interact with MHV nsp3. They, which are N and nsp3 proteins, also mapped basic determinants of this high affinity binding to the SR region of N and acidic α2 helix of nsp3. Moreover, Masters PS, et al. [58] found that there was a correlation between the N-nsp3 interaction and the ability of N protein to stimulate the infectivity of MHV gRNA. These results confirm the critical nature of N and nsp3 interaction and provide further support for its proposed role in the initiation of coronavirus infection. Nicolas E. Grossoehme, et al. [62] created 3D structure of NTD MHV N protein by using X-ray method (PDB code: 3HD4). This structure includes only NTD domain (53-194) but unfortunately, N-nsp3 interaction mechanisms in the virus replication plays an important role in three-dimensional structure of the SR-rich region (amino acids range 195-230), which cannot be illuminated by the experimental methods [62]. In this case, Tok research group [63] generated a 3D structural model of SR-rich region MHV N protein with homology modeling. This method is based on matching the amino acid sequence of the protein to be modeled with other amino acid sequences in the data banks. Amino acid sequence analysis of the N proteins from a variety of coronavirus (SARS-CoV and Human-CoV OC43) strains suggest that a highly conserved structure within NTD domain and SR-rich region (Figure 6) and the 3D structure of the protein of SARS-coronaviruses (pdb code: 1SSK) and Human-CoV OC43 (pdb code: 4J3K) are available (Figure 7). They selected these proteins as template proteins for homology model of the SR-rich region MHV N protein. After all, this model protein has 53-230 amino acids site, which included NTD and SR-rich region (Figure 8). The MHV nsp3 protein 3D structure is available (pdb code: 2M0A) (Figure 8) [62], therefore there was no need to create a homology model, thus this crystal structure is used for docking. Then, this research group has studied protein-protein docking with N and nsp3 complex in order to illuminate amino acids, which play an important role in virus replication, and reveal replication mechanism of those functions in the epitope portions. As a result, binding site of N proteins Arg 125, Tyr 127 and Tyr 190 residues overlap with the experimental study [62,64,65]. At the same time, different residues (Lys 113, Glu 173) were also predicted for this binding site (Figure 9). On the other hand, mutation analyses were done on these residues. The results reveal that these mutations cause destabilization effect of protein structures and the interaction between this protein and the nsp3 protein is disrupted. Consequently, these residues have an important role in virus replication.

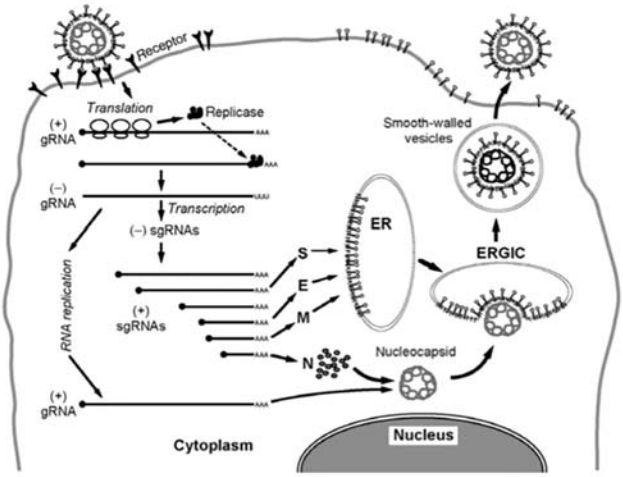

Figure 4: Coronavirus' life cycle [13].

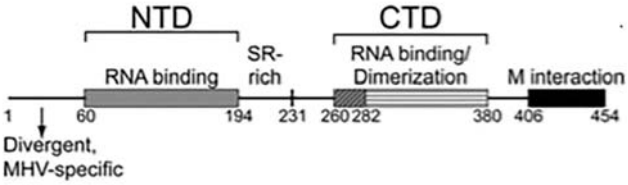

Figure 5: The domain regions of N protein [62].

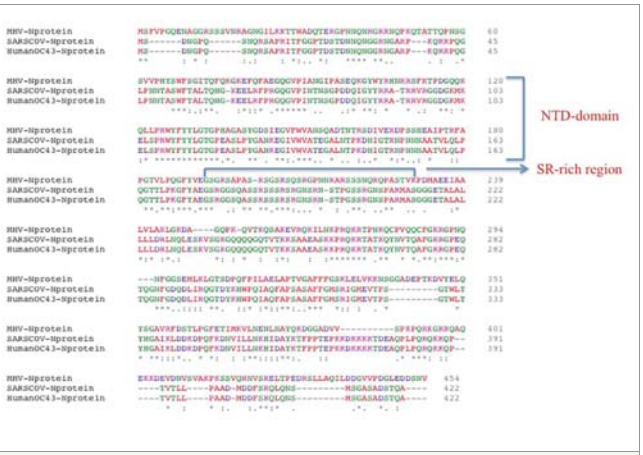

Figure 6: Multiple alignments of N proteins in different coronaviruses. These alignments were calculated on the Clustal Omega server (http://www.ebi.ac.uk/Tools/msa/clustalo/).

CONCLUSIONS AND FUTURE DIRECTIONS

Coronaviruses (CoVs) are a diverse family of viruses that interact at multiple levels with components of host cells taking this advantage of some of the cellular machineries for replication and proliferation. Various are known about the molecular biology of CoVs but more information is needed to learn. For example, many of the non-structural and accessory proteins encoded by these viruses remain uncharacterized with no known function, and it will be important to identify mechanisms of action for these proteins as well as defining their role in viral replication and pathogenesis. The challenge now

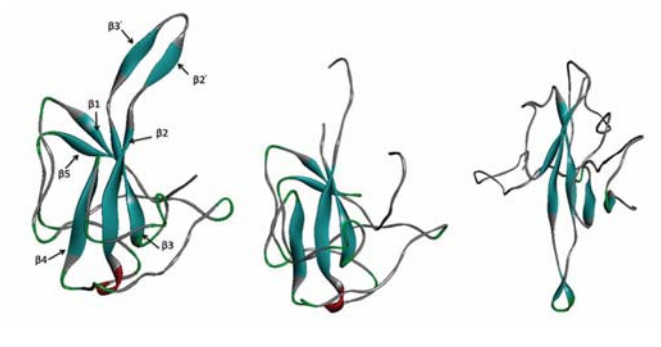

Figure 7: 3D structures of MHV N protein (3HD4) (left side), SARS-CoV (1SSK) (middle side) and Human-CoV OC43 (4J3K) (right side).

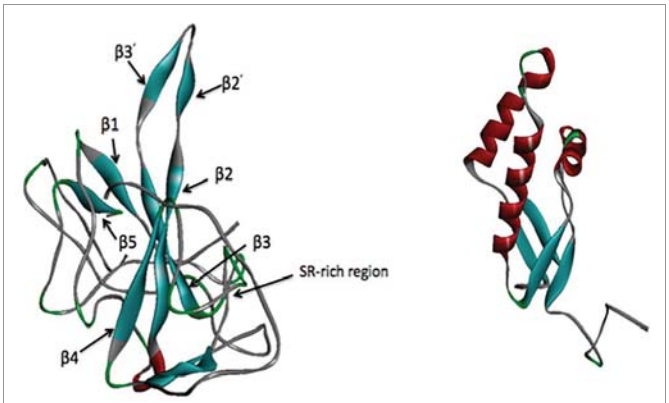

Figure 8: 3D model structure of MHV N-SR rich region (left side) and the crystal structure of nsp3 protein (2M0A) (right side).

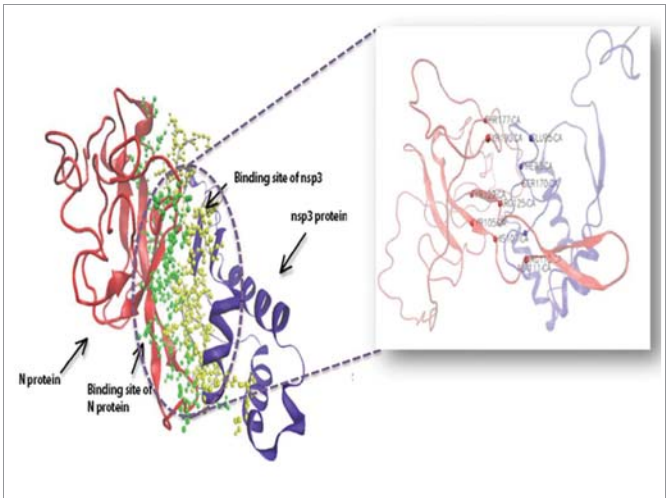

Figure 9: Protein-Protein interactions between N and nsp3 protein.

is to incorporate advance techniques in the investigative efforts done to understand further the biology of CoVs. However, these experimental studies made quite precise, to be dangerous and costly, most importantly 3rd degree with these curity level laboratories, it remains limited around the world. Lately, especially in the United States, Taiwan, Switzerland, European Union member countries, including Computational Molecular Modeling Studies are effective in removing this deficit, and often have an increased use (Bisson, WH, 2012). Due to work and difficulty are developed vaccines that naturally cover yet have no effective medication that has resulted. Developing technology is going to be getting important insight about structure of CoVs protein to define the mechanism of how protein cause disease and understanding the protein-protein and protein-RNA interaction will significantly improve our ability to design vaccines. In the meantime, molecular modeling methods provide important solutions to the struggle.

ACKNOWLEDGMENTS

We acknowledge BAP (*Scientific Research Projects Governing Units)* Project No: FEF.13.20 is funded Gaziantep University.

REFERENCES

1. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV-a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009; 7: 226-236. https://goo.gl/T2kyhu
2. World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) updates. 2012 September 23 to 2016; January 6. 2015: https://goo.gl/NAfKqv
3. Lai MM, Cavanagh D. The molecular biology of coronaviruses. Adv Virus Res. 1997; 48: 1-100. https://goo.gl/AUaxWf
4. Zirkel F, Kurth A, Quan PL, Briese T, Ellerbrok H, Pauli G, et al. An Insect Nidovirus Emerging from a Primary Tropical Rainforest. M Bio. 2011; 2: e00077-11. https://goo.gl/bpBvNn
5. Gorbalenya AE, Enjuanes L, Ziebuhr J, Snijder EJ. Nidovirales: evolving the largest RNA virus genome. Virus Research. 2006; 117: 17-37. https://goo.gl/A2FJCP
6. De Groot RJ, Baker SC, Baric R, Enjuanes L, Gorbalenya AE, Holmes KV, et al. Family *Coronaviridae*. In Virus Taxonomy; Ninth Report of the International Committee on Taxonomy of Viruses. pp. 806–828. Edited by King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Oxford: Elsevier Academic Press.
7. Susanna KPL, Kenneth SML, Alan KLT, Chung Tong S, Ming Wang, Garnet, Choi KV, et al. Recent Transmission of a Novel Alpha coronavirus, Bat Coronavirus HKU10, from Leschenault'sRousettes to Pomona Leaf-Nosed Bats: First Evidence of Interspecies Transmission of Coronavirus between Bats of Different Suborders. J Virol. 2012; 86: 11906-11918. https://goo.gl/Xafpnc
8. Almeida JD, Tyrrell DA. The morphology of three previously uncharacterized human respiratory viruses that grow in organ culture. J. Gen. Virol. 1967; 1: 175-178. https://goo.gl/pPHPE8
9. Van der Hoek, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, et al. Identification of a new human coronavirus. Nat Med. 2004; 10: 368-373. https://goo.gl/X84rqQ
10. Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol. 2005; 79: 884-895. https://goo.gl/Ln6iSB
11. Lissenberg A, Vrolijk MM, van Vliet AL, Langereis MA, de Groot-Mijnes JD, Rottier PJ, et al. Luxury at a cost? Recombinant mouse hepatitis viruses expressing the accessory hem agglutinin esterase protein display reduced fitness in vitro. J Virol. 2005; 79: 15054-63. https://goo.gl/NT5Lp7
12. De Haan CA, Rottier PJ. Molecular interactions in the assembly of coronaviruses. Adv Virus Res. 2005; 64: 165-230. https://goo.gl/CSUCX2
13. Masters PS. The molecular biology of coronaviruses. Adv Virus Res. 2006; 66: 193-292. https://goo.gl/x66XSJ
14. Susan RW, Julian LL. Coronavirus Pathogenesis. Adv Virus Res. 2011; 81: 85-164. https://goo.gl/r0Nydb
15. Graham RL, Baric RS. Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. J Virol. 2010; 84: 3134-3146. https://goo.gl/89BVoZ
16. Bárcena M, Oostergetel GT, Bartelink W, Faas FG, Verkleij A, Rottier PJ, et al. Cryo-electron tomography of mouse hepatitis virus: Insights into the structure of the coronavirion. Proc Natl Acad Sci U S A. 2009; 106: 582-587. https://goo.gl/6eTwmf

17. Tan YJ, Lim SG, Hong W. Characterization of viral proteins encoded by the SARS-coronavirus genome. Antiviral Res. 2005; 65: 69-78. https://goo.gl/J8d3eD

18. Chinese SMEC. Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. Science. 2004; 303: 1666-1669. https://goo.gl/2jO42w

19. Lewicki DN, Gallagher TM. Quaternary structure of coronavirus spikes in complex with carcinoembryonic antigen- related cell adhesion molecule cellular receptors. J Biol Chem. 2002; 277: 19727-19734. https://goo.gl/QTGQoI

20. de Haan CA, Masters PS, Lili Kuo, Harry Vennema, Peter JM, Rottier. Coronavirus particle assembly: primary structure requirements of the membrane protein. J Virol. 1998; 72: 6838-6850. https://goo.gl/czPxHp

21. Holmes KV, Doller EW, Sturman LS. Tunicamycin resistant glycosylation of coronavirus glycoprotein: demonstration of a novel type of viral glycoprotein. Virology. 1981; 115: 334-344. https://goo.gl/bJcBVh

22. Niemann H, Geyer R, Klenk HD, Linder D, Stirm S, Wirth M. The carbohydrates of mouse hepatitis virus (MHV) A59: structures of the O-glycosidically linked oligosaccharides of glycoprotein E1. EMBO J. 1984; 3: 665-670. https://goo.gl/aXoN3d

23. de Haan CA, de Wit M, Kuo L, Montalto-Morrison C, Haagmans BL, Weiss SR, et al. The glycosylation status of the murine hepatitis coronavirus M protein affects the interferogenic capacity of the virus in vitro and its ability to replicate in the liver but not the brain. Virology. 2003; 312: 395-406. https://goo.gl/AYsmyB

24. Alexander S, Elder JH. Carbohydrate dramatically influences immune reactivity of antisera to viral glycoprotein antigens. Science. 1984; 226: 1328-1330. https://goo.gl/lg8Ky5

25. Wissink EH, Kroese MV, Maneschijn Bonsing JG, Meulenberg JJ, van Rijn PA, Rijsewijk FA, et al. Significance of the oligosaccharides of the porcine reproductive and respiratory syndrome virus glycoproteins GP2a and GP5 for infectious virus production. J Gen Virol. 2004; 85: 3715-3723. https://goo.gl/LmtXNO

26. Escors D, Ortego J, Enjuanes L. The membrane M protein of the transmissible gastroenteritis coronavirus binds to the internal core through the carboxy-terminus. Adv Exp Med Biol. 2001; 494: 589-593. https://goo.gl/Erg1Yi

27. Narayanan K, Makino S. Characterization of nucleocapsid-M protein interaction in murine coronavirus. Adv Exp Med Biol. 2001; 494:577-582. https://goo.gl/MkAA2B

28. Raamsman MJB, Locker JK, de Hooge A, de Vries AA, Griffiths G, Vennema H, et al. Characterization of the coronavirus mouse hepatitis virus strain A59 small membrane protein E. J Virol. 2000; 74: 2333-2342. https://goo.gl/GwygyF

29. Baudoux P, Carrat C, Besnardeau L, Charley B, Laude H. Coronavirus pseudo particles formed with recombinant M and E proteins induce alpha interferon synthesis by leukocytes. J Virol. 1998; 72: 8636-8643. https://goo.gl/Fzrwdb

30. Vennema H, Godeke GJ, Rossen JW, Voorhout WF, Horzinek MC, Opstelten DJ, et al. Nucleo capsid-independent assembly of coronavirus-like particles by co-expression of viral envelope protein genes. EMBO J. 1996; 15: 2020-2028. https://goo.gl/tFRLYY

31. Bos EC, Luytjes W, van der Meulen HV, Koerten HK, Spaan WJ. The production of recombinant infectious DI-particles of a murine coronavirus in the absence of helper virus. Virology. 1996; 218: 52-60. https://goo.gl/4NHDYQ

32. DeDiego ML, Alvarez E, Almazan F, Rejas MT, Lamirande E, Roberts A, et al. A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. J Virol. 2007; 81: 1701-13. https://goo.gl/zv5cyu

33. Kuo L, Masters PS. The small envelope protein E is not essential for murine coronavirus replication. J Virol. 2003; 77: 4597-4608. https://goo.gl/RT1og2

34. Stertz S, Reichelt M, Spiegel M, Kuri T, Martinez Sobrido L, Garcia Sastre A, et al. The intracellular sites of early replication and budding of SARS-coronavirus. Virology 2007; 361: 304-15 https://goo.gl/LfTwph

35. Holmes KV, Tresnan DB, Zelus BD. Virus-receptor interactions in the enteric tract. Virus-receptor interactions. Adv Exp Med Biol. 1997; 412: 125-133. https://goo.gl/QBcYVS

36. Bosch BJ, van der Zee R, de Haan CA, Rottier PJ. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J Virol. 2003; 77: 8801-8811. https://goo.gl/cMi6mZ

37. Blau DM, Holmes KV. Human coronavirus HCoV-229E enters susceptible cells via the endocytic pathway. Adv Exp Med Biol. 2001; 494: 193-8. https://goo.gl/nJGLVx

38. Chu VC, McElroy LJ, Ferguson AD, Bauman BE, Whittaker GR. Avian infectious bronchitis virus enters cells via the endocytic pathway. Adv Exp Med Biol. 2006; 581: 309-312. https://goo.gl/UNbb12

39. Eifart P, Ludwig K, Bottcher C, de Haan CA, Rottier PJ, Korte T, et al. Role of endocytosis and low pH in murine hepatitis virus strain A59 cell entry. J Virol. 2007; 81: 10758-68. https://goo.gl/v2e5eH

40. Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, et al. SARS coronavirus entry into host cells through a novel clathrin - and caveolae-independent endocytic pathway. Cell Res. 2008; 18: 290-301. https://goo.gl/xemCsE

41. Lai MM, Baric RS, Brayton PR, Stohlman SA. Studies on the Mechanism of RNA Synthesis of a Murine Coronavirus. Advances in Medicine and Biology. 1984; 173: 187-200. https://goo.gl/B0JChH

42. Lomniczi B. Biological Properties of Avian Coronavirus RNA. J Gen Virol. 1977; 36: 531-533. https://goo.gl/TrV7qz

43. Schochetman G, Stevens RH, Simpson RW. Presence of infectious polyadenylated RNA in the coronavirus avian bronchitis virus. Virology. 1977; 77: 772-782. https://goo.gl/J12VtS

44. Wege H, Müller A, ter Meulen V. Genomic RNA of the murine Coronavirus JHM. J Gen Virol. 1978; 41: 217-227. https://goo.gl/QPwGoy

45. Susan RW, Sonia N. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev. 2005; 69: 635-664. https://goo.gl/dirC5y

46. Baranov PV, Henderson CM, Anderson CB, Gesteland RF, Atkins JF, Howard MT. Programmed ribosomal frame shifting in decoding the SARS-CoV genome. Virology. 2005; 332: 498-510. https://goo.gl/FjF2Sc

47. Snijder EJ, Bredenbeek PJ, Dobbe JC, Thiel V, Ziebuhr J, Poon LL, et al. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J Mol. Biol 2003; 331: 991-1004. https://goo.gl/euDv9C

48. Ziebuhr J. Molecular biology of severe acute respiratory syndrome coronavirus. Curr Opin Microbiol. 2004; 7: 412-9. https://goo.gl/z5xWXN

49. Cynthia SG, Kathleen MT, Thomas GK, Pierre ER, James AC, et al. Ultra structural characterization of SARS coronavirus. Emerg Infect Dis. 2004; 10: 320-326. https://goo.gl/CHVMg5

50. Gosert R, Kanjanahaluethai A, Egger D, Bienz K, Baker SC. RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J Virol. 2002; 76: 3697-708. https://goo.gl/GhSdww

51. Mark RD. Seeking membranes: positive-strand RNA virus replication complexes. PLoS Biol. 2008; 6: e270. https://goo.gl/sPjbGU

52. Miller S, Krijnse-Locker J. Modification of intracellular membrane structures for virus replication. Nat Rev Microbiol. 2008; 6: 363-74. https://goo.gl/K54piM

53. Ahlquist P. Parallels among positive-strand RNA viruses, reverse-transcribing viruses and double-stranded RNA viruses. Nat Rev Microbiol. 2006; 4: 371-382. https://goo.gl/2Axfas

54. Tooze J, Tooze S, Warren G. Replication of coronavirus MHV- A59 in sac-cells: determination of the first site of budding of progeny virions. Eur J Cell Biol. 1984; 33: 281-293. https://goo.gl/AGTAqo

55. Ng ML, Tan SH, See EE, Ooi EE, Ling AE. Proliferative growth of SARS coronavirus in Vero E6 cells. J Gen Virol. 2003; 84: 3291-3303. https://goo.gl/8h1RZ2

56. Stanley GS, Diane Y, Voler T, Stuart GS, Yvonne Meyer, Volker Thiel et al. Functional and Genetic Analysis of Coronavirus Replicase-Transcriptase Proteins. PLoS Pathog. 2005; 1: e39. https://goo.gl/E4wkq6

57. Compton SR, D B Rogers, K V Holmes, D Fertsch, J Remenick, J J McGowan. In vitro replication of mouse hepatitis virus strain A59. J Virol. 1987; 61: 1814-1820. https://goo.gl/EVbz9v

58. Hurst KR, Ye R, Goebel SJ, Jayaraman P, Masters PS. An interaction between the nucleocapsid protein and a component of the replicase transcriptase complex is crucial for the infectivity of coronavirus genomic RNA. J Virol. 2010; 84: 10276-10288. https://goo.gl/eX8eUT

59. Kelley RH, Cheri AK, Paul SM. Characterization of a critical interaction between the coronavirus nucleocapsid protein and nonstructural protein 3 of the viral replicase- transcriptase complex. J Virol. 2013; 87: 9159- 9172. https://goo.gl/eA3kgE

60. Kuo L, Master PS. Genetic evidence for a structural interaction between the carboxy termini of the membrane and nucleocapsid proteins of mouse hepatitis virus. J Virol. 2002; 76: 4987-4999. https://goo.gl/cTQbZy

61. Kelley RH, Lili K, Cheri AK, Rong Y, Bilan H, Paul SM. A major determinant for membrane protein interaction localizes to the carboxy-terminal domain of the mouse coronavirus nucleocapsid protein. J Virol. 2005; 79: 13285-13297. https://goo.gl/doQByY

62. Keane SC, Giedroc DP. Solution of Mouse Hepatit Virus (MHV) nsp3a and determinants of the interaction with MHV nucleocapsid (N) protein. J Virol. 2013; 87: 3502-3515. https://goo.gl/qFHuqt

63. Tatar G, Tok TT. Clarification of Interaction Mechanism of Mouse Hepatitis Virus (MHV) N and nsp3 Protein with Homology Modeling and Protein-Protein Docking Analysis. Current Computer-Aided Drug Design. Epub ahead of print. 2016; 12: https://goo.gl/21A8sV

64. Grossoehme NE, Li L, Keane SC, Liu P, Dann CE 3rd, Leibowitz JL, Giedroc DP. Coronavirus N protein N-terminal domain (NTD) specifically binds the Transcriptional Regulatory Sequence (TRS) and melts TRS- cTRS RNA duplexes. J Mol Biol. 2009; 394: 544-557. https://goo.gl/LqbqDU

65. Keane SC, Liu P, Leibowitz JL, Giedroc DP. Functional transcriptional regulatory sequence (TRS) RNA binding and helix destabilizing determinants of Murine Hepatitis Virus (MHV) nucleocapsid (N) protein. J Bio Chem. 2012; 287: 7063-7073. https://goo.gl/AQqkG8