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LETTER TO THE EDITOR

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Subversion of host stress granules by coronaviruses: Potential roles of π-rich disordered domains of viral nucleocapsids

To the Editor,

A recent article by Benvenuto et al[1] reported that some sites of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike (S) and nucleocapsid (N) proteins are undergoing episodic selection. The S protein directly interacts with angiotensin-converting enzyme 2 (ACE2) receptor, playing a crucial role in virus host cell entry.[2] Through this letter, we would like to highlight potential roles of the N protein in subverting host cellular processes and therefore, contributing to CoV pathogenesis.

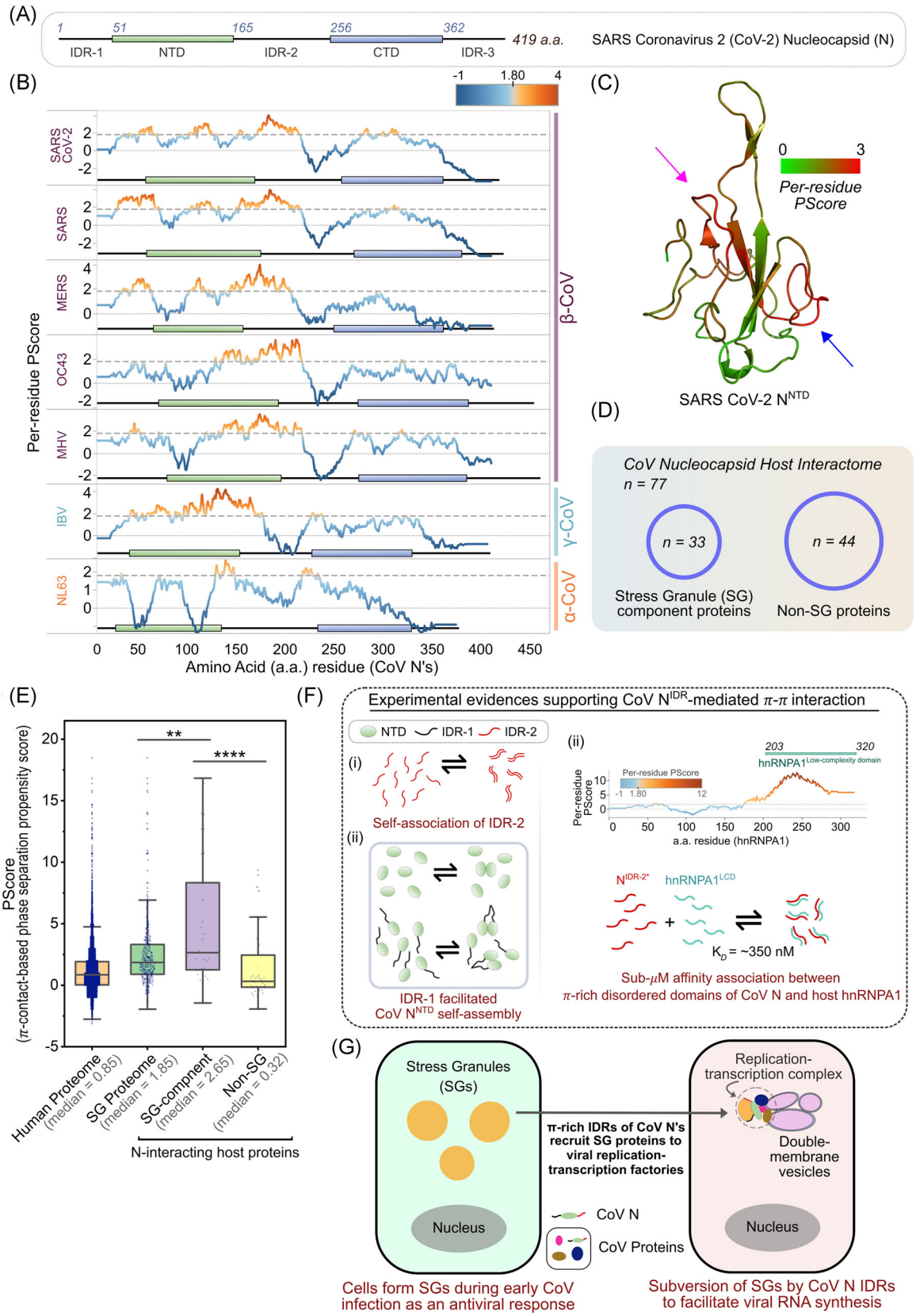
Among the CoV structural proteins, N is unique in the sense that it performs both structural and nonstructural roles during viral life-cycle.[3] In the assembled virion, N binds to the RNA genome in a beads-on-a-string fashion (structural role). Later in the infection-cycle, N associates with viral replication-transcription complex (RTC) to play crucial roles in viral RNA synthesis (nonstructural role).[4]

Bioinformatics analysis of SARS-CoV-2 N indicated that the protein is composed of three intrinsically disordered regions (IDRs) that are separated by two folded domains: the N-terminal domain (NTD) and the C-terminal domain (CTD) (Figure 1A). Since recent advances indicate that many IDRs are involved in liquid-liquid phase separation (a process that drives the formation of many subcellular dynamic assemblies, reversible condensates, and signaling hubs),[5] we analyzed the primary sequences of CoV N's from different CoV genera (*Alphacoronavirus*, *Betacoronavirus*, and *Gammacoronavirus*) for their phase separation potential (Figure 1B). We observe that the first two IDRs of CoV N's exhibit significant phase separation propensity as predicted by the PScore tool.[6] We also observed that two distinct surfaces of folded CoV N NTD are enriched in residues with relatively high per-residue PScore (Figures 1C and S1). Since PScore predicts phase separation propensity based on π/sp^2-contact potential,[6] we infer that π-rich residues of CoV N play crucial roles in viral pathogenesis.

Next, based on previous reports, we identified CoV N-interacting host proteins (n = 77, Table S1; see Supporting Information Method for details). Among these 77 proteins, 33 are known stress granule (SG) components (Figure 1D). SGs are cytoplasimc phase separated membraneless organelles that are part of cellular anti-viral response.[7] Interestingly, these SG-component N-interacting proteins have statistically significant higher PScore distribution when compared with known SG proteins (Figure 1E). Taken together, these analyses suggest that π-π-interactions between CoV N and host proteins are relevant to viral N-mediated host subversion.

Multiple lines of experimental observations support our model (Figure 1F). First, π-rich IDR-2 can self-associate,[8] possibly via homotypic π-π interactions. Second, the presence of IDR-1 facilitates self-interaction of SARS N,[9] again plausibly via $N^{IDR\text{-}1}$-$N^{IDR\text{-}1}$ interactions. Third, the π-rich IDR-2 of CoV N interacts tightly with the π-rich disordered domain of hnRNPA1,[10] an archetypical SG-component protein. Finally, the crucial role of hnRNPA1 in CoV RNA synthesis can be functionally replaced by other hnRNP family members that have similar per-residue PScore profiles (Figure S2). [11]

Based on our bioinformatics analyses and experimental observations reported in literature, we propose that CoV N IDRs recruit host SG-component proteins to viral RTCs (Figure 1G). It is possible that this recruitment is through preferential partitioning of SG proteins into biomolecular condensates of CoV N proteins themselves, attached on double-membrane vesicle surfaces. Alternatively, CoV N IDRs can directly associate with SG proteins as previously observed for hnRNP family members. In conclusion, our analyses point to the crucial roles of CoV N IDRs in the viral hijacking of host machineries.


(A)
1
51
165
256
362
419 a.a.
IDR-1
NTD
IDR-2
CTD
IDR-3
SARS Coronavirus 2 (CoV-2) Nucleocapsid (N)
(B)
-1
1.80
4
SARS CoV-2
SARS
MERS
OC43
MHV
IBV
NL63
β-CoV
γ-CoV
α-CoV
Per-residue PScore
Amino Acid (a.a.) residue (CoV N's)
(C)
0
3
Per-residue PScore
SARS CoV-2 N^NTD
(D)
CoV Nucleocapsid Host Interactome
n = 77
n = 33
Stress Granule (SG) component proteins
n = 44
Non-SG proteins
(E)
PScore
(π-contact-based phase separation propensity score)
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Human Proteome (median = 0.85)
SG Proteome (median = 1.85)
SG-compnent (median = 2.65)
Non-SG (median = 0.32)
N-interacting host proteins
(F)
Experimental evidences supporting CoV N^IDR-mediated π-π interaction
NTD
IDR-1
IDR-2
(i)
Self-association of IDR-2
(ii)
IDR-1 facilitated CoV N^NTD self-assembly
(ii)
203
320
hnRNPA1 Low-complexity domain
Per-residue PScore
a.a. residue (hnRNPA1)
N^IDR-2*
hnRNPA1^LCD
K_D = ~350 nM
Sub-μM affinity association between π-rich disordered domains of CoV N and host hnRNPA1
(G)
Stress Granules (SGs)
Nucleus
π-rich IDRs of CoV N's recruit SG proteins to viral replication-transcription factories
CoV N
CoV Proteins
Replication-transcription complex
Double-membrane vesicles
Nucleus
Cells form SGs during early CoV infection as an antiviral response
Subversion of SGs by CoV N IDRs to facilitate viral RNA synthesis

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CONFLICT OF INTERESTS

All the authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

FIGURE 1 Interaction of coronavirus (CoV) nucleocapsid protein (N) with host proteome. A, Domain organization of SARS-CoV-2 nucleocapsid protein. B, CoV IDRs are enriched in amino acid residues that can partake in π-π interactions. π-π contact score predicted by the PScore prediction tool[6]; C, Two distinct surfaces of the folded NTD of CoV N are enriched in residues with relatively high PScore values. SARS-CoV-2 N^{NTD} (PDB: 6VYO) structure with color coded residues by per-residue PScore. Similar surface PScore patterns were also observed for other CoV N's (Figure S1). D, Stress granule (SG) component proteins account for ~43% (33 out of 77) of CoV N-interacting host proteins (see Table S1). E, CoV N-interacting SG-components have a high phase separation propensity, whereas non-SG proteins have low overall PScore ($P < .01$ for the SG proteome and N-interacting SG-component pair, $P < .0001$ for the SG-component and non-SG component N-interacting protein pair [Mann-Whitney test]). F, Experimental results supporting the role of π-rich IDRs in CoV N molecular associations — (i) murine hepatitis virus (MHV) $N^{IDR\text{-}2}$ can self-associate,[8] (ii) presence of IDR-1 facilitates self-interaction of SARS N[9] and (iii) the high PScore block of IDR-2 (SARS $N^{IDR\text{-}2*}$) associates with the π-rich low-complexity domain of hnRNPA1 (hnRNPA1^{LCD}) with sub-μM affinity.[10] G, A model for nucleocapsid IDR-mediated recruitment of SG-components to viral replication-transcription complex (RTC). Recruitment of SG components to viral RTCs might serve two distinct purposes: (i) SG-component RNA-binding proteins may facilitate viral RNA synthesis through their RNA processing/stabilization roles and (ii) by sequestering SG-component proteins into viral RTCs, CoVs may disrupt or compositionally alter cytoplasmic SGs that are typically antiviral.[7] α-CoV, *alphacoronavirus*; β-CoV, *betacoronavirus*; γ-CoV, gammacoronavirus; CTD, C-terminal domain; IDR, intrinsically disordered region, NTD, N-terminal domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2