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SARS-CoV-2 nucleocapsid protein: Importance in viral infection

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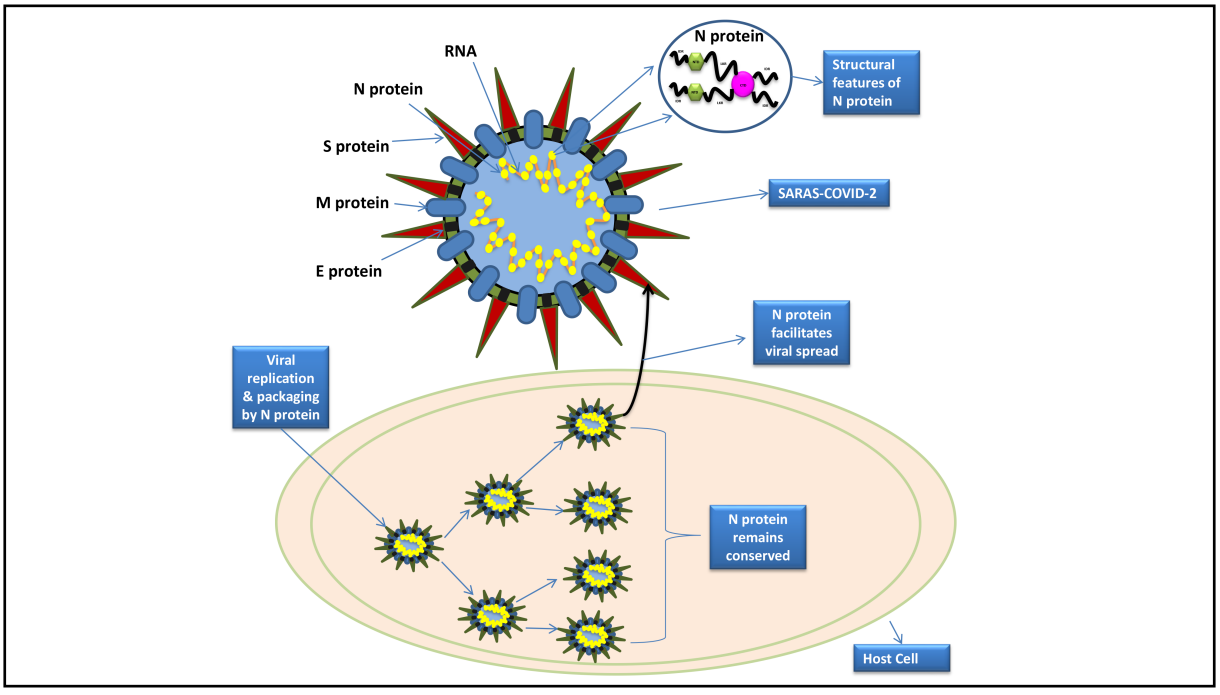
Graphical abstract



Structure and functions of SARS-CoV-2 nucleocapsid protein.

Public summary

- SARS-CoV-2 nucleocapsid (N) protein is an important structural and multifunctional protein.
- N protein of SARS-CoV-2 consists of five major domains including N terminal tail, N-NTD, LKR, N-CTD, and IDR.
- N protein acts as a multifunctional protein by playing roles in genome packaging, RNA chaperoning, protein transport, DNA degradation, interfering with host translation, and limiting host immune responses.
- N protein is an important target for T-cell activation and vaccine design.

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Abstract: The coronavirus disease 2019 (COVID-19) epidemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused millions of deaths worldwide. Therefore, it is critical to understand the biological basis of SARS-CoV-2 to develop novel approaches to control its spread. The SARS-CoV-2 nucleocapsid (N) protein is an important diagnostic and potent therapeutic target of the disease, as it is involved in numerous important functions in the viral life cycle. Several studies have explained the structural and functional aspects of the SARS-CoV-2 N protein. This review summarizes the currently available data on the evolutionarily conserved N protein of SARS-CoV-2 by providing detailed information on the structural and multifunctional characteristics of the N protein.

Keywords: SARS-CoV-2; COVID-19; nucleocapsid protein; vaccine design; therapeutic targets

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1 Introduction

Coronaviruses (CoVs) have been responsible for various diseases in humans and animals for the past two decades, including respiratory complexities in humans and bronchitis in chickens[1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently emerged coronaviruse[2]. To date, SARS-CoV-2 has spread globally, causing the respiratory infection currently known as coronavirus disease-2019 (COVID-19), that is characterized by fever, muscle pain, and dyspnea[3, 4]. Although CoVs infections were reported to cause mild respiratory diseases characteristically, the SARS-CoV-2 outbreak is highly pandemic and deadly. According to the WHO, as of November 26, 2021, 259,502,031 COVID-19 cases with 5,183,003 deaths had occurred worldwide (https://covid19.who.int/)[3, 5]. Recent progress in vaccine development has provided powerful protection against this pathogen. However, owing to the emergence of mutant coronavirus strains, it continues to be a threat to public health and the global economy[6]. Therefore, it is necessary to understand the fundamental biology of SARS-CoV-2 and use advanced therapeutic approaches to target the basic mechanisms of viral infection to provide an open route for first-line intervention to prevent future pandemics.

Coronaviridae family consists of two subfamilies, *Letovirinae* and *Orthocoronavirinae*. *Orthocoronavirinae* contains four genera: α-, β-, γ-, and δ-coronaviruses[7, 8]. Among these, only β-coronaviruses can infect humans and cause severe respiratory diseases[9]. To date, seven human CoVs have been identified, including HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, MERS-CoV, SARS-CoV-1, and SARS-CoV-2[10]. A typical coronavirus genome has a conserved 5 leader sequence and is the largest among different families of RNA viruses. The genomic size of SARS-CoV-2 is approximately 30,000 nucleotides that encode 29 proteins, of which 16 are nonstructural proteins (NSP), 4 are structural proteins, and the remaining 9 are accessory proteins[8, 11, 12].

During the viral life cycle, RNA-dependent RNA polymerase (RdRp) directs the replication and transcription of the positive-sense RNA genome[9, 13]. Generally, during replication, RNA synthesis is a continuous process, as complementary negative-strand RNA is obligatory to act as a template strand to synthesize genomic RNA (gRNA). However, CoVs require irregular sub-genomic RNAs (sgRNAs) for transcription. The RdRp complex synthesizes a set of identical sub-genomic mRNAs (sgmRNAs) in the viral genome via a template switching mechanism[14, 15]. This irregular transcription is under the control of a specific transcriptional regulatory sequence (TRS) that comes after a well-conserved 5′ leader and is present anterior to each open reading frame (ORF) gene (TRS-B). During negative-strand RNA synthesis, base pairing between TRS-L and complementary TRS-B strands occurs[9]. This base-pairing triggers template-switching events, resulting in the synthesis of discontinuous negative-strand RNA, which later serves as a template strand for synthesizing massive amounts of discontinuous positive-strand sgmRNAs,

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which encode the chief structural proteins of the virus, comprising membrane (M), spike (S), envelope (E), nucleocapsid (N), and numerous other auxiliary proteins[9, 16, 17].

Among the four major structural proteins of SARS-CoV-2, the N protein is thought to be highly immunogenic and is abundantly expressed during viral infection[18–20]. According to the WHO, all SARS-CoV-2 variants contain at least one mutation, of which more than 50% lie within seven amino acids (N:199–205) of the N protein[21]. The N protein of CoVs is immunogenic and plays important roles in the packaging of the viral genome, RNA chaperoning, transport of intracellular proteins, degradation of DNA, interfering with the host translation, and limiting host immune responses[22, 23]. According to an estimate, 1,000 copies of N proteins are present in each virion compared to only 100 copies of the S protein[24]. During viral infection, viral RNA and N proteins enter the host cell together, and the N protein eases viral replication by helping to process the assembly of viral particles and their release[25]. As a result, due to its importance in the viral life cycle, abundance in infected cells, and stability, N protein is used for developing vaccines and serological assays[26]. Currently, only a few reports have focused on the SARS-CoV-2 N protein. Consequently, it is imperative to update our understanding of this protein and its therapeutic importance.

2 Composition and structure of SARS-CoV-2 N protein

2.1 Structural domains of SARS-CoV-2 N protein

The N protein of SARS-CoV-2 is highly conserved and has structural and functional homology of 89.7% with that of SARS-CoV-1[27, 28]. The sequence characteristics of the N protein depict a modular organization similar to that of other CoVs and can be allocated as conserved and intrinsically disordered regions (IDRs)[1]. The N protein of SARS-CoV-2 consists of five major domains, including an N-terminal tail encoded by amino acid residues 1–40, an N-terminal domain (N-NTD) encoded by residues 41–173, a linker region (LKR) rich in Ser/Arg encoded by residues 174–249, a C-terminal dimerization domain (N-CTD) coded by residues 250–364, and a C-terminal IDR encoded by residues 365–419 (Fig. 1)[29–32]. N-NTD (which acts as an RNA-binding domain) and N-CTD have a high concentration of positive amino acids that facilitate N protein binding to the viral genome[33, 34]. Ser/Arg-rich LKR domains enhance oligomerization of the protein chain[35]. The structural features of N-NTD and N-CTD from various CoVs have been resolved, but due to complex oligomerization, trouble in upholding protein stability, and high flexibility of the disordered region, the molecular characteristics of full-length N protein remain to be elucidated[1, 36].

2.2 Sequence and localization of SARS-CoV-2 N protein

The genomic size of SARS-CoV-2 (GenBank: MN908947, Wuhan-Hu-1 coronavirus) is 29.9 kb, similar to the 27.9 kb of SARS-CoV-1 and 30.1 kb of the MERS-CoV genome[2, 37, 38]. Complete domain architecture comparison of the SARS-CoV-2 N protein with N proteins of other CoVs, such as SARS-CoV-1, MERS-CoV, and HCoV-OC43, revealed that SARS-CoV-2 N possesses topographies similar to those of other CoVs. Moreover, structural insight into the complete genomic sequence of the SARS-CoV-2 N protein-coding region showed sequence identities of 35.62%, 48.59%, and 89.74% with the genomes of HCoV-OC43, MERS-CoV, and SARS-CoV, respectively[32, 39–42].

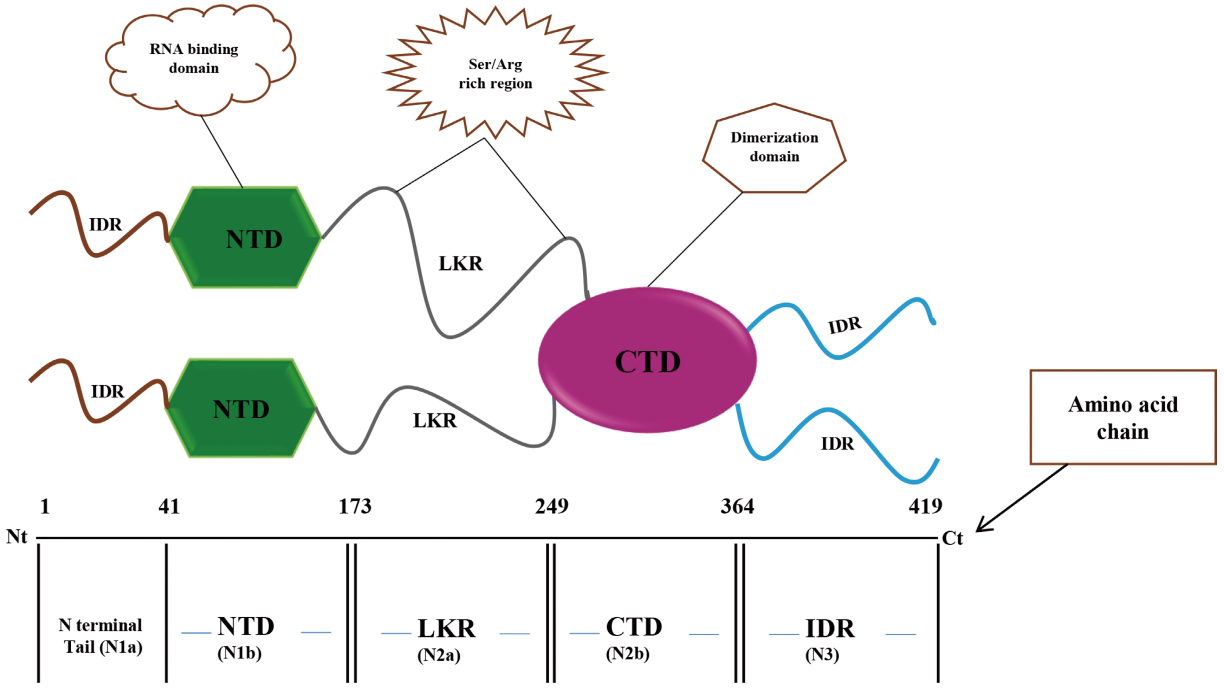

Fig. 1. Structural model of SARS-CoV-2 N protein. Major structural domains of N protein with their sequence of amino acids in protein chain have been represented by different colors in the figure. The N-NTD and N-CTD are represented in green and purple, respectively. While IDR, LKR, and C terminal IDR are represented in brown, black, and blue, respectively.

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N proteins can be found in the cytoplasm or both the cytoplasm and nucleolus in CoVs-infected cells[43]. Subcellular localization analysis revealed that the N protein was chiefly distributed within the nucleus and present in small quantities within the cellular membrane and cytoplasm (Table 1). Moreover, fewer N proteins are expected to be dispersed within cell vesicles, suggesting a distribution of viral particles in the human body using these cell vesicles[41, 42].

2.3 Structural dynamics of SARS-CoV-2 N protein

The secondary structure of the SARS-CoV-2 N protein examined in various studies indicated that it comprises 21.24% α-helix, 16.71% β-fold, 6.92% β-turn, and 55.13% random coils[41, 42, 44]. Of the 419 amino acids of the SARS-CoV-2 N protein chain, 231 were localized within the random coils, forming the chief secondary structure of the N protein. Moreover, the secondary structure comparison of the SARS-CoV-2 N protein with SARS-CoV-1 and MERS-CoV N proteins displayed an extraordinary resemblance[41]. A high-quality tertiary structure of the SARS-CoV-2 N protein designed by global quality estimate results, QMEAN, and local quality estimate scores is worthy of consideration[42].

Structural dynamics studies have revealed significant molecular details of both the N-CTD and N-NTD of the SARS-CoV-2 N protein[45, 46]. In a recent study[47], the crystalline structure of the N-NTD determined at a resolution of 2.7 Å revealed that the overall structure of N-NTD is similar to that of other CoVs, but the values of the surface electrostatic potentials are distinct between them. The overall structural dynamics of each N-NTD is a right-handed fist shape consisting of a β-hairpin, β-sheet core, and long-loop region. The core region comprises U shaped 5 antiparallel β-strands and one short 3_{10} -helix just before β2, whereas the long β-hairpin is a large bulging loop between β2 and β5 strands, as shown in Fig. 2b[1, 8, 47]. The N-NTD has abundant basic and aromatic residues and is structurally similar to a protruded basic finger, acidic wrist, and basic palm[47].

Table 1. Structural composition and cellular localization of SARS-CoV-2 N protein[41].

Formula	$C_{1971}H_{3137}N_{607}O_{629}S_7$
Total amino acids	419
Theoretical pI	10.07
Molecular weight	45.6 kDa
Total charged residues	
Negatively (Asp + Glu)	36
Positively (Arg + Lys + His)	60
Assessed half-life	
Escherichia coli	>10 h (vivo)
Yeast	>20 h (vivo)
Mammalian reticulocytes	30 h (vitro)
GRAVY	− 0.971
Aliphatic index	52.53
Instability index	55.09
Subcellular localization (KNN)	
Nuclear localization	69.6%
Cytoplasmic localization	21.7%
Cytoskeletal localization	4.3%
Secretory vesicles	4.3%

The crystalline structure of the N-CTD of SARS-CoV-2 N protein resolved at 2.0 Å by X-ray crystallography has demonstrated that it also differs in its surface electrostatic potential characteristics, compared to N-CTDs of other CoVs[32], which predicts the role of N-CTD in binding with viral RNA and regulating its transcriptional activities. In SARS-CoV-2 N-CTD, two "C-shaped" monomers combine to form a stable dimer with an overall rectangular slab shape. One monomer of N-CTD consists of five α-helices, two β-strands, and three 3_{10}-helices. Additionally, there is also one α_0-helix in the N-terminus, detected by the partial electron density of the traced molecule[32]. The β-hairpin from one monomer of the N-CTD is interleaved into the cavity of the other monomer, forming four-stranded antiparallel β-sheets at the dimer interface[1, 44].

3 Functional aspects of SARS-CoV-2 N protein

Although all four structural proteins of CoVs play a role in virion assembly and infection, N protein is critical for viral genome replication and dispersion. The two domains of the N protein can bind to the viral genome via different mechanisms. The N protein binds to NSP3, tethers the genome to RTC, and causes genome packaging in virions[30, 48]. Additionally, the N protein functions as an interferon antagonist (IFN) and a virally encoded repressor of RNA interference (RNAi), thus facilitating viral replication. SARS-CoV-2 N proteins play a role in regulating the viral life cycle by interacting with the host's intracellular mechanisms[49].

3.1 Encapsulation of genomic RNA and cell cycle

The primary role of the N protein is to recognize and bind to the viral genome for packaging into large flexible helical ribonucleoprotein (RNP) complexes called nucleocapsid[1]. Both dsRNA and ssRNA bind to the basic β-hairpin and the core of the N-NTD, adopting the same machine where the positively charged Arg residues (R92, R107, and R149) are abundant[50]. Moreover, there is a positively charged groove in SARS-CoV-2 N-CTD consisting of K256, K257, K261, and R262 residues, and the N-terminal IDR and LKR with their RNA-binding ability also facilitate RNA binding and packaging[1]. The viral nucleocapsid shields the genome and ensures its regular replication and transmission. The length of the filamentous nucleocapsid is several hundred nanometers, and its diameter ranges from 10 to 15 nm[51]. The N protein interacts with viral RNA in the viral nucleocapsid, which is known as the N-RNA interaction[52]. This interaction is facilitated by specific binding signals present in the leader RNA sequence[53]. The role of the N protein in genome transcription and translation can be predicted by the interaction of several N proteins with gRNA and sgRNAs during the viral life cycle[25]. A recent study that analyzed the biological functions of SARS-CoV-2 N protein over the cell cycle showed that cells transfected with SARS-CoV-2 N protein showed high

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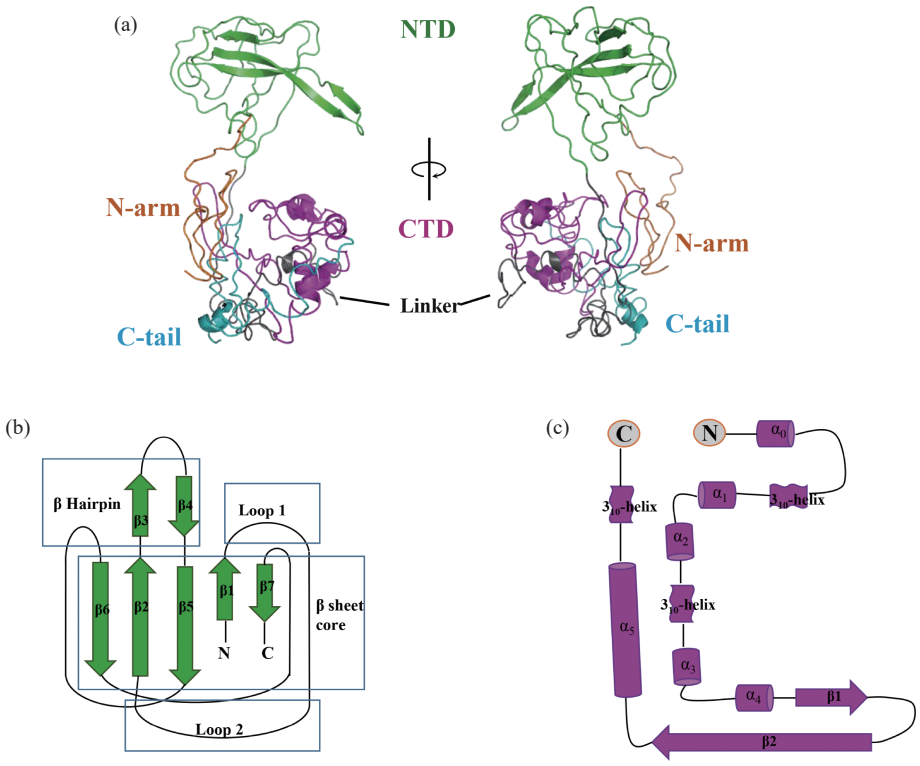

Fig. 2. Structural features and representation of SARS-CoV-2 N protein. (a) Structure of SARS-CoV-2 N protein[42]. The N-NTD and N-CTD are represented in green and purple, respectively. The other structural domain, including N-tail, NTD-CTD linker, and C-tail, are presented by brown, black, and blue, respectively. (b) Schematic representation of N-NTD of SARS-CoV-2 based on the SARS-CoV-2 N protein structure presented in (a). (c) Schematic representation of N-CTD of SARS-CoV-2 based on the SARS-CoV-2 N protein structure presented in (a). (b) and (c) are colored as in (a).

expression of proteins such as TUBA1C TUBB6, which is associated with the cell cycle or mitotic regulation. Cell cycle analysis performed using the SARS-CoV-2 N protein plasmid showed that positively transfected cells had higher rates of G1 and lower rates of S or G2 phases compared with non-transfected cells[41]. This validates the blockage of the G1/S phase of the cell cycle by the involvement of the N protein. The deregulation of the cell cycle caused by the N protein offers a better situation for binding to the viral genome and creating a ribonucleocapsid that ultimately endorses viral replication, transcription, and translation[54].

The dimeric assemblage of the N protein is mainly governed by N-CTD, a characteristic feature necessary for creating viral nucleocapsids[55, 56]. The high-resolution SARS-CoV-2 N-CTD crystal structure verified its compact strand-swapped dimer in solution[44]. CoV structural models have proposed that the N protein forms a helical nucleocapsid and is also present in the inner globular core of the virus[57]. The internal core of CoVs is composed of three components: RNA, the N protein, and the CTD of the M protein. The M protein of CoVs has a 16 amino acid chain (237–252) on its CTD that binds with N protein by ionic interactions, leading to encapsidation of the genome of the freshly budding viral particles[58]. Thus, the N protein is vital for the virion life cycle because of its interaction with both genomic RNA and M protein.

3.2 Role of N protein in RNP formation

In infected cells, RdRp must coordinate with other viral and host factors to produce both viral mRNAs and new genomes[59]. The crystal structures of the NTD and CTD of N proteins of mouse hepatitis virus (MHV), infectious bronchitis virus (IBV), human CoV 229E, and SARS CoV-1 exhibited similar topological organization[60, 61]. N protein dimers arrange themselves into octamers through their CTD, which further assemble into large oligomeric structures that exist constitutively[62]. These oligomers provide the binding surface for the finest entrapping of the large viral gRNA, where the CTD forms the core of the N protein, and the NTD decorates protein surfaces[63, 64]. The resulting ribonucleoprotein complexes (N protein-gRNA complexes) are incorporated into the newly formed viral particles through interactions with the C-terminus of the M protein[65].

3.3 Viral particles protection from host immune response

To protect against microbial infection and dispersion, the host's innate immune system acts as the first line of defense by recognizing and removing infected cells[66]. However, viruses have evolved to protect viral particles from host immune cells by developing antagonists such as glycosylation-based shields or by producing viral proteins that can interfere with the host immune response[67]. Host cells carry antiviral defense mechanisms that inhibit viral RNAi replication by destroying the viral genome[68]. Viral particles produce viral suppressors of RNAi (VSRs) which antagonize the RNAi pathway. Similar to other viral proteins, the SARS-CoV-2 N protein antagonizes RNAi in several ways. The N protein impounds dsRNA in the infected cells by exerting antagonist effects in the effector step to protect viral dsRNA from recogni-

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tion and destruction by Dicer[69].

Moreover, RNA viruses such as SARS-CoV-2 interact with the RIG-I-like receptor and initiate the pathway for IFN-β production, which is a major mechanism of the innate immune system of the host against invading viral particles[70]. SARS-CoV-2 ORF6, ORF8, and N proteins are strong antagonists of interferon[71, 72]. In SARS-CoV-2, N protein interaction with RIG-I receptors is important for immunostimulatory binding of RNAs[73]. Usually, COVID-19 patients produce insufficient interferon type-I (IFN-I) (immunosuppression) during the initial stage of infection, while producing cytokines abundantly in the later stages of infection[74, 75]. Another recent study revealed that a small dose of the SARS-CoV-2 N protein suppresses inflammatory cytokines and inhibits IFN-I signaling. However, a large dose of N protein stimulates IFN-I signaling and the production of inflammatory cytokines[76], suggesting that a low concentration of N protein or the initial stage of COVID-19 infection causes suppression of the IFN response in the host cells by targeting the RIG-I receptors and RNA recognition mechanism, which are the initial steps of IFN activation. Overall, these data suggest that the N protein acts as a VSR in CoVs for evasion from immune cells and thus protects the viral particles.

3.4 Liquid-liquid phase separation (LLPS) of SARS-CoV-2 N protein enhances viral transcription and assembly

LLPS helps organize biological substances into sections. During virion assembly, dense protein-nucleic acid compartments are formed by sequestering host cell proteins, protecting the host immune system, and increasing the replication efficiency[77]. The structural topographies of the N proteins are similar to those of other proteins that undergo LLPS with nucleic acids. In various in vitro studies, the SARS-CoV2 N protein showed phase separation with RNAs tuned by pH, salt, and RNA concentration[29, 78, 79]. Phosphorylation of the SR region of N proteins modifies their RNA-induced phase-separation properties. The non-phosphorylated N protein develops more gel-like condensates by protein-protein and RNA-protein interactions, while the phosphorylated N protein reduces these interactions, producing a more liquid-like compartment that enhances viral genome processing and endorses the protein's transcription, but the underlying mechanism is still unknown[80]. In one study, the formation of LLPS by N protein and RNA relied on the size and concentration of ssRNA[81]. The N protein forms spherical droplets with short ssRNAs and solid-like structures with long ssRNA, suggesting that N protein/RNA LLPS is vital for the viral assembly of SARS-CoV-2 and can be targeted to develop strategies for the prevention of the COVID-19 pandemic.

3.5 SARS-CoV-2 N protein as an important diagnostic target

Rapid detection of pathogens is essential to prevent the spread of infection. The well-known genomic sequence of SARS-CoV-2 has enabled the development of various diagnostic tests for viral detection. One fast diagnostic test employed during the COVID-19 epidemic was reverse transcription-polymerase chain reaction (RT-PCR)[82]. Nevertheless, the sensitivity of the test was not consistent and was dependent on the testing time in relation to time of exposure, thus resulting in false-negative results[83]. Subsequently, serological tests were performed.

Studies on the serum of patients with COVID-19 have shown that the N protein of SARS-CoV-2 can act as a potent diagnostic marker of COVID-19[84–86]. Similar to other CoV N proteins, the SARS-CoV-2 N protein abundantly triggers an early antibody response in patients with COVID-19, making the N protein an efficient target for developing specific immunological tests[26, 87]. Currently, serological tests for COVID-19 are performed using immunoassays to assess immunoglobulin IgG and IgM[88, 89]. Detection of antibodies against N protein can be a useful tool for identifying individuals before COVID-19 infection because it can take 2–3 weeks to produce specific antibodies against viral particles in patients with COVID[90]. Western blot and dot blot analyses of the patient sera with serial dilutions of antibodies specific to the N antigen showed the presence of IgG, IgA, and IgM immunoglobulins specific to the N protein[42]. A clinical study[91] revealed that SARS-CoV-2 N protein can be detected in patient serum with high specificity (97%) and sensitivity (92%), suggesting that N protein detection in plasma/serum can be valuable for the precise and prompt diagnosis of COVID-19. Recently, highly sensitive immunosensors have been developed for assessing N proteins in serum at concentrations as low as 50 pg/mL in total blood serum and at a concentration of 10 pg/mL in 5X diluted serum[85].

Furthermore, more efficient chemiluminescent magnetic bead-based automatic label-free immunoassays have been developed to rapidly detect N proteins with high sensitivity (up to 69 fg/mL), which is far better than the previously described methods for N protein detection[92, 93]. Presently, the FDA had approved two antigen tests, Sofia 2 SARS Antigen FIA and BD Veritor System, to recognize N protein in nasopharyngeal swab samples[85]. Overall, these data demonstrate that the N protein is an efficient diagnostic target for the early and quick detection of SARS-CoV-2 infection, and these strategies should be further validated in more patients.

3.6 SARS-CoV-2 N protein as a component in vaccine

Extensive research has been conducted on COVID-19 treatment; however, effective clinical treatment remains unavailable. The N protein of CoVs is evolutionarily conserved and plays a vital role in replicating and transmitting the virus; therefore, it can be a promising tool for gene-targeted drug discovery. Since the N protein is crucial for RNP formation and replication of the viral genome, inhibiting the RNA-binding activity of the N protein can be an important strategy to control COVID-19. However, a few small molecules such as PJ34 and H3 target the RNA-binding site of N-NTD were proven to prevent HCoV-OC43 replication[44] and can also be considered for inhibiting SARS-CoV-2. Remarkably, some of the important residues of the N protein that are critical for RNA binding, including S51, F53, R107, Y109, Y111, and R149, are preserved among CoVs[1], suggesting an efficient target against COVID-19. In a recent study, 17 different phenanthridine derivatives were synthesized to target the N protein, and two of these compounds were reported to act-

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ively target N-NTD by binding to Y109 and inhibiting SARS-CoV-2 replication in vitro[94].

Additionally, hindering normal RNP formation or blocking oligomerization of the N protein can also be an effective strategy for inhibiting viral growth. Recently, Lin et al. acknowledged a unique inhibitor named 5-benzyloxygramine (P3) for MERS-CoV, which mediates non-native dimerization of N-NTD and causes protein aggregation owing to its hydrophobic properties[44, 95]. Considering that the N protein is conserved among CoVs, this compound can also be used for targeting SARS-CoV-2 and inhibiting viral spread. In a recent study, it was proposed that the N protein chain R203K/G204R has a higher propensity to undergo LLPS, while gallocatechin gallate (GCG), which is a polyphenol compound extracted from green tea, can disturb the formation of LLPS and thus hinder viral replication, suggesting its use to control viral spread[96]. Overall, N protein-based therapeutic strategies should be considered clinically to control the COVID-19 pandemic and prevent future pandemics.

4 Conclusion and perspectives

This review summarizes the current progress in understanding the structural and functional aspects of the N proteins of SARS-CoV-2 and their contribution to viral replication and dispersion of infection. The molecular insight on the N protein could enable us to develop strategies for the efficient treatment of patients with COVID-19 and its epidemiological control.

Considerable insights have been revealed concerning the assembly and function of the N protein of β-CoVs, and subsequent outbreaks of SARS-CoV-1 and MERS-CoV in 2003 and 2012, respectively[8]. SARS-CoV-2 has been recognized as the etiological agent of COVID-19. The modular structural organization of the SARS-CoV-2 N protein is remarkably similar to that of other CoVs, including N-NTD, N-CTD, and IDRs. Recent studies have revealed that the SARS-CoV-2 N protein acts as a multifunctional protein that binds with RNA and is accountable for packaging viral genomic material and regulating innate immune responses[1, 32]. However, it is still unknown how the N protein expresses RNP and performs its functions throughout the virus's life cycle; thus, potential vaccination and antibiotics for the inhibition and management of SARS-CoV-2 remain unavailable.

Extensive research is being conducted worldwide to understand major structural and functional proteins of SARS-CoV-2 that can provide us with major information for the overall understanding of its mechanism of infection and replication[8]. Additionally, for the new therapeutics development against SARS-CoV-2, structure-based drug discovery could be a promising approach. Many ongoing studies have attempted to develop a treatment for COVID-19 by targeting the spike protein and other viral proteases, including papain-like and 3C-like proteases[47]. Several vaccines against COVID-19 have been permitted and are available in the market, but all are specifically based on S protein invasion[97]. However, with the progression of the epidemic, various mutations in the S protein of SARS-CoV-2 have been reported[54] that can jeopardize previously implemented vaccine countermeasures. Therefore, there is a need to pay more attention to a well conserved protein, i.e., the N protein. Recent studies have demonstrated that the N protein of SARS-CoV-2 could be an effective therapeutic target because it performs several critical functions in the viral life cycle and its dispersion from infectious cells[32, 98]. High concentrations of IgG against the N protein were detected in the sera of patients affected by COVID-19; thus, it acts as a diagnostic marker for the infection[99, 100]. Although considerable research has been conducted on SARS-CoV-2 throughout the COVID-19 pandemic, the molecular foundation and many other areas related to the N protein of SARS-CoV-2 still need to be elucidated, which may later play a role in the development of a better tool for preventing such pandemics in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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