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Structures of SARS-CoV-2 RNA-Binding Proteins and Therapeutic Targets

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Abstract
Background: The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) epidemic has resulted in thousands of infections and deaths worldwide. Several therapies are currently undergoing clinical trials for the treatment of SARS-CoV-2 infection. However, the development of new drugs and the repositioning of existing drugs can only be achieved after the identification of potential therapeutic targets within structures, as this strategy provides the most precise solution for developing treatments for sudden epidemic infectious diseases. ***Summary:*** In the current investigation, crystal and cryo-electron microscopy structures encoded by the SARS-CoV-2 genome were systematically examined for the identification of potential drug targets. These structures include nonstructural proteins (Nsp-9; Nsp-12; and Nsp-15), nucleocapsid (N) proteins, and the main protease (M^{pro}). ***Message:*** The structural information reveals the presence of many potential alternative therapeutic targets, primarily involved in interaction between N protein and Nsp3, forming replication-transcription complexes (RTCs) which might be a potential drug target for effective control of current SARS-CoV-2 pandemic. RTCs consist of 16 nonstructural proteins (Nsp1-16) that play the most essential role in the synthesis of viral RNA. Targeting the physical linkage between the envelope and single-stranded positive RNA, a process facilitated by matrix proteins may provide a good alternative strategy. Our current study provides useful information for the development of new lead compounds against SARS-CoV-2 infections.

Introduction

Public health emergencies, in the form of infectious disease outbreaks, epidemics, and pandemics, represent an increasing risk to the world's population. Manage-

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ment requires coordinated responses, across many disciplines and nations, and the capacity to gather proper national and global public health education, infrastructure, and preventive measures. According to the WHO, viral diseases represent a major threat to public health and are continuously emerging. Several viral epidemics have been observed over the past 20 years, and these include severe acute respiratory syndrome coronavirus (SARS-CoV) from 2002 to 2003 and H1N1 influenza in 2009. In 2012, the Middle East respiratory syndrome CoV (MERS-CoV) emerged in Saudi Arabia [1, 2].

Among the infectious agents, HIV emerged as a global threat in the late twentieth century. AIDS, a retrovirus, was first identified in the early 1980s as a disease [3, 4]. HIV-1 was identified as the causative agent, infecting CD4+ T cells. Advances in understanding the molecular biology of HIV together with global effort have led lifesaving screening, surveillance, and antiretroviral therapy [5, 6]. Later on, another viral epidemic in the form of MERS-CoV was found emerged as a threat to global health. A total of 229 MERS-CoV cases, including 70 deaths (30.5%), were recorded in the disease outbreak. The case fatality rate was recorded as 30.5% (70/229). The MERS-CoV occurrence was higher among men than women [7]. Although sufficient research has been conducted to investigate the molecular structure and epidemiology in these previous outbreaks, no significant global health security has been improved nor future possible outbreaks warning has been predicted. In the year 2003, SARS disease caused by a SARS-associated coronavirus emerged. In February 2003, the infectious agent emerged in China and rapidly spread to 4 other countries. WHO, with the assistance of the Global Outbreak Alert and Response Network and international investigation, worked with health system in affected countries to provide clinical support, but the epidemic of SARS spread among 26 countries, which >8,000 cases reported in 2003 (https://www.who.int/ith/diseases/sars/en/). However, no significant improvement has been observed in the form of vaccine to prevent further human loss in future.

Among all viral epidemics, the deadliest 1 emerged in the form of coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2. The primary disease epicenter is currently Wuhan, China, its transmission and spread can be observed globally, where this disease affects >200 countries. This outbreak is affecting the world economy in a drastic manner, as millions of people, including all types of workers, are being quarantined. Currently, no vaccine or drug has been approved to treat COVID-19; however, several treatment options, such as the use of effective antiviral medications, are under consideration [8].

SARS-CoV-2 is a single-stranded positive RNA (+ssRNA) virus of the betacoronavirus family, and which also includes MERS-CoV and SARS-CoV. Coronaviruses are enveloped and nonsegmented positive-sense RNA viruses that belong to the order nidovirales. Among RNA viruses, coronaviruses possess the largest genome (30 kb) that contains structural and accessory genes, ample replicase, and other nonstructural proteins (Nsps) [9–12]. Two-thirds of the SARS-CoV-2 genome consists of the ORF1a/b region that is translated into 2 polyproteins, pp1a (Nsp1-Nsp11) and pp1ab (Nsp1-Nsp16) [13]. The 4 structural proteins that include envelope (E), matrix (M), nucleocapsid (N) phosphoprotein, and spike (S) function together with the viral RNA and Nsp1-16 to facilitate the replication of the virus within the host cell. The 4 structural proteins in combination with the viral ssRNA genome and the E constitute the complete virion (Fig. 1).

Crystal and cryo-electron microscopy (EM) structure are three-dimensional structures that are determined by X-ray crystallography and nuclear magnetic resonance, and these structures can be representative of proteins and nucleic acids. These structures provide a powerful tool in the elucidation of the three-dimensional structure of a molecule at atomic resolution [14–19]. They are also favored for proteins that can provide detailed and accurate information for future research in drug design and development. In the present study, we analyzed the structures of 1 structural protein, N, and 3 Nsps that included Nsp-15, Nsp-9, and the main protease ([M^{pro}] Nsp5) of SARS-CoV-2 that were recently released by the protein data bank to provide for a broader range of possible target identification. All crystal and cryo-EM structures were extracted from protein data banks [20, 21] and were carefully analyzed using PyMOL and Chimera [22, 23]. Structural-related data were also analyzed, and the interacting partners of N, Nsp-15, and Nsp-9 were investigated and compared to SARS-CoVs for potent therapeutic target identification.

Nucleocapsid Protein

Current antiviral drugs have been developed by targeting the S protein. However, the strategy of targeting the S protein does possess some limitations. In certain cases, a number of viruses that possess mutations within the S protein have been observed [24–26], and these mutant viruses are prone to resist therapy due to an altered man-

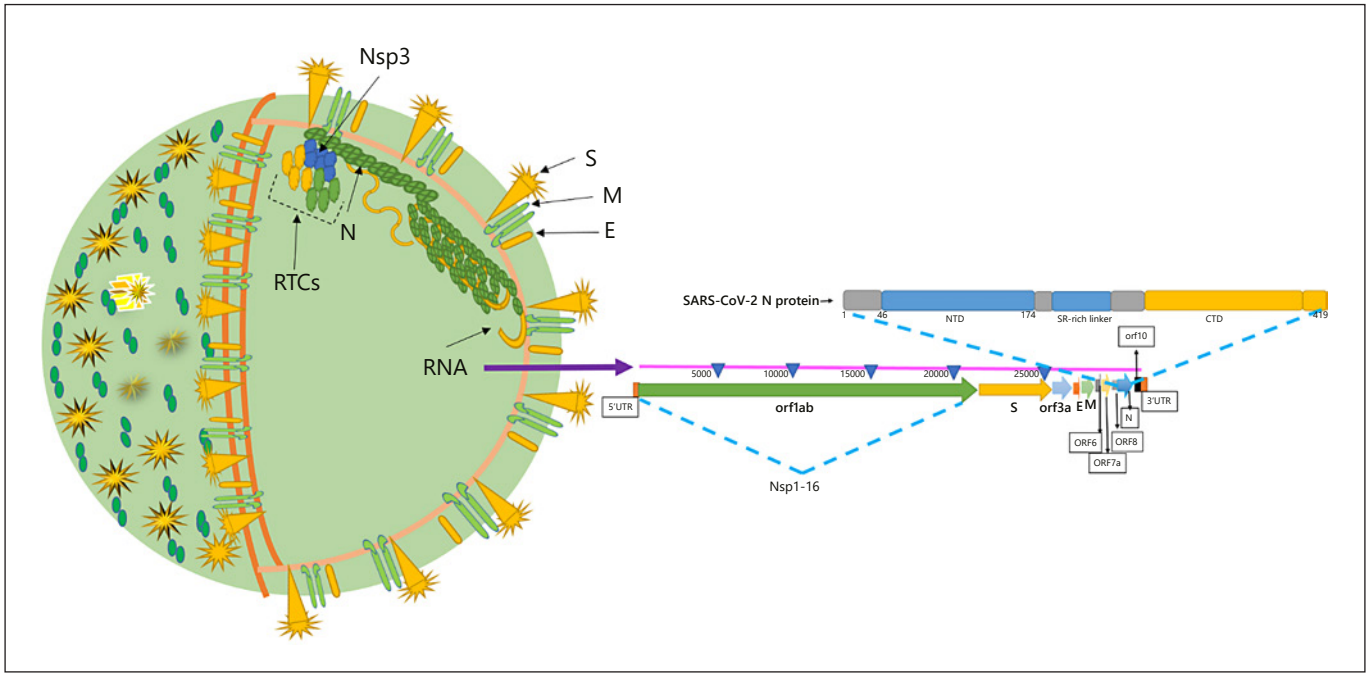

Fig. 1. Structure of SARS-CoV-2 and its genome organization [40, 92–94]. N protein is a major facilitator of viral replication within host cells, where it interacts with viral RNA during replication to form the virion after attachment to Nsp3 of the RTCs. RNA also interacts with M proteins via N. RTCs facilitate viral RNA replication. SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; RTC, replication-transcription complex; Nsp, nonstructural protein; S, spike, E, envelope; M, matrix; EM, electron microscopy.

ner of attachment to the host-cell receptor that results in changes in binding patterns. Furthermore, in cases involving the M^{pro}, nonspecific binding may result in antiviral activity and may act on homologs of the cellular protease, ultimately resulting in host-cell toxicity and severe adverse effects. To avoid these limitations, SARS-CoV-2 essential proteins and RNA must be specifically targeted by novel antiviral strategies.

Among the major targets, the N is an important protein that is involved in RNA binding and is essential for RNA activities such as replication. It also plays an integral role in host-cell metabolism and RNA packaging when the most essential viral processes, including replication and transcription, are at their peak, and N protein also modulates the activities of infected cells [27–29]. The N protein primarily promotes the binding and packing of the RNA ribonucleoprotein complex (N) [30–33].

To facilitate replication and transcription of the viral genome [28, 34], the N protein also maintains a highly ordered RNA conformation that is suitable for RNA activities Figure 1. Previous studies indicated that N protein is responsible for the regulation of host-cell cycle progression, host-pathogen interactions, actin reorganization, and apoptosis [35, 36]. Additionally, the N protein is extremely immunogenic, induces protective immune responses, and is abundantly expressed during infection [37, 38].

The N-phosphoprotein consists of N-terminal (N-NTD) and C-terminal (CTD) domains. Both of these domains exhibit RNA-binding affinity, while the CTD also binds the M protein to establish the physical linkage between the E and ssRNA. The SARS-CoV N proteins also play regulatory roles in the viral life cycle through the use of host intracellular machinery [30]. The N proteins have been demonstrated as key for incorporating viral RNA into viral progeny particles.

Recent studies have shown that the structure of the N protein includes a right hand-like fold composed of a β-sheet core with an extended central loop. The core region adopts a five-stranded U-shaped right-handed antiparallel β-sheet platform with the topology β4-β2-β3-β1-β5, and this structure is flanked by 2 short α-helices. A prominent feature of the structure is a large extending loop that exists between β2–β3 and forms a long basic β-hairpin (β2′ and β3′) [39, 40].

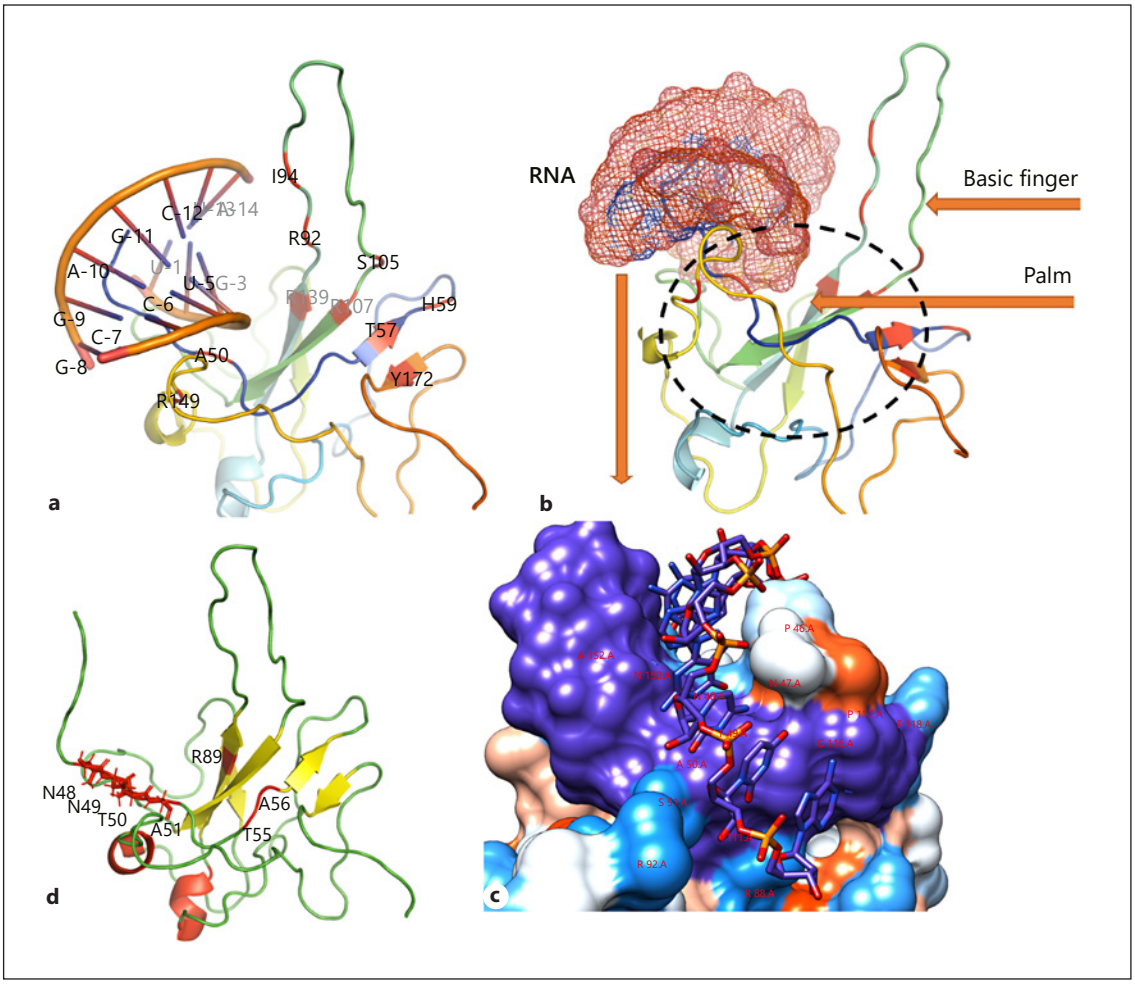

Fig. 2. Structural organization of N proteins. **a** RNA-binding location and residues involved in interactions. **b** Structural components of N proteins (basic finger and palm) that facilitate RNA binding. Surface representation of RNA binding (**c**) N-terminal tail residues N48, N49, T50, and A51 (**d**). G phosphate moiety recognition residues T55, A56, and R89. N, nucleocapsid proteins; G, guanosine.

The crystal structure of the RNA [41] and the N protein was docked using HADDOCK [42]. The RNA duplex was found to be bound between the basic finger and the palm of the N-NTD that contains highly positive arginine residues (R92, R107, and R149) that directly contact the RNA. The model predicts several hydrophobic interactions with side chains of residues I94 and L104 that contribute to RNA binding. Residues A50, T57, H59, R89, R92, I94, S105, R107, R149, and Y172 in the surrounding areas may interact with RNA (Fig. 2a). In vitro studies identified several features of the N-NTD, and these revealed the specific targeting of antiviral agents to SARS-CoV-2 [40] at areas that included conserved regions, N-NTD RNA-binding residues, CTD dimerization, and central Ser/Arg (SR)-rich linker regions. The specific roles of each of these regions have been previously described, where N-NTD exhibits RNA-binding affinity, CTD facilitates oligomerization, and SR-rich linkers perform primary phosphorylation roles [43–47]. Each of these may represent a potential drug target that could be targeted by antiviral inhibitors.

Several residues were identified within the SARS-CoV-2 N-NTD and are involved in the ribonucleotide binding domain functions (Fig. 2). The N-terminal tail residues, N48, N49, T50, and A51 Figure 1d, appear to be more flexible and can open the binding pocket that allows for interaction with SARS-CoV-2 RNA. Residues R89, T55, and A56 are involved in guanosine (G) phosphate

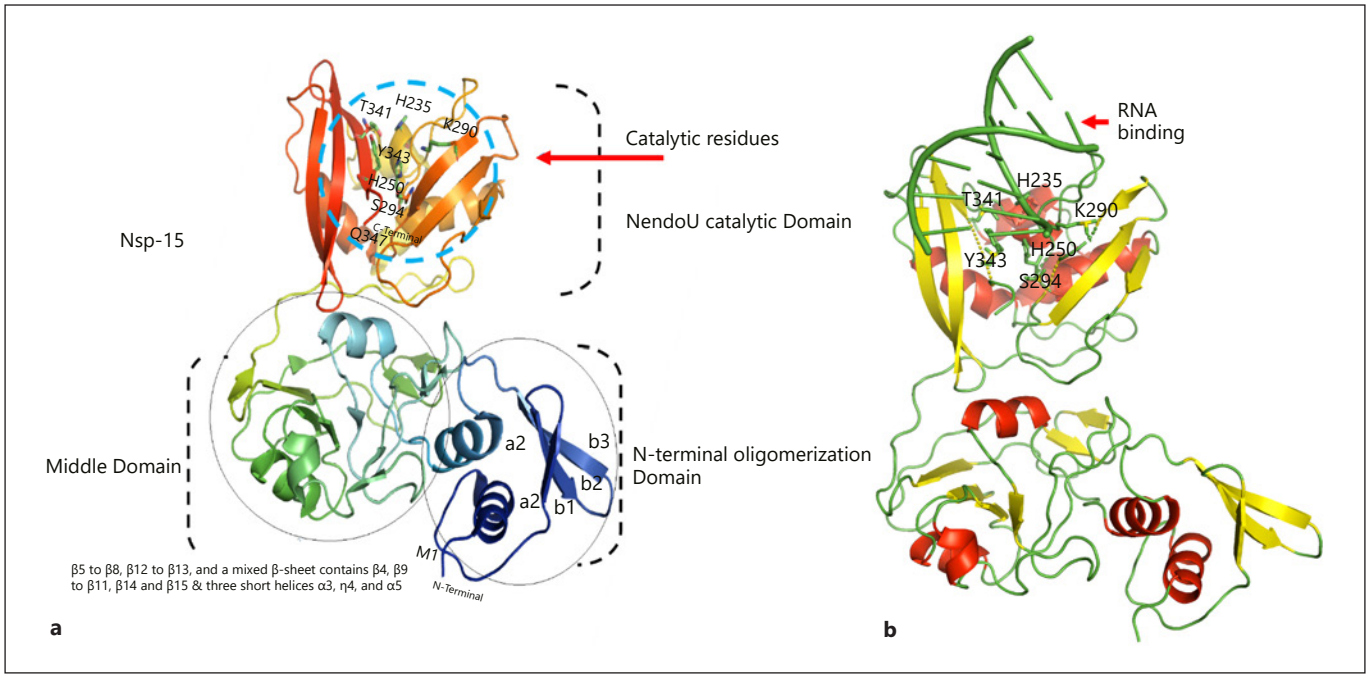

Fig. 3. Domain organization of Nsp15 (**a**) Catalytic residues are indicated by blue circles (H235, H250, K290, T341, Y343, and S294). **b** RNA (PDB ID: 4u37) binding to the NendoU catalytic domain. RNA was docked using the HDOCK webserver [95]. Nsp, nonstructural protein.

moiety recognition [40]. A more recent study [27] reported that the interaction of N proteins with the Nsp3 component of replication-transcription complexes (RTCs), a CoV-RNA synthesis site, plays an essential role in replication (Fig. 1).

In conclusion, inhibitors may be designed to target the RNA-binding site residues. Furthermore, both NTD and CTD are important targets, as they facilitate viral RNA binding and replication. M protein-binding residues may also be a potential target that could be used to break the physical linkage between the E and ssRNA. Inhibition of the N protein-Nsp3 interaction may also block viral replication. In a broader sense, RTCs, which consist of 16 Nsps, play the most essential role in the synthesis of viral RNA and may provide good targets. N proteins may be used as vaccines due to their conservation and immunogenic properties.

Nonstructural Protein 15

The ORFs rep1a and rep1b of SARS-CoV-2 RNA encode 2 large polypeptides (pp1a and pp1ab) [13] that are processed into 3C-like protease and papain-like protease, also called Nsp5 and Nsp3. Processing of pp1a and pp1ab results in 16 viral Nsps [48] that assemble into RTCs. These complexes are involved in multiple functions that range from replication to the processing of polypeptides [49–57].

Among all the Nsps, Nsp15 is a nidoviral RNA uridylate-specific endoribonuclease (NendoU) that belongs to the EndoU family and possesses a C-terminal catalytic domain. EndoU enzymes exist in all types of organisms and function as RNA endonucleases on phosphodiesters and hydroxyl termini [58]. The prototypic member is XendoU (*Xenopus laevis*), and it is involved in small nucleolar RNA maturation [59–62].

NendoU is conserved in several viruses, including coronaviruses, and its function is to respond to the innate immune system [63]. In addition to EndoU activity, evidence in animal models suggests for an immunomodulating property during early viral infection. Nsp15 is playing a leading role in suppressing the IFN-α/β associated innate immune response and avoiding detection of viral mRNA. Nsp15 is a critical component in the life cycle of the coronavirus, is well conserved, and (88% sequence identity) exhibits high similarity (95%) to SARS-CoV-2 and its closest homolog from SARS-CoV [62].

Structural studies have revealed that Nsp15s folds into dimers of trimers, which then form hexamers. A total of ~345 residues combine into the monomeric unit, and this unit, further, folds into the N-NTD, the middle, and the C-terminal (NendoU) catalytic domain.

The structure of SARS-CoV-2 Nsp15 contains 1 chloride and 1 magnesium ion, 3 acetate ions, water molecules (346), and 8 molecules of glycerol. The structure of a given monomer possesses 3 distinct domains. The NTD consists of β1, β2, and β3 strands surrounding helices α1 and α2. The middle domain consists of β5–β8, β12–β13, and a mixed β-sheet containing β4, β9–β11, β14, and β15 along with 3 short helices (α3, η4, and α5). The NendoU domain containing the C-terminal catalytic region possesses 2 β-sheets with edges that contain the active sites. Monomers of NendoU join into a double-ring hexamer (Fig. 3. According to a previous report, hexamer creation is critical for proper catalytic activity. Stabilization of the hexamer requires the interaction of oligomers within each subunit domain and NTD oligomerization. The main differences between SARS-CoV-2 and SARS-CoV exist in the middle domains.

NendoU Active Site

Nsp15 catalytic activity is facilitated by the NendoU domain. The active site residues, H235, H250, K290, T341, Y343, and S294, are conserved among SARS-CoVs. The main framework of the NendoU CTD and the active site residues (except K290) are well conserved among SARS-CoVs. Residues 235H, 250H, and 290K are involved in catalytic triad formation [64].

NendoU specificity is controlled by S294 and Y343, and these residues correspond to the base recognition roles of F120 and T45 (pyrimidine base B1 sub-site specific site) in RNAse A [65]. Mn^{2+} dependency has been suggested for nearly all members of the EndoU family with the exception of Nsp11, which did not show such a response [66]. Although the metal-binding site was not detected, it is still essential to note the lack of a protein/RNA complex structure. From the recently published structure of Nsp15 [62], there are 2 subunits in which the presence of electron density was observed at the peak near the active site, and this was uncertainly modeled as Mg^{2+}, despite the poor coordination sphere. The metal ion is correlated by D283, S262, and P263 followed by R258, a side-chain residue in the neighborhood. These 4 amino acids are conserved, and they constitute the metal-binding site in SARS enzymes that are essential for holding and retaining the active site and configuration substrate during catalytic activity. NendoU binds RNA, while large substrates can approach these 6 sites from the site of the hexamer. These sites, together with metal binding, are potential drug targets (Table 1).

In conclusion, the hexamer clearly represents the active form of Nsp15. Structural comparisons between SARS and MERS-CoVs Nsp15s suggest that druggable compounds targeting Nsp15 might have a good chance of blocking SARS-CoV-2 replication inside the host cells. The CTD of NendoU contains a conserved active site (H235, H250, K290, T341, Y343, and S294), and along with the catalytic triad H235, H250, and K290, it is another potential site that can be targeted by inhibitors. Moreover, S294 and Y343 govern "U" specificity, which corresponds to the base recognition roles of F120 and T45, and the region containing these residues presents another attractive site for druggable compounds.

Nonstructural Protein 9

Nsp9-SARS plays a significant role in viral replication during the infection of human cells [67]. In various coronavirus counts, porcine delta virus (Nsp9 PDCoV), porcine epidemic diarrhea virus (Nsp9 PEDV), avian infectious bronchitis virus (Nsp9 IBV), human coronavirus 229E (Nsp9-HcoV), and SARS-Cov-2 (Nsp9-COV-19), and the homologs of the Nsp9 protein have been recognized. For long oligonucleotides, Nsp9-SARS appears to possess unobtrusive proclivity, thus restricting the idea that function is reliant upon oligomerization state [68, 69]. By means of a conserved α-helical "GxxxG" motif, the Nsp9-SARS dimerizes in solution. Interruption of key buildups within this process weakens RNA-binding [69]. Amongst beta coronaviruses, the order of Nsp9 homologues is preserved, and information regarding their residues can potentially provide useful contrasts among various viruses. Nsp9-COV-19 displays 97% sequence uniqueness to Nsp9-SARS; however, it displays only 44% similarity to Nsp9-HCoV.

The structure of apo-Nsp9-COV-19 displays an infrequent fold similar to other Nsp9 homolog folds that is currently thought to represent the exterior of coronaviruses [69]. From the center of a fold that exists as a small 6-stranded enclosed β-barrel, a sequence of stretched loops ventures outward. The discrete β-strands of the barrel are connected by elongated loops that exist alongside an anticipating N-terminal β-strand and C-terminal α1-helix, and the last 2 components make up the funda-

Table 1. Recently released structures, their active sites, and potential drug targets

Proteins or complex	PDB id	Function	Active site/essential residues	Ref.	Some other drug targets
N	6YI3	RNA binding	Ala50, Thr57, His59, R89, Arg92, Ile94, Ser105, Arg107, Arg149, and Tyr172	[14, 43]	Nsp3-N interaction site RTCs
Nsp9	6W9Q	RNA binding	Asn33, Gly100, Met101, Val102, Leu103, Gly104, and Ser105	[91]	Helical GxxxG interaction motif β2–3- and β3–4-loops are glycine rich and integral for RNA-binding
Nsp15	6VWW	RNA binding	His235, His250, Lys290, Thr341, Tyr343, and Ser294	[65]	Ser294 and Tyr343 govern "U" specificity RNA base recognition residues, Phe120 and Thr45
M^{pro}	6M03, 6LU7	Cleaving pp1a and pp1ab	Cys145 and His41	[83]	Residues involved in dimerization Residues A285 and L286, render enhanced catalytic property Sequence recognition site LQ▼(S, A, and G)
RdRp	7BV1 (apo RdRp complex) 7BV2 (template RNA and Remdesivir bound RdRp complex)	SARS-CoV-2 RNA synthesis	S759, D760, and D761	[86]	Nsp7-Nsp8 heterodimer Catalytic active center (residues 759–761)

SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; RTC, replication-transcription complex; N, nucleocapsid; M^{pro}, main protease; Nsp, nonstructural protein.

mental segments of the dimer interface. From the open face of the barrel, 2 loops extend, and both the β2–3- and β3–4-loops are glycine rich, intricate in RNA-binding, and are positively charged.

In various viruses, the organization of monomers in Nsp9-dimers is well preserved, and this is maintained inside Nsp9-COV-19. The self-connotation of the rationed GxxxG protein-protein restricting motif is the primary component of the inter-subunit interaction that facilitates backbone van der Waals interactions among interfacing copies of the C-terminal α1-helix [70]. These communications were reproduced after a complete helical turn by Gly104 within the separate chains, consequently shaping the molecular basis of the Nsp9-COV-19 dimer interface. When contrasting apo-Nsp9-COV-19 and 3C-Nsp9-COV-19, it is clear that the N-terminal interface strand responsible for separation is in close proximity to L9 (Fig. 4). Within the apo structure, van der Waals interactions occur through the side chains of N33, M101, and S105.

The function of Nsp9 is important for the proliferation of SARS-CoV. It remains to be determined if Nsp9-COV-19 plays a comparable role in SARS-CoV-2; however, the observed 97% sequence similarity suggests a high level of practical preservation. The CoV-Nsp9 proteins apparently exist as dimers that contain a unique fold that that possesses an unusual α-helical GxxxG interaction motif, and this represents a good antiviral target, as it is required for viral replication. When examining Nsp9 antiviral targets, the side chains of N33, M101, and S105 are significant as it closely mimics the rationed protein-binding motif (33N, 100G, 101M, 102V, 103L, 104G, and 105S). Additionally, the β2–3- and β3–4-loops are glycine rich, integral for RNA-binding, positively charged, and exist as potential hot-spot regions.

Main Protease

One of the most attractive drug targets within SARS-CoV-2 is the M^{pro}($3CL^{pro}$) due to its vital role in processing the polyproteins translated from SARS-CoV-2 RNA. M^{pro} remains the best-characterized target protease (M^{pro}, also called $3CL^{pro}$) [71–75] along with papain-like

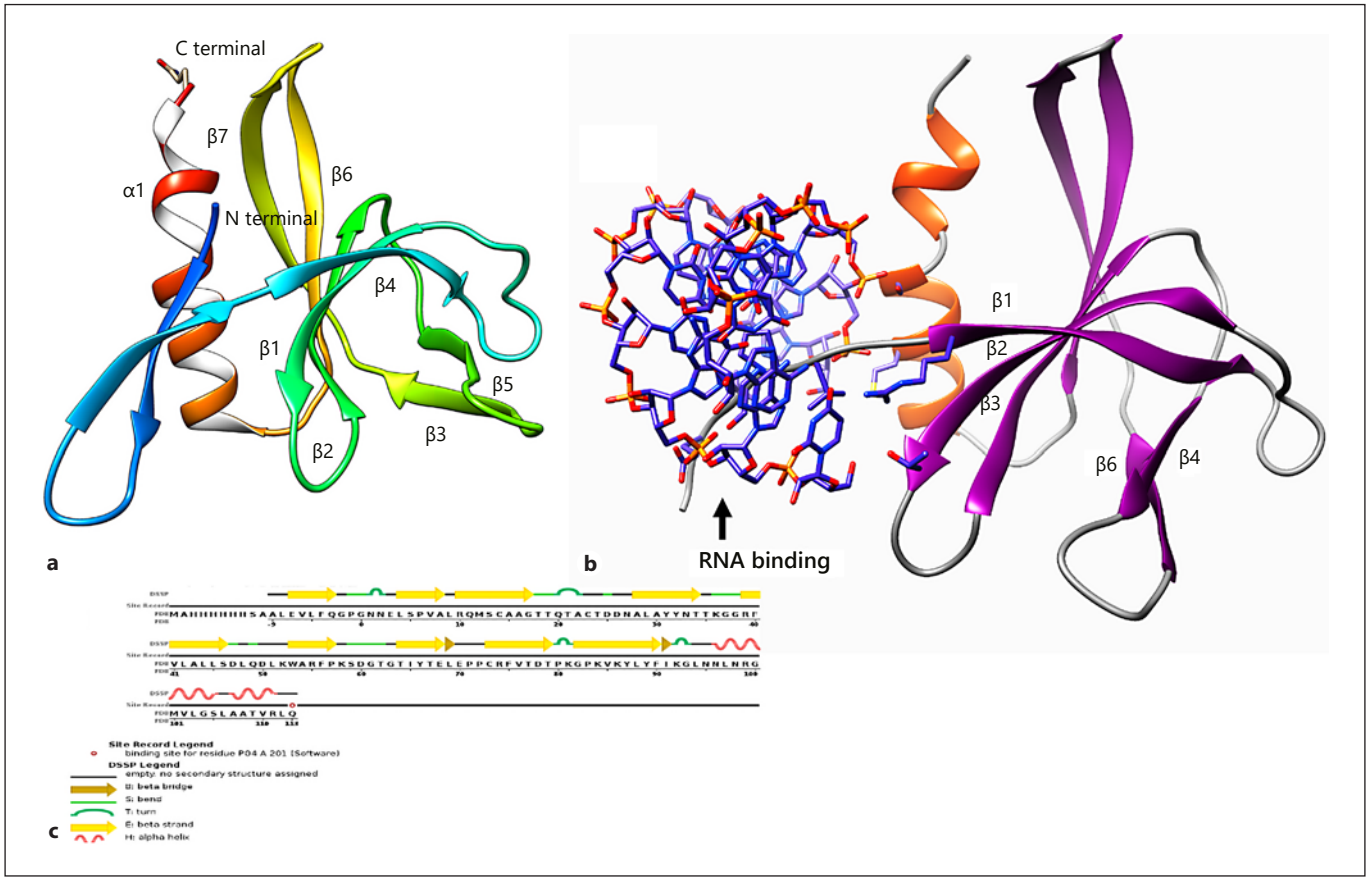

Fig. 4. a Structure of Nsp9. **b** β2–3- and β3–4-loops are glycine rich and integral for RNA-binding. β2–3-and β3–4-loops are glycine rich and are involved in RNA-binding. **c** Secondary structure of Nsp9. Nsp, nonstructural protein.

protease(s) [48, 49, 76]. M^{pro} acts on approximately 11 cleavage locations within polyprotein 1ab. The sequence recognition at most sites consists of LQ▼(S, A, and G) ("▼" shows cleavage site). Inhibitors are likely to be nontoxic to human replication, as human proteases do not share similar cleavage specificity.

The substrate-binding sites, 3C protease-like residues 10–99 and 100–182 (domains I and II) in picornavirus, are six-stranded antiparallel β-barrels that harbor the substrate between them. Residues 198–303 form domain III that consists of 5 helices that regulate the dimerization of the M^{pro} between Glu290 and Arg4 of different protomers primarily through salt bridges [77]. Amino-acids C145 and H41 form the catalytic site. The M^{pro} of SARS-CoV-2 exhibits a tight dimer that creates a contact interface between domain II and the NH2-terminal amino acids ("N-finger") of molecules A and B, respectively (Figure 5. Catalytic activity depends on the dimerization of the enzyme, as the N-finger interacts with Glu166 to facilitate the S1 pocket shape of the substrate-binding site [78].

In CoV-2, residue T285 is substituted by A285, and I286 is substituted by L286 Figure 5 [79]. Substituting S284, T285, and I286 for alanine in M^{pro} led to a threefold increase in enzymatic activity [80].

The SARS-CoV M^{pro} (T285, I286) is different from SARS-CoV-2 (A285, L286) due to the catalytic properties conferred by the residue substitutions at 285 and 286. The catalytic properties may be reduced by designing active inhibitors against these locations. Inhibitors are more likely to be toxic if they block the cleavage site (LQ▼[S, A, and G]) specific to SARS-CoV-2, as human proteases do not share a similar cleavage specificity (Table 1). In a more recent study [81], 2 lead compounds have been synthesized (11a and 11b), targeting M^{pro}, and exhibited good activity as anti-SARS-CoV-2. The crystal structures

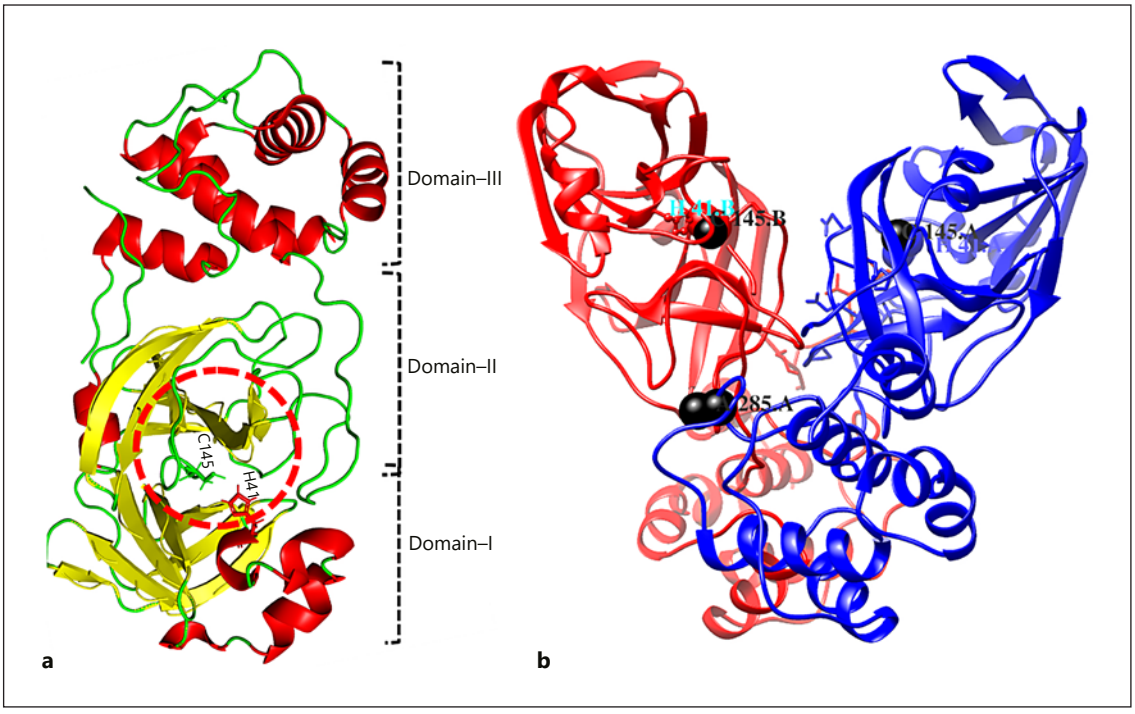

Fig. 5. Domain organization of M^{pro}. **a** The 3 domains are shown as domain I (residues 8–101), domain II (residues 102–184), and domain III (residues 201–303). **b** Dimers. Catalytic residues (C145 and H41) are circled. In SARS-CoV-2, T285 is replaced by A285 (black balls) and Ile286 is replaced by leucine. SARS-CoV-2, severe acute respiratory syndrome coronavirus-2

of M^{pro} in complex with 11a or 11b demonstrated good binding affinity with C145 of SARS-CoV-2 M^{pro}. These compounds also exhibited a good pharmacokinetic result in vivo, suggesting that these are potential drug candidates.

RNA-Dependent RNA Polymerase

The polymerase enzymes called RNA-dependent RNA polymerase (RdRp) is playing a key role in corona viral transcription and replication assembly and thus seems as a foremost target for antiviral drug such as remdesivir [82]. Recently, the cryo-EM structure of SARS-CoV-2 RdRp has been released in the apo form (2.8 Å resolution) and in complex (2.5 Å resolution) with a 50-base template-primer RNA and remdesivir [83]. At the central channel of the RdRp, the partial double-stranded RNA template is inserted in Figure 6. This insertion is basically at the first replicated base pair and terminates chain elongation, where remdesivir is covalently incorporated into the primer strand. This structure gives basic bits of knowledge into the component of viral RNA replication and a balanced format for medicate configuration to battle the viral infection.

The catalytic subunit (Nsp12) of an RdRp is the essential constituent of this complex. Nsp12 alone has a little action and its capacities require adornment factors including Nsp7 and Nsp8 [84], which increment RdRp template binding and processability. RdRp is likewise proposed to be the objective of a class of antiviral medications that are nucleotide analogs, including remdesivir [85]. The remdesivir is a prodrug that is converted to the active drug in the triphosphate form (RTP) [86]. The purified Nsp12 demonstrated little activity in binding to a 50-base partial double-stranded template-primer RNA [87]. The binding of Nsp12 to the template-primer RNA is dramatically expanded by the existence of Nsp7 and Nsp8.

Upon addition of adenosine triphosphate, the Nsp12-Nsp7-Nsp8 complex likewise indicated RNA polymerization activity on a poly-U template. By the addition of the active triphosphate form of remdesivir (RTP), this RNA polymerization activity was viably hindered. The apo RdRp complex is composed of unique structure that

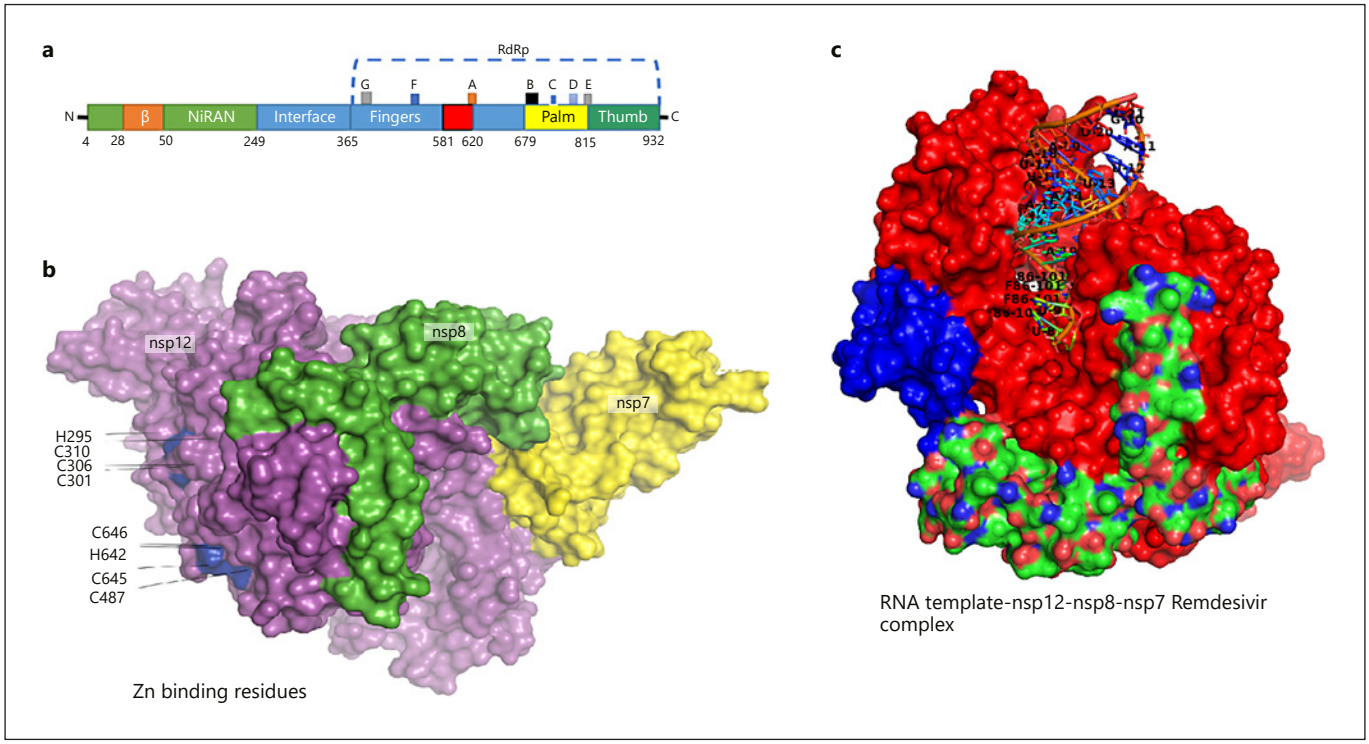

Fig. 6. Structure assembly of Nsp12-Nsp7-Nsp8 complex in SARS-CoV-2. **a** Organization of SARS-CoV-2 domains. **b** Zn binding residues. **c** Complex structure containing active site residues for RNA template access and remdesivir (F88). SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; Nsp, nonstructural protein.

contains 1 Nsp12, 1 Nsp7, and 2 Nsp8. Unique in relation to the SARS-CoV RdRp structure, the SARS-CoV-2 RdRp structure additionally contains an N-terminal β-hairpin (residues 31–50), with 3 β-strands and 7 helices. An interface domain (residues 251–365) is subsequent the NiRAN domain and is comprised of 3 helices and 5 β-strands, which is associated with the RdRp domain (residues 366–920). The canonical cupped right-handed configuration is displayed by the Nsp12 RdRp domain, in which the finger subdomain (resides 397–581 and residues 621–679) creating a closed circle with the thumb subdomain (residues 819–920). Binding of Nsp7 and Nsp8 stabilizes the closed conformation, with 1 Nsp8 molecule sitting on the top of the finger subdomain and, furthermore, collaborating with the interface domain. The Nsp7-Nsp8 heterodimer further stabilizes the closed conformation of Nsp12, which is packed beside the thumb-finger interface. In the conserved metal-binding motifs, 2 zinc ions, which are also observed in the SARS-CoV RdRp structure have been assigned and are composed by H295, C301, C306, C310, C487, H642, C645, and C646. In keeping up the integrity of the RdRp architecture, these zinc ions likely serve as preserved structural components.

The template-RTP RdRp complex has a unique structure composed of 1 Nsp12, 1 Nsp7, and 1 Nsp8. In the final model, the second Nsp8 was not included as it was largely invisible in the EM map of the template-RTP complex. Furthermore, the template-RTP RdRp structure contains inhibitor remdesivir in its monophosphate form (RMP), and it also contains 14-base RNA in the template strand and 11-base RNA in the primer strand. At the primer strand, the inhibitor (RMP) is covalently linked, as well as 3 magnesium ions and a pyrophosphate that may attend as catalytic ions close to the active site. Although the 2 proteins (Nsp7 or Nsp8) are required for RNA binding by RdRp, surprisingly no RNA interactions are mediated by these proteins. The RMP is located at the 3′ end of the primer strand, which is covalently unified into the primer strand at the +1 location. Supplementary nucleotides interrelate with residues from the back of finger subdomain at the +2 and +3 locations of the template strand. Just a single RMP is assembled into the primer strand regardless of the presence of surplus RTP in com-

plex assembly. Accordingly, remdesivir, in the same way as other nucleotide analog prodrugs, hinders the viral RdRp activity through nonobligate RNA chain termination, a process that necessitates the transformation of the parent medication to the active triphosphate form [88, 89]. The catalytic active center is formed by the SDD sequence (residues 759–761) in motif C. At the catalytic center, both D760 and D761 are engaged in coordination of the 2 magnesium ions. The location of motifs F and G is within the finger subdomain Figure 6a and both interrelate with the template strand RNA and direct this strand into the active site. Motif F, thus, stabilizes the incoming nucleotide in the correct position for catalysis as it can interact with the primer strand RNA with the side chains of K545 and R555 contacting the +1 base. Other than remdesivir, a few nucleotide analog drugs, counting galidesivir, favipiravir, EIDD-2801, and Ribavirin, effectively hinder SARS-CoV-2 replication in cell-based measures [90, 91]. These nucleotide analogs are proposed to repress the viral RdRp as remdesivir with the help of nonobligate RNA chain termination, a process that necessitates the alteration of the parent compound to the triphosphate active form.

Conclusion

Although there are many investigations underway to identify potential drugs and vaccines against the SARS-CoV-2 pandemic, drugs, and vaccines that possess desirable properties may only be designed when accurate target information is available. In the current review, multiple targets have been identified after careful analysis of recently released structures, specific to SARS-CoV-2. Despite identifying several drug targets in recent studies, we suggest that there are many more targets that are conserved and should urgently be considered when designing vaccines and potential inhibitors. Further studies are required to define the role of a number of important enzymes and complexes such as RTCs to allow for improved management of the current pandemic.

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Conflict of Interest Statement

The authors declare that they have no competing interests.

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Conceptualization: D.Q.W. and M.T.K. Data curation: M.T.K., A.A., A.C., and S.C. Formal analysis: M.T.K., S.C., A.A., and M.I. Manuscript: A.A., M.I., M.T.K., A.A., H.A., and A.S.K. Funding acquisition: D.Q.W. Supervision and Approval: D.Q.W.

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