

TECHNICAL COMMENT

DNA CHARGE TRANSPORT

Comment on “The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport”

Andrey G. Baranovskiy,¹ Nigar D. Babayeva,¹ Yinbo Zhang,² Luis Blanco,³ Yuri I. Pavlov,^{1,4,5} Tahir H. Tahirov^{1*}

O'Brien *et al.* (Research Article, 24 February 2017, eaag1789) proposed a novel mechanism of primase function based on redox activity of the iron-sulfur cluster buried inside the C-terminal domain of the large primase subunit (p58C). Serious problems in the experimental design and data interpretation raise concerns about the validity of the conclusions.

The human p58C protein used by O'Brien *et al.* (1) contains the I271S mutation and exhibits an anomalous structure at the DNA:RNA-binding interface that is drastically different from the conserved structure of human p58C and its yeast ortholog determined by two independent groups in four different protein assemblies (2–5) (Fig. 1A). Without any explanation or qualification, O'Brien *et al.* assumed that the β -hairpin structure in their mutated p58C binds the RNA:DNA substrate in the same way as the α -helical structure in wild-type

(WT) p58C [see figure 3, A and B, and figure S6 in (1)]. Moreover, the authors concluded that Y345 and Y347, which are adjacent in the mutated p58C (Fig. 1A), participate in the charge transfer (CT) between the iron-sulfur cluster and the DNA substrate. However, these two residues are 16.1 Å apart and oriented differently in the conserved p58C structure (Fig. 1, A and B). Consequently, results of CT and DNA-binding experiments are not applicable to WT primase.

DNA substrates used by O'Brien *et al.* in the binding and CT experiments are biologically ir-

relevant. Both the 5'-triphosphate of a primer and the 3' overhang of a template are critical for DNA:RNA binding by p58C and primase (4, 6) (Fig. 1C, highlighted in blue). However, the substrate used by O'Brien *et al.* lacks these key binding elements (Fig. 1D), which results in affinity for mutated p58C less than 1/150 that of the natural substrate for WT p58C ($K_d = 5.5 \mu\text{M}$ versus 33 nM) [see figure S1 in (1) and table 2 in (6), respectively]. Previously, Chazin's group obtained a K_d of 0.3 μM [table S2 in (7)] instead of 5.5 μM as reported in (1) using the same protein, substrate, and experimental procedure (1). This almost 20-fold difference was not explained.

The only supporting evidence by O'Brien *et al.* for the redox switch involvement in primer synthesis is the rate reduction of de novo RNA synthesis by the Y345F mutant [figure 4A in (1)]. However, the rate reduction can be explained by a completely different mechanism. In the conserved p58C/substrate complex structure, Y345 interacts with the γ -phosphate of the initiating nucleotide triphosphate (NTP) (Fig. 1B) (4), which is rather sensitive to the binding environment (8, 9). For example, mutation of Arg306 analog in yeast primase, which makes two contacts with

¹Eppley Institute for Research in Cancer and Allied Diseases, Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE 68198, USA.

²Department of Immunology, University of Washington, Seattle WA 98195, USA. ³Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid 28049, Spain.

⁴Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198, USA. ⁵Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, USA.

*Corresponding author. Email: ttahirov@unmc.edu

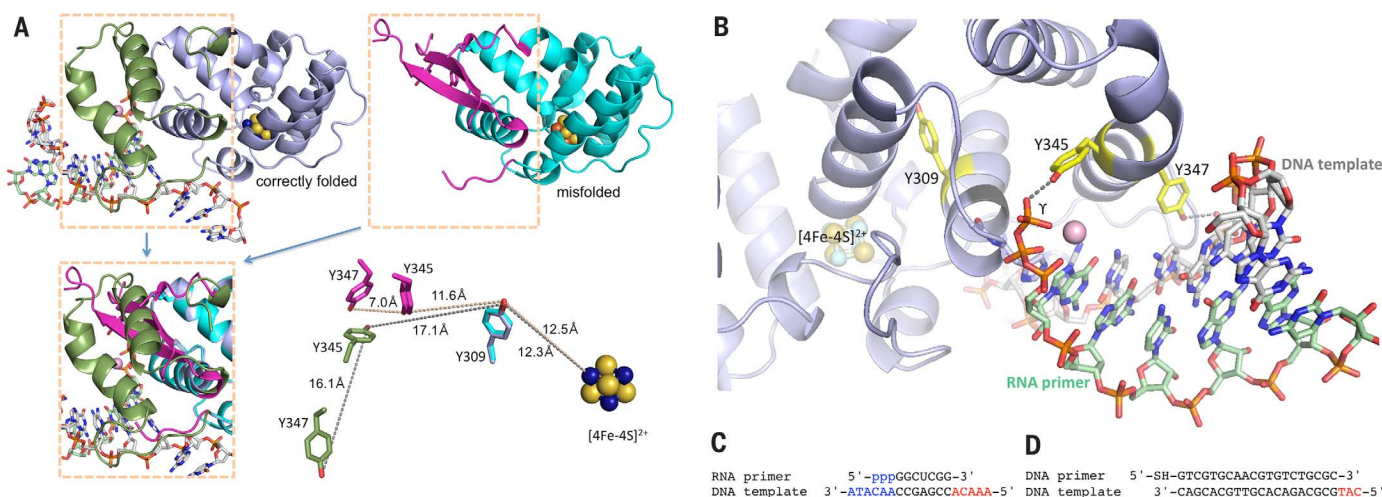


Fig. 1. Comparison of functionally relevant primase substrate and correctly folded p58C (4) with the substrate and misfolded p58C used by O'Brien *et al.* (1). (A) Side-by-side comparison of p58C structures with correctly folded [Protein Data Bank (PDB) code 5FOQ] and misfolded (PDB code 3L9Q) substrate-binding regions highlighted in different colors. These regions are overlapped in the left bottom quarter. The positions of Y309, Y345, and Y347 relative to [4Fe-4S]²⁺ in two structures are shown in the right bottom quarter. The reason for p58C misfolding might be explained by substitution of structurally important I271 by serine in erroneously named

“wild-type” p58C (PDB code 3L9Q) or by asparagine in its mutants Y345F and Y347F (PDB codes 5I7M and 5DQO, respectively). **(B)** Crystal structure of p58C in complex with a duplex containing 5'-triphosphate RNA and 3'-overhang DNA (4). **(C)** Primase substrate (6) with the 5'-triphosphate of a primer and the 3' overhang of a template that are required for p58C binding and are shown in blue. **(D)** Substrate used by O'Brien *et al.* (1) for electrochemistry experiments. In (C) and (D), the red-colored 5' overhangs do not participate in binding of correctly folded p58C. The figure was prepared using the PyMOL Molecular Graphics System (version 1.8, Schrödinger, LLC).

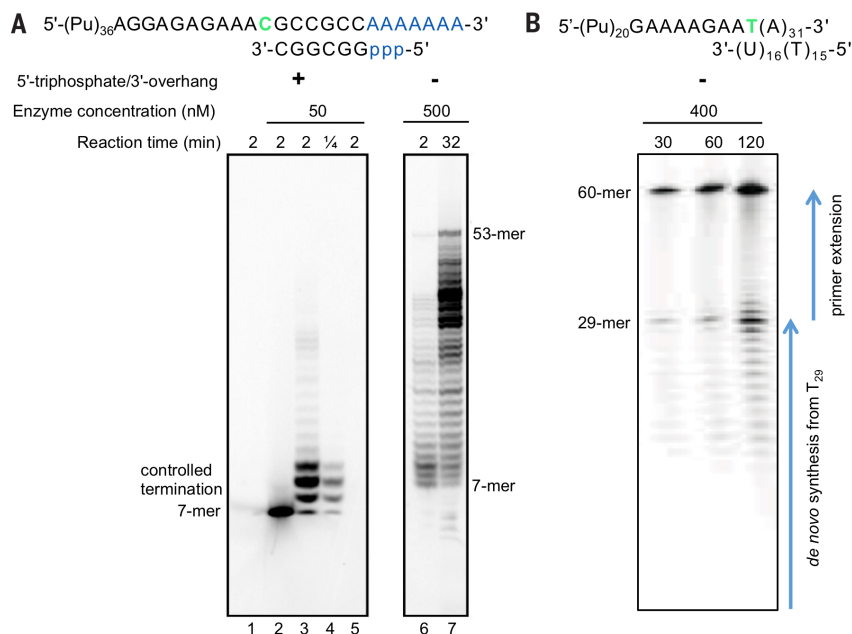


Fig. 2. Side-by-side comparison of experimental setups for examination of RNA synthesis termination by human primase.

(A) Example of primer elongation reactions showing the effect of template:primer structure on the efficiency of RNA synthesis termination by human primase, as described by Baranovskiy *et al.* (6). When using the correct substrate containing 5'-triphosphate and 3' overhang, there is pronounced termination of reaction mainly at 9-nucleotide oligomer primers (lanes 3 and 4), which are optimal for extension by Pol α (4). However, in the case of template:primer without 5'-triphosphate and 3' overhang, primase loses the ability to terminate synthesis at 9-nucleotide oligomer primers (lanes 6 and 7) and has dramatically reduced activity, which requires a higher load of the enzyme and longer reaction time. Primase activity reactions were reproduced as in (6). The products were labeled by incorporation of [α -³³P]GTP (guanosine triphosphate) at the seventh position of primer. The negatively charged triphosphate moiety increases the mobility of RNA primers. Lanes 1 and 5 control incubations in absence of enzyme or primer, respectively. Lane 2 reaction was not supplied with CTP and UTP. (B) Example of incorrectly designed primer elongation reactions, which are not capable of capturing physiologically relevant primer termination. The image was adopted from figure 5A in (1); note the high enzyme concentrations and long reaction time. The products with a length of 29 nucleotides or less are the result of RNA synthesis initiated from T₂₉ in the template [primase initiates RNA synthesis only from a template pyrimidine in the presence of adenosine triphosphate (ATP) or GTP (13)]. Appearance of primers longer than 10 nucleotides is due to the absence of Pol α or its catalytic core, which allows primase to rebind the 9-nucleotide oligomer primer without involvement of p58C and extend further (6, 14). Unfortunately, the gel provided does not allow visualization of the products of de novo synthesis from 2-nucleotide oligomer to 9- to 12-nucleotide oligomer. Counting of bands below 29-nucleotide oligomer product indicates that the lowest visible band corresponds to 18-nucleotide oligomer primer, not to 10-nucleotide oligomer as shown in figure. S14 in (1).

initiating NTP (4), completely abrogates de novo RNA synthesis (9). In comparison, Y345F mutation eliminates only one hydrogen bond (Fig. 1B) and, therefore, impairs the initiation partially. Consequently, the effect of Y345F mutation on synthesis initiation cannot be used as the evidence for Y345 participation in CT. Of the three Tyr residues suggested by O'Brien *et al.* to participate in the CT, only Y309 does not contact the DNA:RNA substrate (Fig. 1B), but the authors did not provide primer synthesis data for the Y309F mutant.

O'Brien *et al.* also claim that CT regulates primer truncation. However, they used a 31-nucleotide oligomer primer that is longer than the functionally relevant 9-nucleotide oligomer primer and lacks the elements that are essential both for reaction efficiency and for the control of synthesis

termination (see Fig. 2, A and B for comparison) (4, 10, 11). In their assay, primase generates a mixture of two product types: 32- to 60-nucleotide oligomer products, synthesized during extension of a 31-nucleotide oligomer primer, and RNA fragments with 5'-triphosphate and lengths of 2 to 31 nucleotides, which originate from RNA synthesis initiation from T₂₉ in the template. Obviously, the labels for 10- and 30-nucleotide oligomer products are not consistent. Two important controls are missing: reaction in the absence of the primer to show only the products of de novo synthesis, and reaction in the absence of cytidine triphosphate/uridine triphosphate (CTP/UTP) allowing the visualization of the exact position of a 32-nucleotide oligomer product on the gel. So the prominent band corresponding to an ~30-nucleotide oligomer product [figures

5A and S14 (1)] is a result of de novo RNA synthesis termination due to reaching the end of the primer [similar to termination in figure 4A in (1)] rather than abrogation of extension of a 31-nucleotide oligomer primer. Indeed, figure S14 in (1) confirms that Y345F substitution reduces the number of de novo synthesized primers, without any relation to CT. In the same manner, the absence of products with a length of ~30 nucleotides or less [as observed in figure 5A (right panel) in (1)] relates to abrogation of de novo primer synthesis and not to disruption of CT, because the introduced mismatch definitely disturbs the short (6 base pair) and AT-rich DNA:RNA duplex.

No direct evidence was provided that the oxidized [4Fe-4S]³⁺ cluster was obtained during electrochemical experiments with p58C. Moreover, the affinity of DNA to p58C with the oxidized cluster was not measured to confirm the statement that this form binds more strongly to the template:primer. Actually, p58C without any electrochemical manipulations binds tightly to the correct substrate under aerobic conditions (6), in which the [4Fe-4S]²⁺ cluster of primase is stable (9, 12). The authors avoided comparison of primer synthesis in aerobic versus anaerobic conditions but claimed the advantage of the latter. In fact, primase efficiently initiates and terminates RNA synthesis under aerobic conditions (6, 8), and the presence of an iron-sulfur cluster in Pol α is still under debate (13). Moreover, Pol α is absent in the primer elongation assay in (1), which renders the conclusion of its proposed role in termination of RNA primer synthesis a mere speculation.

Given the questionable results and the conclusions contradicting the biochemical, structural, and genetic data cumulated over three decades (13), we ask O'Brien *et al.* to re-examine their experiments and reconsider the conclusions.

REFERENCES AND NOTES

1. E. O'Brien *et al.*, *Science* **355**, eaag1789 (2017).
2. V. B. Agarkar, N. D. Babayeva, Y. I. Pavlov, T. H. Tahirov, *Cell Cycle* **10**, 926–931 (2011).
3. A. G. Baranovskiy *et al.*, *J. Biol. Chem.* **290**, 5635–5646 (2015).
4. A. G. Baranovskiy *et al.*, *J. Biol. Chem.* **291**, 10006–10020 (2016).
5. L. Sauguet, S. Klinge, R. L. Perera, J. D. Maman, L. Pellegrini, *PLoS ONE* **5**, e10083 (2010).
6. A. G. Baranovskiy *et al.*, *J. Biol. Chem.* **291**, 4793–4802 (2016).
7. S. Vaithiyalingam, E. M. Warren, B. F. Eichman, W. J. Chazin, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 13684–13689 (2010).
8. W. C. Copeland, T. S. Wang, *J. Biol. Chem.* **268**, 26179–26189 (1993).
9. S. Klinge, J. Hirst, J. D. Maman, T. Krude, L. Pellegrini, *Nat. Struct. Mol. Biol.* **14**, 875–877 (2007).
10. T. Ogawa, T. Okazaki, *Annu. Rev. Biochem.* **49**, 421–457 (1980).
11. R. D. Kuchta, B. Reid, L. M. Chang, *J. Biol. Chem.* **265**, 16158–16165 (1990).
12. B. E. Weiner *et al.*, *J. Biol. Chem.* **282**, 33444–33451 (2007).
13. A. G. Baranovskiy, T. H. Tahirov, *Genes (Basel)* **8**, 62 (2017).
14. Y. Zhang, A. G. Baranovskiy, T. H. Tahirov, Y. I. Pavlov, *J. Biol. Chem.* **289**, 22021–22034 (2014).

ACKNOWLEDGMENTS

We thank W. C. Copeland for scientific comments and K. Jordan for editing this manuscript. Preparation of this Technical Comment was supported by National Institute of General Medical Sciences grant GM101167 to T.H.T.

20 March 2017; accepted 13 June 2017
10.1126/science.aan2396

Comment on "The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport"

Andrey G. Baranovskiy, Nigar D. Babayeva, Yinbo Zhang, Luis Blanco, Youri I. Pavlov and Tahir H. Tahirov

Science **357** (6348), eaan2396.
DOI: 10.1126/science.aan2396

ARTICLE TOOLS

<http://science.sciencemag.org/content/357/6348/eaan2396>

RELATED CONTENT

<http://science.sciencemag.org/content/sci/357/6348/eaan2954.full>
<http://science.sciencemag.org/content/sci/357/6348/eaan2762.full>
<http://science.sciencemag.org/content/sci/355/6327/eaag1789.full>

REFERENCES

This article cites 14 articles, 9 of which you can access for free
<http://science.sciencemag.org/content/357/6348/eaan2396#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)