The coronavirus SARS-CoV-2 uses an RNA-dependent RNA polymerase (RdRp) to replicate and transcribe its genome. Previous structures of the RdRp revealed a monomeric enzyme composed of the catalytic subunit nsp12, two copies of subunit nsp8, and one copy of subunit nsp7. Here we report an alternative, dimeric form of the enzyme and resolve its structure at 5.5 Å resolution. In this structure, the two RdRps contain only one copy of nsp8 each and dimerize via their nsp7 subunits to adopt an antiparallel arrangement. We speculate that the RdRp dimer facilitates template switching during production of sub-genomic RNAs.
neither the catalytic sites nor the RNA duplexes are involved in RdRp dimer formation. It is therefore likely that the two RdRp enzymes remain functional within the dimer structure. The two RdRp enzymes in the dimer may thus be simultaneously involved in RNA-dependent processes. Unfortunately, we could not test whether the RdRp dimer is functional because we were unable to purify it. In particular, we attempted to reconstitute the RdRp with a nsp12:nsp8:nsp7 stoichiometry of 1:1:1, but obtained preparations showed again an apparent stoichiometry of 1:2:1 that was observed in previous RdRp structures. Therefore, the functional relevance of the RdRp dimer reported here needs to be established.

We hypothesize that the RdRp dimer is involved in the production of sub-genomic RNA (sgRNA). In this intricate process, positive-strand genomic RNA (gRNA) is used as a template to synthesize a set of nested, negative-strand sgRNAs that are 5’ and 3’ coterminal with gRNA. The obtained sgRNAs are later used as templates to synthesize viral mRNAs. Production of sgRNAs involves a discontinuous step, a switch of the RdRp from an upstream to a downstream position on the gRNA template26. These positions contain transcription regulatory site (TRS) sequences27–29, but it is enigmatic how a single RdRp enzyme could ‘jump’ between these.

Our dimer structure suggests a model for sgRNA synthesis that extends a recent proposal30,31 (Fig. 2). In the model, one RdRp of the dimer (RdRp 1) synthesizes sgRNA from the 3’ end of the gRNA template until it reaches a TRS in the template body (TRS-B). Due to the lack of one nsp8 subunit, the dimeric RdRp is predicted to have lower processivity than monomeric RdRp16,14 and this may facilitate TRS recognition. The viral helicase nsp13 could then cause backtracking of the RdRp30,31. Backtracking exposes the 3’-end of the nascent sgRNA, which is complementary to the TRS and may hybridize with another TRS located in the leader (TRS-L) at the 5’-end of the template. The resulting RNA duplex could then bind to the active center of the second RdRp (RdRp 2) to continue sgRNA synthesis.

In our model, it is not the RdRp that switches to a second RNA position, but instead the RNA switches to a second RdRp. After the switch, RdRp 1 may backtrack further, whereas RdRp 2 could move forward until it reaches the 5’-end of the template. These movements occur on the same template but in opposite directions and would be facilitated by the antiparallel arrangement of the polymerases. Superpositions show that only one copy of the template-stand engaged nsp1332 can be modeled on our dimer structure without clashes (Supplementary Fig. 3d). However, the interaction of this nsp13 copy to the monomeric RdRp is partially mediated by the nsp8 copy that is absent in the dimeric form. Thus, how backtracking in a dimeric complex may be facilitated remains unclear and it is possible that the second nsp13 copy that was previously not observed to be engaged with template RNA is involved in this process (Supplementary Fig. 3e).

Although the functional relevance of the RdRp dimer remains to be established, we note that RdRp dimerization and oligomerization has been reported for many other viruses including Influenza, Polio, Hepatitis C, Norovirus, and others33. RdRp oligomerisation can be important for cooperative template binding34 and can be critical for the viral life cycle35,36. Future work should therefore concentrate on the preparation and functional analysis of the coronavirus RdRp dimer and possible additional higher-order structures of the enzyme.

Methods

Cryo-EM sample preparation. We reused the already processed data set 3 from our previous publication11. Briefly, RNA sequence for the scaffold used was:

rUUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrU
**Fig. 1** Structure of antiparallel RdRp dimer. **a** Two views of a ribbon model of the antiparallel RdRp–RNA dimer. Color code for nsp7, nsp8, nsp12 domains (NiRAN, interface, fingers, palm, and thumb). RNA template (blue), and RNA product (red) is used throughout. Nsp7 subunits in the two RdRp monomers are colored slightly differently for the two monomers (dark and light blue, respectively). Views are related by a 90° rotation around the vertical axis. **b** Close-up view of nsp7–nsp7 dimerization interface. View is as in the left structure of panel **a**. The final cryo-EM density is shown as a black mesh.

**Fig. 2** Hypothetical model of subgenomic RNA production for viral transcription. **a** Genomic positive-strand (⊕) RNA (gRNA) is used as a template to produce the 5′ region of negative-strand (⊖) sgRNA until TRS-B is reached by the RdRp monomer 1. **b** Backtracking is mediated by nsp13 helicase and exposes the newly synthesized, complementary TRS sequence. **c** The complementary sequence in sgRNA can pair with the downstream TRS-L in gRNA and is then loaded into RdRp monomer 2. **d** RdRp 2 then completes ⊖ sgRNA synthesis while RdRp 1 backtracks further.
rGUrA and rGGrA rGGrC rUrGC rUrArC rGGrC rAr/r -rGGrUrG. RdRp–RNA complexes were formed by mixing 1.6 nmol of purified nsp12 with an equimolar amount of RNA scaffolds (4.8 nmol of each nsp8 and nsp7). The mixture was incubated for 10 min and afterwards applied to a Superdex 200 3.2/300 size exclusion chromatography column, which was equilibrated in complex buffer (20 mM Na-HEPES pH 7.4, 100 mM NaCl, 1 mM MgCl2, 1 mM TCEP) at 4 °C. Peak fractions corresponding to the RdRp–RNA complexes were pooled and diluted to 1 mg/ml. The concentration of the concentrated RdRp–RNA complexes was mixed with 0.5 µl of octyl β-d-glucopyranoside (0.0033% final concentration) and applied to freshly glow discharged R 2/1 holey carbon grids (Quantifoil). The grids were blotted for 5 s using a Vitrobot MarkIV (Thermo Fischer Scientific) at 4 °C and 100% humidity and plunge frozen in liquid ethane.

Providing the cryo-EM data. Data collection and preprocessing was the same as previously described. Briefly, data was collected using SerialEM39 on a 300 kV Titan Krios transmission electron microscope (Thermo Fischer Scientific) and a K3 direct electron detector (Gatan). Inelastically scattered electrons were filtered out prior to detection using a GIF quantum energy filter (Gatan) using a slit width of 20 eV. Images were acquired at a nominal magnification of 105,000x and a calibrated pixel size of 0.834 Å/pixel. Due to previously observed preferred orientation when imaging RdRp complexes, data was collected using a 30° tilt to obtain more particle orientations. 7043 raw micrographs were acquired in total and preprocessed on-the-fly in Warp for automatic contrast transfer function (CTF) estimation, averaging, motion correction, and particle prediction and extraction. 2.2 million individual RdRp particles were predicted and extracted by Warp and imported into cryoSPARC and subjected to a Hetero Refinement job using five ‘Junk’ classes and one class representing monomeric RdRp as described previously. The resulting particle set was used for a 3D homogenous refinement to obtain refined poses and positions for each RdRp monomer.

Initial detection of RdRp dimers in cryo-EM data. To analyze the statistical distribution of RdRp monomers in our cryo-EM data, we calculated the nearest-neighbor (NN) distances and the relative orientations for all neighboring RdRp complexes using the previously refined monomer poses. To account for the tilted data acquisition, we treated the influence on distances observed on the micrograph as a 30° rotation around the x-axis. We chose to express relative orientation through a single angle by calculating the angle of the rotation around the eigenvector of the rotation matrix. This showed that certain distances and relative orientations between two monomers were highly prevalent (Supplementary Fig. 1) and indicated the presence of dimeric particles where the two RdRps would adopt a specific distance and relative orientation with respect to each other. We then located such RdRp dimers in micrographs by identifying pairs of RdRps within a narrow range of NN distances and orientations. We observed an overrepresented RdRp distance of 80 Å and relative angle of 180°. Furthermore, we could observe that the overrepresented distance and relative orientation correlated with one another, indicating the occurrence of a defined RdRp dimer (Supplementary Fig. 1). We initially obtained ~31,000 dimeric particles using a distance <90 Å and relative orientation larger than 166° as a filtering criterion.

Detection of additional dimeric particles. Because the yield of dimeric particles depended on both halves of a dimer being first detected as monomer, we aimed to detect more RdRp monomers using two strategies. First, we carried out template-based picking and particle extraction in RELION38 using the monomeric RdRp–RNA structural model as a reference to detect RdRp–RNA monomers without a gap between them. After 2D classification, we used 27,673 particles for an ab initio refinement with three classes. For the subsequent 3D homogenous refinement job in cryoSPARC, we chose a reconstruction from the ab initio refinement that had two clearly resolved RdRp monomers close to one another as a reference. A molecular model was obtained by placing two copies of RdRp–RNA complexes (PDB:7B3D, structure 3 from our previous publication)31, removal of mobile regions and fitting of domains as rigid bodies was done in UCSF Chimera40.

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Author contributions

F.A.J. carried out data analysis, assisted by D.T. H.S.H. and G.K. helped with modeling. H.S.H., G.K., J.S. and C.D. helped with structure interpretation. P.C. supervised the project and wrote the manuscript, with input from all authors.

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Competing interests

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Correspondence and requests for materials should be addressed to P.C.

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