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Research Focus

Unique SARS-CoV protein nsp1: bioinformatics, biochemistry and potential effects on virulence

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Viruses have evolved a myriad of strategies for promoting viral replication, survival and spread. Sequence analysis of the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) genome predicts several proteins that are unique to SARS-CoV. The search to understand the high virulence of SARS-CoV compared with related coronaviruses, which cause lesser respiratory illnesses, has recently focused on the unique nsp1 protein of SARS-CoV and suggests evolution of a possible new virulence mechanism in coronaviruses. The SARS-CoV nsp1 protein increases cellular RNA degradation and thus might facilitate SARS-CoV replication or block immune responses.

SARS-CoV and conservation of nsp1

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) caused a pandemic with >800 deaths worldwide in only a few months [1]. Its genome was rapidly sequenced [2] and analysis of the identified predicted proteins is advancing. Bioinformatic analysis predicts that the SARS-CoV nonstructural protein 1 (nsp1) is translated from the most 5' coding region of the SARS-CoV genome [2–4]. It is predicted to be cleaved from a large viral polypeptide to form a mature peptide of ~20 kDa, in agreement with recent reports [3,5]. The conservation of a viral protein indicates its importance in the virus life cycle. The nsp1 sequence is highly conserved in SARS-CoV isolates sequenced from humans, civet cats and bats, implying that it is crucial to replication of the virus, survival in the host or spread in susceptible populations. The full-length nsp1 protein is reported to be conserved in all 130 (to date) SARS-CoV isolates [4], in which the replicase proteins are predicted to be expressed. Therefore, the nsp1 protein seems to be absolutely conserved in all replication-competent SARS-CoV virus isolates.

Here, we discuss the SARS-CoV nsp1 protein from the following perspectives: first, bioinformatics analysis shows that it is a unique and highly conserved protein; second, biochemical studies by Kamitani *et al.* show the first functional data for nsp1, which implicate it in enhancing RNA degradation [5]; and third, we discuss how nsp1 might contribute to the unique virulence of SARS-CoV.

Uniqueness of SARS-CoV nsp1

Whereas the SARS-CoV nonstructural proteins 3–16 are predicted to be conserved in both approximate size and sequence homology in all groups of coronaviruses [4], bioinformatics analyses show that the SARS-CoV nsp1 is a unique and novel sequence. BLASTP E-value scores for nsp1 compared between SARS-CoV isolates are typically 1×10^{-99} (high percentage identity) but after all SARS-CoV hits are considered, only poor BLAST E values (0.85) remain, which indicates that nsp1 has no significant similarity to any known sequences [6]. The closest hits are to vertebrate, not viral, genes. Similarly, BLASTn and tBLASTn showed no significant similarity (score 0.13 and 1.3) between SARS-CoV nsp1 nucleotide sequence and any other nucleotide sequence in the NCBI databases, coronavirus or non-coronavirus. Thus, the SARS-CoV nsp1 is unique among coronaviruses [7] and could contribute to the exceptional pathogenesis of SARS-CoV in humans [1], although direct demonstration of an nsp1 effect on virulence is yet to be shown. Because there is no significant similarity to other genes, a function for nsp1 cannot be inferred from knowledge of related gene functions. Similarly, the nsp1 tertiary NMR structure [8] showed a novel fold (M.A. Johnson, personal communication) with no significant similarity to other proteins and thus, the structure provided little insight into protein function. Finally, motif analysis of SARS-CoV nsp1 showed that no significant protein functional motifs were present [9,10]. These data all attest to the uniqueness of the SARS-CoV nsp1 protein and make it especially important and interesting to characterize.

Biochemistry of nsp1

Recently, two groups have begun to characterize the SARS-CoV nsp1 protein, showing that it is present in infected cells as a 20 kDa protein, as expected, and demonstrating that the nsp1 protein is localized in the cytoplasm of infected or uninfected cells in which it is ectopically expressed [3,5]. In an exciting turn of events, it has now been reported that plasmid-driven expression of nsp1 causes a sharp reduction in the expression of proteins driven by promoters from simian virus 40 (SV40), cytomegalovirus (CMV) and interferon (IFN)β, without necessarily affecting cell viability [5]. Further investigation indicated the specific mRNA was also decreased, whereas rRNA remained unaffected. Transfected nsp1 RNA (capped and polyadenylated) decreased host protein synthesis, and the inclusion of actinomycin D (to block new transcription) showed a much stronger inhibition of protein synthesis in the presence of nsp1. This indicated that while translation of new transcripts was proceeding (in cells not treated with actinomycin D), translation from

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pre-existing transcripts was blocked by nsp1 [5]. This suggests, but does not directly prove, that nsp1 increases RNA degradation. Decreased mRNA levels and decreased translation of pre-existing mRNA (presumably as a result of degradation) were also seen during infection with SARS-CoV. Together, these data provide the first evidence of a function for the SARS-CoV nsp1 protein.

Mechanism of action of nsp1

The mechanism of action of nsp1 remains mysterious. It is possible that the observed effect of nsp1 protein on infected cells is caused by induction of a generalized 'stress response', which causes host shut-off rather than specifically causing RNA degradation. For example, cells transfected with nsp1 RNA showed reduced viability (33%) compared with cells transfected with chloramphenicol acetyl transferase RNA (99%). However, it seems likely that degradation of RNA would plausibly cause cell death. Evidence suggesting that the presence of nsp1 inside cells does not simply induce a non-specific host cell shutdown includes the following observations: normal actin RNA levels, normal Sendai virus N-protein expression and replication in nsp1-transfected cells, stable rRNA levels and, finally, the fact that 293 cells transfected with plasmid expressing nsp1 maintained 98% viability after 48 h [5].

Among the possible hypotheses for nsp1 function are that nsp1 might: (i) have nuclease activity, either alone or as part of a complex with host proteins; (ii) alter RNA-interacting proteins or RNA to make it more nuclease sensitive; (iii) be required for the formation or activity of a cellular RNase; or (iv) affect the localization of RNA, which could subsequently affect its stability and/or degradation. Because there are no identifiable nucleotide-binding sites, nuclease homologies or motifs [6,10], perhaps the latter mechanisms are more likely. Nsp1 has been localized intracellularly with other viral replication proteins [11]; however, the nsp1 effect on RNA has been demonstrated in the absence of other viral proteins. Thus, the functional significance of this association should be further studied. Furthermore, the specificity of nsp1 remains to be elucidated. If nsp1 decreases all RNA (viral and cellular), is it still an advantage to the virus because viral transcription occurs at a much higher rate? Or is nsp1 somehow targeted to degrade cellular transcripts or even certain cell transcripts specifically? The fact that it promotes degradation of several mRNA sequences from different organisms but not rRNA suggests some form of specificity. However, in some cases, nsp1 does not decrease host protein levels appreciably, and yet it seems to decrease even its own expression [5]. Certainly, these questions will be the focus of future studies.

SARS-CoV virulence

Virus depression of host protein synthesis might give a competitive advantage to viral protein production and hence replication. Many viruses, with both DNA and RNA genomes, dampen host protein synthesis by various mechanisms [12–14]. Interestingly, however, not all of the identified genes that decrease host protein synthesis affect viral replication *in vitro* – in the case of the herpes virus vhs protein, an effect is seen solely in virulence *in vivo* [14]. These data suggest that, at least in some cases, the effects of host protein shut-off might not be competitive for protein production as much as subversive of the immune response. For example, a cell that is not expressing adequate levels of MHC class I or II molecules will be impaired in its antigen presentation capacity, both in initiating an adaptive immune response and in being targeted by it [cytotoxic T lymphocyte (CTL) killing] (Figure 1). Indeed, it has been shown that the herpes protein that causes RNA degradation inhibits antigen presentation by dendritic cells [15] and directly decreases the specific immune response to the virus [16]. Additionally, production of proinflammatory cytokines, which alert the innate immune system to the presence of virus, can also be blocked [17]. The kinetics of the response also suggest involvement of the innate immune response because the presence of a gene that decreases host protein production can enhance viral replication *in vivo* before measurable adaptive immunity [14]. Because SARS-CoV is IFN-sensitive [18,19], nsp1 control of IFN might be crucial for SARS-CoV survival [5].

How SARS-CoV nsp1 functions remains to be answered. Removal of the nsp1 protein function from the virus [20] will enable determination of its effects on replication and/or virulence *in vivo*; however, these experiments will require careful design and analysis because indiscriminate deletion of nsp1 sequence would remove *cis*-acting elements required for replication. Nsp1 effects on MHC

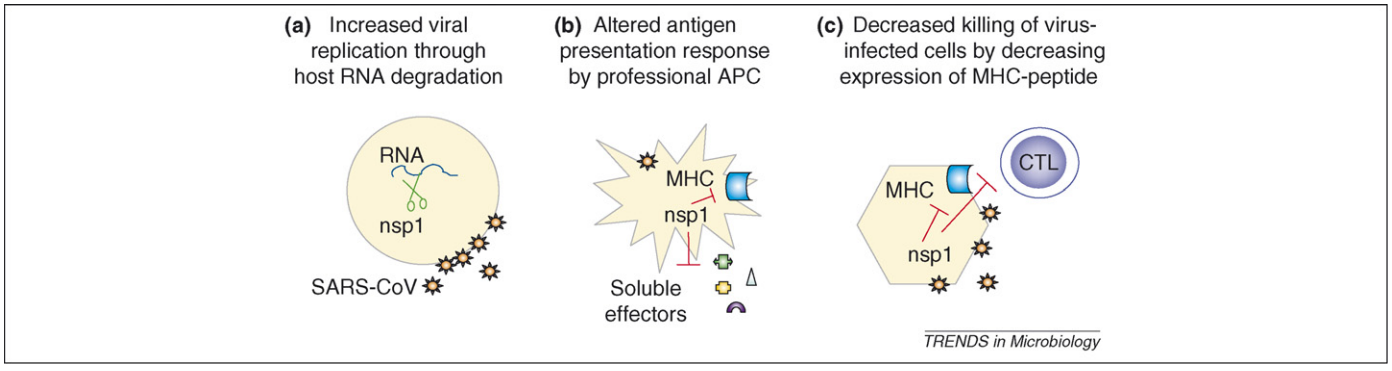

Figure 1. Potential mechanisms of SARS-CoV nsp1 in virulence. **(a)** Nsp1 can cause degradation of host RNA (directly or indirectly), thus increasing viral replication. **(b)** Nsp1 degradation of RNA might decrease the ability of professional antigen presenting cells (APC) to present viral peptides to T cells by decreasing host major histocompatibility complexes (MHC), antigen processing, co-stimulatory molecules or expression of soluble effectors such as cytokines. **(c)** Nsp1 RNA degradation could block MHC presentation from virally infected cells, decreasing CTL killing.

Box 1. Unanswered questions regarding SARS-CoV nsp1

(i) Does nsp1 directly cause specific RNA degradation? Biochemical evidence in a defined *in vitro* system is necessary to confirm the putative nsp1 activity.
(ii) Are other cellular proteins required for nsp1 function? Are there other proteins that nsp1 interacts with in cells?
(iii) Are there other mechanisms mediated by nsp1 that cause host protein shut-off, separate from RNA degradation?
(iv) Does nsp1 affect SARS-CoV replication? Deletion or suppression of nsp1 function during SARS-CoV growth in tissue culture will answer whether nsp1 affects replication of the virus. Careful design of the recombinant construct will be necessary to isolate the effect of nsp1 protein function from the effects of adjacent *cis*-acting nucleotide sequences required for viral replication.
(v) Does removal of nsp1 function decrease virulence? To answer this question, suitable animal models will be necessary to evaluate multiple aspects of virulence. Several animal models have been studied including mice, ferrets and primates [1,24].
(vi) How is the immune system affected? How does nsp1 affect cell surface proteins that are immunologically important for antigen presentation and CTL killing?

expression, antigen presentation, cytokine production and CTL killing all need to be explored. Nsp1 could block induction of specific immunity by blocking host protein synthesis and antigen presentation or cytokine synthesis from SARS-CoV infected cells [21–23], or it could block presentation of viral peptides on infected cells, thus masking and protecting infected cells from CTL killing. Determining this difference is essential to predicting SARS-CoV vaccine success. If nsp1 blocks generation of specific immunity, a well-designed SARS-CoV vaccine will circumvent this problem. It is certainly possible to generate both good neutralizing antibody titer and T lymphocyte responses to SARS-CoV vaccines [24]. However, vaccination could be ineffective if cells harboring virus do not present viral peptides so that they can be recognized and eliminated by CTL killing.

Concluding remarks and future perspectives

Evidence has been presented that SARS-CoV nsp1, a novel protein unique to SARS-CoV, induces degradation of RNA and diminishes subsequent protein synthesis, similar to the reduction of RNA seen in infected cells [5]. This is an exciting step that will enable the field of SARS-CoV research to confirm and extend – or refute – these observations. The specificity and biochemical mechanism of action is still to be elucidated, as are the potential effects of the RNA degradation on virulence and the immune response to SARS-CoV (Box 1). Creation of a virus with a functional nsp1 knockout [20] will enable direct demonstration of nsp1 effects on replication *in vitro* and virulence in animal models [1,24]. These experiments will answer whether the SARS-CoV nsp1 is an important factor in the high virulence of SARS-CoV compared with related coronaviruses.

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