Status: Preprint has been published in a journal as an article
DOI of the published article: https://doi.org/10.1590/1678-4685-gmb-2020-0212

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https://doi.org/10.1590/SciELOPreprints.1045

Submitted on (YYYY-MM-DD): 2020-07-31
Posted on (YYYY-MM-DD): 2020-11-24

Molecular mechanisms and pharmacological interventions in the replication cycle of human coronaviruses

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Abstract

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), as well as SARS-CoV from 2003 and MERS-CoV from 2012, is a member of the Betacoronavirus genus of the Nidovirales order and is currently the cause of the pandemic called COVID-19 (or Coronavirus disease 2019). COVID-19 is characterized by cough, fever, fatigue, and severe cases of pneumonia and has affected more than 9.1 million people worldwide until June 22nd, 2020. Here we present a review of the cellular mechanisms associated with human coronaviruses replication, including the unique molecular events related to the replication transcription complex (RTC) of coronaviruses. Information regarding the interactions between each viral protein and cellular proteins is presented, associating to known host-pathogen implications for the coronavirus biology. Finally, a specific topic addresses the current attempts for pharmacological interventions against COVID-19, highlighting the possible effects of each drug on the molecular events of viral replication. This review intends to contribute to future studies for a better understanding of the SARS-CoV-2 replication cycle and the development of pharmacological approaches targeting COVID-19.

Running title: Replication of human coronaviruses

Keywords: coronavirus; SARS-CoV-2; COVID-19; viral replication; RNA virus

Number of words, excluding references: 6949

1. Introduction

In 2002 and 2003, the first coronavirus outbreak started in Guangdong province in China, causing a total of 8,098 cases and 774 deaths worldwide, with a mortality rate of around 9% (Fehr and Perlman 2015). This outbreak was responsible for causing Severe Acute Respiratory Syndrome or SARS. In 2012, another outbreak started in the middle-east, called MERS (Middle East Respiratory Syndrome), with an initial mortality rate of close to 50%, getting controlled in the following years. In 2014, a total of 855 cases and 333 deaths were reported by the MERS-CoV, with a 40% mortality rate, according to the European Center for Disease Prevention and Control (Fehr and Perlman 2015). Before these two outbreaks, coronaviruses were believed to cause only self-limiting mild respiratory tract infections in humans.

In December 2019, a group of patients from the city of Wuhan, Hubei province, China, was initially diagnosed with pneumonia of unknown etiology and epidemiologically linked to a seafood and wildlife market in the city (Rothan and Byrareddy 2020). Afterward, reports predicted the appearance of a potential coronavirus outbreak, given the reproduction number for the new 2019 coronavirus disease (COVID-19, as named by WHO on 11 February 2020) significantly greater than 1, estimated between intervals of 2.24 to 3.58 (Zhao et al. 2020). Since then, until June 22nd, 2020, the number of infected people has reached 9,154,232 cases and 473,849 deaths worldwide (Coronavirus Resource Center, John Hopkins University).

Symptoms of COVID-19 infection appear after an incubation period of approximately 5.2 days. The period between the onset of COVID-19 symptoms and death has a median of 14 days, varying from 6 to 41 days. The most common symptoms at the beginning of COVID-19 disease are cough, fever, and fatigue, in some cases including sputum production, headache, hemoptysis, diarrhea, dyspnoea, and lymphopenia (Rothan and Byrareddy 2020).

2. Taxonomy and genomic organization of coronaviruses

The Coronaviridae family is part of the Nidovirales order and can be divided into 2 subfamilies: Coronavirinae and Torovirinae. The Coronavirinae subfamily has 4 genera: alpha-, beta-, gamma- and delta-coronaviruses. SARS-CoV-2 (also called 2019-nCoV) is the coronavirus that causes COVID-19, and together with SARS-CoV and

MERS-CoV, are part of the genus Betacoronavirus. A study showed that SARS-CoV-2 is more similar (88% identity) to two SARS-like coronaviruses derived from bats (bat-SL-CoVZC45 and bat-SL-CoVZXC21), collected in 2018 in Zhoushan, eastern China, than with SARS-CoV (about 79%) and MERS-CoV (about 50%) (Lu et al. 2020). Another study has shown that SARS-CoV-2 genome is 91.02% identical to Pangolin-CoV, despite a higher identity of 96.2% between SARS-CoV-2 and another bat coronavirus (RaTG13) (Zhang et al. 2020c). The coronaviruses genome is composed of a single-stranded positive RNA (ssRNA+), therefore included in class IV of the Baltimore classification (Baltimore 1971). The genomic organization of coronaviruses can be divided into two main parts, the genes encoding the non-structural poly-proteins pp1a and pp1b and the genes encoding the structural genes, including the S, E, M and N genes, as shown in figure 1.

3. The replication and the non-structural proteins (nsps) of coronaviruses

The replication of SARS-CoV-2 begins with the translation of the pp1a and pp1b polyproteins from the single-stranded positive polarity genomic RNA (ssRNA+), which is 5'-capped and 3'-polyadenylated (figure 1). The pp1b polyprotein is produced in fusion with pp1a, through a -1 frameshift mechanism, generating 2 polyproteins, called pp1a (without frameshift) and pp1ab (with frameshift) (Brierley et al. 1989). The frameshift occurs due to a slippery sequence in the genome (5'-UUUAAAC-3') and a pseudoknot structure in the secondary structure of the RNA before the STOP codon of pp1a ORF, causing a pause in the ribosome reading and the translation of pp1b ORF in fusion with pp1a ORF, thus generating pp1ab (Fehr and Perlman 2015).

Once produced, the pp1a and pp1ab polyproteins undergo proteolytic cleavage, forming a total of 16 proteins, detailed in table 1 and outlined in figure 1. The cleavage is made by 2 proteases, nsp3 which is a Papain-like protease (PL^{pro}) and nsp5 which is a 3C-like protease ($3CL^{pro}$). Nsp3 cleaves the sites between nsp2 to nsp4, generating, therefore, nsp1, nsp2, and nsp3. On the other hand, nsp5 cleaves the other sites generating the other non-structural proteins (Ziebuhr et al. 2000; Báez-Santos et al. 2015).

Table 1. Non-structural proteins of coronaviruses and their functions.

Non-structural proteins (nsps)	**Functions**	**References**
nsp1	Promotes cell mRNA degradation and blockage of host cell translation and innate immune response	(Huang et al. 2011; Tanaka et al. 2012)
nsp2	Unknown function, binds to prohibitins	(Cornillez-Ty et al. 2009)
nsp3	Papain-like protease (PL^{pro}), cleaves the viral polyproteins and blocks the innate immune response, has multiple domains	(Lei et al. 2018)
nsp4	Transmembrane scaffold protein, formation of DMVs (Double Membrane Vesicles)	(Gadlage et al. 2010)
nsp5	3C-like protease ($3CL^{pro}$), cleaves viral polyproteins, inhibits IFN signaling by cleaving STAT2	(Stobart et al. 2013; Zhu et al. 2017)
nsp6	Transmembrane scaffold protein, formation of DMVs (Double Membrane Vesicles), inhibits autophagosome	(Angelini et al. 2013; Cottam et al. 2014)
nsp7	Forms a hexadecameric complex with nsp8	(te Velthuis et al. 2012)
nsp8	Forms a hexadecameric complex with nsp7, can act as primase	(te Velthuis et al. 2012)
nsp9	Dimerization and RNA binding	(Zeng et al. 2018)
nsp10	Cofactor for nsp14 and nsp16	(Decroly et al. 2011)
nsp11	In pp1a, it consists of a small peptide with unknown function. In pp1ab polyprotein, nsp11 is now translated into nsp12 due to the -1 frameshift between pp1a and pp1b	(Fehr and Perlman 2015)
nsp12	RNA-dependent RNA polymerase (RdRp)	(te Velthuis et al. 2010)
nsp13	RNA helicase, 5’ triphosphatase	(Jia et al. 2019)

nsp14	Exo-ribonuclease 3'-5' proofreading, N7-methyltransferase	(Chen et al. 2009b; Bouvet et al. 2012)
nsp15	Endo-ribonuclease, evasion of apoptosis and dsRNA cell sensors	(Bhardwaj et al. 2006; Deng et al. 2017)
nsp16	2'-O-methyltransferase; inhibits RIG-I and MDA5, negatively regulating innate immunity	(Decroly et al. 2011; Shi et al. 2019)

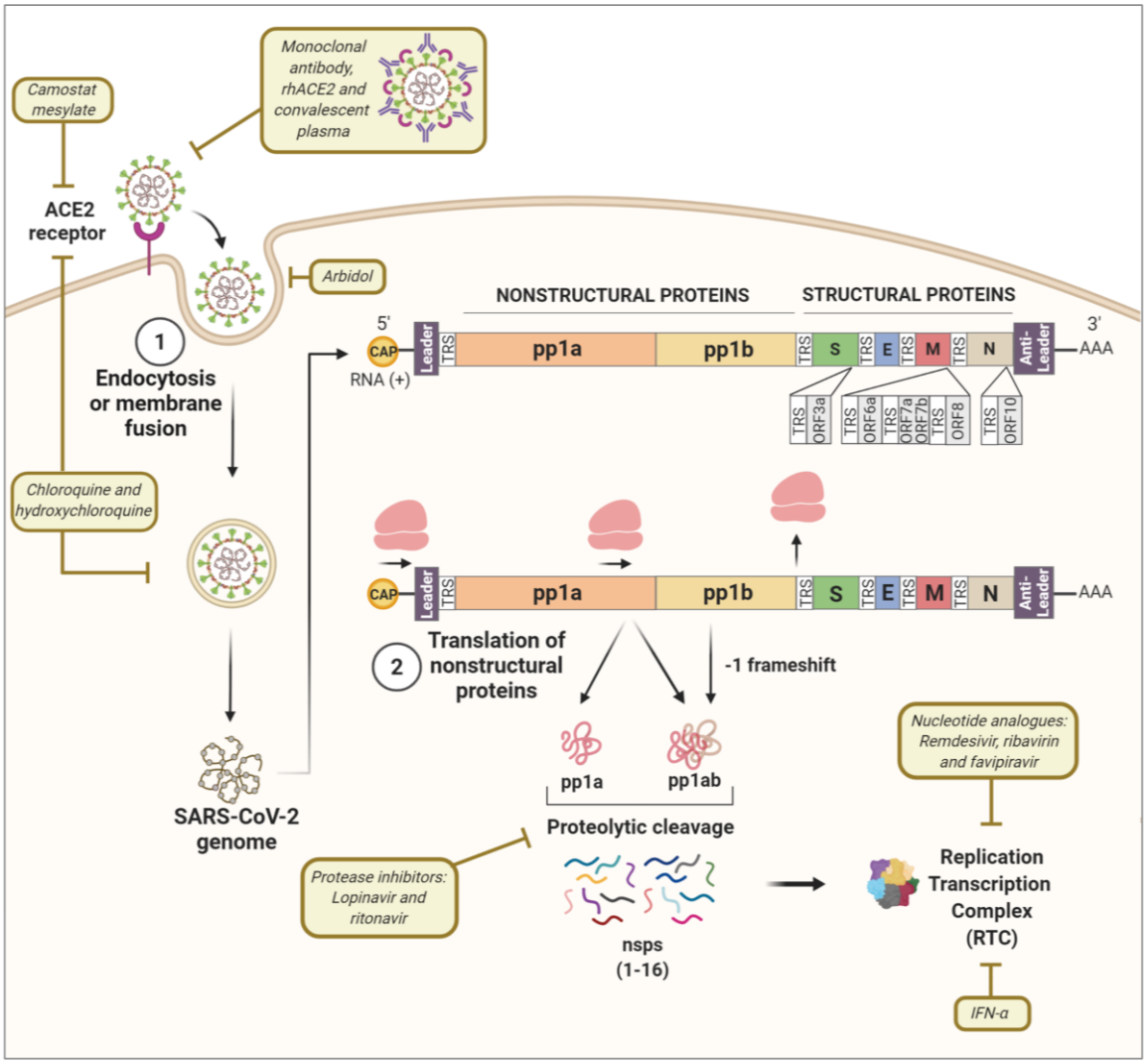

Figure 1. Molecular mechanisms related to the production of non-structural proteins (nsps) and assembly of the SARS-CoV-2 replication and transcription complex (RTC). Process 1: after recognition of the ACE2 (Angiotensin Converting Enzyme 2) cell receptor, the viral nucleocapsid is released into the cytoplasm by endocytosis or fusion of the viral envelope with the cell membrane. Process 2: the

translation of the pp1a and pp1b genes from the 5’-capped and 3’-polyadenylated genomes (+) of the virus produces the pp1a or pp1ab polyproteins, the latter being generated by a -1 frameshift of ribosomes. These polyproteins are then cleaved by viral proteases generating 16 virus nonstructural proteins (nsps), of which some are used to assemble the RTC, including the RNA-dependent RNA polymerase (RdRp or nsp12). Pharmacological interventions targeting specific points of the replication cycle of coronaviruses are highlighted. RTC: Replication and transcription complex; RdRp: RNA-dependent RNA polymerase.

The next processes of coronavirus replication and transcription are outlined in figure 2 below. Once produced and processed, part of the non-structural proteins together with nsp12, the RNA-dependent RNA polymerase (RdRp), assemble the Replication and Transcription Complex (RTC). RTC acts primarily by producing a set of single-stranded negative RNAs (ssRNA-), including copies of the genomic RNA and subgenomic RNAs, which will then serve as templates for the production of the genome and mRNA, respectively. These intermediate negative RNA molecules are about 1% as abundant as their respective positive counterparts and contain anti-leader sequences, present in the 5’ untranslated region (UTR) of the antigenome and in the 3’-UTR of the genome (Sethna et al. 1991). On the other hand, in the 5’-UTR of the viral genome and 3'-UTR of the antigenome there are leader sequences. The leader and anti-leader sequences are used by the RTC to initiate replication and transcription. In the 5’-UTR of the genome and at the beginning of each ORF of the structural genes there are other regulatory regions, called Transcriptional Regulatory Sequences (TRS), as shown in figures 1 and 2. During the transcription of ssRNA molecules, two mechanisms may happen, according to the currently established model of replication of coronaviruses (Pasternak et al. 2006; Sawicki et al. 2007):

1) The RTC, after binding to the 3’ anti-leader sequence of the viral genome, initiates the synthesis of negative RNA throughout the molecule until it finds the leader region in the 5’ end, generating a complete copy of negative polarity of the genome, called antigenome. This antigenome will serve as a template for the synthesis of the genome (+) (figure 2, processes 1a and 1b).

2) The RTC may, however, temporarily pause the transcription in each of the TRS regions of each ORF and continue in the 5’-UTR of the genome, given the complementarity of the TRS regions. Therefore, a leader region is incorporated in each RNA, generating subgenomic RNAs of negative polarity. These subgenomic RNAs will

serve as a template for mRNA (+) synthesis, containing their 3' regions co-terminal to the genomic RNA (figure 2, processes 2a and 2b). This process is often called "copy-choice" mechanism and is presented in other viruses, allowing recombination, as it will be discussed later (Simon-Loriere and Holmes 2011).

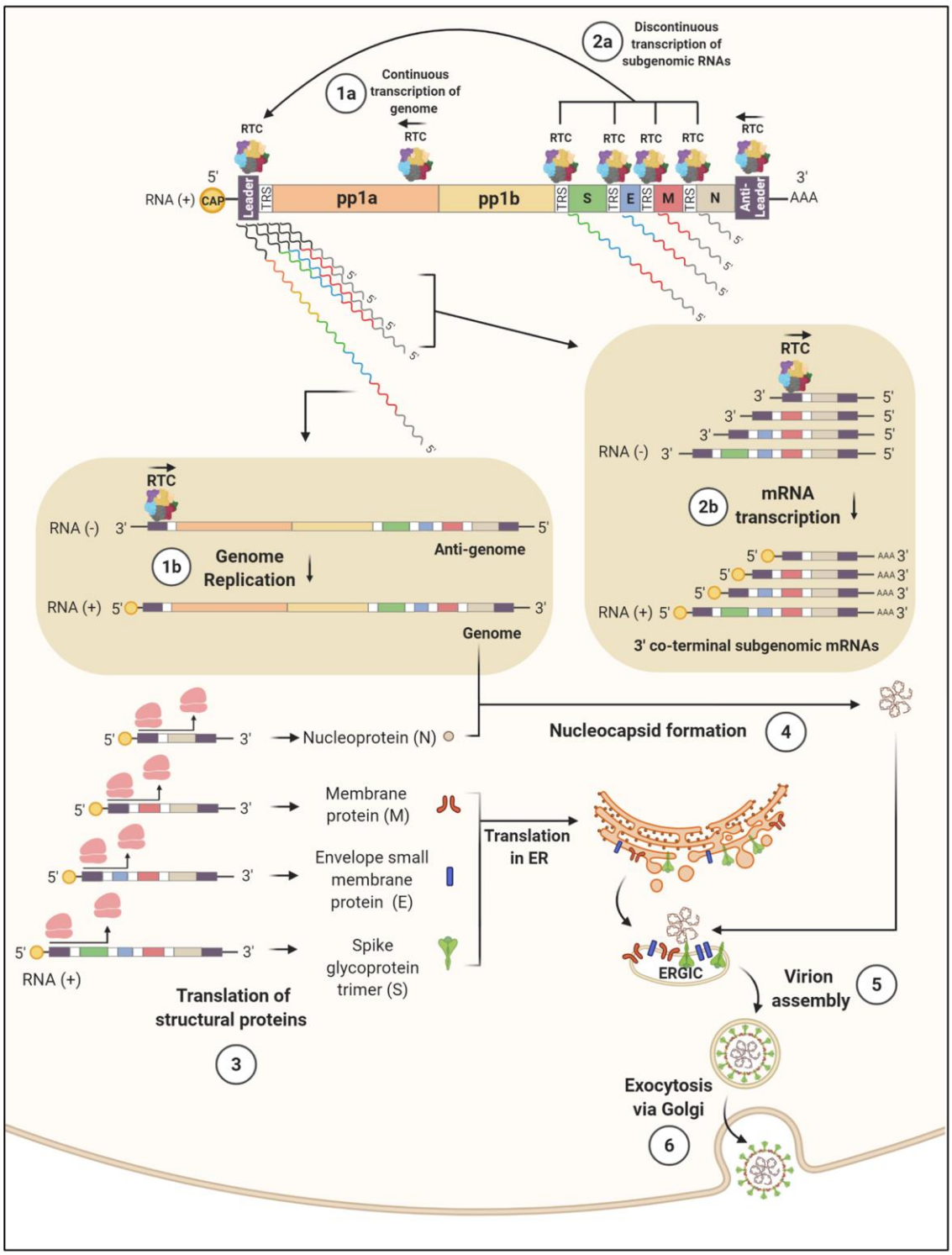

Figure 2. Molecular events related to the expression of structural proteins, replication of the genome, and assembly of the SARS-CoV-2. Processes 1a and 1b: the synthesis of RNA (-) by RTC, initiated in the 3' anti-leader sequence of the genome (+), may occur continuously, generating a complete copy of the genome called antigenome (-). The antigenome is then used by RTC to produce multiple copies of the genome (+). Processes 2a and 2b: RNA synthesis by RTC may, however, be temporarily interrupted when a TRS is copied. The newly synthesized RNA (-) is then transferred to the 5' end of the genome, where the complementarity of sequences allows the RNA (-) synthesis to continue in the leader TRS, merging the sequences between body and leader TRSs. In turn, these subgenomic chimeric RNAs (-) serve as templates for the continuous synthesis of subgenomic mRNAs (+). Process 3: the structural S, E, M, and N proteins are then translated from the 3'-co-lateral subgenomic mRNAs (+), where S, E, and M proteins are produced in the rough endoplasmic reticulum. Process 4: the N protein produced in the cytosol interacts with the viral genome (+), forming the nucleocapsid. Process 5: membrane proteins S, M, and E then interact with viral nucleocapsids to form virions in the ERGIC. Process 6: finally, the virions are externalized from the cell by exocytosis via the Golgi pathway. TRS: Transcriptional Regulatory Sequences; ER: endoplasmic reticulum; RTC: Replication Transcription Complex. ERGIC: Endoplasmic Reticulum - Golgi Intermediate Compartment.

Once the complete genomic RNA and mRNAs of each structural ORFs are produced, the translation of these genes begins to occur (figure 2, process 3), generating the S, E, M and N proteins, which are essential for the assembly of the viral particles, among others (ORFs 3a, 6, 7a / b, 8 and 9). The specific functions of these proteins will be described later. The viral replication cycle then continues through the interaction of the N protein with viral genomic RNA, forming the nucleocapsid in the cytosol (figure 2, process 4). On the other hand, the production of S, E, and M proteins are directed to the rough endoplasmic reticulum (RER) (Fehr and Perlman 2015). Finally, interactions mediated between these structural proteins culminate in the recruitment of nucleocapsids into the compartment between RER and the Golgi apparatus called ERGIC (Endoplasmic Reticulum - Golgi Intermediate Compartment) and finally in the exocytosis of the viral particles (figure 2, processes 5 and 6) (Fehr and Perlman 2015).

An important feature related to the replication of coronaviruses is the high rates of mutation and recombination, altering viral protein properties, host range, and pathogenicity. For example, the heterologous recombination between subgroup A Betacoronavirus and other virus is reported, since some of these coronaviruses have the hemagglutinin esterase gene, derived from influenza C virus (Zeng et al. 2008). The recombination between coronaviruses targeting different species is also largely reported and may explain the similarity between the genome sequence of human SARS-CoV-2

and bat and pangolin coronaviruses (Lu et al. 2020; Lau et al. 2020). Three aspects may explain this increased capacity for recombination/mutation:

1) the RdRp of coronaviruses has low fidelity. Although a 3'-5' exonuclease proofreading activity is reported, the mutation rate of this polymerase is about 2.0×10^{-6} mutations per site, per replication cycle (Eckerle et al. 2010);

2) the unique RNA replication mechanism using the TRS motifs, known as the "copy-choice" mechanism, may induce homologous RNA recombination between genes of different coronaviruses (Simon-Loriere and Holmes 2011);

3) Coronaviruses have the largest genome (26–32 kb) among RNA viruses (Terada et al. 2014).

Several studies have shown the modulation of cellular pathways by coronavirus proteins, favoring the viral cycle or impacting the viral pathogenesis, which is summarized in table 2. Among these studies, interactions were found between the coronavirus nsp1 protein with the cyclophilins PPIA, PPIG, PPIH, and FKBP1A, FKBP1B, which are capable of modulating the Calcineurin/NFAT pathway, which plays an important role in the activation of immune cells (Pfefferle et al. 2011). The same study, which used the yeast two-hybrid system to demonstrate those interactions, showed that the inhibition of cyclophilins by cyclosporine A (CspA) blocked the replication of different CoVs, including the human coronaviruses SARS-CoV, CoV-229E and -NL-63, the feline CoV and the avian infectious bronchitis virus (IBV). Another study has demonstrated the interaction between the SARS-CoV nsp2 and cellular prohibitins, suggesting that this nsp may be involved in the disruption of intracellular host signaling (Cornillez-Ty et al. 2009).

Table 2. Interactions between coronaviruses proteins and cellular proteins and/or pathways.

Viral protein	Interactions		References
	Viral	Cellular	
Nsp1		Cyclophilin (PPIA, PPIB, PPIH, PPIG, FKBP1A, FKBP1B)	(Pfefferle et al. 2011)

Nsp2		Prohibitin	(Cornillez-Ty et al. 2009)
Nsp3	N protein	TGF-β1 (indirect), RCHY1, p53 and IRF3	(Hurst et al. 2013; Chen et al. 2014; Ma-Lauer et al. 2016; Li et al. 2016)
Nsp5		STAT2	(Zhu et al. 2017)
Nsp6		Autophagosome	(Cottam et al. 2014)
Nsp7	Nsp8		(te Velthuis et al. 2012)
Nsp8	Nsp7		(te Velthuis et al. 2012)
Nsp10	Nsp14 and Nsp16		(Decroly et al. 2011)
Nsp15		apoptosis and dsRNA cell sensors; Rb	(Bhardwaj et al. 2012; Deng et al. 2017)
Nsp16		RIG-I and MDA5 (innate immunity)	(Shi et al. 2019)
RTC		Translation initiation factors (eIF3E, eIF3F and eIF3I); Intracellular transport (SNARE proteins; SRP54a and SRP68 proteins); autophagy-related factors and ubiquitin-dependent ERAD components	(V'kovski et al. 2019)
S	M	ACE2; apoptosis	(Yeung et al. 2008; Neuman et al. 2011;

			Yan et al. 2020)
E	M protein	PALS1 (tight junction); palmitoylations	(Boscarino et al. 2008; Chen et al. 2009a; Teoh et al. 2010)
M	E protein; N protein; S protein		(Chen et al. 2009a; Hurst et al. 2009; Neuman et al. 2011)
N	M protein; Nsp3	RNA interference machinery; NCL; NPM; NONO; PABP; HNRNPs; ribosomal proteins; caprin-1; G3BPs; GSK3; PACT; TRIM25; cyclin D	(Chen et al. 2002; Surjit et al. 2006; Hurst et al. 2009; Emmott et al. 2013; Hurst et al. 2013; Cui et al. 2015; Ding et al. 2017)
ORF3a		TRAF3 and ASC; caveolin-1; eIF2α and PERK	(Padhan et al. 2007; Minakshi et al. 2009; Siu et al. 2019)
ORF6	Nsp8	karyopherin alpha 2 and karyopherin beta 1	(Kumar et al. 2007; Frieman et al. 2007)
ORF7a	ORF3	Type I IFN response; BST-2; cyclin D3/pRb pathway	(Yuan et al. 2006; Dedeurwaerder et al. 2014;

			Taylor et al. 2015)

The PL^{pro} protein (nsp3) from SARS-CoV significantly triggered the activation of the TGF-β1 promoter through ROS/p38-MAPK/STAT3, correlating with the positive regulation of pro-fibrotic responses *in vitro* and *in vivo* (Li et al. 2016). Another study showed that p53 downregulates SARS-CoV replication and is a target of nsp3 via an E3 ubiquitin ligase (Ma-Lauer et al. 2016). The other viral protease, $3CL^{pro}$ (nsp5), was proven to cleave STAT2, but not JAK1, TYK2, STAT1, and IRF9, which are key molecules of the JAK-STAT pathway, antagonizing the type I interferon signaling (Zhu et al. 2017).

Coronaviruses nsp6 is known to interfere with autophagy, limiting the autophagosomes diameter at the point of omegasome formation, which may favor viral infection by compromising the ability of the autophagy system to degrade viral components via lysosomes (Cottam et al. 2014). Another study reported that the coronavirus endoribonuclease nsp15 is required for evasion of dsRNA sensors and apoptosis since the loss of nsp15 activity is related to attenuation of the disease in mice and stimulated a protective immune response (Deng et al. 2017). Nsp16 can downregulate the activities of RIG-I and MDA5, inhibiting innate immunity to promote viral proliferation (Shi et al. 2019).

Finally, an important study recently showed cellular pathways related to the coronaviruses RTC, using MHV (mouse hepatitis virus) as a model (V'kovski et al. 2019). The study identified that RTC interacts with translation initiation factors, which may not persist throughout the replication cycle but may be of transitory importance during specific phases of the replication cycle. In addition, the knockdown of some of these factors, such as eIF3E, eIF3F, and eIF3I, impacted in the MHV replication. Proteins related to transport and intracellular organization were also related to RTC in this study.

4. The structural proteins of coronaviruses

4.1. The S protein

Among all the structural proteins of coronaviruses, S, E, M, and N proteins are considered essential and their functions are described below. Homotrimers of S protein form the spikes in the viral surface, which give the virion a crown or corona aspect and the name of the family Coronaviridae, being responsible for binding to host receptors (Beniac et al. 2006). S protein has about 150 kDa and contains an N-terminal signal sequence that gives access to the endoplasmic reticulum (RER) for its synthesis, being strongly N-terminal glycosylated. Trimeric S glycoprotein is a class I fusion protein and mediates host receptor binding (Bosch et al. 2003). A recent study showed that the angiotensin-converting enzyme 2 (ACE2) is the cell receptor for SARS-CoV-2, as well as for SARS-CoV, being trimeric protein S its ligand (Yan et al. 2020). In addition, the serine protease TMPRSS2, targeting ACE2, facilitates the cellular entry of SARS-CoV and SARS-CoV-2 and a TMPRSS2 inhibitor, camostat mesylate, partially inhibits *in vitro* SARS-CoV-2 infection (Hoffmann et al. 2020).

4.2. The E protein

E protein plays a role in the assembly and release of virions from cells, being involved in viral pathogenesis (DeDiego et al. 2007). It is a small protein (~8–12 kDa) and is found in small amounts within the virion. Protein E is a homopentameric transmembrane protein, possessing an N-terminal ectodomain, a C-terminal endodomain, and ion channel activity, and such activity in SARS-CoV is not necessary for viral replication but has an impact on viral pathogenesis (Nieto-Torres et al. 2014). Recombinant viruses without protein E are viable but attenuated, unlike other structural proteins, although this effect is dependent on the virus type (DeDiego et al. 2007). Interacts with M membrane protein in the budding compartment of the cell, located in the ERGIC (Chen et al. 2009a). SARS-CoV E protein interacts with PALS1 protein (Protein Associated with Lin-Seven 1) via the PDZ binding motif in its C-terminal domain and delays the formation of tight junctions, altering epithelial morphogenesis (Teoh et al. 2010). Another study showed that palmitoylation of E protein is crucial for the assembly of murine coronavirus (Boscarino et al. 2008).

4.3. The M protein

The M protein presents three transmembrane domains and is responsible for the shape of virions, promoting the membrane curvature and binding to the nucleocapsid

(Neuman et al. 2011). It is the most abundant structural protein in the virion, having approximately 25–30 kDa and presenting a small glycosylated ectodomain at the N-terminal and a larger C-terminal endodomain that extends from 6 to 8 nm in the viral particle (Nal et al. 2005). M protein forms a dimer in the virion and can adopt two different conformations, interacting with nucleocapsid N protein and S protein (Neuman et al. 2011). The interaction with the nucleocapsid N protein promotes the complete assembly of the virion and was mapped to occur between the C-terminal of the M protein endodomain and the N protein CTD (Hurst et al. 2005). As already reported, its interaction with protein E has also been demonstrated (Chen et al. 2009a). M protein directs most of the protein-protein interactions necessary for the assembly of coronaviruses. A study showed that the expression of M protein alone is not sufficient for the formation of virus-like particles (VLPs) (Bos et al. 1996). However, when M protein was expressed together with E protein, the formation of VLPs occurred, suggesting the important role of the two proteins in producing the envelopes of coronaviruses. A study showed that the surface proteins of coronaviruses S, M, and E show differential subcellular locations when expressed alone, suggesting that additional cellular or viral factors may be necessary for coordinated traffic to the viral assembly site in the ERGIC (Nal et al. 2005).

4.4. The N protein

N protein is the only viral nucleocapsid protein and contains two domains. The two structural domains of N protein, the N-terminal RNA binding domain (RBD) (residues 45-181) and the C-terminal dimerization domain (DD) (residues 248-365), do not interact with each other and are surrounded by flexible linkers (Chang et al. 2006). It is reported that N protein can bind to nsp3 to assist the binding of the viral genome to the RTC and the packaging of the encapsidated genome in virions (Hurst et al. 2009; Chen et al. 2020b). The interaction between N and nsp3 supports a model in which this interaction tethers the genome to newly translated RTCs at an early stage of infection (Hurst et al. 2013). Besides, one of the N protein domains is critical for the recognition of the M protein during virus assembly in cells. The interaction of N protein and viral nucleocapsid with the membrane proteins S, E, and M for viral packaging takes place in the ERGIC, forming the mature virions that are then extruded from the cells by exocytosis via Golgi (de Haan and Rottier 2005). The expression of N protein increases the formation of VLPs,

suggesting that the fusion of encapsidated genomes in the ERGIC improves viral envelope formation (Siu et al. 2008).

Regarding cellular interactions, several targets of N protein have been proposed. A study has shown that coronavirus N protein is a viral suppressor of RNA silencing (VSR) since the ectopic expression of SARS-CoV N protein could promote MHV-A59 coronavirus replication in RNAi-active cells but not in RNAi-depleted cells (Cui et al. 2015). Besides, a proteomics study has demonstrated interactions between the coronavirus N protein and several cellular components, including ribosomal proteins, translation initiation factors, nucleolar proteins, helicases, and hnRNPs (Emmott et al. 2013). Some of those interactions, like NONO and poly(A)-binding protein (PABP), were potentially mediated by RNA and the interactions with caprin-1, G3BP-1, and G3BP-2, which are involved in the formation of cytoplasmic stress granules, explain the localization of N protein in these cell structures. The interaction between N protein and NCL is a possible explanation of how coronavirus N proteins can localize to the nucleolus of cells (Chen et al. 2002). Finally, the impact of some cellular targets for viral replication was evaluated by RNA-interference depletion, demonstrating the functional importance of NCL, RPL19, or GSK3 proteins in the biology of coronavirus (Emmott et al. 2013).

5. Other ORFs of coronaviruses

Besides pp1ab, S, E, M, and N proteins, SARS-CoV-2 present at least 5 more ORFs, called ORF3a, ORF6a, ORF7a/7b, ORF8, and ORF10, which much less information is known regarding their molecular mechanisms of action in the viral replication cycle. SARS-CoV ORF3a is reported to bind to TRAF3 and ASC, promoting TRAF3 ubiquitination and activation of the NLRP3 inflammasome (Siu et al. 2019). Other studies have demonstrated that ORF3a presents binding affinities for caveolin-1 and calcium (Padhan et al. 2007; Minakshi et al. 2014). SARS-CoV ORF6 is localized in the ER and Golgi membranes in infected cells, binding to karyopherin alpha 2 and karyopherin beta 1 proteins and hindering STAT1 nuclear import and its function (Frieman et al. 2007). Another study showed that nsp8 interacts with ORF6, suggesting that ORF6 protein plays a role in virus replication (Kumar et al. 2007).

Coronavirus ORF7a has been recognized as a type I IFN antagonist only when in the presence of ORF3 protein, protecting the virus from the antiviral state induced by this

cytokine (Dedeurwaerder et al. 2014). ORF7a protein also binds to BST-2 (Bone marrow stromal antigen 2 or tetherin), an antiviral protein that restricts SARS-CoV infection, blocking its glycosylation, whereas the loss of ORF7a leads to a much greater restriction (Taylor et al. 2015). ORF7a expression has been also associated with cell cycle arrest at the G0/G1 phase in HEK 293 cells via the cyclin D3/pRb pathway (Yuan et al. 2006). Another study has found that the translation of SARS-CoV ORF7b may be mediated by leaky scanning of ribosomes and that it localizes in the Golgi compartment and is incorporated into viral particles (Schaecher et al. 2007). Finally, a study has demonstrated that SARS-CoV ORF8 may have originated through recombination from SARS-related coronavirus from bats, which may have an impact on animal-to-human transmission (Lau et al. 2015).

6. Analysis of cellular pathways related to coronavirus replication

To further analyze the cellular proteins related to coronaviruses biology, we performed Ingenuity Pathway Analysis, using the list of proteins presented in table 2. This allowed the generation of a canonical pathways list, presented in table 3, that may be important or modulated during the replication cycle of coronaviruses. The top 10 pathways were selected based on their associated p-value. The modulation of key molecular players, such as p53 and mTOR pathways, the inhibition of the host immune response by restraint of IFN induction and changes in cell cycle and cell growth, has been highly associated with the proteins of coronaviruses. These events may create a proliferative state that favors viral replication and inhibits apoptosis, facilitating viral cycle progression.

Table 3. Ingenuity Pathway Analysis (IPA) reveals the top 10 canonical pathways related to the cellular proteins that interact with coronavirus proteins, as summarized in Table 2.

Ingenuity Canonical Pathways	**-log (p-value)**	**Ratio (strength of association)***	**Genes/Proteins (total number)**
Role of PKR in Interferon Induction and Antiviral Response	8,84E00	5,93E-02	DDX58, IFIH1, NPM1, PRKRA, STAT2, TP53, TRAF3 (7)

Activation of IRF by Cytosolic Pattern Recognition Receptors	7,06E00	7,94E-02	DDX58, IFIH1, PPIB, STAT2, TRAF3 (5)
Cell Cycle: G1/S Checkpoint Regulation	6,92E00	7,46E-02	CCND3, GSK3B, RB1, TGFB1, TP53 (5)
Cyclins and Cell Cycle Regulation	6,51E00	6,17E-02	CCND3, GSK3B, RB1, TGFB1, TP53 (5)
Systemic Lupus Erythematosus In B Cell Signaling Pathway	6,26E00	2,5E-02	CCND3, GSK3B, IFIH1, MTOR, STAT2, TGFB1, TRAF3 (7)
EIF2 Signaling	5,56E00	2,64E-02	EIF3E, EIF3F, EIF3I, GSK3B, PABPC1, RPL19 (6)
Autophagy	5,28E00	6,15E-02	LAMP2, MAP1LC3B, MTOR, SQSTM1 (4)
FAT10 Signaling Pathway	5,18E00	1,43E-01	MAP1LC3B, PSMD4, SQSTM1 (3)
Regulation of eIF4 and p70S6K Signaling	5,04E00	3,11E-02	EIF3E, EIF3F, EIF3I, MTOR, PABPC1 (5)
Role of p14/p19ARF in Tumor Suppression	4,74E00	1,03E-01	NPM1, RB1, TP53 (3)

*number of molecules in the pathway present in the input divided by the total number of proteins in that pathway

6.1. Immune system pathways

SARS-CoV infects poorly monocytes/macrophages, even though viral proteins are expressed, replication is incomplete in these cell types, that respond secreting low levels of IFN-β and high levels of chemokines like IP-10 and MCP-1 and may be part of the inflammatory response that participates in the pathogenesis of the disease (Cheung et al. 2005); dendritic cells infected by SARS-CoV induce low levels of IFN-α, IFN- β, IFN-γ, IL12p40, moderate levels of TNF- α and IL-6 and high levels of MIP-1A, IP-10, and MCP-1 (Law et al. 2005). SARS-CoV shows a delayed induction of IFN- α (Spiegel

2006). Comparison of SARS-CoV with Vesicular Stomatitis Virus (VSV) and Newcastle virus indicates that SARS-CoV induces lower levels of IFN-α, β, and γ, independently of viral replication. Differently from SARS-CoV, MERS-CoV is able to establish a productive infection in human macrophages and induce higher levels of IL-12, IFN- γ, IP-10, MCP-1, MIP-1A, RANTES, and IL-8 (Zhou et al. 2014).

The low activation of IFN pathway is mediated by viral regulation of IRF3, a transcription factor activated by phosphorylation or polyubiquitination, and then translocates to the nucleus and induces the IFN response genes (Chattopadhyay et al. 2016). The SARS-CoV PL^{pro} inhibits IRF3 phosphorylation, preventing its nuclear translocation and disrupting IFN response, probably through inhibition of STING (stimulator of interferon genes), that is responsible for IRF3 phosphorylation (Chen et al. 2014). Nsp3 DUB domain of MHV-A59 and SARS-CoV promotes deubiquitination of IRF3 and also prevents its activation, blocking NF-κBsignaling (Frieman et al. 2009). PL^{pro} of MERS-CoV has also been described to inhibit IRF3 nuclear translocation (Yang et al. 2014). Interferon inhibition makes PL^{pro} an important determinator of virulence of coronavirus (Niemeyer et al. 2018). SARS-CoV Nsp1 protein also participates in IFN inhibition through decreasing STAT1 phosphorylation (Wathelet et al. 2007). While ORF3a protein induces ER stress, activates PERK (PKR-like ER Kinase), and promotes phosphorylation, ubiquitination, and degradation of IFNAR1, attenuating interferon response (Minakshi et al. 2009). The SARS-CoV N protein has also been described to inhibit IFN production at an early step, by sequestering PACT (protein activator of the dsRNA activated protein kinase R) and TRIM25 (tripartite motif protein 25), which bind to RIG-I (retinoic acid-inducer gene I) and MDA5 (melanoma differentiation gene 5) and activate IFN production (Ding et al. 2017). Finally, ORF4b of MERS-CoV is another protein that has been characterized to inhibit IFN and NF-κB signaling (Matthews et al. 2014).

STAT3 modulation plays an important role in pro- and anti-inflammatory responses. As already mentioned, SARS-CoV PL^{pro} activates TGF-β1 through p38MAPK/ERK1-2 pathway, promoting STAT3 activation (Li et al. 2016). MERS-CoV strains with mutations in the NSP3 and ORF4a display differential STAT3 activation and different inflammatory cytokines profiles (Selinger et al. 2014). In SARS-CoV infection it is observed a reduction of IL-4, which participates in humoral protection, an increase of IFN- γ, that participates in a potent cell-mediated immune response and also the elevation of IL-10, that plays a part in disease susceptibility (Zhu 2004).

441

442 6.2. Cell cycle pathways

443

444 SARS-CoV has also been described to arrest the cell cycle. Nucleocapsid protein
445 was shown to arrest cell cycle at S phase, through direct interaction with cyclin D and
446 inhibition of the CDK4/Cyclin D complex, preventing phosphorylation of Rb
447 (Retinoblastoma) protein, a central player in cell cycle control (Surjit et al. 2006). IBV
448 infection also reduces Cyclin D1, which participates in G2/M transition, inducing cell
449 cycle arrest at G2/M (Harrison et al. 2007).

450 The blockage of G0/G1 progression has been observed by SARS-CoV ORF7a and
451 ORF3a proteins through the reduction of cyclin D3 expression, decreased activity of
452 cyclin D/CDK4/6, and inhibition of Rb phosphorylation (Yuan et al. 2006). Nsp15 is able
453 to alter cellular localization of Rb and function, promoting pRb ubiquitination and
454 degradation, increasing the proportion of S-phase cells, while overexpression of ORF4
455 (3b) protein arrests cell cycle at G0/G1 and promotes apoptosis (Yuan et al. 2005;
456 Bhardwaj et al. 2012).

457 Expression of viral proteins regulates cell fate, not only cell cycle, but also
458 controls apoptosis, since its importance for viral replication. SARS-CoV S protein
459 suppresses extrinsic apoptotic pathway, downregulating TRAIL and FasL, and activates
460 of the intrinsic apoptotic pathway, through upregulation of Bax and down-regulation of
461 Bcl-2, Mcl-1, Bcl-xL, and MDM2, leading to increased levels of p53 and p21 induction
462 and G1/S arrest (Yeung et al. 2008). ORF9b protein when accumulated in the nucleus
463 induces caspase 3-mediated apoptosis (Sharma et al. 2011). Inhibition of apoptosis is also
464 mediated by SARS-CoV E protein, which down-regulates IRE-1 (inositol-requiring
465 enzyme-1) and DUSP1/10 proteins, critical regulators of innate immune response and
466 apoptosis (DeDiego et al. 2011). SUD domain of PL^{pro} interacts with RCHY1 and
467 promotes p53 degradation, playing a role in cell cycle and apoptosis control, whereas p53
468 overexpression was able to inhibit viral replication (Ma-Lauer et al. 2016). SARS-CoV
469 promotes the expression of a truncated form of p53, that inhibits apoptosis mediated by
470 wild-type p53 (Leong et al. 2005). This is supported by the observation that the Porcine
471 epidemic diarrhea vírus (PEDV) production is increased in p53 knockout cells (Hao et al.
472 2019).

473

474 6.3. Protein synthesis control pathways

Protein synthesis pathways are often modulated by viruses. The activation of the PKR pathway by RNA viruses is an important cellular defense mechanism, which is in several cases counteracted by viruses, including coronaviruses. Dengue virus for example sustains activation of the cap-dependent machinery at early stages of infection, switching the protein synthesis to a cap-independent process in the late stages by downregulation of p70-S6K, 4E-BP1 and eIF4 factors (Villas-Bôas et al. 2009). The SARS-CoV ORF3a is known to cause endoplasmic reticulum stress and activation of eIF2α (eukaryotic initiation factor 2 alpha) and PERK, affecting innate immunity by suppression of type 1 IFN signaling (Minakshi et al. 2009). PKR and PERK, which promote phosphorylation of EIF2a that may suppress host translation, are expressed at high levels during SARS-CoV replication, although knockdown of PKR does not affect viral replication, suggesting that SARS-CoV possesses a mechanism to overcome the inhibitory effects of phosphorylated eIF2α on viral mRNA translation (Krähling et al. 2009). On the other hand, another study has shown that depletion of the antiviral PKR pathway enhanced virus replication, increasing SARS-CoV protein expression and virus production (de Wilde et al. 2015).

Metformin and rapamycin are known modulators of viral infection and translation control pathways, such as mTOR. It is reported for example that in the 1971 influenza outbreak, diabetic patients treated with phenformin and buformin presented a lower incidence of infection compared to diabetics treated with sulfonylureas or insulin (Lehrer 2020). The immunoregulation of COVID-19 with mTOR inhibitors such as rapamycin has been recently proposed (Zheng et al. 2020).

7. Pharmacological interventions for the treatment of diseases associated with coronaviruses

Severe coronavirus infection leads to epithelial cell proliferation, macrophage infiltration in the lung (Nicholls et al. 2003) and can cause pulmonary fibrosis, which can still be present in recovered patients (Antonio et al. 2003). About 15% of COVID-19 patients progress to acute respiratory distress syndrome (ARDS), the most severe cases should be treated in intensive care units (ICU) and receive oxygen therapy and mechanical ventilation (Li et al. 2020). In extreme cases of COVID-19, lung transplantation is a viable option (Chen et al. 2020a). The pharmacological interventions against coronaviruses, as

summarized in figure 1 and table 4, are reviewed regarding their molecular mechanisms of action, effectiveness *in vitro* and *in vivo*, and ongoing clinical trials. There are two important aspects in the clinical outcome of COVID-19: one is viral entry/replication and the second is host response. Both are intimately linked and can be targeted by different compounds.

Table 4. Pharmacological interventions targeting human coronaviruses replication cycle

Pharmacological interventions	**Targeting mechanism**	**Reference**
Human recombinant ACE2	Virus entry: inhibition of virus binding	(Monteil et al. 2020)
Arbidol	Virus entry: envelope fusion and endocytosis blockage	(Teissier et al. 2011; Blaising et al. 2013)
Remdesivir	Replication: adenosine analog	(Sheahan et al. 2017; Brown et al. 2019; Wang et al. 2020b)
Ribavirin, Sofosbuvir, Galidesivir, Tenofovir Favipiravir	Replication: nucleotides analogs	(Elfiky 2020; Cai et al. 2020)
Lopinavir and Ritonavir	Protease inhibitors	(Chu et al. 2004; Nutho et al. 2020; Choy et al. 2020)
Chloroquine, Hydroxychloroquine	Virus entry: alkalinization of acid vesicles, inhibition of virus binding	(Simmons et al. 2004; Rolain et al. 2007)

7.1. Interventions on viral entry/replication

Strategies to hinder viral binding have been investigated. Human recombinant ACE2 reduced SARS-CoV-2 recovery *in vitro* and protected mice from acute lung injury caused by SARS-CoV (Monteil et al. 2020). A chimeric protein composed of the extracellular domain of ACE2 fused with the Fc region of IgG1 exhibited pharmacological properties in mice (Lei et al. 2020). SARS-CoV-2 has two possible entry mechanisms, through endosome or membrane fusion. Arbidol is a potent broad-spectrum antiviral, that blocks viral envelope fusion (Teissier et al. 2011) and clathrin-mediated endocytosis (Blaising et al. 2013), suppressing the replication of SARS-CoV *in vitro* (Khamitov et al. 2008). Patients treated with Arbidol had a shorter period of SARS-CoV-2 infection compared to patients treated with lopinavir/ritonavir (Zhu et al. 2020).

Several drugs have recently been used to inhibit SARS-CoV-2 replication, including the adenosine analog remdesivir, which targets the RNA dependent RNA polymerase and is incorporated into viral RNA chains, resulting in premature termination. Remdesivir was firstly shown to be effective against the Ebola virus (Warren et al. 2016) but presents activity against other viruses, including members of the *Filoviridae, Paramyxoviridae, Pneumoviridae,* and *Orthocoronavirinae* families (Brown et al. 2019). Remdesivir inhibits SARS-CoV and MERS-CoV (Sheahan et al. 2017), even though the identity among the coronaviruses RdRps range from 70-90%, remdesivir shows a broad spectrum of activity (Brown et al. 2019). Recently, a study showed that remdesivir also acts against SARS-CoV-2, according to its potential antiviral mechanism as a nucleotide analog (Wang et al. 2020b). Other nucleotide analogs, such as Ribavirin, Sofosbuvir, Galidesivir, and Tenofovir, can bind to SARS-CoV-2 RdRp (Elfiky 2020). Favipiravir, a purine analog used against Influenza, is being tested against COVID-19 and reduced the time for viral clearance compared to patients treated with lopinavir/ritonavir (Cai et al. 2020). There are currently ongoing clinical trials to evaluate Remdesivir and Ribavirin against COVID-19, regardless of its cost and administration routes there are also concerns regarding its side effects and efficacy (Khalili et al. 2020).

Furthermore, studies reported that the protease inhibitors lopinavir and ritonavir, used as HIV antivirals, also appear to have effects against SARS-CoV and SARS-CoV-2 (Chu et al. 2004; Choy et al. 2020). Both drugs can interact with the protease $3CL^{pro}$; ritonavir has a higher binding affinity compared with lopinavir (Nutho et al. 2020). Animal experiments against SARS-CoV and MERS-CoV showed that the combination

of lopinavir/ritonavir (LPV/r) with IFN-β significantly reduced viral load and improved pulmonary function. The combination of LPV/r shows a synergistic effect in the treatment of SARS patients (Yao et al. 2020). A clinical trial (NCT02845843) is currently testing a combination of lopinavir, ritonavir, and interferon-β1b against MERS (Arabi et al. 2020). Combined LPV/r reduced the time for the patients to become negative for SARS-CoV-2 (Yao et al. 2020), and increased eosinophils, indicating an improvement in COVID-19 clinical outcome (Liu et al. 2020). Another study, however, reported no difference in the administration of lopinavir and ritonavir in a group of patients with COVID-19 already in an advanced stage (Cao et al. 2020), or shortening of the duration of SARS-CoV-2 shedding (Cheng et al. 2020). A retrospective analysis of adverse drug reactions (ADRs) from patients with COVID-19 admitted at the First Hospital of Changsha in China revealed that about 64% of the observed ADRs were correlated with the use of LPV/r (Sun et al. 2020). Patients treated with LPV/r presented a significantly higher proportion of abnormal liver function (Fan et al. 2020). There are about 211 clinical trials ongoing, considering these antivirals, using Lopinavir, Ritonavir, Remdesivir, Favipiravir, in combination, alone or with other drugs in SARS-CoV-2 patients (Cochrane COVID-19 Study register).

Chloroquine (CQ) has been tested as well against SARS-CoV. It promotes alkalinization of acid vesicles in cells infected by intracellular pathogens (Rolain et al. 2007) and emerged as a substitute to quinine against malaria. CQ and hydroxychloroquine (HCQ) have been tested against viral hepatitis (PAREJA-CORONEL 1963), dengue virus (Farias et al. 2015), HIV (Paton et al. 2002), other viruses, and also against other pathogens, such as intracellular bacteria (*Coxiela burnetii* and *Tropheryma whipplei*), bacteria-like *Legionella pneumophila* and *Mycobacterium spp,* and fungal infections by *Histoplasma capsulate* and *Cryptococcus neoformans* (Rolain et al. 2007). CQ is active in Vero E6 and Huh7 cells infected with MERS-CoV (De Wilde et al. 2014), but not in dendritic cells and monocyte-derived macrophages (Cong et al. 2018). CQ is also active *in vitro* against SARS-CoV either before or after virus exposure, interfering with ACE2 glycosylation and inhibiting viral binding (Keyaerts et al. 2004; Vincent et al. 2005). In addition, CQ induces alteration of endosomal pH that inhibits viral infection (Simmons et al. 2004).

Moreover, CQ and HCQ have shown some *in vivo* effects against SARS-CoV-2 (Wang et al. 2020b). A group of Chinese researchers recently reported beneficial effects of chloroquine in the treatment of COVID-19, however, without yet publishing data (Gao

et al. 2020). Another group of French researchers reported that hydroxychloroquine decreased SARS-CoV-2 levels in a small group of tested patients, and the administration of azithromycin appears to improve such effects (Gautret et al. 2020). A recent review analyzed several ongoing clinical trials and indicates there are paradoxical results, some have shown beneficial results, others point to the toxicity issues (Sharma 2020). One important point is that there are different strains of SARS-CoV-2 circulating (Wang et al. 2020a). Importantly, CQ has pro-apoptotic activity and the prophylactic use of CQ has been linked to the selection of intracellular pathogen strains that promote cell resistance to apoptosis and enhanced lethality, as observed for HIV and SARS-CoV (Parris 2004). Despite being a low-cost drug, it is a consensus among health agencies such as the WHO that further studies are needed for the clinical use of CQ and HCQ for COVID-19 treatment. Until now, 390 clinical trials are registered using HCQ or CQ to enlighten their role in SARS-CoV-2 infection treatment (Cochrane COVID-19 Study register).

7.2. Interventions on host cell response

The host response to viral infection is another important factor in COVID-19. SARS-CoV-2 induces secretion of IFN-γ, IL-1β, IL-4, IL-10, IP-10, and MCP-1 (Huang et al. 2020). Patients in intensive care units show higher levels of IL-2, IL-7, GCSF, IP-10, MCP-1, MIP-1A, and TNF-α that may induce cytokine storm and exacerbated inflammatory response (Huang et al. 2020). SARS-CoV infects not only alveolar epithelial cells, but also vascular endothelial cells, macrophages, monocytes, and lymphocytes. Rapid viral replication causes endothelial cell damage and vascular leakage, leading to the release of pro-inflammatory cytokines. Seroconversion of the host leads to the presence of IgG anti-S protein, which may promote the accumulation of proinflammatory monocyte/macrophage and release of MCP-1 and IL-8 and have been linked to severe lung injury (Fu et al. 2020). Viral clearance depends on the activation of both innate and adaptive immune responses. IFN-γ and IL-6 contribute to neutrophil recruitment and transition to the adaptive response. However, exacerbated levels of IL-6 and reduced expression of IFN-γ may decrease $CD4^+$, $CD8^+$, and NK cells and may be connected to cytokine storm (Lagunas-Rangel and Chávez-Valencia 2020).

Tocilizumab is a humanized anti-IL6R monoclonal antibody that prevents IL-6 signaling. Preprint studies indicate that it is safe and shows good efficiency against COVID-19. As there is a need for more clinical trial data, it is suggested that it is used

only in critically ill patients with a profile of high levels of IL-6 (Zhang et al. 2020a). Early clinical data recommends the use of repeated doses (Luo et al. 2020). Some of the concerns that have been raised are about the development of osteonecrosis of the jaws and the development of acute hypertriglyceridemia (Bennardo et al. 2020; Morrison et al. 2020).

Interferon release is one of the most important natural defense mechanisms against viral infection. *In vivo* experiments showed that treatment with IFN-β1b reduced pulmonary infiltrates, bronchointerstitial pneumonia, and viral load against MERS-CoV (Chan et al. 2015). IFN-α, a mismatched double-stranded RNA Interferon inducer, and the IFN inducer Ampligen, inhibited SARS-CoV replication in the lungs (Barnard et al. 2006). IFN-λ also showed activity against SARS-CoV and MERS-CoV, establishing an antiviral state and presenting minimal systemic inflammation (Prokunina-Olsson et al. 2020). Antibodies against cytokines are presented in 88 studies, while 34 focus on IFN, either by inhibiting them or by giving their recombinant form to treat patients (Cochrane COVID-19 Study register).

CQ and HCQ have also shown anti-inflammatory activity and have been used in inflammatory diseases like rheumatoid arthritis and osteoarthritis (Sharma 2020). CQ and HCQ intervene with lysosomal acidification, inhibiting antigen presentations, phospholipase A2, Toll-Like Receptors (TLRs), T and B cell receptors, and production of cytokines, like IL-1 and IL-6 (Sinha and Balayla 2020). The inhibition of GSK3β by CQ may also be responsible for its immunomodulatory activity against COVID-19 (Embi et al. 2020).

Metronidazole is a redox-active prodrug that reduces the levels of pro-inflammatory cytokines, increases circulating lymphocytes, and decreases ROS produced by neutrophils and has also been suggested for the treatment of COVID-19 (Gharebaghi et al. 2020). Another class of anti-inflammatory drugs is the Statins, and they have been included in some treatment protocols. However, as Statins also modulate TLR response, the use of Statins in animal experiments against SARS-CoV and MERS-CoV resulted in increased viral load, severe lung damage, and death (Dashti-Khavidaki and Khalili 2020). Nitazoxanide is used against protozoan and helminthic infection. Tizoxanide, the active form of nitazoxanide inhibits 16 strains of Influenza A and one strain of Influenza B, rotavirus, HCV, Yellow fever virus, HBV, HIV, norovirus, and others (Rossignol 2014). Nitazoxanide reduces the viral load from different coronaviruses (Cao et al. 2015) and

suppresses IL-6 production in mice (Hong et al. 2012), being suggested for COVID-19 treatment.

In viral RNA infections, the use of nutraceuticals has been suggested to inhibit NOX2, which in turn, restores TLR7 response to single-stranded viral RNA infection and induces IFN; nutraceuticals could also up-regulate mitochondrial antiviral-signaling proteins (MAVS) and reduce pro-inflammatory signaling (McCarty and DiNicolantonio 2020). Besides nutraceuticals, Vitamins A and D, Selenium, Zinc, and probiotics may be beneficial for COVID-19 patients, by enhancing immunity and preventing respiratory infections (Grant et al. 2020; Jayawardena et al. 2020). Thus, the nutritional status of COVID-19 patients may be of further interest in future therapies since it might have an impact on disease development.

The clinical progression of COVID-19 indicates that the initial symptoms are due to increased viral load and, in the following weeks of infection, seroconversion of IgG reduces viral load at the same time that some patients present worsening of the symptoms related to immunopathological damage (Peiris et al. 2003). Convalescent plasma (CP) has been used for SARS, MERS, Ebola virus and Chikungunya virus disease to improve survival rate (Alzoughool and Alanagreh 2020). Different groups have tested critically ill COVID-19 patients with CP and obtained good recovery with no severe adverse effects (Duan et al. 2020; Shi et al. 2020; Zhang et al. 2020b). FDA has approved CP to treat critical patients (Tanne 2020), although it has the risk of aggravating hyperimmune response, presenting a better response if administered in the early onset of the disease (Zhao and He 2020). Key points to the use of CP are: establishing eligibility criteria of donor COVID-19 convalescent patients, pre-screening tests of the donors, criteria for CP collection, and treatment of plasma (Epstein and Burnouf 2020). Currently, CP is being tested in 212 clinical trials worldwide against COVID-19 (Cochrane COVID-19 Study register).

8. Conclusions

In summary, the cellular mechanisms associated with coronaviruses replication form a complex and integrated network of molecular events, starting from the translation of Nsps, proteolytic cleavage of polyproteins, assemble of RTC, transcription of antigenome, genome and subgenomic RNAs, translation of structural proteins and finally viral particles assembly and budding. The analysis of the cellular proteins related to

coronaviruses proteins reveals the modulation of key cellular pathways related to innate immunity, cell cycle, and protein synthesis. The current therapeutic approaches for COVID19 are partially related to these molecular events and pathways, but future pharmacological interventions may take advantage of a better understanding regarding the replication cycle of SARS-CoV-2.

9. Acknowledgments

Financial support was received from the São Paulo Research Foundation, FAPESP (FMS: 2018/14818-9; FMS: 2020/05346-6; RET: 2019/15619-2; ICBP: 2018/08391-2; MGM: 2016/25139-0). The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

10. Author's contributions

FMS: conceptualization, writing-original draft and writing-review & editing; RET: writing-original draft and writing-review & editing; ICBP: conceptualization, formal analysis and writing-review & editing; MGM: formal analysis, writing-review & editing; AMV: writing-original draft and writing-review & editing.

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