Coronaviruses (CoVs) are positive-stranded RNA viruses known to cause highly prevalent respiratory and enteric diseases in humans and animals. About two-thirds of their genomes encode the viral replicase that mediates viral RNA synthesis (19). The replicase gene is comprised of two open reading frames at the 5′ end of the genome, termed ORF1a and ORF1b (26). The upstream ORF1a encodes polyprotein pp1a (450 to 500 kDa), while ORF1a and ORF1b together encode pp1lab (750 to 800 kDa). Expression of the ORF1b-pp1a (450 to 500 kDa), while ORF1a and ORF1b together and ORF1b (26). The upstream ORF1a encodes polyprotein

Prior to the global severe acute respiratory syndrome (SARS) outbreak in 2003, scant attention was paid to coronaviruses by researchers, as this genus of viruses causes severe diseases predominantly in animals and only comparatively mild diseases in humans. In the wake of the SARS outbreak, greater attention has been focused on the replicate proteins with a view to understanding the replication/transcription machinery and to identify new therapeutic targets. To date, the three-dimensional structures of a series of nonstructural proteins, which then assemble to form the replicase complex required for viral replication and transcription. The severe acute respiratory syndrome coronavirus (SARS-CoV) nonstructural proteins nsp1 to nsp16 have been implicated by genetic analysis in the assembly of a functional replication/transcription complex. We report the crystal structure of nsp10 from SARS-CoV at 2.1-Å resolution. The nsp10 structure has a novel fold, and 12 identical subunits assemble to form a unique spherical dodecameric architecture. Two zinc fingers have been identified from the nsp10 monomer structure with the sequence motifs C-(X)2-C-(X)5-H-(X)6-C and C-(X)2-C-(X)7-C-(X)-C. The nsp10 crystal structure is the first of a new class of zinc finger proteins three-dimensional structures to be revealed experimentally. The zinc finger sequence motifs are conserved among all three coronavirus antigenic groups, implicating an essential function for nsp10 in all coronaviruses. Based on the structure, we propose that nsp10 is a transcription factor for coronavirus replication/transcription.

**MATERIALS AND METHODS**

**Protein expression, purification, and characterization.** The coding sequence for SARS-CoV nsp10-nsp11 was amplified by PCR from the SARS-CoV B301 strain (corresponding to Ala4231 to Val4382 of the ORF1a polyprotein) and inserted into pGEX-fp1 plasmid DNA (Amersham Biosciences) using BamHI and XhoI sites. The forward and reverse PCR primers used for amplification were 5′-CGGATCCGCTGGAAATGCTACAGAAGT-3′ and 5′-CGGGATCCGCTGGAAATGCTACAGAAGT-3′, respectively. The protein was expressed in *Escherichia coli* strain BL21 (DE3) as a thioglutathione S-transferase (GST) fusion protein and purified using glutathione affinity chromatography. The GST tag was removed by PreScission protease (Amersham Biosciences), leading to five additional residues (GPLGS) at the N terminus. The protein was further purified by a G25 desalting column in 2 mM dithiothreitol (DTT) and concentrated to 10 mg ml\(^{-1}\) in preparation for crystallization screening.

*Dodecamer Structure of Severe Acute Respiratory Syndrome Coronavirus Nonstructural Protein nsp10*

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Coronaviruses (CoVs) are positive-stranded RNA viruses known to cause highly prevalent respiratory and enteric diseases in humans and animals. About two-thirds of their genomes encode the viral replicase that mediates viral RNA synthesis (19). The replicase gene is comprised of two open reading frames at the 5′ end of the genome, termed ORF1a and ORF1b (26). The upstream ORF1a encodes polyprotein pp1a (450 to 500 kDa), while ORF1a and ORF1b together encode pp1lab (750 to 800 kDa). Expression of the ORF1b-encoded half of pp1lab requires a −1 ribosomal frameshift during translation. The polyproteins pp1a and pp1lab undergo extensive proteolytic processing by viral proteases to produce 16 functional subunits known as nonstructural proteins, which then assemble to form the replicase complex required for viral replication and transcription.

Prior to the global severe acute respiratory syndrome (SARS) outbreak in 2003, scant attention was paid to coronaviruses by researchers, as this genus of viruses causes severe diseases predominantly in animals and only comparatively mild diseases in humans. In the wake of the SARS outbreak, greater attention has been focused on the replicate proteins with a view to understanding the replication/transcription machinery and to identify new therapeutic targets. To date, the three-dimensional structures of a series of nonstructural proteins, which then assemble to form the replicase complex required for viral replication and transcription. The severe acute respiratory syndrome coronavirus (SARS-CoV) nonstructural proteins nsp1 to nsp16 have been implicated by genetic analysis in the assembly of a functional replication/transcription complex. We report the crystal structure of nsp10 from SARS-CoV at 2.1-Å resolution. The nsp10 structure has a novel fold, and 12 identical subunits assemble to form a unique spherical dodecameric architecture. Two zinc fingers have been identified from the nsp10 monomer structure with the sequence motifs C-(X)2-C-(X)5-H-(X)6-C and C-(X)2-C-(X)7-C-(X)-C. The nsp10 crystal structure is the first of a new class of zinc finger proteins three-dimensional structures to be revealed experimentally. The zinc finger sequence motifs are conserved among all three coronavirus antigenic groups, implicating an essential function for nsp10 in all coronaviruses. Based on the structure, we propose that nsp10 is a transcription factor for coronavirus replication/transcription.
TABLE 1. Data collection and refinement statistics

<table>
<thead>
<tr>
<th>Statistical element</th>
<th>nsp10</th>
<th>nsp10 in the absence of zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Data collection statistics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell parameters</td>
<td>$a = 159.1 \AA$, $b = 321.8 \AA$, $c = 161.9 \AA$, $\alpha = \beta = \gamma = 90^\circ$</td>
<td>$a = 159.7 \AA$, $b = 322.7 \AA$, $c = 162.2 \AA$, $\alpha = \beta = \gamma = 90^\circ$</td>
</tr>
<tr>
<td>Space group</td>
<td>C222</td>
<td>C222</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>50 (2.2)–2.1</td>
<td>50 (2.6)–2.7</td>
</tr>
<tr>
<td>No. of all reflections</td>
<td>1,545,407</td>
<td>845,526</td>
</tr>
<tr>
<td>No. of unique reflections</td>
<td>276,259</td>
<td>114,838</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.5 (99.4)</td>
<td>100.0 (99.9)</td>
</tr>
<tr>
<td>Average I/σ(I)</td>
<td>10.5 (4.2)</td>
<td>6.6 (3.8)</td>
</tr>
<tr>
<td>$R_{merge}$ (%)</td>
<td>6.1 (36.3)</td>
<td>10.4 (40.1)</td>
</tr>
<tr>
<td><strong>Refinement statistics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of reflections used ($σ(F) &gt; 0$)</td>
<td>468,890</td>
<td>249,037</td>
</tr>
<tr>
<td>$R_{work}^{a}$ (%)</td>
<td>21.9</td>
<td>21.6</td>
</tr>
<tr>
<td>$R_{free}^{a}$ (%)</td>
<td>24.7</td>
<td>25.1</td>
</tr>
<tr>
<td>RMSD bond distance (Å)</td>
<td>0.013</td>
<td>0.008</td>
</tr>
<tr>
<td>RMSD bond angle (°)</td>
<td>1.67</td>
<td>1.79</td>
</tr>
<tr>
<td>Average B value (Å$^2$)</td>
<td>31.8</td>
<td>34.5</td>
</tr>
<tr>
<td><strong>Ramachandran plot (excluding Pro and Gly)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Res. in most favored regions</td>
<td>2,039 (87.6%)</td>
<td>1,932 (83%)</td>
</tr>
<tr>
<td>Res. in additionally allowed regions</td>
<td>278 (12.0%)</td>
<td>375 (16.1%)</td>
</tr>
<tr>
<td>Res. in generously allowed regions</td>
<td>0</td>
<td>18 (0.8%)</td>
</tr>
</tbody>
</table>

$^{a}$ $R_{merge} = \sum \Sigma |I_{h} - \langle I_{h} \rangle| / \sum \Sigma |I_{h}|$, where $\langle I_{h} \rangle$ is the mean of the observations $I_{h}$ of reflection $h$.

RESULTS AND DISCUSSION

**Overall structure.** The cDNA coding for SARS-CoV nsp10-nsp11 was amplified using PCR. The coded protein consists of amino acid residues 4231 to 4382 of pp1a, which are renumbered 1 to 152 hereafter for convenience, and includes both nsp10 and nsp11 regions. The recombinant protein was expressed in *E. coli* as a GST fusion protein, purified, separated from the GST tag, and crystallized in both the presence and absence of additional zinc ion. Crystals grown under these two conditions have the same morphology, and their crystal cell parameters are similar to each other. Unless otherwise mentioned, the higher resolution crystal (with supplemental zinc salt) will be used in the following structural description. The crystal form belongs to an orthogonal space group with 24 molecules of nsp10-nsp11 per crystallographically asymmetric unit and a $V_M$ of 3.1 Å$^3$ Da$^{-1}$, where $V_M$ is the Matthews’ coefficient. The crystal structure was determined using SAD phasing from the bound zinc ions. The structure model was refined to a final $R_{work}$ of 21.9% and $R_{free}$ of 24.7%. Refinement statistics are detailed in Table 1. All 24 crystallographically independent copies of nsp10-nsp11 have very similar structures, with all pair-wise Ca root mean square deviations (RMSD) being less than 0.5 Å. Residues before Ala9 (including five leading residues left from the tag) and Pro86-Gly88 and after Ser129 could not be traced due to lack of interpretable electron density. Since the latter region includes the entire nsp11 peptide, the crystallized protein is nsp10 per se; it is called such hereafter. The absence of Cys130 and subsequent residues is a result of incidental cleavage, as indicated by (i) the reduced molecular weight band from so-
The N-terminal region and on the inner surface of the dodeca-
structure of each nsp10 monomer. The first one is located in
outer radius of 42 Å and an inner radius of 18 Å (Fig. 2). Two
(Fig. 1). The dodecamer is hollowed in the center, with an
try and can be viewed as the assembly of four nsp10 trimers
structural spherical dodecamers related by a local twofold symmetry.
the crystal lattice.
C-terminal peptide deletion is required for the formation of
clear. However, analysis of the crystal packing suggests that the
of the loss of Cys130 and its C-terminal residues remains un-
ivalence mass spectrometry analysis (data not shown). The cause
and (ii) matrix-assisted laser desorption ionization–time of
alysis compared with that of a freshly prepared protein sample
of the total SAS of an nsp10 monomer), with 65% contributed
continuous, except that the region Pro86-Gly88 is missing in
the α4-β3 connecting loop. Nevertheless, in the 2.7-Å structure
which was solved using crystals grown in the absence of addi-
tional zinc, we were able to build the Pro86-Gly88 loop region
for some nsp10 monomers, suggesting that this region is flex-
ible in general. The central core of the nsp10 monomer is an
antiparallel β-sheet formed by strands β1 (residues 55 and 56),
β2 (65 to 69), and β3 (96 to 100). The central β-sheet is flanked
on one side by helices α3 (residues 70 to 73) and α4 (75 to 79),
while helices α1 (residues 10 to 18), α2 (23 to 32) at the N
terminus, helix α5 (107 to 113), and the extended C-terminal
coil shy away from the central core. Residues on the α4 helix
and α4-β3 loop constitute the N-terminal zinc binding site, and
the C-terminal coil contributes to the C-terminal zinc binding
site. A DALI (http://www.ebi.ac.uk/dali/) search indicated no
similar match to the nsp10 monomer in the current Protein
Data Bank (PDB), suggesting a novel fold for nsp10.

nsp10 is cysteine-rich, with 13 cysteines in its sequence,
mainly Cys17, Cys41, Cys46, Cys73, Cys74, Cys77, Cys79,
Cys90, Cys103, Cys117, Cys120, and Cys128. Of these, Cys74, Cys77, and Cys90 form the N-terminal zinc binding
site, while Cys117, Cys120, and Cys128 chelate the C-terminal zinc ion (Fig. 4). Cys130 is missing from the refined model due to
the loss of the C-terminal peptide. Although Cys17-Cys46 and
Cys41-Cys73 pairs are close in the three-dimensional structure,
with Sy-Sy distances of 5.5 Å and 4.7 Å, respectively, they do
not form disulfide linkages. This observation is consistent with
the presence of high concentrations of reducing agents in the
crystallization reservoir.

The trimeric assembly. Three monomers are related by a
noncrystallographic threefold symmetry to form a trimeric sub-
unit (Fig. 1A). The trimer is stabilized largely by hydrophobic
interactions. The buried solvent-accessible surface (SAS) of an
nsp10 monomer within a trimer is about 620 Å² (or about 9% of
the total SAS of an nsp10 monomer), with 65% contributed
from hydrophobic atoms. Residue Val21 in the α1-α2 loop of
one monomer forms hydrophobic interactions with residues
Val57 and Thr58 in strand β1 of an adjacent monomer; resi-
dues Thr115 and Thr118 of one monomer also form hydro-
phobic interactions with Thr118 and Val119 of an adjacent
monomer. Additional hydrogen bonds are formed between the
Nε atom of Lys25 in one molecule and the Oζ atom of Glu60
in an adjacent molecule, with a distance of 2.6 Å, and between
the main chain O atom of Pro84 in one molecule and the Nε
atom of Lys95 in an adjacent molecule, with a distance of 3.3
Å. Furthermore, the three monomers are oriented such that
their C-terminal zinc fingers are clustered around the threefold
axis, with their zinc ions separated by approximately 14 Å.

Trimer-trimer interactions and the dodecamer architecture.
The assembly of the 24 copies of nsp10 into two identical

FIG. 1. Ribbon representation of the nsp10 crystal structure. A.
One nsp10 trimer is viewed along the noncrystallography threefold axis
from the outside of a dodecamer. The three protomers are colored in
magenta, gold, and green. B. The remaining three trimers of the
dodecamer are shown in the same orientation as that for panel A using
the same color scheme. C. The same as panel B but rotated by 90°. D.
The relationship between threefold axes. The twist angle between pairs
of threefold axes is approximately 108°. E. The complete dodecamer
are shown in the same orientation as that for panel A using
magenta, gold, and green. This figure was prepared with the
programs Molscript (8), Bobscript (5), and Raster3D (10).
dodecamers indicates that the dodecamer is a stable structural unit of nsp10 under the crystallization conditions. The four nsp10 trimers (named trimers 1 to 4) in a dodecamer are related by a tetrahedral symmetry (Fig. 1). Any combination of three trimers is related by a local threefold symmetry, and so is a combination of two trimers related by a local twofold symmetry. The buried SAS of a trimer in the dodecamer is about 3,040 Å² (or about 16% of the total SAS of an nsp10 trimer), with 69% contributed by hydrophobic atom groups. The crystal structure shows that helix H9251 and residues in the H9251-H9252 loop (residues 42 to 46) play a key role in trimer-trimer interactions. In the first (threefold symmetry-related) interaction region, Leu14, Cys17, Ala18, and Cys79 of trimer 1 form a hydrophobic base, which is directed towards Phe19 of trimer 2. At the same time, Phe19 of trimer 1 forms hydrophobic interactions with the equivalent hydrophobic base of trimer 3, and so on. In the second (twofold symmetry-related) interaction region, Met44 and Leu45 of trimer 1 are oriented to interact directly with their counterparts in trimer 2; Val42 and Tyr96 of trimer 1 interact with Tyr96 and Val42 of trimer 2, respectively. Both Cys17 and Cys79 contribute to stability of the dodecamer architecture, although they do not form a disulfide bridge in the reduced conformation state. Following this protocol, the 12 molecules can assemble to form a pseudododecahedron.

The zinc ion binding sites. Zinc binding is a major structural feature of nsp10. Spectral analysis of the nsp10 crystal during synchrotron data collection clearly demonstrated the presence of zinc, evident by a clear peak near the zinc absorption edge. It allows the nsp10 crystal structure to be solved by the SAD method. Furthermore, the 2.7-Å resolution structure of nsp10 determined from crystals prepared in the absence of additional zinc shows the same monomer fold, dodecameric architecture, and conformation in the zinc binding sites, with an RMSD of 0.4 Å for all Cα atoms in a dodecamer.

Two bound zinc ions were identified from the crystal structure of nsp10 with unambiguous electron density: one located in the N-terminal region on the inner surface of the dodecamer and the other one at the C-terminal region on the outer surface (Fig. 2 and 4). The first zinc binding site is formed by residues on helix α4 and the α4-β3 loop. This zinc ion is tetrahedrally chelated by the Sγ atoms of three cysteine residues (Cys74, Cys77, and Cys90) and the Nε2 atom of His83, which have bond distances of 2.3 Å, 2.3 Å, 2.4 Å, and 2.2 Å, respectively (Fig. 2).
This binding site constitutes a CCHC-type zinc finger with a C-(X)_2-C-(X)_5-H-(X)_6-C sequence motif. The second zinc ion is located at the C terminus and is also tetrahedrally coordinated (Fig. 4B). Three zinc-chelating cysteine residues (Cys117, Cys120, and Cys128) have distances of 2.4 Å from the zinc ion to their side chain S atoms. The fourth ligand is a water molecule, with clearly defined electron density, whose distance to the zinc ion is 2.5 Å. This chelating water molecule also interacts with Ser129 through a perfect hydrogen bond to form a stabilized hydrogen bond network. All bond lengths related to zinc binding are summarized in Table 2. Noteworthy is the observation that Cys130 neighbors Ser129 in the nsp10 sequence but did not exist in the crystallized protein. Otherwise, the presence of Cys130 might suitably position it to chelate the zinc ion, suggesting the second zinc binding site should also be a zinc finger of a C-(X)_2-C-(X)_7-C-(X)_C sequence motif. This CCCC-type zinc finger is distributed on the surface of the dodecamer and is relatively flexible in our structure compared to the CCHC-type zinc finger (Table 2). However, the flexibility of this region may not reflect the conformation of the full-length protein.

A multiple-sequence alignment of nsp10 from SARS-CoV with coronaviruses from groups I, II, and III of the genus Coronavirus indicates that all seven observed zinc-chelating residues, plus Cys130, are strictly conserved (Fig. 5), implying their importance in the functional replicase-transcriptase complex. In contrast, a number of other cysteine residues in SARS nsp10 are not conserved at all.

**Functional implications.** The finding of zinc binding sites in nsp10 provides a strong implication for nsp10 biological functions. The zinc finger is a common DNA binding motif found as part of transcription-regulatory proteins, which can often include more than one zinc finger in the same peptide chain. Moreover, increasing evidence suggests that zinc fingers can more widely be used to recognize RNA or even in protein-protein recognition.

Although the DALI search did not find any candidate structure similar to SARS-CoV nsp10 from PDB, a PFAM search (http://www.sanger.ac.uk/Software/Pfam) for similar sequence motifs identified several members of the HIT-type zinc finger family as nsp10 homologous candidates. Named after the first protein that originally defined the domain, the yeast HIT1

### TABLE 2. Zinc chelation

<table>
<thead>
<tr>
<th>Zinc ion</th>
<th>Chelating residue</th>
<th>Chelating atom</th>
<th>Bond lengtha (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn 1</td>
<td>Cys74</td>
<td>Sγ</td>
<td>2.29 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Cys77</td>
<td>Sγ</td>
<td>2.27 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>His83</td>
<td>Nε2</td>
<td>2.18 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Cys90</td>
<td>Sγ</td>
<td>2.40 ± 0.03</td>
</tr>
<tr>
<td>Zn 2</td>
<td>Cys117</td>
<td>Sγ</td>
<td>2.41 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Cys120</td>
<td>Sγ</td>
<td>2.45 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Cys128</td>
<td>Sγ</td>
<td>2.38 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Wat997</td>
<td>H2O</td>
<td>2.51 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Wat997</td>
<td>H2O</td>
<td>2.76 ± 0.16</td>
</tr>
</tbody>
</table>

a The listed bond length is an average value over the 24 monomers with standard deviations.
protein, the HIT-type zinc finger contains seven conserved cysteines and one histidine that can potentially coordinate two zinc atoms. While the function of the HIT-type zinc finger is unknown, this motif is mainly found in nuclear proteins involved in gene regulation and chromatin remodeling. To date, there are no three-dimensional structures of HIT-type zinc finger domains reported in the PDB. Therefore, our nsp10 crystal structure serves as the first example of a three-dimensional structure of this novel class of double zinc finger-containing motif.

This finding provides strong structural evidence that, like other better studied nsp proteins, nsp10 also likely plays a role in RNA synthesis, as suggested by other researchers (26). nsp10 is involved in network interactions with other nsp proteins, and the integrity of its zinc fingers seems important for such interactions. Experiments on mouse hepatitis virus (MHV), a group II coronavirus along with SARS-CoV, demonstrated the colocalization of nsp10 with nsp7, nsp8, and nsp9, providing solid evidence for their interaction in the coronavirus life cycle (2). Our chemical cross-linking experiment further demonstrated that SARS-CoV nsp10 can be cross-linked with nsp9 (data not shown), which itself interacts with nsp8 (17). Furthermore, an MHV ts mutant, Alb ts6, encoding a mutant form of nsp10 with a Gln65-to-Glu mutation, was shown to have a defect in negative-strand RNA synthesis (15). The Gln65 residue, conserved in all three groups of the genus Coronavirus, is located on strand B2 of the SARS nsp10 structure and hydrogen bonds via the Nε2 atom to the main chain carbonyl oxygen of Gly52. Gln65 is thus important for the conformational stability of nsp10 and particularly for the α4 helix which forms part of the N-terminal CCHC zinc finger. Therefore, mutation of Gln65 might be expected to perturb the folding of pp1a into a less productive conformation that would prevent it from participating in the formation of a replicase-transcriptase complex with negative-strand activity.

Conclusions. The scientific significance of the SARS-CoV nsp10 structure is at least threefold. First, nsp10 has a novel protein fold. A search with the DALI web engine (http://www.ebi.ac.uk/dali/) for structural homologs failed to yield any match to the nsp10 fold, suggesting a novel function for the nsp10 family members. Second, nsp10 possesses two zinc fingers, located in the N-terminal region and at the C-terminal region, with C-(X)2-C-(X)7-H-(X)6-C and C-(X)12-C-(X)7-C-(X)-C sequence motifs, respectively. These motifs are conserved in all three groups of the genus Coronavirus, and our crystal structure illustrates for the first time the significance of these conserved residues. Further sequence analysis suggests that nsp10 is related to the HIT-type zinc finger family, which is often found in nuclear proteins involved in gene regulation and chromatin remodeling. Thus, our nsp10 crystal structure becomes the first of a new class of zinc finger protein three-dimensional structures to be revealed experimentally. Third, the molecular assembly of nsp10 is a hollow dodecamer with an outer diameter of 84 Å and an inner diameter of 36 Å. Twelve C-terminal zinc fingers stick out from the outer surface of the sphere, and another 12 zinc fingers are distributed around the inner surface. The strong positive electrostatic potential found on both the inner and outer surfaces of the dodecamer is intriguing, consistent with the probable function of nsp10 in the RNA synthesis machinery.

To date, the structures and functions of several components of the replication/transcription machinery have been determined, including the nsp3 ADP ribose 1-phosphatase domain (14); nsp5 (23, 24), nsp7, and nsp8 in complex (25); and nsp9 (4, 17). nsp5 is the main protease for cleavage of the replicase polyproteins, nsp7 and nsp8 are proposed to function as processivity factors for nsp12 (the RNA-dependent RNA polymerase), and nsp9 is a single-strand RNA binding protein. The crystal structure reported here will help to clarify the function(s) of nsp10, in which the presence of two zinc fingers should enable it to play an important role in RNA synthesis. Elucidation of the nsp10 structure will provide further insights into the sophisticated replication/transcription mechanism of SARS-CoV and other coronaviruses, such as mouse hepatitis virus (MHV), human coronavirus strain 229E, and human coronavirus strain HKU1.

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