committees are exploring strategic prioritization plans. Health care workers are a common first-tier group (*14*), which in turn preserves health care systems by protecting those who run them. A next priority is to directly protect those who are at highest risk of death or hospitalization when infected: specifically, those over 65 and people with certain comorbid conditions. This strategy may be optimal for reducing mortality even if the vaccine is somewhat less effective in these groups (*2*). But if a vaccine offers little to no protection in high-risk groups yet is able to reduce infection or infectiousness in younger adults, an indirect strategy could be preferred as vaccine supplies become large enough (*1*, *2*). A worst-case scenario for an effective vaccine is one that reduces disease in younger adults but provides neither direct nor indirect protection to high-risk groups, leaving the most vulnerable at risk. Knowing these vaccine characteristics is important when evaluating the relative merits of other products. Fortunately, there are many vaccine candidates in development that use a mixture of innovative and existing technologies. Although vaccines may vary in their characteristics, having reliable evidence on direct and indirect protection can help plan how to use these vaccines in a coordinated way. ■

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CORONAVIRUS

Enhancing host cell infection by SARS-CoV-2

Neuropilin-1 binds the furin-processed spike protein of SARS-CoV-2 to promote virus entry

By **Margaret Kielian**

The current global pandemic of coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A critical initial step of infection is the interaction of the virus with receptors on host cells. In the case of SARS-CoV-2 and other coronaviruses, this receptor binding occurs through the spike (S) protein on the virus surface. Both SARS-CoV-2 and the related SARS-CoV, which caused an outbreak in 2003, bind to angiotensin-converting enzyme 2 (ACE2) on human cells. However, the observed differences in tissues that are infected by these two viruses (tropism) suggests that additional host factors may be involved. On pages 861 and 856 of this issue, Daly *et al.* (*1*) and Cantuti-Castelvetri *et al.* (*2*), respectively, show that the membrane protein neuropilin-1 (NRP1) promotes SARS-CoV-2 entry and explain how NRP1 interacts with the SARS-CoV-2 S protein. The results suggest the S protein–NRP1 interaction as a potential antiviral target.

Coronaviruses are enveloped RNA viruses that can cause human diseases that range from the common cold to severe and fatal respiratory infections. It is thought that both SARS-CoV-2 and SARS-CoV bind to ACE2 on the host cell surface, are internalized by endocytosis, and fuse with the endolysosome membrane to deliver the viral genome for replication in the host cell (*3*, *4*) (see the figure). The viral S protein mediates this key membrane fusion reaction, but its activity requires several processing steps. S is synthesized as a large membrane protein that is cleaved into two components, S1 and S2, which remain noncovalently associated (*5*, *6*). Cleavage is required for infection and can occur during virus particle production or virus entry into the target cell. The S1 protein forms the "head" of the molecule and mediates binding to ACE2. The S2 protein is anchored in the virus membrane and mediates membrane fusion. S2, like the fusion proteins of influenza virus and HIV-1, inserts a hydrophobic fusion peptide at its amino terminus into the cell membrane and then folds back to merge the host and virus membranes (*7*). The S2 protein needs a further proteolytic step to "liberate" its fusion peptide, and this is carried out by transmembrane protease serine 2 (TMPRSS2) or other proteases (*3*).

A potentially important difference between SARS-CoV-2 and SARS-CoV is the mechanism of S protein cleavage into S1 and S2. In SARS-CoV, this is caused by host cell proteases called cathepsins, which are located within endocytic compartments. However, the sequence of the SARS-CoV-2 S protein contains a series of basic amino acids at the S1-S2 junction. Such polybasic sites can be substrates for furin, a protease that is present in the secretory pathway and endocytic compartments (*8*). Studies with SARS-CoV-2 show that its S protein is cleaved by furin during virus production and that this cleavage promotes subsequent virus infection (*3*, *6*). Thus, a notable difference between the S proteins of these two coronaviruses is the protease that carries out the S1-S2 cleavage reaction. Furin cleavage also produces a potentially important remnant: the polybasic site that remains at the carboxyl terminus of SARS-CoV-2 S1 after cleavage.

Neuropilins are a family of membrane proteins that were originally identified because of their involvement in angiogenesis (blood vessel formation) and axon guidance (*9*). The neuropilins are co-receptors for molecules such as vascular endothelial growth factors (VEGFs) and semaphorins, and recent studies have demonstrated their upregulation during tumor angiogenesis and their potential as anticancer targets. Both NRP1 and NRP2 can bind the carboxyl-terminal sequences generated by furin processing of molecules such as VEGFs, with the sequences fitting into a pocket on the b1 domain of the NRP (*9*). Detailed studies of such NRP1-peptide interactions show that binding to the b1 pocket requires the sequence Arg/Lys-X-X-Arg/Lys (R/K-XX-R/K, where X can be any amino acid) at the carboxyl terminus of the protein or peptide (*10*). This "C-end rule," or CendR, can thus predict whether a protein is a candidate for binding to NRPs.

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Daly *et al.* and Cantuti-Castelvetri *et al.* found that the sequence of the S1-S2 junction of virus isolates from human patients suggested that they fit the C-end rule, with Arg-Arg-Ala-Arg (RRAR) predicted to form the carboxyl-terminal sequence of the furin-cleaved S1. They showed that NRP1 promoted infection of human cell lines by SARS-CoV-2 and by lentivirus pseudotypes that contained the SARS-CoV-2 S protein on their surface. NRP1 was not the only host factor that promoted SARS-CoV-2 infection, but even when both ACE2 and TMPRSS2 were present, NRP1 gave an additional increase. This was due to an increase in virus uptake into the cell rather than an increase in virus binding to the cell surface. The promotion of virus infection by NRP1 was inhibited by the addition of a soluble NRP1 or by an antibody that mapped to the binding pocket on NRP1. Further analyses revealed that S1 or its carboxyl-terminal region interact with NRP1, and this was inhibited by mutations in the NRP b1 pocket. SARS-CoV-2 mutants in which the polybasic cleavage site was deleted or S was made resistant to furin cleavage were insensitive to NRP1 expression.

Daly *et al.* showed that a peptide derived from the S1 carboxyl terminus binds to the NRP1 b1 domain with micromolar affinity. They solved the crystal structure of the complex, which revealed that the peptide is positioned in the b1 pocket, similar to a VEGF peptide that was crystallized with b1 (*9*). A small-molecule antagonist of NRP1 that inhibits VEGF binding (*11*) also inhibited the b1-S1 peptide interaction and virus infection.

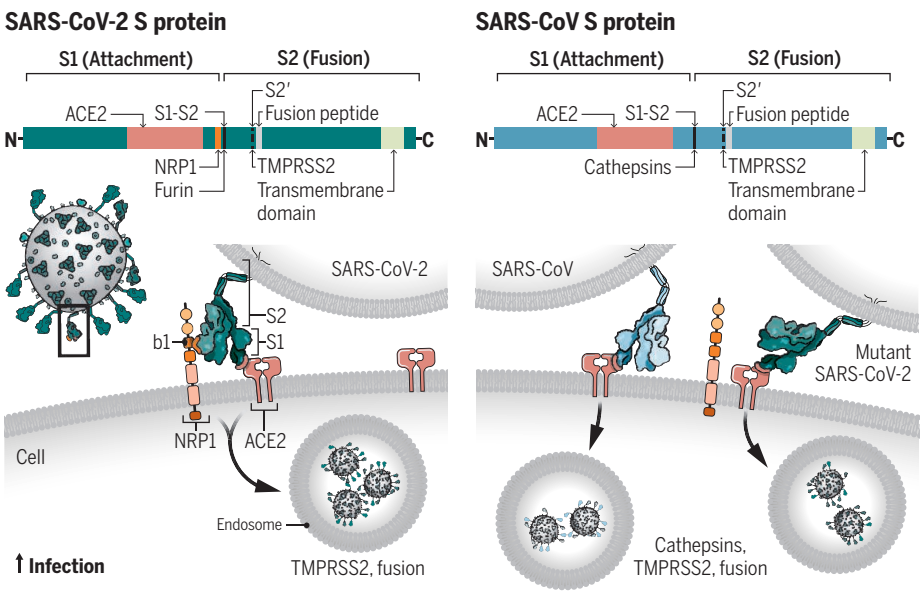
C-end rule peptides had previously been shown to mediate uptake of particles or bacteriophages by cells and tissues (*10*). Cantuti-Castelvetri *et al.* conjugated the S1 peptide onto nanoparticles and administered them intranasally to mice. The mouse olfactory epithelium expresses NRP1, and the authors observed significant uptake of the peptide-conjugated particles in this site. Similar to previous results (*10*), the S1 peptide–coated particles were observed to also travel into the neurons and blood vessels of the cortex. To address the possible role of NRP1 in human SARS-CoV-2 infection, the authors used available data to confirm expression of both *NRP1* and *NRP2* RNA in human lung and olfactory epithelial tissue. They also showed that five out of six autopsy samples of olfactory epithelia from human COVID-19 patients were positive for both S protein and NRP1. These results from mice and humans are intriguing given that many COVID-19 patients lose their sense of smell.

Together, these papers establish NRP1 as a host factor for SARS-CoV-2 and suggest interesting parallels and differences with the roles of NRPs in infection by other viruses. For example, both the human T cell lymphotropic virus SU protein and the Epstein Barr virus (EBV) gB protein are processed by furin, and infection by these viruses is promoted by NRP1 (*12*, *13*). EBV, which infects nasopharyngeal epithelial cells, shows an apparent reciprocal effect in which NRP1 promotes infection, whereas NRP2 inhibits it (*13*). Although Daly *et al.* showed that S1 protein can also bind NRP2, its role in SARS-CoV-2 infection is unknown. NRP2 is a receptor for Lujo virus, but this does not involve binding to the b1 pocket (*14*). These and other examples indicate that a number of viruses have evolved to use NRPs during infection, but much remains unclear.

There is more to learn about the promotion of SARS-CoV-2 infection by NRP1. The virus can propagate in the absence of furin cleavage in some cultured cells, where cathepsin cleavage may be sufficient, but in vivo, the virus relies on furin processing, which enhances viral pathogenesis in a hamster model (*15*). How does NRP1 binding affect the virus internalization pathway, and does it act as a co-receptor with ACE2? C-end rule peptide binding to NRP1 can promote vascular leakage and tissue penetration, especially when the peptides are presented on a multivalent particle (*10*). Does NRP1 similarly help to promote SARS-CoV-2 dissemination and spread? Together, Daly *et al.* and Cantuti-Castelvetri *et al.* show that furin processing, which has an important role in the maturation and fusion activity of the SARS-CoV-2 S protein, also acts to generate ligands on the virus particle that bind NRP1. The availability of small molecule inhibitors of the NRP1-C-end rule peptide interaction suggests a potential antiviral strategy. Defining the importance of this interaction in vivo will be a vital next step. ■

Model for SARS-CoV-2 processing and entry

Proteolytic processing of SARS-CoV and SARS-CoV-2 S proteins facilitates virus entry. SARS-CoV and SARS-CoV-2 bind to ACE2 at a region on S1. Furin cleavage at the S1-S2 junction exposes the C-end rule peptide on SARS-CoV-2 S1 and allows binding to NRP1. Subsequent processing by cathepsins and TMPRSS2 allows S2 fusion peptide–mediated membrane insertion and merging of membranes. The absence of a furin cleavage site in SARS-CoV S1 and a SARS-CoV-2 S1 mutant prevents binding to NRP1 and limits virus entry and infection.



ACE2, angiotensin-converting enzyme 2; NRP1, neuropilin 1; S, spike; SARS-CoV, severe acute respiratory syndrome coronavirus; TMPRSS2, transmembrane protease serine 2.

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