

Antiviral immunity via telomerase-like reverse transcription in bacteria

Corresponding Author: Dr Samuel Sternberg

Any redactions in this file are there to maintain patient confidentiality, the confidentiality of unpublished data, or to remove third-party material.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)

Tang and colleagues present a mechanistic dissection of DRT10, a previously uncharacterized member of the UG (unknown group) reverse transcriptase family, and report several findings of genuine significance to the RT field. The central discovery is that DRT10 uses a C-terminal serine residue as the priming nucleophile. To my knowledge this is the first reported instance of serine-primed reverse transcription, and it represents a departure from the tyrosine-priming chemistry seen in every protein-primed RT studied to date, the Abi family included. Truncation, substitution, and rescue mutagenesis of the ncRNA template define an A-B-A' architecture in which a templating segment is flanked by a downstream homology element. This element drives iterative dissociation and realignment of the nascent cDNA to prime successive rounds of extension. Phylogenetic analysis groups TERT, PLE, and Class 2 UG RTs into a single clade and raises the intriguing possibility that telomerase-like repeat addition has bacterial origins. Consistent with this view, chimeric ncRNAs bearing telomerase RNA template segments direct synthesis of the cognate telomeric repeats in both *E. coli* and HEK293T cells.

Despite these strengths, the manuscript falls short of the bar for Nature because it leaves the two questions most central to its own framing unanswered. First, no structural data are reported for the DRT10 RT-ncRNA complex. This is a striking omission, given that the serine-priming claim sits at the heart of the paper's novelty, and given that comparable systems (DRT2, DRT9) have been solved by cryo-EM in recent contemporaneous work. A structure resolving Ser456 in the active site, ideally trapped at the priming step or with the ncRNA template threaded through the enzyme, would convert the serine-priming assignment from a strong inference grounded in mass spectrometry depletion and AlphaFold modeling into a definitive mechanistic statement. Second, and in my view the more serious issue, the immune function of the tandem-repeat cDNA product is not established. The system is framed throughout as an antiviral defense, yet no experiment links the unusual RT chemistry to the protective phenotype. The proposed model in which cDNA regulates the SLATT pore is not tested. The authors acknowledge that cDNA abundance decreases rather than increases during phage infection, opposite to the behavior of DRT2 and DRT9, but offer no mechanistic explanation. Absent either a structural account of the priming chemistry or more importantly, a functional connection between cDNA synthesis and host protection, the contribution amounts to a careful description of an unusual enzymatic activity that cannot by itself carry a paper in a generalist venue. The work would be more appropriately placed at Nature Communications, where the mechanistic depth would be appreciated and where the absence of a defined defense mechanism would not undermine the principal claims.

Minor comments:

Page 4 line 63 references Fig. 1b but describes a plaque assay shown in Fig. 1c.

Page 14 line 379 references Extended Data Fig. 7c but describes in vitro assays shown in Extended Data Fig. 8e.

Page 27 line 781 and page 28 line 809 both reference Fig. 3h, but there is no Figure 3h.

Referee #2

(Remarks to the Author)

In this study, Tang et al. investigate a candidate anti-phage defense system from the UG17 group of reverse-transcriptase which they call DRT10. This system is particularly intriguing for its relationship to eukaryotic telomerases.

They reveal a ncRNA through RIP-seq, and identified conserved sequences and structural features of the ncRNA and were able to experimentally validate their importance in anti-phage defense, as well as that of a SLATT protein next to the RT. Sequencing extrachromosomal DNA revealed cDNA products of the RT with a 9nt repeat motif present a median of 15 times. The authors then reconstituted the RT activity in vitro, and confirmed its ability to generate ssDNA repeats of 9nt yielding products of kilobase size.

The authors then elegantly show how synthesis is protein-primed at a serine residue using a combination of mass-spectrometry and genetics. They further describe a mechanism of repeat creation through iterative cycles of extension, dissociation, and template resetting, which they nicely demonstrate by mutating the A and A' segments involved in resetting. They describe rules that enable a complete reprogramming of the repeat sequences (up to a certain length). The authors further propose convincingly that the ABA' resetting mechanism is shared by other DRT systems.

They then note the parallel between the mechanism they describe here and telomerase repeat addition and further investigate whether this is due to convergent evolution or shared ancestry. The conclusions of this analysis are key to raising the impact to this work to the level of a publication in Nature. The authors seem to tackle this through a combination of approaches that lend credibility to the conclusions that telomerase RT branch within the bacterial Class 2 UG RT. While I find the results exciting and believable, I must however note that I'm not an expert in phylogeny and can only provide a superficial evaluation of this part of the work. They further demonstrate that while DRT10 does protein-primed reverse-transcription, it can also prime on DNA 3' ends and work in human cells, further adding credibility that this family of RT had all the mechanistic properties required to act as telomerase and could have been co-opted to protect linear genomes against replication-associated DNA loss.

The paper is an important contribution to the field. It is overall very well written, all the genetics and molecular biology experiments are performed to a high standard and support the conclusions.

I only have minor comments:

The ssDNA size in vitro seems to easily reach 3kb or more, what limits its size to 15 repeats in vivo?

Beyond eukaryotic telomerase, could the same mechanism of repeat addition be involved in the maintenance of linear phage genomes or linear bacterial genome ?

While not needed for publication of the study, I'm be curious to know if the DRT10 can catalyze repeat addition to 3' ends after DNA breaks in E. coli or to the end of a phage genome upon DNA injection?

Referee #3

(Remarks to the Author)

In this manuscript, Tang et al. investigated a new antiviral defense system called DRT10. Using bioinformatics and cell-based assays, they identified its core components, including an ncRNA, a SLATT protein, and a reverse transcriptase (RT). The study focused primarily on the ncRNA and RT. In an extensive biochemical analysis, they clarified how the RT uses ncRNA as a template to synthesize long strings of single-stranded repeat-sequence DNA. They pinpointed a serine residue essential to its function and provided evidence that it serves as a primer for reverse transcription. Notably, they showed that the RT uses the A-B-A' motif to synthesize DNA, a feature shared with telomerases that allows synthesis of sequence repeats. Furthermore, bioinformatic and biochemical data suggested a possible functional and evolutionary connection between DRT10 and telomerase, implying that they may have originated from a common ancestor. Overall, the manuscript is well-written. Because the findings bridge two formerly disparate fields, bacterial phage defense and eukaryotic telomere replication, there will be a large audience for these unexpected and intriguing results.

Major comment:

1. Interpreting the phylogenetic tree would benefit from more caution, particularly when the branch point remains unidentified. Lines 51-52 say the RT "originated in bacteria before being co-opted" for telomerase, which could be interpreted to suggest horizontal gene transfer. Lines 398 ("common bacterial ancestor") and 405 ("bacterial origin of telomerase") might be more conservatively expressed as the DRT10 and telomerase having a common ancestor, just as LUCA cannot be classified as bacterial or archaeal or eukaryotic. In other words, the tree in Fig. 5d seems completely justified by the data, with the ancestral RT preceding the divergence of bacteria from eukaryotes, whereas the word "bacterial" on line 1249 could be deleted.

Minor comments:

1. Lines 23-24, TERT was unknown to the cited authors, and was discovered a decade later by the Cech and Lundblad labs (Science, 1997).
2. Line 64, "mutation of the RT catalytic residues" could be interpreted to mean that they're in SLATT! Help the reader by adding a sentence about the DD motif, catalytic Mg++ ions, cite reference.
3. Figure 1f and lines 87-90. While the step-loop structure is presumably perturbed, this has not been experimentally confirmed. Caution is advised when interpreting the results, given the dynamics of RNA structures. Additionally, the sequences following the scramble could not be found in the manuscript. The same issue applies to Figure 3g.

4. Figure 2i. Line 176 states "... a high-molecular-weight smear upon dNTP incubation that was unaffected by RNase treatment but largely eliminated by DNase treatment". Rather than eliminating the bands, Exol treatment produced additional bands, and a significant high-molecular-weight smear persisted. The additional bands imply that Exol had an effect, whereas RNase A did not. The results in Extended Figure 3f are much more convincing than those in Figure 2i.
5. Lines 224 – 225, although the authors have not yet tested if the extension is distributed or processive, the synthesis of long repeats suggests that it will be processive, i.e., product not completely dissociated from the enzyme after each repeat. It would help reader to add a phrase or a sentence to cover this.
6. Lines 421-423 seem redundant with next paragraph (and lines 437-438). Considering how little is known, less could be said here.
7. It took me some time to fully understand Figure 3d, particularly the arrows and the labels "-1, -2, -3". If the "5' truncation (-1) and two arrows" indicates one nucleotide truncation from both A and A', I would suggest describing each component separately in the figure legend.
8. Figure 1f and Figure 3a. Please clarify whether the 3 technical replicates are from the same experiment or from 3 biological replicates.

Referee #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #5

(Remarks to the Author)

Review of: Antiviral immunity via a telomerase-like reverse transcription in bacteria.

This interesting paper identifies a novel form of bacterial defence associated reverse transcriptase (DRT10) that generate DNA repeat additions. The paper identifies many mechanistic aspects of these reverse transcription function and demonstrates function of this system associated with SLATT proteins can generate phage protection (although does not directly demonstrate how phage protection is achieved – which would also be cool). The work then goes on to demonstrate that these enzymes show some functional similarity to telomerases, while phylogenetic analyses show that the DRT10s and telomerases are phylogenetically related to the exclusion of many other reverse transcription systems. Based on these analyses the authors put forward an exciting idea that the shared subsection of the wider RT gene family led to viral protection in bacteria and linear chromosome function through telomere capping in the early eukaryotes. This is an exciting paper.

The editorial team asked me to review the comparative evolutionary biology and phylogenetic aspect of this paper given my 'expertise' lies in this area rather than viral defence or reverse transcription mechanistic biology which is reported here.

Minor comments:

1) I do not recognise either the terms 'local support value' or 'ensemble bootstrap value' as common methods for analysing topology support for these types of phylogenies. Both are notated as 0.X in the figures suggesting they are branch-specific likelihood ratio test (LRT) or posterior probabilities. If they are bootstraps, they are notated incorrectly. Such values usually appear as % values indicating they have been derived from parallel analysis of pseudo-replicates. My guess is that they were not from alignment pseudo-replicates (note bootstrap pseudo replicates are not the same as TBE). Discussed more below.

2) Similarly details of what type of bootstrap analysis conducted were absent from the methods section. They do however report a TBE analysis in the methods which is quite nice. But it is not recorded how this set of results relates to either the 'local support value' or 'ensemble bootstrap value' notated on the figure legends. This all needs to be clarified and at some point, proper bootstrap analysis with pseudo-replicated alignments should be incorporated if not done already. This may show reduced support for the DRT10 and telomerase clustering pattern. I think this is fine and the authors can contrast this with the TBE analysis (aLRTs and Bayesian posteriors?) given the nature of the alignments, i.e., a large number of sequences and few informative alignment positions. I still suspect that they would arrive at a similar conclusion but they would have demonstrated a full phylogenetic analysis process to widest field of evolutionary biologists present in the journal readership.

3) Throughout, the authors use a '-wag' '-gamma model' for modelling sequence change in their phylogenetic analysis. Usually, model selection is tested using either a separate program or the phylogenetic program itself derives a model comparison. I do not know if FastTree 2 does this? If it does the details of model comparisons and selection should be included in the methods. If not, the authors should subject their alignment to alternative analysis to identify appropriate sequence substitution models and then incorporate these models in their analyses. Assuming a -wag -gamma model excluding parameters such as I and F may lead to errors in their tree topology. Also WAG as a substitution matrix is a little dated. It may be the best matrix but it all needs testing.

4) I found figure 5D problematic. i) I am not sure the emergence of linear genomes (in the sense of eukaryotic linear chromosomes) placement is correct. I know some prokaryotes have linear chromosomes but I do not think this is the point the authors care about. I don't think they are 'homologous' to eukaryotic centromeric/telomeric chromosomes. So, should

linear centromeric/telomeric chromosomes be placed at the base of eukaryotes? ii) The figure shows Animal TERT is a product of multiple genes shared with plants and separately fungi, which is not possible unless there was a HGT or complex paralogue loss, this needs clarification or simplification. Animal TERT cannot be on the end of two separate polyphyletic branches without explanation. iii) I do not understand the topology given current tree of life data. Flagellates and plants seem to be in the wrong place. This tree looks like a 1977 Woese tree...

5) I think the authors should carefully reword some text in places because they seem to suggest that the bacterial phage defence system was the ancestral function which led to telomerase. Their trees currently do not show this. Although this is formally possible it is equally possible that an unknown common functional property independently led to two different functions (phage defence and telomerase). This slight correction in wording in several places does not distract from the novelty of this work in my opinion, but would make the interpretation a little more robust.

Very general comment. I wonder if the authors are aware of a prevailing idea that group II introns gave rise to eukaryotic spliceosomal introns. If this theory is true. That would suggest that two parallel domestications of ancestrally RT-capable prokaryotic systems occurred in the origin of the eukaryotes. I find this interesting given the radical change in gene complement and genome evolutionary dynamics that characterises the origin of the eukaryotes.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)

My previous two main critiques were: 1) lack of a high resolution cryo-EM structure and 2) the mechanism connecting enzyme activity to phage defense was unknown. The authors addressed criticism #1 very well with multiple cryo-EM structures illuminating the priming mechanism. However, #2 remains unaddressed. Phage defense papers in this type of generalist venue typically connect unusual enzyme activity to phage defense with a well delineated mechanism. That component is still missing. The work is very thorough and well done, but lacks the mechanistic significance expected of Nature.

Referee #2

(Remarks to the Author)

The authors have done substantial work to improve the manuscript and have addressed all my comments. I support the publication of this article.

Referee #3

(Remarks to the Author)

Sternberg and colleagues have submitted a revised manuscript on the mechanism of action of a fascinating bacterial defense system consisting of a noncoding RNA and a reverse transcriptase protein. The revision is extensive. First, they address the reviewers' comments in great detail. As one major example, the weak claim that their data support eukaryotic telomerases as having a bacterial ancestry is now judiciously restated, much to the benefit of the paper. Second, they solve and analyze the molecular structures of two of the bacterial RT-RNA complexes, which adds two powerful main figures to the presentation. The structures support the conclusions presented in the initial submission and also extend the story in new directions, revealing 2:1 RT:RNA complexes with mechanistic implications. All told, this is an exciting and rigorous contribution that will attract a wide audience and is eminently suitable for publication without further revision.

I will take this opportunity to voice a concern about Nature editorial policy. I considered the initial submission of the Sternberg paper to require more analysis and rewriting, but not additional laboratory experiments. Although the reviewers had a range of comments, their consensus appeared to be that the lab experiments were sufficient. Thus, it was discouraging to me to see that the paper was rejected. Now the cryo-EM structures have been added, which strengthens the paper but is detrimental to the careers of grad students and postdocs who deserved to have a separate publication on the structures. I recommend that the editors consider the current trend of moving the bar for publication higher and higher, to the point that each paper represents 20 person-years of work.

Referee #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #5

(Remarks to the Author)

This is my second review. I found that the authors have dealt with all the reviewers comments adequately. I have no further

comments.

I also agree with the reviewers that work reporting exactly how the DRT10 manifests phage protection is a huge subject and worthy of a separate major paper. This separation does not distract from the novelty and interest of this paper, which are both very strong.

Open Access This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

August 10, 2026

Tang, Ramirez, He *et al.*

Response to reviewers' comments: Nature manuscript 2026-03-09614

We thank the editor and referees for their careful evaluation of our manuscript, "Antiviral immunity via telomerase-like reverse transcription in bacteria" (2026-03-09614). We are grateful for the opportunity to submit this appeal of the July 10, 2026 decision, in accordance with the editorial team's instructions of July 15, 2026, and following our subsequent correspondence regarding combining this study with our companion cryo-EM structural analysis of the DRT10 RT–ncRNA complex. We apologize to the referees for the substantial resulting increase in the length of this manuscript, and we are enormously grateful for their willingness to consider this expanded body of work.

In revising the manuscript, we made several substantial improvements, which we believe respond directly to the three principal concerns raised in the decision letter:

- We have determined three new cryo-EM structures of the DRT10 RT–ncRNA complex — apo structures of *Eco*₁DRT10 and *Eco*₃DRT10 (3.2 and 3.3 Å, respectively), and a dNTP-soaked, pre-catalytic *Eco*₃DRT10 structure (3.0 Å) — which directly address Referee #1's concern regarding the absence of structural data supporting the serine-priming mechanism (**Fig. 3a–d, Fig. 4a–d**). The structures reveal an unanticipated 2:1 architecture in which two RT monomers engage opposite faces of a single, pseudo-symmetric ncRNA, resolve the C-terminal domain harboring the priming serine, and visualize the trajectory of the nascent cDNA relative to incoming nucleotides. Combined with a new radiolabeled nucleotide-transfer assay providing direct biochemical evidence of a covalent RT–DNA conjugate, and additional new biochemical experiments, these data substantially strengthen support for the serine-priming mechanism, they clarify how the template boundary is defined, and they explain why only one monomer generates the long repetitive product.
- We have performed substantial additional phylogenetic analyses in response to Referee #5, including explicit testing and reporting of substitution model selection across parallel phylogenetic reconstructions (rather than relying solely on a WAG+Γ model used in the principal analysis) with calculation of conventional column-resampling bootstrap values using pseudo-replicated alignments. We have also improved the manuscript text by clarifying the branch-support metrics reported in the figures (distinguishing SH-like local support and alignment-ensemble TBE support from posterior probabilities) and revising the **Figure 6d** schematic to address the unsupported placement of linear centromeric/telomeric chromosomes and the implied polyphyly of animal TERT.
- Per Referee #3's major comment, we have softened our language throughout regarding the directionality of the DRT10–telomerase relationship (e.g., replacing "bacterial origin of telomerase" with more conservative language regarding a shared ancestor), consistent with the phylogenetic evidence, which supports common ancestry but not a specific rooting.

- We have corrected the identified figure-citation errors and revised the text, figures, and legends throughout for clarity and consistency, incorporating the new cryo-EM data alongside the existing mechanistic and phylogenetic findings.

Collectively, we believe these revisions substantively resolve two of the three principal concerns raised in the decision letter — the lack of structural data and the rigor of the phylogenetic support for a common DRT10–telomerase origin — and directly engage with the third, the incomplete functional connection between the tandem-repeat cDNA product and phage defense. For this remaining question, we propose a specific, testable hypothesis (multivalent regulation of the SLATT pore by the repetitive cDNA product) and explain in our response to Referee #1 why we believe rigorously establishing this mechanism warrants a dedicated, focused follow-up study rather than additional experiments appended to an already substantially expanded manuscript. Together with the extensive new structural, biochemical, and phylogenetic data described above, we believe these revisions result in a substantially stronger and more complete manuscript, and we are grateful for the opportunity to have this combined study reconsidered by the same referees.

As requested by the editor, this submission includes: (1) the revised manuscript; (2) this point-by-point response to the referees' comments; (3) the completed Reporting Checklist; (4) the standard cryo-EM data collection, refinement, and validation statistics (**Supplementary Table 4**); and (5) accession codes for the underlying raw data — cryo-EM maps deposited in the EMDB (EMD-57959, EMD-57969, and EMD-57972) and atomic coordinates deposited in the PDB (30QM, 30QU, and 30QX) — with sequencing data to be deposited in the NCBI Sequence Read Archive prior to publication.

In order to guide reviewers to the portion of the manuscript that have been substantially changed or added anew since the previous submission, we list here the new Results sections and figures that were absent in the previous submission:

- Result sections:
 - 2:1 stoichiometry of the RT–ncRNA complex
 - Functional asymmetry within a pseudo-symmetric RT–ncRNA complex
 - Insights into the RT–ncRNA priming mechanism
 - Geometric definition of template boundaries during tandem-repeat synthesis
 - Conserved logic for tandem-repeat cDNA synthesis across Class 2 RT systems
- Figures:
 - Figures 3–5
 - Extended Data Figures 5–12

We look forward to the editorial team's and referees' assessment of this revised, combined manuscript.

Referee expertise:

Referee #1: RNA structure, cryoEM, retroelements

Referee #2: anti-phage defence systems

Referee #3: telomere biology

Referee #4: co-reviewer

Referee #5: evolution and phylogenetics

Referees' comments:**Referee #1 (Remarks to the Author):**

Tang and colleagues present a mechanistic dissection of DRT10, a previously uncharacterized member of the UG (unknown group) reverse transcriptase family, and report several findings of genuine significance to the RT field. The central discovery is that DRT10 uses a C-terminal serine residue as the priming nucleophile. To my knowledge this is the first reported instance of serine-primed reverse transcription, and it represents a departure from the tyrosine-priming chemistry seen in every protein-primed RT studied to date, the Abi family included. Truncation, substitution, and rescue mutagenesis of the ncRNA template define an A-B-A' architecture in which a templating segment is flanked by a downstream homology element. This element drives iterative dissociation and realignment of the nascent cDNA to prime successive rounds of extension. Phylogenetic analysis groups TERT, PLE, and Class 2 UG RTs into a single clade and raises the intriguing possibility that telomerase-like repeat addition has bacterial origins. Consistent with this view, chimeric ncRNAs bearing telomerase RNA template segments direct synthesis of the cognate telomeric repeats in both *E. coli* and HEK293T cells.

Despite these strengths, the manuscript falls short of the bar for Nature because it leaves the two questions most central to its own framing unanswered. First, no structural data are reported for the DRT10 RT-ncRNA complex. This is a striking omission, given that the serine-priming claim sits at the heart of the paper's novelty, and given that comparable systems (DRT2, DRT9) have been solved by cryo-EM in recent contemporaneous work. A structure resolving Ser456 in the active site, ideally trapped at the priming step or with the ncRNA template threaded through the enzyme, would convert the serine-priming assignment from a strong inference grounded in mass spectrometry depletion and AlphaFold modeling into a definitive mechanistic statement. Second, and in my view the more serious issue, the immune function of the tandem-repeat cDNA product is not established. The system is framed throughout as an antiviral defense, yet no experiment links the unusual RT chemistry to the protective phenotype. The proposed model in which cDNA regulates the SLATT pore is not tested. The authors acknowledge that cDNA abundance decreases rather than increases during phage infection, opposite to the behavior of DRT2 and DRT9, but offer no mechanistic explanation. Absent either a structural account of the priming chemistry or more importantly, a functional connection between cDNA synthesis and host protection, the contribution amounts to a careful description of an unusual enzymatic activity that cannot by itself carry a paper in a generalist venue. The work would be more appropriately placed

at Nature Communications, where the mechanistic depth would be appreciated and where the absence of a defined defense mechanism would not undermine the principal claims.

We thank the reviewer for this careful assessment and for recognizing the significance of serine-primed reverse transcription and the A–B–A' templating mechanism. We agree that structural data would strengthen the story, and have made substantial progress on this front, which is outlined below; on host protection, we believe the question is better addressed in a dedicated follow-up study.

The revised manuscript adds extensive new data that support the biochemical and genetics findings of the original submission, while offering exciting new advances. Specifically, we report cryo-EM structures of the *Eco*₁DRT10 and *Eco*₃DRT10 RT–ncRNA complexes at 3.2 Å and 3.3 Å (**Fig. 3a–d, Extended Data Figs. 5–8**), revealing an unanticipated 2:1 architecture in which two RT protomers engage opposite faces of a single pseudo-symmetric ncRNA. These structures also resolve the C-terminal domain that harbors the priming residue within the intact complex. A dNTP-soaked, 3.0 Å pre-catalytic state (**Fig. 4a–d**) further shows incoming nucleotides paired with the ncRNA template and the CTD helix positioned along the trajectory of the nascent cDNA.

We appreciate the reviewer's emphasis on the serine-priming claim, though we respectfully disagree that this finding represents the heart of our study's novelty — which lies more in the functional and evolutionary links to telomerase. We also note that protein-primed reverse transcription has been described for almost ten distinct UG/Abi systems, and serine-primed DNA polymerases have also been extensively documented elsewhere (e.g. in phage polymerases like ϕ 29 and PRD1, adenovirus replication-initiation machinery, and fungal mitochondrial plasmids, among others). In the present study, serine priming is supported by mass spectrometry depletion, S456A loss-of-function, and reciprocal serine/tyrosine swaps (S456Y, Y444S), which together establish that hydroxyl chemistry at this position is required. Furthermore, the direct transfer of radioactive [α -³²P]dCTP onto the enzyme — detected as a radiolabeled species migrating at the exact mobility of the RT protein by SDS-PAGE — provides direct biochemical proof of a covalent, radioactive protein–DNA conjugate (**Extended Data Fig. 3e,i**). Our new structural data are in excellent agreement with these findings, and while we do not have an intermediate structure showing cryo-EM density directly connecting this side chain to the DNA, trapping such an intermediate will be a natural centerpiece of a focused follow-up study.

We share the reviewer's interest in tying this chemistry to a defined mechanism of host protection, and we flag this explicitly in the revised Discussion as a central open question, together with a specific hypothesis implicating multivalent SLATT regulation by the repetitive cDNA. Rigorously testing that model will require direct SLATT–cDNA binding assays, assessment of SLATT oligomeric or pore state as a function of cDNA abundance, and infection time-course experiments relating cDNA dynamics to the protective phenotype — a body of work substantial enough, in our view, to warrant its own dedicated manuscript, particularly given that the present study already combines what were originally two independent submissions (mechanistic and structural), at the editor's request.

Minor comments:

Page 4 line 63 references Fig. 1b but describes a plaque assay shown in Fig. 1c.

We appreciate the reviewer pointing out this mistake, which has been updated in the revised manuscript. The statement now refers to **Figure 1b and Extended Data Figure 1c**.

Page 14 line 379 references Extended Data Fig. 7c but describes in vitro assays shown in Extended Data Fig. 8e.

This mistake has been corrected in the revised manuscript, and the statement now refers to **Extended Data Fig. 15e**.

Page 27 line 781 and page 28 line 809 both reference Fig. 3h, but there is no Figure 3h.

This mistake has been corrected in the revised manuscript, and both statements now refer to **Figure 2h**.

Referee #2 (Remarks to the Author):

In this study, Tang et al. investigate a candidate anti-phage defense system from the UG17 group of reverse-transcriptase which they call DRT10. This system is particularly intriguing for its relationship to eukaryotic telomerases.

They reveal a ncRNA through RIP-seq, and identified conserved sequences and structural features of the ncRNA and were able to experimentally validate their importance in anti-phage defense, as well as that of a SLATT protein next to the RT. Sequencing extrachromosomal DNA revealed cDNA products of the RT with a 9nt repeat motif present a median of 15 times. The authors then reconstituted the RT activity in vitro, and confirmed its ability to generate ssDNA repeats of 9nt yielding products of kilobase size.

The authors then elegantly show how synthesis is protein-primed at a serine residue using a combination of mass-spectrometry and genetics. They further describe a mechanism of repeat creation through iterative cycles of extension, dissociation, and template resetting, which they nicely demonstrate by mutating the A and A' segments involved in resetting. They describe rules that enable a complete reprogramming of the repeat sequences (up to a certain length). The authors further propose convincingly that the ABA' resetting mechanism is shared by other DRT systems.

They then note the parallel between the mechanism they describe here and telomerase repeat addition and further investigate whether this is due to convergent evolution or shared ancestry. The conclusions of this analysis are key to raising the impact to this work to the level of a publication in Nature. The authors seem to tackle this through a combination of approaches that lend credibility to the conclusions that telomer RT branch within the bacterial Class 2 UG RT. While I find the results exciting and believable, I must however note that I'm not an expert in phylogeny and can only provide a superficial evaluation of this part of the work. They further demonstrate that while DRT10 does protein-primed reverse-transcription, it can also prime on DNA 3' ends and work in human cells, further adding credibility that this family of RT had all the mechanistic properties required to act as telomerase and could have been co-opted to protect linear genomes against replication-associated DNA loss.

The paper is an important contribution to the field. It is overall very well written, all the genetics and molecular biology experiments are performed to a high standard and support the conclusions.

We thank the reviewer for this thorough and generous read of our study, and for highlighting its central advances — the ncRNA- and SLATT-dependent basis of defense, the serine-primed reverse transcription chemistry, the A-B-A' resetting mechanism underlying repeat synthesis and reprogramming, and the functional and evolutionary parallels to telomerase, including DRT10's ability to prime on DNA 3' ends and function in human cells.

We are grateful for the reviewer's positive assessment of the writing, rigor, and conclusions, and address their specific comments below.

I only have minor comments:

The ssDNA size *in vitro* seems to easily reach 3kb or more, what limits its size to 15 repeats *in vivo*?

We thank the reviewer for this question. The 15-repeat figure from *in vivo* experiments is purely a reflection of DNA high-throughput sequencing limitations, not a biological limit: reads containing the 9-nt motif were capped at 15 consecutive repeats because a 150-cycle sequencing run cannot resolve more than 16 repeats of a 9-nt unit (**Extended Data Fig. 2e**). We have ensured that this point is clearly emphasized in the main text, figure legends, and Methods. As the reviewer points out, our *in vitro* data strongly suggest that the repeat number *in vivo* is likely far greater than sequencing alone can capture.

Beyond eukaryotic telomerase, could the same mechanism of repeat addition be involved in the maintenance of linear phage genomes or linear bacterial genome ?

We thank the reviewer for this interesting question. Linear bacterial genomes have indeed been studied at length, and are thought to be stabilized by molecular mechanisms distinct from telomerase-type reverse transcription. One is invertron-type, protein-primed replication, used by *Streptomyces* linear chromosomes and plasmids (and by ϕ 29-like phages and adenoviruses), in which terminal inverted repeats and a covalently bound terminal protein prime synthesis from each end. A second is protelomerase-mediated hairpin formation, exemplified by *Borrelia burgdorferi* (ResT) and temperate phages such as N15, PY54, and ϕ KO2 (TelN/TelY/TelK), in which a tyrosine-recombinase-like enzyme resolves replicated “telomere” junctions into covalently closed hairpin ends. (Note that these are not classical telomeres in the sense of repetitive sequence arrays added *de novo* to chromosome ends, but rather single covalently closed hairpin loops formed by resolution of a specific junction sequence. In a similar vein, protelomerase, despite its name, is not a reverse transcriptase: it belongs to the tyrosine-recombinase/topoisomerase-IB superfamily and bears no molecular or biochemical resemblance to telomerase.)

Regarding phage genomes specifically, the examples we are aware of are stabilized by exactly these two systems, rather than an RT-based mechanism: N15 and its relatives use a protelomerase to convert their linear genome into a hairpin-capped linear plasmid prophage, while ϕ 29-like phages instead rely on protein-primed replication with terminal proteins, the same invertron logic used by *Streptomyces*. We are not aware of any phage or free-living bacterial replicon that uses a reverse-transcriptase-based, telomerase-like repeat-addition mechanism to maintain its genome ends, though this would certainly be of great interest if it existed! (We are currently exploring this possibility bioinformatically.)

Interestingly, RT-based end synthesis does reappear in a different context: linear mitochondrial “retroplasmids” of filamentous fungi (e.g., the pFOXC plasmids of *Fusarium oxysporum*) replicate their telomere-like terminal repeats using a dedicated reverse transcriptase, and it has been proposed that such elements contributed more broadly to the evolution of linear mitochondrial genomes. This suggests that RT-mediated repeat synthesis for genome-end maintenance, while so far unobserved in bacteria or phages, has been convergently exploited elsewhere in nature — consistent with DRT10 representing a distinct, bacterial instance of a related molecular strategy.

While not needed for publication of the study, I'm be curious to know if the DRT10 can catalyze repeat addition to 3' ends after DNA breaks in *E. coli* or to the end of a phage genome upon DNA injection?

We thank the reviewer for this excellent question — which is one we also wondered about ourselves. Early in this study, after resolving the mechanism of tandem-repeat cDNA synthesis, we specifically mined our sequencing data for chimeric reads that might indicate cDNA-tagged phage genome ends, reasoning that such tagging could inhibit phage genome replication or packaging as a distinct antiviral strategy. However, we found no evidence of such species above the background noise inherent to sequencing.

We have not directly tested whether DRT10 can catalyze repeat addition at free 3' ends generated by targeted double-strand breaks in the *E. coli* genome. However, given the comparatively inefficient, DNA-primed nature of tandem-repeat synthesis relative to protein-primed priming mechanisms (**Extended Data Fig. 15e**), we suspect this activity, if present at all, is unlikely to occur to any appreciable extent in cells.

More broadly, we find these ideas exciting and suspect that other UG/Abi RT-family systems may have evolved to "tag" phage genome ends as a distinct immune strategy — a possibility we are actively pursuing.

Referee #3 (Remarks to the Author):

In this manuscript, Tang et al. investigated a new antiviral defense system called DRT10. Using bioinformatics and cell-based assays, they identified its core components, including an ncRNA, a SLATT protein, and a reverse transcriptase (RT). The study focused primarily on the ncRNA and RT. In an extensive biochemical analysis, they clarified how the RT uses ncRNA as a template to synthesize long strings of single-stranded repeat-sequence DNA. They pinpointed a serine residue essential to its function and provided evidence that it serves as a primer for reverse transcription. Notably, they showed that the RT uses the A-B-A' motif to synthesize DNA, a feature shared with telomerases that allows synthesis of sequence repeats. Furthermore, bioinformatic and biochemical data suggested a possible functional and evolutionary connection between DRT10 and telomerase, implying that they may have originated from a common ancestor. Overall, the manuscript is well-written. Because the findings bridge two formerly disparate fields, bacterial phage defense and eukaryotic telomere replication, there will be a large audience for these unexpected and intriguing results.

We are grateful for the reviewer's generous summary of our work, and for recognizing the broad interest these findings may hold across the phage defense and telomere biology communities.

Major comment:

1. Interpreting the phylogenetic tree would benefit from more caution, particularly when the branch point remains unidentified. Lines 51-52 say the RT "originated in bacteria before being co-opted" for telomerase, which could be interpreted to suggest horizontal gene transfer. Lines 398 ("common bacterial ancestor") and 405 ("bacterial origin of telomerase") might be more conservatively expressed as the DRT10 and telomerase having a common ancestor, just as LUCA cannot be classified as bacterial or archaeal or eukaryotic. In other words, the tree in Fig. 5d seems completely justified by the data, with the ancestral RT preceding the divergence of bacteria from eukaryotes, whereas the word "bacterial" on line 1249 could be deleted.

We thank the reviewer for this important point, and agree that our original language should be more conservative, particularly that, as the reviewer notes, an ancestral lineage predating the divergence of bacteria and eukaryotes cannot itself be assigned to either domain. We have revised the manuscript to remove language implying a specifically bacterial origin or directional horizontal transfer, replacing it with wording describing a common ancestor that predates this divergence, as follows:

The Introduction (originally lines 51–52) now reads: "These findings raise the intriguing possibility that telomerase-like reverse transcription originated in a common ancestor, prior to further elaboration in bacteria for phage defense and co-option in eukaryotes for the maintenance of linear genomes."

The end of the Results section (originally lines 382–383) now reads, "...telomerase-like DNA repeat addition may have arisen in a common ancestor of bacteria and eukaryotes prior to the emergence of linear chromosomes."

The Discussion (originally line 398) now reads “...phylogenetic analyses suggest that these mechanisms trace back to a common ancestor predating the divergence of bacteria and eukaryotes.” The subsequent text (originally line 405) has been revised to remove “bacterial origin of telomerase,” and now reads: “...provides support for a common evolutionary origin of the DRT and TERT lineages.”

Finally, we have deleted “bacterial” from the **Figure 6d** legend, which now reads “RNA-templated DNA repeat addition first arose in an ancient RT lineage...” rather than “an ancient bacterial RT.”

Minor comments:

1. Lines 23-24, TERT was unknown to the cited authors, and was discovered a decade later by the Cech and Lundblad labs (Science, 1997).

We apologize for the oversight, and are grateful that the reviewer caught this mistake. We have now corrected the citation and added references to both foundational 1997 studies from the Cech and Lundblad labs (Lingner *et al.*, *Science* 1997; Nakamura *et al.*, *Science* 1997).

2. Line 64, “mutation of the RT catalytic residues” could be interpreted to mean that they’re in SLATT! Help the reader by adding a sentence about the DD motif, catalytic Mg⁺⁺ ions, cite reference.

We thank the reviewer for flagging this ambiguity. We have revised the text to specify that defense was abolished by mutation of the catalytic aspartate residues of the RT itself (YVDD→YVAA), and have added a citation (Steitz, *Nature*, 1998) supporting the general two-metal-ion mechanism by which these aspartates coordinate the catalytic Mg²⁺ ions required for nucleotidyl transfer.

3. Figure 1f and lines 87-90. While the step-loop structure is presumably perturbed, this has not been experimentally confirmed. Caution is advised when interpreting the results, given the dynamics of RNA structures. Additionally, the sequences following the scramble could not be found in the manuscript. The same issue applies to Figure 3g.

We thank the reviewer for this cautionary note. We agree that the effect of these mutations on RNA secondary structure was not independently validated, and have revised the text to present the data more objectively without additional interpretive claims about the resulting structural perturbation, by deleting the phrase “underscoring the importance of these ncRNA structural elements for DRT10 function.” We note, however, that the specific mutations chosen were designed to disrupt the base-pairing patterns that covariance modeling clearly identifies as evolutionarily conserved, making a bona fide structural disruption highly likely even without direct experimental confirmation.

Regarding the reviewer's point that the scrambled sequences could not be located in the manuscript: these plasmids are listed in **Supplementary Table 9** (renumbered from Table 8 in the original submission), which includes the scrambled constructs tested in the context of phage defense (pSL9702–pSL9706) as well as those used in the miniprep-seq experiments presented in **Figure 2g** (formerly Fig. 3g; pSL9309–pSL9313). We have also added explicit reference to these plasmids in the revised Methods, for clarity.

4. Figure 2i. Line 176 states "... a high-molecular-weight smear upon dNTP incubation that was unaffected by RNase treatment but largely eliminated by DNase treatment". Rather than eliminating the bands, Exol treatment produced additional bands, and a significant high-molecular-weight smear persisted. The additional bands imply that Exol had an effect, whereas RNase A did not. The results in Extended Figure 3f are much more convincing than those in Figure 2i.

We agree, and thank the reviewer for this excellent suggestion. We have reordered the presentation of these results so that the radioactive labeling experiment (new **Fig. 1m**), which provides the most direct and unambiguous evidence for a covalent RT–cDNA linkage, is now the primary supporting data in the main text, with the nuclease-sensitivity experiment moved to **Extended Data Fig. 3e** as corroborating evidence that excludes the existence of a covalent RNA–DNA hybrid, due to the absence of an effect from RNase treatment.

5. Lines 224 – 225, although the authors have not yet tested if the extension is distributed or processive, the synthesis of long repeats suggests that it will be processive, i.e., product not completely dissociated from the enzyme after each repeat. It would help reader to add a phrase or a sentence to cover this.

We thank the reviewer for raising this important point.

Rather than adding a brief phrase in the indicated location, we designed a dedicated experiment to directly test whether tandem-repeat synthesis is processive or distributive. In brief, we incubated the RT enzyme with two ncRNA variants harboring nearly identical template regions but with neutral 'watermark' mutations to the B motif within the A–B–A' pattern, reasoning that a distributive mechanism — in which intermediate cDNA products dissociate and re-engage a different template molecule — would generate chimeric reads bearing motifs templated by both variants. We detected no such chimeric products above the background level of artifactual PCR recombination observed in negative controls (**Extended Data Fig. 9i**), indicating that intermolecular template jumping is rare or nonexistent.

This result demonstrates that tandem-repeat cDNA synthesis instead proceeds through repeated engagement of a single A-B-A' template within an individual RT–ncRNA complex, consistent with a processive mechanism in which the product remains associated with the enzyme across successive rounds of repeat addition. These results and interpretations are described in **lines 340–358** of the revised manuscript.

6. Lines 421-423 seem redundant with next paragraph (and lines 437-438). Considering how little is known, less could be said here.

We appreciate this suggestion and have streamlined this section of the Discussion to avoid redundancy.

7. It took me some time to fully understand Figure 3d, particularly the arrows and the labels “-1, -2, -3”. If the “5’ truncation (-1) and two arrows” indicates one nucleotide truncation from both A and A’, I would suggest describing each component separately in the figure legend.

We thank the reviewer for flagging this ambiguity in **Figure 2d** (formerly Figure 3d). The reviewer's reading is correct: each truncation variant removes the indicated number of nucleotides simultaneously from the same relative end (5' or 3') of both A and A' (e.g., the “-1” variant removes one nucleotide from the 5' end of A and the paired 5' end of A'). We have revised the figure legend to state explicitly that A and A' are truncated simultaneously, in incremental steps ranging from 1–3 nt.

8. Figure 1f and Figure 3a. Please clarify whether the 3 technical replicates are from the same experiment or from 3 biological replicates.

These experimental results (now **Extended Data Fig. 1g** and **Fig. 2a**) are technical replicates from a single experiment, and we have revised the corresponding figure legends throughout the manuscript to state this explicitly (“n = 3 technical experimental replicates”).

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

[We thank the reviewer for their time and effort in co-reviewing this manuscript.](#)

Referee #5 (Remarks to the Author):

Review of: Antiviral immunity via a telomerase-like reverse transcription in bacteria.

This interesting paper identifies a novel form of bacterial defence associated reverse transcriptase (DRT10) that generate DNA repeat additions. The paper identifies many mechanistic aspects of these reverse transcription function and demonstrates function of this system associated with SLATT proteins can generate phage protection (although does not directly demonstrate how phage protection is achieved – which would also be cool). The work then goes on to demonstrate that these enzymes show some functional similarity to telomerases, while phylogenetic analyses show that the DRT10s and telomerases are phylogenetically related to the exclusion of many other reverse transcription systems. Based on these analyses the authors put forward an exciting idea that the shared subsection of the wider RT gene family led to viral protection in bacteria and linear chromosome function through telomere capping in the early eukaryotes. This is an exciting paper.

The editorial team asked me to review the comparative evolutionary biology and phylogenetic aspect of this paper given my ‘expertise’ lies in this area rather than viral defence or reverse transcription mechanistic biology which is reported here.

We thank the reviewer for their careful and positive assessment of the evolutionary and phylogenetic aspects of our work, as well as for their constructive suggestions. We are pleased that their evaluation raised only minor concerns and agree that certain aspects of our analyses and their presentation could have been explained more clearly. The reviewer’s comments have helped us improve both the methodological description and the overall clarity of the manuscript. We address each point in detail below.

Overall, we have clarified the methodological terminology, revised our depiction of the proposed evolutionary trajectory of telomerase-like repeat addition, and expanded the relevant figure legends — particularly the legend of **Figure 6b** — to make the phylogenetic analyses more accessible to the journal's broad readership. We have also added explicit cross-references to the detailed methodological descriptions in the **Methods** section (“RT phylogenetic tree analyses”) and the **Supplementary Text**.

Minor comments:

1) I do not recognise either the terms ‘local support value’ or ‘ensemble bootstrap value’ as common methods for analysing topology support for these types of phylogenies. Both are notated as 0.X in the figures suggesting they are branch-specific likelihood ratio test (LRT) or posterior probabilities. If they are bootstraps, they are notated incorrectly. Such values usually appear as % values indicating they have been derived from parallel analysis of pseudo-replicates. My guess is that they were not from alignment pseudo-replicates (note bootstrap pseudo replicates are not the same as TBE). Discussed more below.

We thank the reviewer for raising this point and agree that our original terminology needed further clarification. The two values shown in **Figure 6b** represent distinct measures of branch support, neither of which is a Bayesian posterior probability.

The first value is the SH-like local support calculated internally by VeryFastTree, following the method implemented in FastTree 2. This measure evaluates alternative local topologies around each internal branch using resampled site likelihoods. FastTree and VeryFastTree conventionally report SH-like local support as a proportion between 0 and 1. We have therefore replaced the generic term “local support value” with the more precise term “SH-like local support” throughout the manuscript and figure legends.

The second value assesses the robustness of the reference topology to uncertainty in the multiple sequence alignment. As described in the **Methods** section “RT phylogenetic tree analyses,” we generated an ensemble of 1,000 independently perturbed alignments using the MUSCLE5 -perturb procedure and reconstructed a phylogenetic tree from each alignment using the same filtering and VeryFastTree inference pipeline applied to the original alignment. We then mapped these replicate trees onto the reference topology and calculated branch support using the Transfer Bootstrap Expectation (TBE) metric implemented in RAxML-NG. By default, TBE is reported by RAxML-NG as a proportion between 0 and 1, which we maintained for consistency. In summary, “alignment ensemble” describes how the replicate alignments and corresponding trees were generated, whereas TBE is the statistic used to summarize support for each branch of the reference tree across those replicates. To make this distinction explicit, we have revised the term “ensemble bootstrap value” to “alignment-ensemble TBE support” throughout the manuscript and figure legends.

The revised **Figure 6b** legend now states: “Values at selected nodes are shown as SH-like local support // alignment-ensemble TBE support; both support measures are reported as proportions between 0 and 1.”

In the revised manuscript, we also included the relevant methodological references for SH-like local support (ref. 79), MUSCLE5 alignment ensembles (ref. 120), TBE (ref. 103), and RAxML-NG (ref. 104).

2) Similarly details of what type of bootstrap analysis conducted were absent from the methods section. They do however report a TBE analysis in the methods which is quite nice. But it is not recorded how this set of results relates to either the ‘local support value’ or ‘ensemble bootstrap value’ notated on the figure legends. This all needs to be clarified and at some point, proper bootstrap analysis with pseudo-replicated alignments should be incorporated if not done already. This may show reduced support for the DRT10 and telomerase clustering pattern. I think this is fine and the authors can contrast this with the TBE analysis (aLRTs and Bayesian posteriors?) given the nature of the alignments, i.e., a large number of sequences and few informative alignment positions. I still suspect that they would arrive at a similar conclusion but they would have demonstrated a full phylogenetic analysis process to widest field of evolutionary biologists present in the journal readership.

We thank the reviewer for this comment and apologize that this was not sufficiently clear in the original manuscript. The replicate-based TBE analysis was indeed performed and corresponds to the second of the two support metrics shown in **Figure 6b** (as we described in our response to point 1 above, starting at “The second value assesses...”).

To be more specific, the reference topology was first reconstructed from the original structure-guided alignment using VeryFastTree. The SH-like local support values calculated directly by VeryFastTree constitute the first support metric displayed in **Figure 6b**.

Independently, as described in the **Methods** section “RT phylogenetic tree analyses,” we generated 1,000 alternative alignments by perturbing the MUSCLE5 alignment model using random seeds from 1 to 1,000. Each alignment was processed using the same trimming, sequence-filtering, and tree-building pipeline as the reference alignment. The resulting 1,000 trees were mapped onto the reference topology using RAXML-NG, and support for each reference branch was quantified using TBE. This constitutes the second aforementioned support metric displayed in **Figure 6b**.

This analysis was specifically designed to evaluate a major source of uncertainty in deep protein phylogenies, namely uncertainty in the alignment of highly divergent sequences. A conventional bootstrap based on a single fixed MSA evaluates sampling variation in the aligned columns while treating the original alignment as fixed. By generating replicate alignments under perturbed alignment parameters, our analysis additionally tests whether the inferred groupings are robust to plausible variation in the MSA itself.

The complete workflow is described in the **Methods** section “RT phylogenetic tree analyses” and its rationale is discussed further in the **Supplementary Text** sections “Methodological considerations for phylogenetic reconstruction” and “Choice of tree-building algorithm.” These sections specify:

- (1) the SH-like local support calculated by VeryFastTree;
- (2) the generation of 1,000 perturbed MUSCLE5 alignments;
- (3) the reconstruction of one tree from each alignment; and
- (4) the calculation of TBE support by mapping the replicate trees onto the reference topology with RAXML-NG.

The **Supplementary Text** also explains why alignment uncertainty is particularly relevant for the present dataset, which contains thousands of highly divergent RT domains and a comparatively limited number of confidently homologous positions.

We also performed an additional sensitivity analysis by computing conventional bootstrap analysis with pseudo-replicated alignments on selected curated and downsampled datasets using IQ-TREE. One hundred bootstrap pseudo-replicated alignments were generated by column resampling for each selected analysis, and the bootstrap trees were mapped onto the corresponding maximum-likelihood reference tree using RAXML-NG and TBE. These analyses are described in more detail in our response to point 3 below.

Thus, the analyses provide complementary assessments of uncertainty arising from both alignment variation and conventional column resampling. Both approaches support the broad placement of TERT among bacterial RTs recovered in the principal phylogenetic analysis.

3) Throughout, the authors use a ‘-wag’ ‘-gamma model’ for modelling sequence change in their phylogenetic analysis. Usually, model selection is tested using either a separate program or the phylogenetic program itself derives a model comparison. I do not know if FastTree 2 does this? If it does the details of model comparisons and selection should be included in the methods. If not, the authors should subject their alignment to alternative analysis to identify appropriate sequence substitution models and then incorporate these models in their analyses. Assuming a -wag -gamma model excluding parameters such as I and F may lead to errors in their tree topology. Also WAG as a substitution matrix is a little dated. It may be the best matrix but it all needs testing.

We thank the reviewer, and we agree that substitution-model choice should be justified by testing rather than assumed. We did test this extensively; the rationale is set out in **Methods** (“RT phylogenetic tree analyses”) and the **Supplementary Text** (“Methodological considerations for phylogenetic reconstruction” and “Choice of tree-building algorithm”), which we have expanded upon below for clarity.

Two considerations frame our strategy. First, the dataset is intrinsically difficult, comprising 6,734 highly divergent RT domains with a limited number of confidently homologous positions. Pythia scores it at approximately 0.7 (“difficult”), predicting slow or ambiguous convergence and the recovery of alternative, near-equal-likelihood topologies depending on the program, starting tree, alignment treatment, or random seed. Second, VeryFastTree was selected for the principal reconstruction because it enabled us to analyze the complete dataset, and to reconstruct trees across all 1,000 perturbed alignments used for support estimation. The use of a FastTree-based method with WAG, rate heterogeneity, and pseudocounts is also consistent with previous large-scale analyses of prokaryotic RT evolution, facilitating comparison with established RT classifications (e.g. see PMID 35648479, 31276437, 33275130, 28769116, 25423096, 28698278).

The analysis accounts for among-site rate variation. VeryFastTree uses the CAT approximation during topology optimization, while the -gamma option recalculates the final likelihood using a discrete gamma model. These parameters, together with the use of pseudocounts for highly divergent sequences, are specified in the **Methods** section “RT phylogenetic tree analyses.”

Importantly, the VeryFastTree reconstruction was not the only phylogenetic analysis we performed. As mentioned in our response to point 2 above, we systematically tested the effects of dataset composition, alignment trimming, tree-building software, and substitution model. These results are summarized below, and although they further support our broader conclusions, we did not include these analyses in the original submission because they were not essential to the manuscript’s central argument and would have added considerable complexity.

In total, we generated 75 alternative trees, which were generated by testing every combination of five datasets, three alignment-trimming thresholds, and five inference configurations, as below:

- (1) The five datasets included the complete dataset of 6,734 RTs, a manually curated dataset of 4,761 RTs, and three differently downsampled or compositionally balanced datasets containing 2,972, 2,101, and 1,029 RTs.

(2) The three alignment-trimming thresholds included the removal of columns containing more than 50%, 90%, or 99% gaps.

(3) The five inference configurations comprised VeryFastTree, IQ-TREE under WAG+F+I+G4, IQ-TREE under Q.pfam+F+I+G4, RAxML-NG under WAG+F+I+G4, and RAxML-NG under Q.pfam+F+I+G4.

Notably, Q.pfam+F+I+G4 was selected by ModelFinder as the best-fitting model for the relevant alignments.

Across these parallel analyses, the broad result relevant to the central evolutionary conclusion was consistently recovered: TERT was separated from the major eukaryotic LTR and non-LTR retrotransposon RT lineages and was instead placed among bacterial RT lineages (see **Figure R1**), albeit with lower bootstrap support.

However, the precise internal topology among the ancient bacterial RT families was unstable across datasets, alignment-filtering thresholds, substitution models, inference programs, and starting conditions. In several IQ-TREE and RAxML-NG reconstructions, established RT families were placed in unexpected relationships. For example, in one analysis, Class 1 UG RTs were split into separate clades, one of which was grouped together with Class 3 UG RTs (**Figure R1A**). In another, retron RTs were nested within the Class 2 Unknown Group lineage, despite previous phylogenetic analyses consistently recovering retron and Unknown Group RTs as distinct groups (**Figure R1B**).

ModelFinder identified models with an improved statistical fit to the corresponding input alignments, but these models did not eliminate the deeper topological instability or consistently recover established relationships among RT families. This is consistent with the Pythia difficulty assessment and illustrates that substitution-model selection alone cannot resolve uncertainty resulting from extreme sequence divergence, alignment ambiguity, substitutional saturation, limited phylogenetic signal, and the exceptionally large tree-search space.

Among the alternative reconstructions, the VeryFastTree topology presented in **Figure 6b** most consistently recovered the established monophyly and separation of the known principal RT families, including retrons, group II introns, DGRs, LTR retrotransposons, and non-LTR retrotransposons. Together with its scalability to the complete dataset and the 1,000 perturbed alignments, this motivated its use as the principal phylogenetic representation.

Taken together, the principal VeryFastTree reconstruction, the parallel IQ-TREE, RAxML-NG, and ModelFinder analyses, and the alignment-ensemble TBE analysis converge on the same broad conclusion: TERT and PLE share a closer evolutionary relationship with bacterial UG RTs than with the major eukaryotic LTR and non-LTR retrotransposon RT lineages. At the same time, the precise order of the deepest internal branches remains difficult to resolve, though our conclusions do not depend on any single detailed branching arrangement.

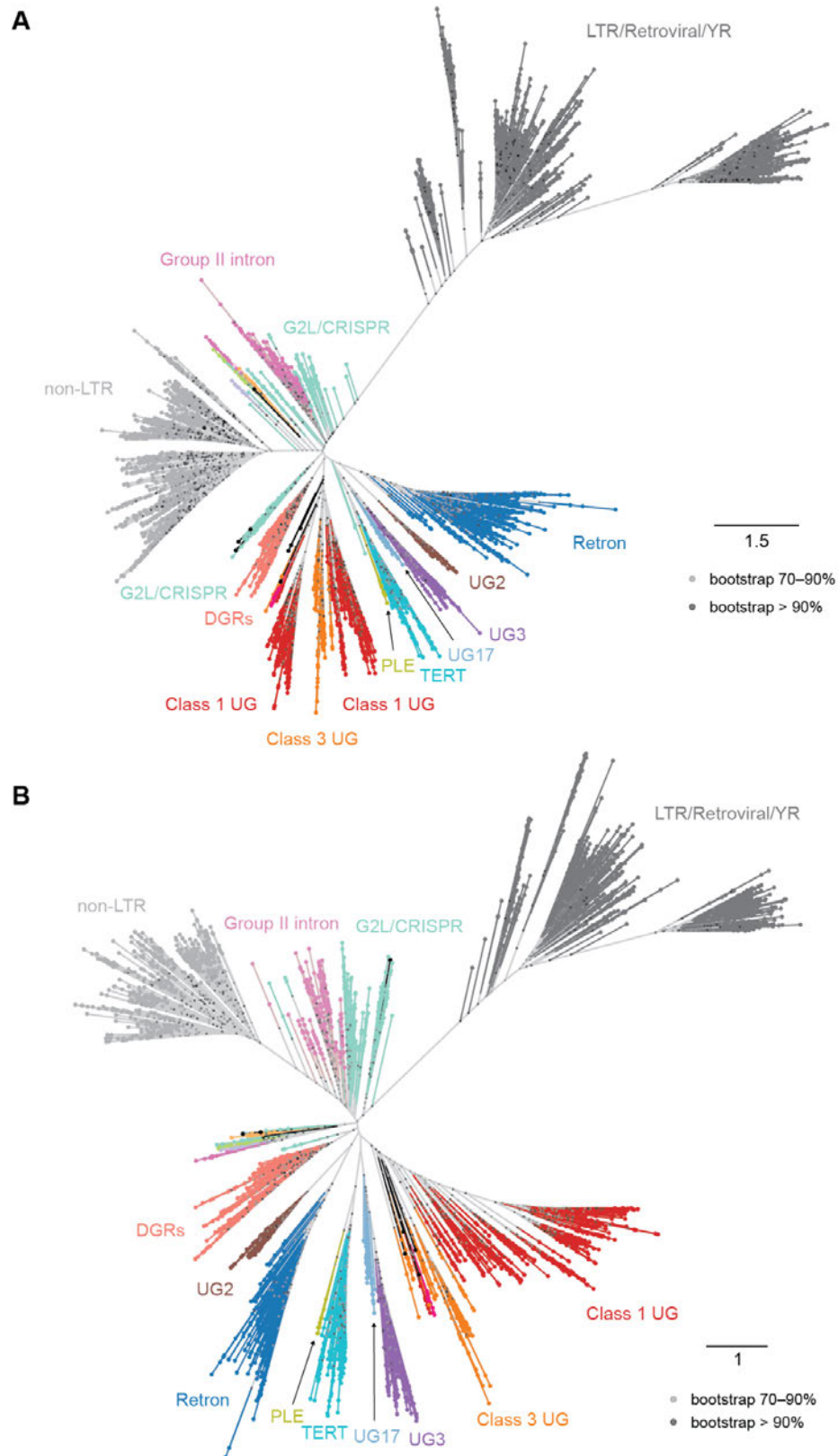


Figure R1. Maximum-likelihood phylogenetic reconstructions based on curated and compositionally balanced RT datasets. Phylogenetic trees were inferred with IQ-TREE under

the Q.pfam+F+I+G4 substitution model, selected as the best-fitting model by ModelFinder, using reduced versions of the RT dataset in **Supplementary Data 1**. **(A)** Tree inferred from a manually curated multiple sequence alignment in which sequences with a disrupted DDD catalytic triad, poor alignment quality, or highly gapped motifs, were removed. **(B)** Tree inferred from the same curated alignment after down-sampling three over-represented clades (LTR retrotransposons, non-LTR retrotransposons, and retrons) by clustering aligned sequences at 40% sequence identity with CD-HIT and retaining representative sequences prior to phylogenetic inference. Branch support was estimated using the following bootstrap procedure implemented in IQ-TREE: One hundred bootstrap replicates were generated using the -bo 1 option (one replicate per independent run). Bootstrap trees were mapped onto the best-scoring maximum-likelihood tree using RAxML-NG and the transfer bootstrap expectation (TBE) metric (--support --bs-metric tbe). Internal nodes are colored by TBE support, as indicated in the figure.

4) I found figure 5D problematic. i) I am not sure the emergence of linear genomes (in the sense of eukaryotic linear chromosomes) placement is correct. I know some prokaryotes have linear chromosomes but I do not think this is the point the authors care about. I don't think they are 'homologous' to eukaryotic centromeric/telomeric chromosomes. So, should linear centromeric/telomeric chromosomes be placed at the base of eukaryotes? ii) The figure shows Animal TERT is a product of multiple genes shared with plants and separately fungi, which is not possible unless there was a HGT or complex paralogue loss, this needs clarification or simplification. Animal TERT cannot be on the end of two separate polyphyletic branches without explanation. iii) I do not understand the topology given current tree of life data. Flagellates and plants seem to be in the wrong place. This tree looks like a 1977 Woese tree...

We thank the reviewer for identifying these potentially confusing aspects of **Figure 6d** (formerly Figure 5d). This panel was intended as a conceptual model of the functional diversification of telomerase-like repeat addition, rather than as a formal or fully resolved organismal phylogeny. We have now revised it to remove all three sources of confusion pointed out by the reviewer.

(1) Linear genomes: We agree that the original placement of "linear genomes" could be interpreted as including the independently evolved linear chromosomes found in some prokaryotes. Our argument concerns the emergence of linear, telomere-bearing eukaryotic chromosomes and the associated end-replication problem. We have therefore placed this event within the early eukaryotic lineage. The revised figure no longer implies homology between eukaryotic telomere-bearing chromosomes and the linear bacterial chromosomes that are maintained through unrelated mechanisms.

(2) Animal TERT: We have also simplified the representation of eukaryotic TERT evolution. Animal TERT is no longer shown at the end of two separate branches. Instead, the revised model shows a single ancestral eukaryotic TERT lineage followed by its subsequent diversification among eukaryotic groups. This avoids implying independent origins, horizontal transfer, or an unexplained pattern of paralogue loss.

(3) Organismal topology: we have removed the outdated and potentially misleading arrangement of "flagellates," plants, fungi, and animals. Figure 5d is a conceptual evolutionary model, and the organismal relationships have been

simplified so that the panel does not imply a detailed eukaryotic topology that is not relevant to the proposed evolutionary history of the RT mechanism.

5) I think the authors should carefully reword some text in places because they seem to suggest that the bacterial phage defence system was the ancestral function which led to telomerase. Their trees currently do not show this. Although this is formally possible it is equally possible that an unknown common functional property independently led to two different functions (phage defence and telomerase). This slight correction in wording in several places does not distract from the novelty of this work in my opinion, but would make the interpretation a little more robust.

We appreciate this comment and fully agree with the reviewer: the phylogenetic analyses do not establish that antiviral defense was necessarily the ancestral biological function that directly gave rise to telomere maintenance. Our data support a common evolutionary origin for the RNA-templated DNA repeat-addition mechanisms of TERT, PLE, and bacterial UG RTs. However, the biological function of their common ancestor cannot be inferred directly from the present phylogeny. Antiviral defense and telomere maintenance may represent separate functional specializations of an ancestral repeat-addition activity, and another, presently unknown ancestral function may have preceded both.

We have carefully reviewed our text throughout the manuscript and made refinements to avoid any errant assumptions that the ancestral enzyme to both DRT-family systems and eukaryotic TERTs would have also functioned as a phage defense system. To quote a few specific excerpts:

- **Abstract:** “This mode of iterative, boundary-delimited cDNA synthesis exhibits direct parallels to the mechanism of RNA-templated telomeric repeat addition by eukaryotic telomerase, and phylogenetic analyses of RTs across domains of life place telomerase in a monophyletic clade with the DRT enzyme family, suggesting a common origin for their unique DNA polymerization activities.”
- **Introduction:** “These findings raise the intriguing possibility that telomerase-like reverse transcription originated in a common ancestor, prior to further elaboration in bacteria for phage defense and co-option in eukaryotes for the maintenance of linear genomes...”
- **Results:** “Collectively, our findings suggest that telomerase-like DNA repeat addition may have arisen in a common ancestor of bacteria and eukaryotes prior to the emergence of linear chromosomes. We propose that in prokaryotes, this mechanism was further elaborated to protect cells against genetic parasites, while in early eukaryotes, it was co-opted to protect linear genomes against replication-associated DNA loss (Fig. 6d).”
- **Discussion:** “The identification of a bacterial RT family with mechanistic parallels to telomerase, together with phylogenetic analyses placing these enzymes within a monophyletic clade, provides support for a common evolutionary origin of the DRT and TERT lineages ... The cellular function of a putative bacterial progenitor to modern telomerase, however, remains unknown.”

While we find it plausible that the ancient telomerase-like activity could have also been linked to antiviral defense, we agree that this ancestral biological function cannot be resolved from the available data.

Very general comment. I wonder if the authors are aware of a prevailing idea that group II introns gave rise to eukaryotic spliceosomal introns. If this theory is true. That would suggest that two parallel domestications of ancestrally RT-capable prokaryotic systems occurred in the origin of the eukaryotes. I find this interesting given the radical change in gene complement and genome evolutionary dynamics that characterises the origin of the eukaryotes.

We thank the reviewer for this insightful and exciting observation. We are indeed aware of the prevailing hypothesis that group II introns contributed to the origin of spliceosomal introns and the spliceosome, and we considered this broader evolutionary context when interpreting our analyses. Indeed, our proposal is fully compatible with this model. Rather than representing mutually exclusive scenarios, the origins of the spliceosome and telomerase may reflect parallel examples of prokaryotic retroelement-derived machinery being recruited, domesticated, and elaborated during early eukaryotic evolution.

More broadly, the evolutionary diversification of RTs appears to have repeatedly involved the loss or modification of an ancestral mobile-element function, followed by the acquisition of useful cellular roles. This general concept was discussed by Zimmerly and Wu in their survey of bacterial RT diversity, and was subsequently emphasized in a review of prokaryotic RT domestication co-authored by one of the authors of the present study (Zimmerly and Wu, 2015; González-Delgado *et al.*, 2021). These studies highlighted that, beyond their canonical roles in selfish genetic elements, RTs have repeatedly been recruited for specialized biological processes, including antiviral defense, genome diversification, chromosome maintenance, and RNA processing.

From this perspective, the group II intron-spliceosome relationship forms part of a wider history of RT-associated innovations that extend across prokaryotes and eukaryotes. Group II intron-derived machinery is thought to have contributed to spliceosomal introns and the spliceosome, while other bacterial RT lineages may have contributed to eukaryotic innovations including telomerase, Penelope-like elements, and other non-LTR retroelements. Additional organellar examples illustrate the diversity of possible evolutionary outcomes. The chloroplast-encoded MatK protein is a degenerated group II intron maturase that has been retained as a trans-acting splicing factor for multiple chloroplast group II introns. Similarly, the mitochondrial MatR protein promotes the splicing of multiple mitochondrial introns. By contrast, the free-standing mitochondrial reverse-transcriptase-like (RTL) protein of *Chlamydomonas* is thought to derive from an ancient group II intron, but its precise function remains unresolved. Fungal mitochondrial retroplasmids provide another related example, encoding RTs closely related to group II intron RTs and using reverse transcription for autonomous plasmid replication, although their broader significance to the host remains unclear.

The repeated recruitment of RTs for cellular functions is particularly striking in prokaryotic immunity. CRISPR-associated RTs have originated independently from several distinct RT lineages. Most derive from group II intron-related RTs, but additional CRISPR-associated RT clades have been recruited independently from retron/retron-like and Abi-

P2-related RTs. Thus, even within a single immune pathway, evolution has repeatedly recruited related enzymatic activities from distinct retroelement lineages. Similarly, retransposons and UG/Abi RTs represent at least two evolutionarily distinct recruitments of RT-based systems for antiviral defense. These examples reinforce the broader principle that mobile genetic elements and their molecular components are recurrently exapted for host functions, particularly in biological conflict. This is consistent with the “guns-for-hire” framework proposed by Koonin, Krupovic, and colleagues, in which components move repeatedly between mobile elements, host defense systems, and counter-defense systems.

We have expanded the Discussion to highlight this broader context:

“This diversification is itself part of a much broader history of RT domestication that extends well beyond antiviral defense. A striking parallel is the prevailing model in which group II introns gave rise to spliceosomal introns and the spliceosome — a domestication event that, like the one proposed here for telomerase, appears to trace back to an ancestral, mobile-element-associated RT activity later repurposed for an essential cellular function. More broadly, RTs have been independently recruited for useful cellular roles across both prokaryotes and eukaryotes: telomerase for chromosome-end maintenance, group II intron-derived maturases and the spliceosome for RNA splicing, non-LTR and LTR retroelements for genome diversification, and — in at least three separate instances — retransposons, UG/Abi systems, and CRISPR-associated RTs for antiviral defense. This recurrent exaptation is consistent with a “guns-for-hire” model, in which mobile genetic elements serve as a persistent reservoir of molecular components for the evolution of cellular defense and fundamental biological pathways.”

September 28, 2026

Tang, Ramirez, He *et al.*

Response to reviewers' comments: Nature manuscript 2026-03-09614A-Z

We thank the editor and referees for evaluating our revised manuscript, "Antiviral immunity via telomerase-like reverse transcription in bacteria." Referees #2 and #5 support publication, with no further concerns. Referee #3 describes the revision as "exciting and rigorous," recommends publication without further revision, and raises concerns about the increasing editorial burden on authors. Referee #4 notes their participation as a co-reviewer. Referee #1 considers the structural concern "very well" addressed, while maintaining the request for a mechanistic link between DRT10 activity and phage defense. We address this remaining concern below.

Referees' comments:

Referee #1 (Remarks to the Author):

My previous two main critiques were: 1) lack of a high resolution cryo-EM structure and 2) the mechanism connecting enzyme activity to phage defense was unknown. The authors addressed criticism #1 very well with multiple cryo-EM structures illuminating the priming mechanism. However, #2 remains unaddressed. Phage defense papers in this type of generalist venue typically connect unusual enzyme activity to phage defense with a well delineated mechanism. That component is still missing. The work is very thorough and well done, but lacks the mechanistic significance expected of Nature.

We thank the referee for recognizing that the new cryo-EM structures address the structural concern well, and for their assessment of the revised work as thorough.

The revised manuscript establishes a previously unrecognized mechanism of antiviral reverse transcription: serine-primed, iterative synthesis of tandem-repeat cDNA by an RNA-templated RT complex. By integrating structural, biochemical, genetic, and evolutionary evidence, this study unifies our understanding of how RNA-templated UG RTs function in bacterial immunity and reveals a shared mechanistic logic with telomerase, connecting antiviral defense to a fundamental process of chromosome maintenance. The work also directly establishes how DRT10 synthesizes repetitive cDNA and demonstrates that this activity is required for defense. Together, these findings constitute a substantial mechanistic and conceptual advance. How the cDNA product engages SLATT to confer protection is an important, distinct question, and we acknowledge that the proposed SLATT-cDNA connection remains a model. Resolving that downstream mechanism warrants a focused follow-up study, but we do not believe its current uncertainty diminishes the impact and novelty established here.

Referee #2 (Remarks to the Author):

The authors have done substantial work to improve the manuscript and have addressed all my comments. I support the publication of this article.

We thank the referee for their constructive comments and support for publication. We are pleased that the revision addresses all their concerns.

Referee #3 (Remarks to the Author):

Sternberg and colleagues have submitted a revised manuscript on the mechanism of action of a fascinating bacterial defense system consisting of a noncoding RNA and a reverse transcriptase protein. The revision is extensive. First, they address the reviewers' comments in great detail. As one major example, the weak claim that their data support eukaryotic telomerases as having a bacterial ancestry is now judiciously restated, much to the benefit of the paper. Second, they solve and analyze the molecular structures of two of the bacterial RT-RNA complexes, which adds two powerful main figures to the presentation. The structures support the conclusions presented in the initial submission and also extend the story in new directions, revealing 2:1 RT:RNA complexes with mechanistic implications. All told, this is an exciting and rigorous contribution that will attract a wide audience and is eminently suitable for publication without further revision.

We are especially grateful to the referee for their supportive assessment of the revision as exciting and rigorous, and for recommending publication without further revision.

I will take this opportunity to voice a concern about Nature editorial policy. I considered the initial submission of the Sternberg paper to require more analysis and rewriting, but not additional laboratory experiments. Although the reviewers had a range of comments, their consensus appeared to be that the lab experiments were sufficient. Thus, it was discouraging to me to see that the paper was rejected. Now the cryo-EM structures have been added, which strengthens the paper but is detrimental to the careers of grad students and postdocs who deserved to have a separate publication on the structures. I recommend that the editors consider the current trend of moving the bar for publication higher and higher, to the point that each paper represents 20 person-years of work.

We sincerely thank the referee for their thoughtful perspective on manuscript scope and editorial burden, and especially for recognizing the impact on student and postdoctoral researchers.

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

We thank the reviewer for their time and effort in co-reviewing this manuscript.

Referee #5 (Remarks to the Author):

This is my second review. I found that the authors have dealt with all the reviewers comments adequately. I have no further comments.

I also agree with the reviewers that work reporting exactly how the DRT10 manifests phage protection is a huge subject and worthy of a separate major paper. This separation does not distract from the novelty and interest of this paper, which are both very strong.

We thank the referee for their supportive assessment and for confirming that the previous comments have been adequately addressed. We particularly appreciate their recognition that resolving the downstream mechanism of phage protection merits a separate study.