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April 8, 2019

Subject: Human DNA Polymerase η is a Reverse Transcriptase

Dear Editors in Chief of the *Journal of Biological Chemistry*,

We are molecular immunologists and immunogeneticists, and we have spent decades working with our collaborators on the mechanism of immunoglobulin somatic hypermutation. We bring to your attention an important issue of scientific priority and thus maintenance of the integrity of the published scientific record. We have just become aware of two articles published recently in the *Journal of Biological Chemistry* that describe the reverse transcriptase activity of human DNA polymerase η (Su *et al*., 2017; Su *et al*., 2019). We would like it to be noted that clear priority for the demonstration of the reverse transcriptase activity of human DNA polymerase η was published previously by us in the journal *Immunology and Cell Biology* (Franklin *et al*., 2004). Please find the abstracts of all three articles below. We would appreciate that a note regarding this precedence be published without delay in the *Journal of Biological Chemistry* and linked to both articles (Su *et al*., 2017; Su *et al*., 2019) so that the scientific record is corrected. Our collaborators and colleagues in the international scientific community are following developments closely with us (see shortlist of supporting academics below), and we look forward to this matter being resolved promptly by publication of a clarifying statement.

Yours sincerely,

Andrew Franklin PhD, Edward J. Steele PhD

References

Franklin A., Milburn P.J., Blanden R.V., Steele E.J. (2004). Human DNA polymerase-η, an A-T mutator in somatic hypermutation of rearranged immunoglobulin genes, is a reverse transcriptase. *Immunol Cell Biol* 82, 219–25.

Su Y., Egli M., Guengerich F.P. (2017). Human DNA polymerase η accommodates RNA for strand extension. *J Biol Chem* 292, 18044–51.

Su Y., Ghodke P.P., Egli M., Li L., Wang Y., Guengerich F.P. (2019). Human DNA polymerase η has reverse transcriptase activity in cellular environments. *J Biol Chem*, in press (published on March 6, 2019).

Franklin A., Milburn P.J., Blanden R.V., Steele E.J. (2004). Human DNA polymerase-η, an A-T mutator in somatic hypermutation of rearranged immunoglobulin genes, is a reverse transcriptase. *Immunol Cell Biol* 82, 219–25.

Abstract: We have proposed previously that error-prone reverse transcription using pre-mRNA of rearranged immunoglobulin variable (IgV) regions as templates is involved in the antibody diversifying mechanism of somatic hypermutation (SHM). As patients deficient in DNA polymerase-η exhibit an abnormal spectrum of SHM, we postulated that this recently discovered Y-family polymerase is a reverse transcriptase (RT). This possibility was tested using a product-enhanced RT (PERT) assay that uses a real time PCR step with a fluorescent probe to detect cDNA products of at least 27-37 nucleotides. Human pol-η and two other Y-family enzymes that are dispensable for SHM, human pols-ι and -κ, copied a heteropolymeric DNA-primed RNA template in vitro under conditions with substantial excesses of template. Repeated experiments gave highly reproducible results. The RT activity detected using one aliquot of human pol-η was confirmed using a second sample from an independent source. Human DNA pols-β and -μ, and T4 DNA polymerase repeatedly demonstrated no RT activity. Pol-η was the most efficient RT of the Y-family enzymes assayed but was much less efficient than an HIV-RT standard in vitro. It is thus feasible that pol-η acts as both a RNA- and a DNA-dependent DNA polymerase in SHM in vivo, and that Y-family RT activity participates in other mechanisms of physiological importance.

Running title: RT activity of human DNA pol-η

Keywords: affinity maturation, human DNA polymerase-eta, immunoglobulin variable region genes, reverse transcription, somatic hypermutation

PMID: 15061777

DOI: 10.1046/j.0818-9641.2004.01221.x

Su Y., Egli M., Guengerich F.P. (2017). Human DNA polymerase η accommodates RNA for strand extension. *J Biol Chem* 292, 18044–51.

Abstract: Ribonucleotides are the natural analogs of deoxyribonucleotides, which can be misinserted by DNA polymerases, leading to the most abundant DNA lesions in genomes. During replication, DNA polymerases tolerate patches of ribonucleotides on the parental strands to different extents. The majority of human DNA polymerases have been reported to misinsert ribonucleotides into genomes. However, only PrimPol, DNA polymerase α, telomerase, and the mitochondrial human DNA polymerase (hpol) γ have been shown to tolerate an entire RNA strand. Y-family hpol η is known for translesion synthesis opposite the UV-induced DNA lesion cyclobutane pyrimidine dimer and was recently found to incorporate ribonucleotides into DNA. Here, we report that hpol η is able to bind DNA/DNA, RNA/DNA, and DNA/RNA duplexes with similar affinities. In addition, hpol η, as well as another Y-family DNA polymerase, hpol κ, accommodates RNA as one of the two strands during primer extension, mainly by inserting dNMPs opposite unmodified templates or DNA lesions, such as 8-oxo-2'-deoxyguanosine or cyclobutane pyrimidine dimer, even in the presence of an equal amount of the DNA/DNA substrate. The discovery of this RNA-accommodating ability of hpol η redefines the traditional concept of human DNA polymerases and indicates potential new functions of hpol η in vivo.

Running title: Reverse transcriptase activity of DNA polymerase η

Keywords: DNA polymerase, RNA, reverse transcriptase, DNA damage, replication initiation, DNA enzymes

PMID: 28972162

DOI: 10.1074/jbc.M117.809723

Su Y., Ghodke P.P., Egli M., Li L., Wang Y., Guengerich F.P. (2019). Human DNA polymerase η has reverse transcriptase activity in cellular environments. *J Biol Chem*, in press (published on March 6, 2019).

Abstract: Classical DNA and RNA polymerase (pol) enzymes have defined roles with their respective substrates, but several pols have been found to have multiple functions. We previously reported that purified human DNA pol η (hpol η) can incorporate both deoxyribonucleoside triphosphates (dNTPs) and ribonucleoside triphosphates (rNTPs) and can use both DNA and RNA as substrates. X-ray crystal structures revealed that two pol η residues, Phe-18 and Tyr-92, behave as steric gates to influence sugar selectivity. However, the physiological relevance of these phenomena has not been established. Here, we show that purified hpol η adds rNTPs to DNA primers at physiological rNTP concentrations and in the presence of competing dNTPs. When two rATPs were inserted opposite a cyclobutane pyrimidine dimer, the substrate was less efficiently cleaved by human RNase H2. Human XP-V fibroblast extracts, devoid of hpol η, could not add rNTPs to a DNA primer, but the expression of transfected hpol η in the cells restored this ability. XPV cell extracts did not add dNTPs to DNA primers hybridized to RNA, but could when hpol η was expressed in the cells. HEK293T cell extracts could add dNTPs to DNA primers hybridized to RNA, but lost this ability if hpol η was deleted. Interestingly, a similar phenomenon was not observed when other translesion synthesis (TLS) DNA polymerases-hpol ι, κ, or ζ-were individually deleted. These results suggest that hpol η is one of the major reverse transcriptases involved in physiological processes in human cells.

Running title: Activities of human DNA polymerase η

Keywords: DNA polymerase, RNA polymerase, reverse transcription, DNA transcription, DNA replication, DNA enzyme, DNA damage, DNA pol eta, translesion synthesis (TLS) enzyme

PMID: 30842261

DOI: 10.1074/jbc.RA119.007925

List of colleagues and co-authors who support this Letter to the Editor and who are prepared for their names to be published for said purpose:

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Outcome of Priority Letter to Editors J. Biol. Chem as 16 April 2019

The Original uncorrected version of Su et al 2019 can be found at
http://www.jbc.org/content/early/2019/03/06/jbc.RA119.007925.full.pdf

With the receipt of our letter the Editor in Chief Lila Gierasch responded (email chain appended to the end of this Outcome report).

Then on 16 April J. Biol Chem published the paper with a **Note In Proof** :

Note added in proof—Following acceptance of our manuscript, our attention was called to a paper (Franklin, A., Milburn, P. J., Blanden, R. V., and Steele, E. J. (2004) Human DNA polymerase-h an A-T mutator in somatic hypermutation of rearranged immunoglobulin genes, is a reverse transcriptase. Immunol. Cell Biol. 82, 219 –225) by one of the authors, in which pol η had been reported to show activity in a product- enhanced reverse transcriptase assay using a 4 mM mixture of dNTPs, and a mechanism had been proposed. Reference to this work had been included in an earlier draft of our manuscript but had been inadvertently deleted in the final version. We believe that our biochemical and cell extract work in Ref. 43 and here in this paper goes beyond this early study in demonstrating the significance of pol η as a reverse transcriptase, but apologize for the oversight.

J Biol Chem. 2019 Apr 12;294(15):6073-6081. doi: 10.1074/jbc.RA119.007925. Epub 2019 Mar 6.

Human DNA polymerase η has reverse transcriptase activity in cellular environments.

Su Y[1], Ghodke PP[1], Egli M[1], Li L[2], Wang Y[2], Guengerich FP[3].
Author information

Abstract
Classical DNA and RNA polymerase (pol) enzymes have defined roles with their respective substrates, but several pols have been found to have multiple functions. We reported previously that purified human DNA pol η (hpol η) can incorporate both deoxyribonucleoside triphosphates (dNTPs) and ribonucleoside triphosphates (rNTPs) and can use both DNA and RNA as substrates. X-ray crystal structures revealed that two pol η residues, Phe-18 and Tyr-92, behave as steric gates to influence sugar selectivity. However, the physiological relevance of these phenomena has not been established. Here, we show that purified hpol η adds rNTPs to DNA primers at physiological rNTP concentrations and in the presence of competing dNTPs. When two rATPs were inserted opposite a cyclobutane pyrimidine dimer, the substrate was less efficiently cleaved by human RNase H2. Human XP-V fibroblast extracts, devoid of hpol η, could not add rNTPs to a DNA primer, but the expression of transfected hpol η in the cells restored this ability. XP-V cell extracts did not add dNTPs to DNA primers hybridized to RNA, but could when hpol η was expressed in the cells. HEK293T cell extracts could add dNTPs to DNA primers hybridized to RNA, but lost this ability if hpol η was deleted. Interestingly, a similar phenomenon was not observed when other translesion synthesis (TLS) DNA polymerases-hpol ι, κ, or ζ-were individually deleted. These results suggest that hpol η is one of the major reverse transcriptases involved in physiological processes in human cells.

KEYWORDS:
DNA damage; DNA enzyme; DNA pol eta; DNA polymerase; DNA replication; DNA transcription; RNA polymerase; reverse transcription; translesion synthesis (TLS) enzyme
PMID:
30842261
DOI:
10.1074/jbc.RA119.007925

From: Ted Steele <e.j.steele@bigpond.com>
Date: Wednesday, 10 April 2019 at 9:49 am
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Subject: FW: Urgent matter -Priority Franklin et al 2014 ICB v Su et al 2017, 2019 JBC

Dear Friends and Colleagues:

That matter of priority of Franklin, Milburn, Blanden and Steele 2004 that Human DNA Polymerase eta is a reverse transcriptase looks like it has been resolved by the Editor in Chief of *Journal of Biological Chemistry*, Professor Lila Gierasch. See our letter at clickable site http://viXra.org/abs/1904.0166 and the email exchanges of yesterday and today
Yours
Ted Steele
……
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From: Lila Gierasch <gierasch@biochem.umass.edu>
Date: Wednesday, 10 April 2019 at 1:21 am
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Subject: Re: Urgent matter -Priority Franklin et al 2014 ICB v Su et al 2017, 2019 JBC

A note added in proof will be published with the redacted version of this paper. The authors were very cooperative and regret tgat this citation was inadvertantly removed at some point in their final editing.

We thank you for helping to correct the citations in this manuscript.

Lila Gierasch
Editor, JBC

Sent from my iPhone

On Apr 8, 2019, at 7:13 PM, Ted Steele <e.j.steele@bigpond.com> wrote:

Click to see attachment Attachment : Franklin A, Steele EJ 2019 Human DNA Polymerase -η is a Reverse Trancriptase http://viXra.org/abs/1904.0166 Submitted *Journal Biological Chemistry* April 8 2019

Dear Professors Gierasch, Guengerich and Tabor (and co-authors of Professor Guengerich, Dr Egli and Wang):

Events have just taken place at *J Biol Chem* concerning the publication of Su et al 2017, 2019, particularly the 2019 JBC paper (see attached). We have only just become aware of this work still In Press at JBC. Accordingly the scientific priority for Human DNA polymerase *h* (eta) as a reverse transcriptase requires recognition, by approriate citation by Su et al, and thus assisted by JBC editirs viz. of the prior work by Andrew Franklin, Peter J Milburn, Robert V Blanden, Edward J Steele (2004). Human DNA polymerase-η, an A-T mutator in somatic hypermutation of rearranged immunoglobulin genes, is a reverse transcriptase. *Immunol Cell Biol* 82, 219–25.

The work was from the PhD thesis of Andrew Franklin at the John Curtin School of Medical Research in 2001-2003. Indeed the first public presentation of the data was at a *Frank and Bobbie Fenner Conference* in late 2003 (to honour the retirement of our colleague Professor Robert V Blanden) at the John Curtin School prior to the publication of the Franklin et al paper a few months later.

We would greatly appreciate publication of our priority letter in JBC as soon as possible appropriately linked to the Su et al 2019 paper in particular. Our scientific colleagues who have read both sets of papers agree with our claim (see the list in the attached Letter) . Indeed Peter A Bretscher and Gerald W Both were both present at the 2003 conference where Andrew first presented his final PhD work, and where the data on DNA Polymerase *h (*eta) as a reverse transcriptase was first publicly ventilated.

Science, as we have understood it, and been brought up with, actually depends on the recognition of priority for its credibility and public support.

Yours sincerely

……

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