Biochemical Society Transactions (2024) **52** 481–490
https://doi.org/10.1042/BST20231119

PORTLAND PRESS

Review Article

The art of hijacking: how Nsp1 impacts host gene expression during coronaviral infections

Evangelos D. Karousis[1,2]

[1]Multidisciplinary Center for Infectious Diseases, University of Bern, Bern, Switzerland; [2]Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Bern, Switzerland

Correspondence: Evangelos D. Karousis (evangelos.karousis@unibe.ch)

Non-structural protein 1 (Nsp1) is one of the first proteins produced during coronaviral infections. It plays a pivotal role in hijacking and rendering the host gene expression under the service of the virus. With a focus on SARS-CoV-2, this review presents how Nsp1 selectively inhibits host protein synthesis and induces mRNA degradation of host but not viral mRNAs and blocks nuclear mRNA export. The clinical implications of this protein are highlighted by showcasing the pathogenic role of Nsp1 through the repression of interferon expression pathways and the features of viral variants with mutations in the Nsp1 coding sequence. The ability of SARS-CoV-2 Nsp1 to hinder host immune responses at an early step, the absence of homology to any human proteins, and the availability of structural information render this viral protein an ideal drug target with therapeutic potential.

Introduction

In recent years, the emergence of SARS-CoV-2, the coronavirus responsible for the COVID-19 pandemic, has drawn significant attention to the intricate interactions between viruses and their host cells [1]. All viruses rely on host mechanisms to produce new viruses, and they have evolved strategies to hijack multiple steps of gene expression. After a eukaryotic mRNA is transcribed and processed in the nucleus, it is transported to the cytoplasm, where ribosomes are located. The mRNA export is tightly regulated and ensures that only correctly processed transcripts are translated. Translation, the process by which ribosomes synthesize proteins, initiates when mRNA is threaded through the entry channel of the small 40S ribosomal subunit and is scanned until aligning at an initiation mRNA codon with the P site of the ribosome [2]. Joining of a 60S subunit yields a functional 80S ribosome, which catalyzes multiple elongation steps to form a peptide. Following the recognition of a termination codon, the two ribosomal subunits dissociate. After several rounds of translation, the life cycle of mRNA molecules is completed by degradation, a step that acts as another important contributor to gene expression regulation [3]. Ribonucleases degrade RNA molecules at specific sites (endonucleases) or exonucleolytically, leading to mRNA decay. The coordination of mRNA export, translation, and degradation is crucial for proper protein synthesis and overall cellular function, enabling the cell to respond effectively to various signals and maintain homeostasis [4]. Viruses have evolved intricate mechanisms to use the host gene expression machinery to their benefit [5].

RNA viruses, including SARS-CoV-2 have evolved impressive mechanisms to inhibit host cell translation and promote viral protein synthesis [5–7]. When SARS-CoV-2 infects a host cell, the 30 kb-long single-stranded capped and polyadenylated viral RNA genome is translated to produce non-structural proteins (Nsps) (Figure 1) [8]. Opposite to structural proteins that form new viral particles, Nsps are essential for synthesizing genomic RNA and propagating viral replication by modulating and often inhibiting cellular processes [8].

Nsp1, a notable non-structural protein of SARS-CoV-2, exemplifies this by inhibiting host cell translation, stimulating the degradation of host cell mRNA and affecting the export of mRNA from the nucleus to the cytoplasm (Figure 1).

Received: 18 January 2024
Revised: 14 February 2024
Accepted: 14 February 2024

Version of Record published: 22 February 2024

Biochemical Society Transactions (2024) **52** 481–490
https://doi.org/10.1042/BST20231119

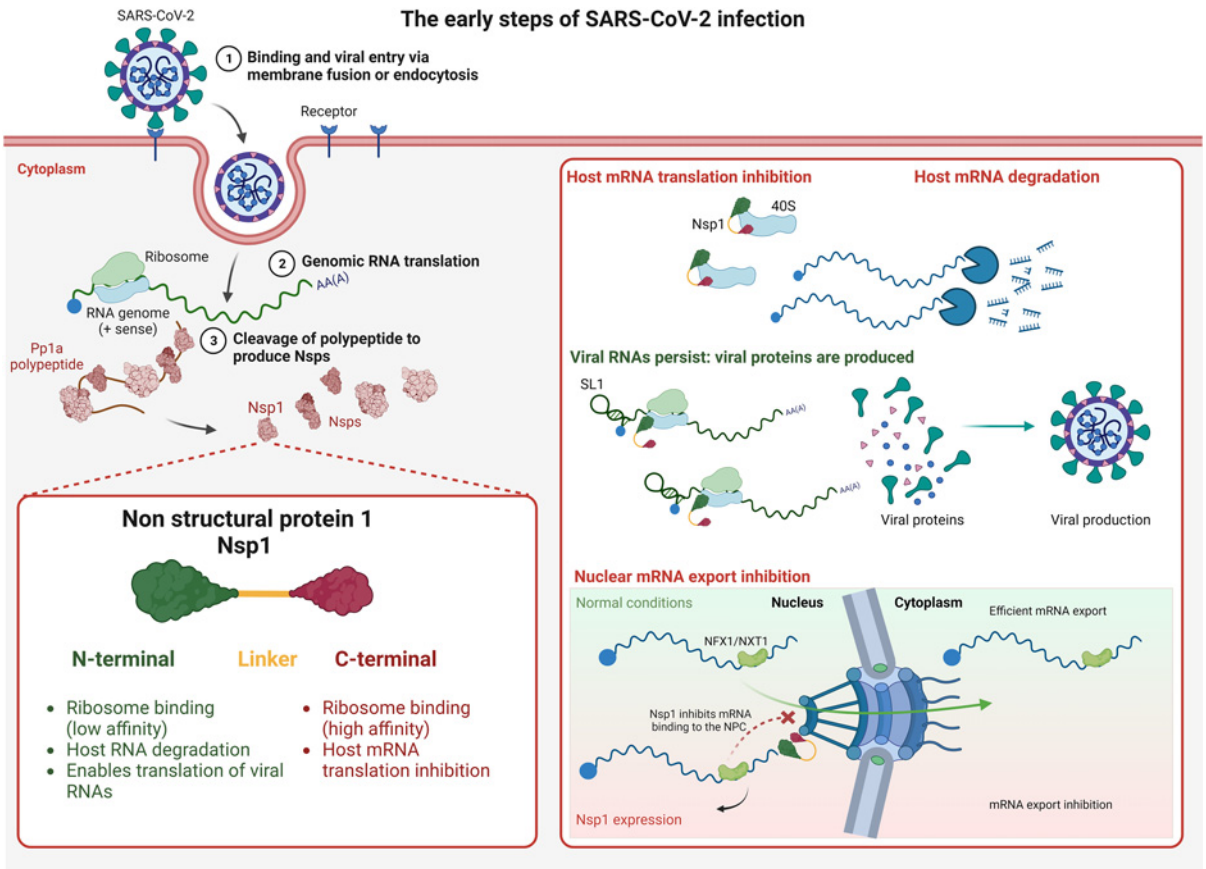


Figure 1. Structural and functional features of Nsp1 (left) and biological functions of Nsp1 (right).

Different biochemical and molecular biology techniques have contributed to understanding how viral biomolecules affect the host molecular mechanisms. RNA sequencing and ribosome profiling report on host and viral mRNA abundance and translation status, providing a holistic overview of host cell gene expression when viral components are expressed [9,10]. Mass spectrometry allows the characterization of the protein interactome [11,12], and single-molecule fluorescence assays monitor individual molecular events, revealing nuanced interactions between viral components and host cell machinery [13]. Other biochemical experiments like cell-free translation allow the careful titration of viral components and observe their effects [14,15]. These are only some of the approaches that have been put forward to dissect the various roles of Nsp1 *in vitro* and *in vivo*.

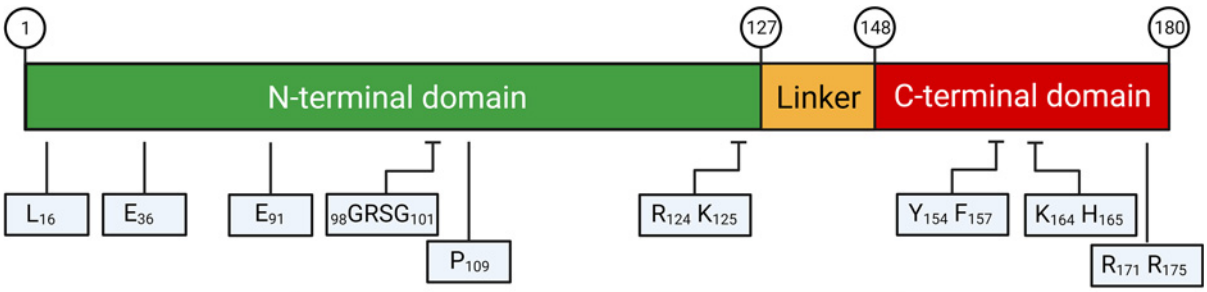
This review delves into the complex role of SARS-CoV-2 Nsp1, and expands in discussing the features of Nsp1 from different Betacoronaviruses including the highly pathogenic relatives SARS-CoV and MERS-CoV [16].

A functional and biochemical portrait of Nsp1

Nsp1 is the first protein produced upon infection, and among other non-structural coronaviral proteins, it is the most toxic when it is overexpressed in human cells [17]. This toxicity is attributed to the capacity of Nsp1 to inhibit host cell translation and stimulate host cell mRNA degradation [18–22], observations that were first made for the SARS-CoV Nsp1 counterpart [23,24]. A primary goal of host translation inhibition is to reduce the expression of antiviral proteins, including interferons (IFN) and interferon-stimulated genes (Figure 1) [18,22]. At the same time, viral RNAs are immune to Nsp1 translation inhibition and degradation, shifting the gene expression output of the host cell to the benefit of viral propagation [25,26]. Nsp1 can also block nuclear RNA export, and there are indications that it affects host mRNA splicing [26].

The SARS-CoV-2 Nsp1 protein sequence is divided into three parts: an N-terminal domain (NTD) (residues 1–128), a 20 amino acid-long linker (128–148) and the C-terminal domain (CTD) (148–180) (Figure 2). The short N-terminal and long C-terminal tails (residues 1–9 and 128–180, respectively) are mostly unstructured

Biochemical Society Transactions (2024) **52** 481–490
https://doi.org/10.1042/BST20231119



Residues	Function	Source
L_{16}	Translation inhibition	Shehata et al., 2023
E_{36}	RNA decay	Shehata et al., 2023
E_{91}	RNA decay	Abaeva et al., 2023
$_{98}GRSG_{101}$	Translation inhibition	Schubert et al., 2023
P_{109}	RNA decay	Shehata et al., 2023
R_{124} K_{125}	Translation inhibition RNA decay	Mendez et al., 2021 Schubert et al., 2023
Y_{154} F_{157}	Translation inhibition RNA decay	Schubert et al., 2020
K_{164} H_{165}	Translation inhibition RNA decay	Thoms et al., 2020 Schubert et al., 2020
R_{171} R_{175}	Translation inhibition RNA decay	Schubert et al., 2020

Figure 2. Functional residues of Nsp1 associated with mRNA translation or mRNA degradation.
A table listing the relevant residues follows a color code, with green corresponding to NTD residues and red related to CTD residues.

and flank a globular core domain formed by the NTD residues 10–127 [19,27]. When the Nsp1 CTD binds the 40S ribosomal subunit, amino acids 154–180 form two short α helices [17–19] (Numbering refers to the accession NC_045512, version NC_045512.2).

The mechanism of host translation shutdown

The Nsp1 CTD binds the mRNA entry channel of the 40S subunit, hindering the accessibility of host mRNAs and leading to translation initiation inhibition (Figure 2) [18,19,28]. Because eukaryotic mRNA translation relies on recruiting the 40S ribosomal subunit to the template mRNA [2], Nsp1 suppresses the global translation of the host with direct consequences on viral propagation because the host cell translation machinery is employed to promote the production of viral proteins [20,22]. This strategy is shared among different Betacoronaviruses, as shown by structural and biochemical studies using Nsp1 from MERS [29,30] and the Bat-Hp-CoV_Zhejiang2013, referred to as Bat-Hp Coronavirus [30]. In all cases, the C-terminal end anchors Nsp1 with high affinity to the mRNA entry channel in a conformation that clashes with the translated mRNA. As a result, the global translation of host mRNAs is suppressed, including mRNAs that are important for antiviral responses, such as the host IFN response [18,22,26].

Biochemical data from Nsp1 proteins from different Betacoronaviruses show that the N-terminal domain of Nsp1 also contributes to translation inhibition [30–32]. Recently, an additional interaction of Nps1 with the 40S ribosomal subunit was reported. Cryo-EM structures revealed that the N-terminal part of Nsp1 from Bat-Hp Coronavirus interacts with the 40S decoding center, a crucial region for accurately matching tRNA molecules with the appropriate codons on the mRNA strand. Mutations of the corresponding amino acids in SARS-CoV-2 and MERS-CoV decrease the inhibition efficiency of Nsp1, implying that this interaction is conserved among the three Betacoronaviruses [30]. Nsp1 binds the 40S in a bipartite mode of interaction: stably through the CTD at the mRNA entry channel and, more weakly, NTD with the decoding center (Figure 1).

Biochemical Society Transactions (2024) 52 481–490
https://doi.org/10.1042/BST20231119

During canonical eukaryotic translation initiation, before mRNA recruitment, the 40S subunit is bound by several eukaryotic initiation factors (eIFs), including eIF1, eIF1A, eIF3, eIF5, and the ternary complex (TC) of eIF2–GTP–methionine initiator transfer RNA (Met-tRNA$_i^{Met}$) [33]. Single-molecule fluorescence experiments revealed an early association between Nsp1 and ribosomal pre-initiation complexes. This association is promoted by eIF1, which is involved in the correct positioning of the mRNA and the scanning process during translation initiation. The Nsp1-40S association can be outcompeted by eIF3j, a part of the eIF3 complex crucial for translation initiation [28]. Translation initiation factors (eIFs) allosterically modulate the interaction of Nsp1 with ribosomal pre-initiation complexes in the absence of mRNA, supporting the affinity of Nsp1 with early pre-initiation complexes, and in particular with eIF3g [34]. Nsp1 is also associated with pre-assembled 80S ribosomes on mRNA [18,19] but less efficiently [28]. Despite the association of Nsp1 with 80S ribosomes, the global elongation rate does not seem to be affected in cells overexpressing Nsp1 outside the context of infection [35,36].

Viral 5′UTRs escape Nsp1 translation inhibition

Translation of viral RNAs is not inhibited by Nsp1 [25,26,30,37]. All viral RNAs contain a 72-nt leader sequence at the very 5′ terminus, allowing them to bypass Nsp1-mediated translation inhibition. This portion of the genomic viral 5′UTR is also present in subgenomic RNAs and acts like a viral signature organized into three stem loops: SL1, SL2 and SL3 [38–41]. A sequence included in the stem-loop 1 (SL1), a *cis*-acting element encoded in all viral RNAs, is sufficient for evasion of Nsp1 inhibition [25,26,31,37,41]. The importance of SL1 is highlighted by the absence of single-nucleotide variants with >1% in frequency and the lack of known mutations among variants of concern among SARS-CoV-2 genomes. This evolutionary constraint renders SL1 an attractive therapeutic target against immune-evasive or particularly infectious strains [25]. The presence of SL1 at a specific distance from the 5′ cap acts as a switch that allows the translation of all viral RNAs that include SL1 [25,26,42]. Three cytosine residues at positions 15, 19, and 20 of SL1 and the amino acid residue R124 within Nsp1 are crucial for viral evasion [42]. At high concentrations of Nsp1, SL1-containing reporter mRNAs are also repressed, but at lower amounts, Nsp1 stimulates their expression [22,30,36,42]. This observation may explain the fact that in some cases Nsp1 was not shown to induce translation of viral-harbouring 5′UTRs [25]. Along these lines it would be interesting to relate these results, which originate mostly from *in vitro* assays with the intracellular concentration of Nsp1 during different stages of infection which remains elusive. Initiation factors eIF1 and eIF1A are important to promote accurate translation initiation on viral RNAs in the presence of Nsp1 [43]. Additionally, the global repression of host translation by Nsp1 may increase the pool of available translation factors and ribosomes supporting viral translation, further explaining the increase of viral mRNA translation in the presence of Nsp1. Such behavior is similar to other viruses that hijack host cell components to support their metabolism [5]. Interestingly, SL1 is the most variable region of the 5′UTRs among different β-Coronaviruses, implying a possible co-evolution with the corresponding Nsp1 proteins [44].

Different models could explain how SL1 of the leader sequence can allow translation of viral mRNAs: (1) SL1 could directly recruit free ribosomes, bypassing the need of scanning 40S subunits, (2) Nsp1 could bind to the viral RNA through the leader independently of its interaction with the ribosome and enhance recruitment of the ribosome, or (3) SL1 could allosterically modulate the Nsp1-ribosome interaction. According to the first hypothesis, SL1 would protect the mRNA from Nsp1 independently of its precise position within the 5′UTR. Contradicting this model, it was found that mRNA requires the 5′ leader to be precisely positioned relative to the Nsp1-bound 40S ribosome to enable translational initiation: changing the position of the leader led to translation inhibition in the presence of Nsp1 [26]. In addition, sequences in SARS-CoV-2 5′UTR cannot directly bind the 40S, unlike IRES-containing sequences from other viruses [37]. Interaction studies revealed that Nsp1 has no affinity for RNA [25,27,37], and Nsp1 evasion requires the presence of SL1 in *cis* on the mRNA [37].

Evidence shows that both the C-terminal and the N-terminal Nsp1 domains are required to shift from host to viral translation [25,30,31]. Indeed, structural evidence from Bat-Hp Nsp1 and biochemical evidence from SARS-CoV-2 and MERS-CoV indicating a bi-partite interaction with the ribosome with different affinities [30] supports the hypothesis that the leader may compete with one of the two interactions to alleviate translation inhibition. Polysome gradients from rabbit reticulocyte lysates and SARS-CoV-2 infected cells show that Nsp1 remains bound on the ribosome during translation of viral RNAs [37,43], however, additional experiments are needed to clarify the mode of interaction.

Despite the global translational shutdown, the translation of a subpopulation of host mRNAs persists in the presence of Nsp1 [35]. Matched RNA-seq and ribosome profiling experiments in Nsp1-expressing cells revealed

that a subset of genes that contain 5′-terminal oligopyrimidine (TOP) motifs escape translation inhibition. Transcripts that evade inhibition include translation machinery components like initiation factors, ribosomal proteins, cytoplasmic PolyA-binding protein 1 (PABPC1) and other factors needed for viral propagation. Experiments with reporter mRNAs verified the capacity of the TOP motif to evade Nsp1 inhibition, and additional evidence suggests that the resistance is mediated in an mTOR (Mammalian target of rapamycin)-related manner [35].

Host ribosomes can initiate translation from multiple different start codons in the 5′UTR of SARS-CoV-2 and not all of them are in frame with the main ORF [45–47]. Nsp1 likely interacts with a subset of translation pre-initiation complexes [18,28], affecting translation start site selection in an eIF1 and eIF1A-dependent manner [43].

Nsp1 may help balance virus and host protein production to keep the host cell functional enough for the virus to replicate. Along these lines, translation output may be tuned by Nsp1 and other mechanisms [6] to produce the maximally allowable viral copy number that could still avoid triggering antiviral responses [25].

Nsp1 degrades endogenous mRNAs

Besides inhibiting cellular translation, Nsp1 also induces host mRNA degradation, but SARS-CoV and SARS-CoV-2 Nsp1 alone do not seem to possess a ribonucleolytic activity [34,48]. The expression of Nsp1 in human cells is sufficient to target most of the host mRNAs for degradation [21,22,35,36,49]. Consistent with this observation, SARS-CoV-2-infected cells show an accelerated degradation of mature cytosolic cellular but not viral mRNAs in an Nsp1-dependent manner [22,50]. Similar to evasion from translation inhibition, the SL1 hairpin correctly positioned at the 5′ end of the viral transcripts is sufficient to protect from Nsp1-mediated degradation [50].

Diverse evidence suggests that Nsp1 binding to a translating ribosome is a prerequisite for host mRNA degradation. Nsp1 mutations that block binding to the ribosome also inhibit mRNA degradation (Figure 2) [31]. Furthermore, several studies showed that Nsp1 requires the mRNA to interact with ribosomes to induce degradation: Nsp1-induced mRNA degradation is more pronounced in actively translated mRNAs [22], and non-translated cytosolic long non-coding RNAs (lncRNAs) are resistant to Nsp1 degradation [49]. Data from cell culture experiments support that Nsp1-induced mRNA degradation requires the engagement of 40S or 80S ribosomes on mRNAs [49], and the N-terminal part of the protein is crucial for inducing mRNA degradation [50]. Amino acid substitutions at the NTD of SARS-CoV-2 Nsp1 (R125A/K126A) abrogate Nsp1-mediated mRNA cleavage while still inhibiting translation [31] and mutational analysis identified a positively charged surface on the NTD of Nsp1 that is essential for cleavage [34]. Nsp1 induces cleavage mainly within the 5′UTR and the proximal coding region of capped non-viral mRNAs [34,50]. The cleavage pattern does not seem to depend on the sequence but rather the relative position to the 5′cap [50].

The host cell exonuclease Xrn1 induces 5′–3′ mRNA degradation after SARS-CoV Nsp1 expression [51], and interactome studies from SARS-CoV-2 Nsp1 propose that the protein interacts with several cellular endo- and exonucleases [42]. However, Nsp1, 40S subunits and eIF3g suffice to induce mRNA cleavage in a reconstituted *in vitro* system [34]. It remains to be clarified which cellular factors are required for Nsp1-mediated endonucleolytic cleavage on non-viral mRNAs during an infection.

Overall, SARS-CoV-2 Nsp1 inhibits protein synthesis using a dual strategy: inhibiting translation initiation and inducing the degradation of host cell mRNAs. This dual activity of Nsp1 enables viral mRNAs to outcompete and dominate the mRNA pool, diverting host translation towards producing viral proteins. However, the precise mechanism of Nsp1-mediated mRNA cleavage remains largely unclear.

Nsp1 inhibits nuclear mRNA export

The export of mRNAs from the nucleus to the cytoplasm is an important step in mammalian gene expression as it is a prerequisite for the access of newly synthesized transcripts to the cytoplasmic translation machinery. Several viruses have evolved strategies to target this step [52]. SARS-CoV-2 infection blocks the nuclear export of host cell mRNAs [21], and this activity was attributed to Nsp1 [22,53], ORF6 [54–56] and Nsp14 [57]. Upon Nsp1 transfection, Nsp1 is mainly located in the cytoplasm, but a small subpopulation was found at or near the nuclear pore complex (NPC). Nsp1 directly binds the mRNA nuclear export factor NXF1, a protein that binds mRNA and interacts with the nucleoporins. Nsp1 weakens the interactions of NXF1 with the nuclear pore complex [42,53]. Specifically, Nsp1 impedes the binding of NXF1 to export adaptors and docks at the nuclear pore complex to prevent the export of cellular mRNAs, including IFN-encoding mRNAs (Figure 1)

Biochemical Society Transactions (2024) 52 481–490
https://doi.org/10.1042/BST20231119

[53]. Infection experiments with WT and virus with a mutant Nsp1 that cannot bind the ribosome showed that inhibition of nuclear mRNA export represents a distinct function of Nsp1 that is independent of its ability to induce mRNA degradation and translation inhibition [22]. These observations suggest that Nsp1 has evolved to target an additional step in the lifecycle of the cellular mRNAs, reducing the host cell protein synthesis even further.

Evolutionary insights and variants

Genomic monitoring revealed that the Nsp1 ORF is highly conserved within SARS-CoV-2 populations. The analysis of almost 6 million viral sequences indicated that 93% of the viral genomes did not show any Nsp1 mutations, and the vast majority of mutated Nsp1 sequences contained only one mutation. However, a few variants have been reported, with a mostly unclear impact on the protein function and the viral properties. Within these variants, a higher frequency of mutations has been observed in the NTD of Nsp1, spanning the residues 72–126. The variants E87D, H110Y, and R24C were identified as the most common mutations, and their frequency varied between different continents [58].

In an earlier work, SARS-CoV-2 genome sequencing and phylogenetic analyses identified 35 recurrent Nsp1 mutations associated with different clinical phenotypes. All these mutations were located in the region 79–89 of the Nsp1 coding region. Mutation of the full region in experiments with infected cells induced a lower IFN-I response, correlated with lower viral load and serum IFN-β [59]. Deleting this region reduced the translation inhibition efficiency of Nsp1 [60].

A viral SARS-CoV-2 strain with a deletion of three amino acids (KSF) in the linker was also detected in several geographical areas [61], but the consequences on the protein function are unknown.

Implications for immune evasion

By inducing translation inhibition, SARS-CoV-2 Nsp1 also inhibits the synthesis of IFNs and other pro-inflammatory proteins with antiviral activity [18]. As a result, IFN response inhibition contributes to the pathogenesis of SARS-CoV-2 [62] and promotes the evasion of the virus from the host cell innate immune mechanisms at the early steps of infection. There is evidence that the functional consequences of Nsp1 in SARS-CoV-2 infections rely mainly on blocking the IFN response: Infection experiments with viruses expressing Nsp1 that cannot bind the ribosome showed a viral attenuation only in IFN-competent cell lines, supporting that Nsp1 toxicity heavily relies on interferon responses [22]. Along these lines, it was observed that Nsp1-mutant virus replicons showed a higher sensitivity to interferon-α (IFNα) and interferon-β (IFNβ) compared with the WT virus [63]. However, the toxic effect of Nsp1 ectopic expression is not related to the IFN response [17].

Nsp1 from different coronaviruses

From the four Coronavirus genera, Nsp1 is only expressed in the α and β Coronaviruses. While α-CoVs encode Nsp1 counterparts with a similar length of 110 aa, β-CoVs cover a wide range of sizes, reaching ∼250 aa in counterparts of the lineage A (MHV CoV) to 175 aa in the lineage C (MERS-CoV). Nsp1 of SARS-CoV and SARS-CoV-2 (180 aa) share 84.4% sequence identity and 93.9% sequence similarity, reflected by their structural and functional similarities [64]. α-CoV Nsp1 proteins show a very low sequence identity compared with the β-CoVs [65].

Nsp1 has been studied thoroughly in SARS-CoV, and as expected by the high sequence similarity with SARS-CoV-2 Nsp1, many features of Nsp1 are shared between the two viral proteins. The global inhibition of host cell translation [23,66,67] attributed to Nsp1 binding the 40S subunit of host cells and the induction of host cell mRNA degradation [23,48] are key shared features of Nsp1 from the two closely related viruses.

MERS-CoV originating from the lineage C also inhibits host cell translation and induces mRNA degradation [30,68,69]. Translation inhibition is also mediated through interaction with the 40S ribosomal subunit similar to SARS-CoV-2, and the viral 5′UTRs can rescue mRNAs from translation inhibition [30].

Bioinformatic and structural data from different α-CoVs revealed that they share common structural features despite the lack of apparent sequence homology. Along these lines, Nsp1 from Human coronavirus 229E (HCoV-229E), Transmissible gastroenteritis virus (TGEV), Porcine epidemic diarrhea virus (PEDV) and Human coronavirus NL63 (HCoV-NL63) also inhibit host cell translation [65,70,71]. In two cases, HCoV-229E and HCoV-NL63 Nsp1 interact with the small ribosomal protein S6 and induce host cell RNA degradation, providing additional evidence for similar impacts on host gene expression regulation [65,70].

On the quest for Nsp1 inhibitors

The ability of Nsp1 from SARS-CoVs to hinder host immune responses at an early step, the absence of homology to any human proteins, and the availability of structural information render it an ideal drug target with therapeutic potential. Nsp1 mutations impede the viral replication potential, and the shared structural features among Nsp1 from different Coronaviruses render the quest for Nsp1 inhibitors a possible treatment option to combat coronaviral infections.

In silico screenings of repurposed drugs revealed several Nsp1 inhibitor candidates. However, only combinatory treatments with Ponatinib, Rilpivirine, and Montelukast could reverse the Nsp1 effects on cell toxicity assays [72]. A different *in silico* study also demonstrated that Montelukast sodium hydrate (FDA-approved leukotriene receptor antagonist for asthma) can bind to the C-terminal of Nsp1 [73]. However, further experiments showed that Montelukast did not inhibit the Nsp1 function in cells [49] or in RNA degradation assays [34].

Mitoxantrone dihydrochloride is an anticancer drug that can inhibit the SARS-CoV and SARS-CoV-2 virus entry into cells [74]. According to *in silico* and spectroscopy analyses, mitoxantrone can bind the Nsp1 CTD [75] and inhibit Nsp1-mediated cleavage *in vitro* at a concentration of 10 μM [34]. Two structurally related anthracenedione compounds, ametantrone and pixantrone, were more potent inhibitors of Nsp1-mediated RNA degradation than mitoxantrone *in vitro*. Notably, the three molecules could inhibit Nsp1-induced RNA degradation without affecting the capacity of Nsp1 to inhibit translation initiation [34].

Outlook

The biochemical, structural and functional insights into the art of Nsp1 hijacking host cells allowed us to understand many features of the host gene expression reprogramming and its consequences during Coronaviral infections. Contradictory and unexpected results highlight the complexity of Nsp1 activities that remain to be explored. Understanding the relationship between mRNA translation inhibition and degradation induced by Nsp1, as well as the physiological role and range of the concentration of viral components during infection, is an open field of research. Undoubtedly, the discovery or development of Nsp1-specific inhibitors will be an instrumental tool for further discoveries with therapeutic potential. Lastly, the wealth of information and timely description of intricate molecular mechanisms a few years after the appearance of SARS-CoV-2 Nsp1 underlines the value of basic research in producing new knowledge with immediate social impact.

Perspectives

- The study of SARS-CoV-2 Nsp1 is crucial, as it offers insights into the mechanisms viruses use to hijack host cellular machinery and suppress host immune responses.
- Recent research emphasizes the role of Nsp1 in inhibiting host protein translation and degrading host mRNA, underscoring its function in facilitating viral replication and evading host immunity.
- Future research should focus on exploring the potential of Nsp1 as a target for therapeutic interventions and understanding the interaction of Nsp1 with host cellular components to develop novel strategies for combating SARS-CoV-2 and other Coronaviruses.

Competing Interests

The author declares that there are no competing interests associated with this manuscript.

Funding

This work was supported by grants of EDK from the Swiss National Science Foundation (SNSF CRSK-3_220624), the National Center of Excellence in Research (NCCR) on RNA and Disease funded by the SNSF (51NF40-205601), the Multidisciplinary Center of Infectious Diseases from the University of Bern (MCID), and the Fondation Claude et Giuliana and the Forschungsstiftung of the University of Bern.

Biochemical Society Transactions (2024) **52** 481–490
https://doi.org/10.1042/BST20231119

Author Contributions

E.D.K. conceptualized, researched and wrote the review and designed the figures.

Acknowledgements

Sofia Nasif, Lukas Gurzeler, Emilie Bäumlin, Dominic Andenmatten and Jonas Luginbühl critically read and provided comments on the manuscript. Figures were made using Biorender.

Abbreviations

CTD, C-terminal domain; eIFs, eukaryotic initiation factors; IFN, interferons; IFNα, interferon-α; IFNβ, interferon-β; lncRNAs, long non-coding RNAs; NPC, nuclear pore complex; Nsp1, non-structural protein 1; Nsps, non-structural proteins; NTD, N-terminal domain; PEDV, porcine epidemic diarrhea virus; SL1, stem-loop 1; TC, ternary complex; TGEV, transmissible gastroenteritis virus; TOP, 5′-terminal oligopyrimidine.

References

1 Steiner, S., Kratzel, A., Barut, G.T., Lang, R.M., Aguiar Moreira, E., Thomann, L. et al. (2024) SARS-CoV-2 biology and host interactions. *Nat. Rev. Microbiol.* 1–20 https://doi.org/10.1038/s41579-023-01003-z
2 Brito Querido, J., Díaz-López, I. and Ramakrishnan, V. (2023) The molecular basis of translation initiation and its regulation in eukaryotes. *Nat. Rev. Mol. Cell Biol.* 1–19 https://doi.org/10.1038/s41580-023-00624-9
3 Hershey, J.W.B., Sonenberg, N. and Mathews, M.B. (2019) Principles of translational control. *Cold Spring Harb. Perspect. Biol.* **11**, a032607 https://doi.org/10.1101/cshperspect.a032607
4 Bicknell, A.A. and Ricci, E.P. (2017) When mRNA translation meets decay. *Biochem. Soc. Trans.* **45**, 339–351 https://doi.org/10.1042/BST20160243
5 Rozman, B., Fisher, T. and Stern-Ginossar, N.) Translation—a tug of war during viral infection. *Mol. Cell* **2**, 481–495 https://doi.org/10.1016/j.molcel.2022.10.012
6 Karousis, E.D., Schubert, K. and Ban, N. (2024) Coronavirus takeover of host cell translation and intracellular antiviral response: a molecular perspective. *EMBO J.* **43**, 151–167 https://doi.org/10.1038/s44318-023-00019-8
7 de Breyne, S., Vindry, C., Guillin, O., Condé, L., Mure, F., Gruffat, H. et al. (2020) Translational control of coronaviruses. *Nucleic Acids Res.* **48**, 12502–12522 https://doi.org/10.1093/nar/gkaa1116
8 V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. and Thiel, V. (2021) Coronavirus biology and replication: implications for SARS-CoV-2. *Nat. Rev. Microbiol.* **19**, 155–170 https://doi.org/10.1038/s41579-020-00468-6
9 Ingolia, N.T., Hussmann, J.A. and Weissman, J.S. (2019) Ribosome profiling: global views of translation. *Cold Spring Harb. Perspect. Biol.* **11**, a032698 https://doi.org/10.1101/cshperspect.a032698
10 Westermann, A.J. and Vogel, J. (2021) Cross-species RNA-Seq for deciphering host–microbe interactions. *Nat. Rev. Genet.* **22**, 361–378 https://doi.org/10.1038/s41576-021-00326-y
11 Iwasaki, S. and Ingolia, N.T. (2017) The growing toolbox for protein synthesis studies. *Trends Biochem. Sci.* **42**, 612–624 https://doi.org/10.1016/j.tibs.2017.05.004
12 Greco, T.M., Diner, B.A. and Cristea, I.M. (2014) The impact of mass spectrometry–based proteomics on fundamental discoveries in virology. *Annu. Rev. Virol.* **1**, 581–604 https://doi.org/10.1146/annurev-virology-031413-085527
13 Prabhakar, A., Puglisi, E.V. and Puglisi, J.D. (2019) Single-molecule fluorescence applied to translation. *Cold Spring Harb. Perspect. Biol.* **11**, a032714 https://doi.org/10.1101/cshperspect.a032714
14 Garenne, D., Haines, M.C., Romantseva, E.F., Freemont, P., Strychalski, E.A. and Noireaux, V. (2021) Cell-free gene expression. *Nat. Rev. Methods Primer* **1**, 1–18 https://doi.org/10.1038/s43586-021-00046-x
15 Gurzeler, L.-A., Ziegelmüller, J., Mühlemann, O. and Karousis, E.D. (2021) Production of human translation-competent lysates using dual centrifugation. *RNA Biol.* **19**, 78–88 https://doi.org/10.1080/15476286.2021.2014695
16 Hilgenfeld, R. and Peiris, M. (2013) From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. *Antiviral Res.* **100**, 286–295 https://doi.org/10.1016/j.antiviral.2013.08.015
17 Yuan, S., Peng, L., Park, J.J., Hu, Y., Devarkar, S.C., Dong, M.B. et al. (2020) Nonstructural protein 1 of SARS-CoV-2 is a potent pathogenicity factor redirecting host protein synthesis machinery toward viral RNA. *Mol. Cell* **80**, 1055–1066.e6 https://doi.org/10.1016/j.molcel.2020.10.034
18 Thoms, M., Buschauer, R., Ameismeier, M., Koepke, L., Denk, T., Hirschenberger, M. et al. (2020) Structural basis for translational shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2. *Science* **369**, 1249–1256 https://doi.org/10.1126/SCIENCE.ABC8665
19 Schubert, K., Karousis, E.D., Jomaa, A., Scaiola, A., Echeverria, B., Gurzeler, L.-A. et al. (2020) SARS-CoV-2 Nsp1 binds the ribosomal mRNA channel to inhibit translation. *Nat. Struct. Mol. Biol.* **27**, 959–966 https://doi.org/10.1038/s41594-020-0511-8
20 Finkel, Y., Gluck, A., Nachshon, A., Winkler, R., Fisher, T., Rozman, B. et al. (2021) SARS-CoV-2 uses a multipronged strategy to impede host protein synthesis. *Nature* **594**, 240–245 https://doi.org/10.1038/s41586-021-03610-3
21 Burke, J.M., St Clair, L.A., Perera, R. and Parker, R. (2021) SARS-CoV-2 infection triggers widespread host mRNA decay leading to an mRNA export block. *RNA* **27**, 1318–1329 https://doi.org/10.1261/rna.078923.121
22 Fisher, T., Gluck, A., Narayanan, K., Kuroda, M., Nachshon, A., Hsu, J.C. et al. (2022) Parsing the role of NSP1 in SARS-CoV-2 infection. *Cell Rep.* **39**, 110954 https://doi.org/10.1016/j.celrep.2022.110954
23 Kamitani, W., Narayanan, K., Huang, C., Lokugamage, K., Ikegami, T., Ito, N. et al. (2006) Severe acute respiratory syndrome coronavirus nsp1 protein suppresses host gene expression by promoting host mRNA degradation. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12885–12890 https://doi.org/10.1073/pnas.0603144103

24 Kamitani, W., Huang, C., Narayanan, K., Lokugamage, K.G. and Makino, S. (2009) A two-pronged strategy to suppress host protein synthesis by SARS coronavirus Nsp1 protein. *Nat. Struct. Mol. Biol.* **16**, 1134–1140 https://doi.org/10.1038/nsmb.1680
25 Vora, S.M., Fontana, P., Mao, T., Leger, V., Zhang, Y., Fu, T.-M. et al. (2022) Targeting stem-loop 1 of the SARS-CoV-2 5′ UTR to suppress viral translation and Nsp1 evasion. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2117198119 https://doi.org/10.1073/pnas.2117198119
26 Banerjee, A.K., Blanco, M.R., Bruce, E.A., Honson, D.D., Chen, L.M., Chow, A. et al. (2020) SARS-CoV-2 disrupts splicing, translation, and protein trafficking to suppress host defenses. *Cell* **183**, 1325–1339.e21 https://doi.org/10.1016/j.cell.2020.10.004
27 Wang, Y., Kirkpatrick, J., Lage, S.Z. and Carlomagno, T. (2023) Structural insights into the activity regulation of full-length non-structural protein 1 from SARS-CoV-2. *Structure* **31**, 128–137.e5 https://doi.org/10.1016/j.str.2022.12.006
28 Lapointe, C.P., Grosely, R., Johnson, A.G., Wang, J., Fernández, I.S. and Puglisi, J.D. (2021) Dynamic competition between SARS-CoV-2 NSP1 and mRNA on the human ribosome inhibits translation initiation. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2017715118 https://doi.org/10.1073/pnas.2017715118
29 Devarkar, S.C., Vetick, M., Balaji, S., Lomakin, I.B., Yang, L., Jin, D. et al. (2023) Structural basis for translation inhibition by MERS-CoV Nsp1 reveals a conserved mechanism for betacoronaviruses. *Cell Rep.* **42**, 113156 https://doi.org/10.1016/j.celrep.2023.113156
30 Schubert, K., Karousis, E.D., Ban, I., Lapointe, C.P., Leibundgut, M., Bäumlin, E. et al. (2023) Universal features of Nsp1-mediated translational shutdown by coronaviruses. *Mol. Cell* **83**, 3546–3557.e8 https://doi.org/10.1016/j.molcel.2023.09.002
31 Mendez, A.S., Ly, M., González-Sánchez, A.M., Hartenian, E., Ingolia, N.T., Cate, J.H. et al. (2021) The N-terminal domain of SARS-CoV-2 nsp1 plays key roles in suppression of cellular gene expression and preservation of viral gene expression. *Cell Rep.* **37**, 109841 https://doi.org/10.1016/j.celrep.2021.109841
32 Frolov, I., Agback, T., Palchevska, O., Dominguez, F., Lomzov, A., Agback, P. et al. (2023) All domains of SARS-CoV-2 nsp1 determine translational shutoff and cytotoxicity of the protein. *J. Virol.* **97**, e01865-22 https://doi.org/10.1128/jvi.01865-22
33 Aitken, C.E. and Lorsch, J.R. (2012) A mechanistic overview of translation initiation in eukaryotes. *Nat. Struct. Mol. Biol.* **19**, 568–576 https://doi.org/10.1038/nsmb.2303
34 Abaeva, I.S., Arhab, Y., Miścicka, A., Hellen, C.U.T. and Pestova, T.V. (2023) In vitro reconstitution of SARS-CoV-2 Nsp1-induced mRNA cleavage reveals the key roles of the N-terminal domain of Nsp1 and the RRM domain of eIF3g. *Genes Dev.* **37**, 844–860 https://doi.org/10.1101/gad.350829.123
35 Rao, S., Hoskins, I., Tonn, T., Garcia, P.D., Ozadam, H., Cenik, E.S. et al. (2021) Genes with 5′ terminal oligopyrimidine tracts preferentially escape global suppression of translation by the SARS-CoV-2 Nsp1 protein. *RNA* **27**, 1025–1045 https://doi.org/10.1261/rna.078661.120
36 Slobodin, B., Sehrawat, U., Lev, A., Hayat, D., Zuckerman, B., Fraticelli, D. et al. (2022) Cap-independent translation and a precisely located RNA sequence enable SARS-CoV-2 to control host translation and escape antiviral response. *Nucleic Acids Res.* **50**, 8080–8092 https://doi.org/10.1093/nar/gkac615
37 Tidu, A., Janvier, A., Schaeffer, L., Sosnowski, P., Kuhn, L., Hammann, P. et al. (2021) The viral protein NSP1 acts as a ribosome gatekeeper for shutting down host translation and fostering SARS-CoV-2 translation. *RNA* **27**, 253–264 https://doi.org/10.1261/rna.078121.120
38 Huston, N.C., Wan, H., Strine, M.S., de Cesaris Araujo Tavares, R., Wilen, C.B. and Pyle, A.M. (2021) Comprehensive in vivo secondary structure of the SARS-CoV-2 genome reveals novel regulatory motifs and mechanisms. *Mol. Cell* **81**, 584–598.e5 https://doi.org/10.1016/j.molcel.2020.12.041
39 Rangan, R., Zheludev, I.N. and Das, R. (2020) RNA genome conservation and secondary structure in SARS-CoV-2 and SARS-related viruses: a first look. *RNA* **076141**, 120 https://doi.org/10.1261/rna.076141.120
40 Sun, L., Li, P., Ju, X., Rao, J., Huang, W., Ren, L. et al. (2021) In vivo structural characterization of the SARS-CoV-2 RNA genome identifies host proteins vulnerable to repurposed drugs. *Cell* **184**, 1865–1883.e20 https://doi.org/10.1016/j.cell.2021.02.008
41 Miao, Z., Tidu, A., Eriani, G. and Martin, F. (2021) Secondary structure of the SARS-CoV-2 5′-UTR. *RNA Biol.* **18**, 447–456 https://doi.org/10.1080/15476286.2020.1814556
42 Bujanic, L., Shevchuk, O., von Kügelgen, N., Kalinina, A., Ludwik, K., Koppstein, D., et al. (2022) The key features of SARS-CoV-2 leader and NSP1 required for viral escape of NSP1-mediated repression. *RNA* **28**, 766–779 https://doi.org/10.1261/RNA.079086.121
43 Aviner, R., Lidsky, P.V., Xiao, Y., Tassetto, M., Kim, D., Zhang, L. et al. (2024) SARS-CoV-2 Nsp1 cooperates with initiation factors EIF1 and 1A to selectively enhance translation of viral RNA. *PLOS Pathog.* **20**, e1011535 https://doi.org/10.1371/journal.ppat.1011535
44 Sosnowski, P., Tidu, A., Eriani, G., Westhof, E. and Martin, F. (2022) Correlated sequence signatures are present within the genomic 5′UTR RNA and NSP1 protein in coronaviruses. *RNA* **28**, 729–741 https://doi.org/10.1261/RNA.078972.121/-/DC1
45 Finkel, Y., Mizrahi, O., Nachshon, A., Weingarten-Gabbay, S., Morgenstern, D., Yahalom-Ronen, Y. et al. (2021) The coding capacity of SARS-CoV-2. *Nature* **589**, 125–130 https://doi.org/10.1038/s41586-020-2739-1
46 Kim, D., Kim, S., Park, J., Chang, H.R., Chang, J., Ahn, J. et al. (2021) A high-resolution temporal atlas of the SARS-CoV-2 translatome and transcriptome. *Nat. Commun.* **12**, 5120 https://doi.org/10.1038/s41467-021-25361-5
47 Aviner, R., Lidsky, P.V., Xiao, Y., Tasseto, M., Kim, D., Zhang, L. et al. (2024) SARS-CoV-2 Nsp1 cooperates with initiation factors EIF1 and 1A to selectively enhance translation of viral RNA. *PLoS Pathog.* **20**, e1011535. https://doi.org/10.1371/journal.ppat.1011535
48 Huang, C., Lokugamage, K.G., Rozovics, J.M., Narayanan, K., Semler, B.L. and Makino, S. (2011) SARS coronavirus nsp1 protein induces template-dependent endonucleolytic cleavage of mRNAs: viral mRNAs are resistant to nsp1-induced RNA cleavage. *PLOS Pathog.* **7**, e1002433 https://doi.org/10.1371/JOURNAL.PPAT.1002433
49 Shehata, S.I. and Parker, R. (2023) SARS-CoV-2 Nsp1 mediated mRNA degradation requires mRNA interaction with the ribosome. *RNA Biol.* **20**, 444–456 https://doi.org/10.1080/15476286.2023.2231280
50 Tardivat, Y., Sosnowski, P., Tidu, A., Westhof, E., Eriani, G. and Martin, F. (2023) SARS-CoV-2 NSP1 induces mRNA cleavages on the ribosome. *Nucleic Acids Res.* **51**, 8677–8690 https://doi.org/10.1093/nar/gkad627
51 Gaglia, M.M., Covarrubias, S., Wong, W. and Glaunsinger, B.A. (2012) A common strategy for host RNA degradation by divergent viruses. *J. Virol.* **86**, 9527–9530 https://doi.org/10.1128/JVI.01230-12
52 Guo, J., Zhu, Y., Ma, X., Shang, G., Liu, B. and Zhang, K. (2023) Virus infection and mRNA nuclear export. *Int. J. Mol. Sci.* **24**, 12593 https://doi.org/10.3390/ijms241612593
53 Zhang, K., Miorin, L., Makio, T., Dehghan, I., Gao, S., Xie, Y. et al. (2021) Nsp1 protein of SARS-CoV-2 disrupts the mRNA export machinery to inhibit host gene expression. *Sci. Adv.* **7**, eabe7386 https://doi.org/10.1126/sciadv.abe7386

Biochemical Society Transactions (2024) **52** 481–490
https://doi.org/10.1042/BST20231119

54 Addetia, A., Lieberman, N.A.P., Phung, Q., Hsiang, T.-Y., Xie, H., Roychoudhury, P. et al. (2021) SARS-CoV-2 ORF6 disrupts bidirectional nucleocytoplasmic transport through interactions with Rae1 and Nup98. *mBio* **12**, 10.1128/mbio.00065-21 https://doi.org/10.1128/mbio.00065-21

55 Hall, R., Guedán, A., Yap, M.W., Young, G.R., Harvey, R., Stoye, J.P. et al. (2022) SARS-CoV-2 ORF6 disrupts innate immune signalling by inhibiting cellular mRNA export. *PLOS Pathog.* **18**, e1010349 https://doi.org/10.1371/journal.ppat.1010349

56 Miorin, L., Kehrer, T., Sanchez-Aparicio, M.T., Zhang, K., Cohen, P., Patel, R.S. et al. (2020) SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 28344–28354 https://doi.org/10.1073/pnas.2016650117

57 Katahira, J., Ohmae, T., Yasugi, M., Sasaki, R., Itoh, Y., Kohda, T. et al. (2023) Nsp14 of SARS-CoV-2 inhibits mRNA processing and nuclear export by targeting the nuclear cap-binding complex. *Nucleic Acids Res.* **51**, 7602–7618 https://doi.org/10.1093/nar/gkad483

58 Ghaleh, S.S., Rahimian, K., Mahmanzar, M., Mahdavi, B., Tokhanbigli, S., Sisakht, M.M. et al. (2023) SARS-cov-2 Non-structural protein 1(NSP1) mutation virulence and natural selection: evolutionary trends in the six continents. *Virus Res.* **323**, 199016 https://doi.org/10.1016/j.virusres.2022.199016

59 Lin, J., Tang, C., Wei, H., Du, B., Chen, C., Wang, M. et al. (2021) Genomic monitoring of SARS-CoV-2 uncovers an Nsp1 deletion variant that modulates type I interferon response. *Cell Host Microbe* **29**, 489–502.e8 https://doi.org/10.1016/j.chom.2021.01.015

60 Kumar, P., Schexnaydre, E., Rafie, K., Kurata, T., Terenin, I., Hauryliuk, V. et al. (2022) Clinically observed deletions in SARS-CoV-2 Nsp1 affect its stability and ability to inhibit translation. *FEBS Lett.* **596**, 1203–1213 https://doi.org/10.1002/1873-3468.14354

61 Benedetti, F., Snyder, G.A., Giovanetti, M., Angeletti, S., Gallo, R.C., Ciccozzi, M. et al. (2020) Emerging of a SARS-CoV-2 viral strain with a deletion in nsp1. *J. Transl. Med.* **18**, 329 https://doi.org/10.1186/s12967-020-02507-5

62 Kim, Y.-M. and Shin, E.-C. (2021) Type I and III interferon responses in SARS-CoV-2 infection. *Exp. Mol. Med.* **53**, 750–760 https://doi.org/10.1038/s12276-021-00592-0

63 Ricardo-Lax, I., Luna, J.M., Thao, T.T.N., Le Pen, J., Yu, Y., Hoffmann, H.-H. et al. (2021) Replication and single-cycle delivery of SARS-CoV-2 replicons. *Science* **374**, 1099–1106 https://doi.org/10.1126/science.abj8430

64 Simeoni, M., Cavinato, T., Rodriguez, D. and Gatfield, D. (2021) I(nsp1)ecting SARS-CoV-2–ribosome interactions. *Commun. Biol.* **4**, 1–5 https://doi.org/10.1038/s42003-021-02265-0

65 Wang, Y., Shi, H., Rigolet, P., Wu, N., Zhu, L., Xi, X.G. et al. (2010) Nsp1 proteins of group I and SARS coronaviruses share structural and functional similarities. *Infect. Genet. Evol.* **10**, 919–924 https://doi.org/10.1016/J.MEEGID.2010.05.014

66 Narayanan, K., Huang, C., Lokugamage, K., Kamitani, W., Ikegami, T., Tseng, C.-T.K. et al. (2008) Severe acute respiratory syndrome coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in infected cells. *J. Virol.* **82**, 4471–4479 https://doi.org/10.1128/JVI.02472-07

67 Lokugamage, K.G., Narayanan, K., Huang, C. and Makino, S. (2012) Severe acute respiratory syndrome coronavirus protein nsp1 is a novel eukaryotic translation inhibitor that represses multiple steps of translation initiation. *J. Virol.* **86**, 13598–13608 https://doi.org/10.1128/JVI.01958-12

68 Terada, Y., Kawachi, K., Matsuura, Y. and Kamitani, W. (2017) MERS coronavirus nsp1 participates in an efficient propagation through a specific interaction with viral RNA. *Virology* **511**, 95–105 https://doi.org/10.1016/j.virol.2017.08.026

69 Lokugamage, K.G., Narayanan, K., Nakagawa, K., Terasaki, K., Ramirez, S.I., Tseng, C.-T.K. et al. (2015) Middle East respiratory syndrome coronavirus nsp1 inhibits host gene expression by selectively targeting mRNAs transcribed in the nucleus while sparing mRNAs of cytoplasmic origin. *J. Virol.* **89**, 10970–10981 https://doi.org/10.1128/JVI.01352-15

70 Shen, Z., Ye, G., Deng, F., Wang, G., Cui, M., Fang, L. et al. (2018) Structural basis for the inhibition of host gene expression by porcine epidemic diarrhea virus nsp1. *J. Virol.* **92**, e01896-17 https://doi.org/10.1128/JVI.01896-17

71 Shen, Z., Wang, G., Yang, Y., Shi, J., Fang, L., Li, F. et al. (2019) A conserved region of nonstructural protein 1 from alphacoronaviruses inhibits host gene expression and is critical for viral virulence. *J. Biol. Chem.* **294**, 13606–13618 https://doi.org/10.1074/JBC.RA119.009713

72 Kao, H.-T., Orry, A., Palfreyman, M.G. and Porton, B. (2022) Synergistic interactions of repurposed drugs that inhibit Nsp1, a major virulence factor for COVID-19. *Sci. Rep.* **12**, 10174 https://doi.org/10.1038/s41598-022-14194-x

73 Afsar, M., Narayan, R., Akhtar, M.N., Das, D., Rahil, H., Nagaraj, S.K. et al. (2022) Drug targeting Nsp1-ribosomal complex shows antiviral activity against SARS-CoV-2. *eLife* **11**, e74877 https://doi.org/10.7554/eLife.74877

74 Zhou, Y., Hou, Y., Shen, J., Huang, Y., Martin, W. and Cheng, F. (2020) Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discov.* **6**, 14 https://doi.org/10.1038/s41421-020-0153-3

75 Kumar, P., Bhardwaj, T. and Giri, R. (2022) Mitoxantrone dihydrochloride, an FDA approved drug, binds with SARS-CoV-2 NSP1 C-terminal. *RSC Adv.* **12**, 5648–5655 https://doi.org/10.1039/D1RA07434B