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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

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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

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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

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