DNA Repair 29 (2015) 127–138

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Alternative solutions and new scenarios for translesion DNA synthesis by human PrimPol

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ARTICLE INFO

Article history:
Received 10 November 2014
Received in revised form 12 February 2015
Accepted 13 February 2015
Available online 23 February 2015

Keywords:
PrimPol
DNA primase
DNA polymerase
8oxoG
Lesion bypass
Translesion synthesis

ABSTRACT

PrimPol is a recently described DNA polymerase that has the virtue of initiating DNA synthesis. In addition of being a *sensu stricto* DNA primase, PrimPol's polymerase activity has a large capacity to tolerate different kind of lesions. The different strategies used by PrimPol for DNA damage tolerance are based on its capacity to "read" certain lesions, to skip unreadable lesions, and as an ultimate solution, to restart DNA synthesis beyond the lesion thus acting as a TLS primase. This lesion bypass potential, revised in this article, is strengthened by the preferential use of moderate concentrations of manganese ions as the preferred metal activator. We show here that PrimPol is able to extend RNA primers with ribonucleotides, even when bypassing 8oxoG lesions, suggesting a potential new scenario for PrimPol as a TLS polymerase assisting transcription. We also show that PrimPol displays a high degree of versatility to accept or induce distortions of both primer and template strands, creating alternative alignments based on microhomology that would serve to skip unreadable lesions and to connect separate strands. In good agreement, PrimPol is highly prone to generate indels at short nucleotide repeats. Finally, an evolutionary view of the relationship between translesion synthesis and primase functions is briefly discussed.

1. Introduction

1.1. The priming problem and solutions

It is commonly accepted that DNA polymerases require a primer to initiate DNA synthesis, whereas RNA polymerases do not. Strictly, RNA polymerases do also require a "primer", but that requirement can be fulfilled by a NTP molecule, whose 3′-OH acts as the attacking nucleophyle on a second NTP, to form a dinucleotide. Most DNA polymerases cannot do that and requires a pre-existing primer to provide the free hydroxyl group to transfer the next nucleotide [1]. Because of that limitation, a variety of independent solutions have evolved to address the "priming problem". To initiate DNA replication, some DNA polymerases can use special, non-orthodox primers as tRNA (reverse transcriptases) or terminal proteins (DNA polymerases from ø29-like phages, adenoviruses, and some linear phages), which provide the 3′-OH; thus, the very same enzyme performs the initiation reaction and the subsequent elongation of the DNA chain [2]. A simpler solution is the use of a "nick" introduced in double-stranded circular DNA (as in parvoviruses, geminiviruses and circoviruses and many phages and plasmids) to obtain the needed "primer" to trigger rolling-circle replication [3]. Another solution is valid for DNA polymerases involved in DNA repair and damage tolerance: different DNA intermediates (*i.e.* a nick, a gap, or a DSB) provide their 3′-end as a primer [4]. However, in all cellular life forms and many DNA viruses, phages and plasmids, a short piece of RNA is the prevalent primer used for replicative DNA polymerases. Who makes these primers? Exceptionally, those RNA primers can be directly provided by the transcription activity of monomeric RNA polymerases (as in the case of plasmid ColE1 replication or mitochondrial DNA replication), but in most cases they are produced by specialized polymerases named "primases" [5]. Primases can be divided in two evolutionarily unrelated families: DnaG-like primases (bacterial), having a "Toprim" fold, and AEP-like primases (Archaea and Eukaryotes), having a "RRM" fold [6,7]. About 30

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http://dx.doi.org/10.1016/j.dnarep.2015.02.013
1568-7864/

years ago, a specific mitochondrial primase was proposed to exist [8–10], but its identification and functional role in mitochondrial DNA replication has been a matter of debate [11,12]. More recent work provided evidence that the mtRNA polymerase is the enzyme acting as a primase, not only to prime leading strand synthesis at OriH, but also to prime the lagging strand at OriL [13].

1.2. PrimPol, an ancient solution to start DNA synthesis

A tighter coupling between primase and polymerase function has been described in certain plasmids [14], archea [15] and even bacteria [16], in which a single protein, named **PrimPol**, performs both RNA polymerase (primer synthesis) and DNA polymerase (DNA synthesis) activities. Amino acid sequence comparisons and 3D-structural analysis [7,16,17] indicated that these bifunctional DNA primase–polymerases (PrimPol) belong to the AEP superfamily, although in these cases, the AEP domain is fused to a helicase domain that can belong to different superfamilies (revised by Ref. [16]). It is worth noting that not all members belonging to the AEP-family function as a primase (or have primase activity). The polymerization domain of *Mycobacterium tuberculosis* LigD (MtPolDom) is a very interesting example. MtPolDom belongs to the AEP family of primases, but is not able to make a dinucleotide primer, as it cannot bind a ribonucleoside triphosphate at the 5′-side of the reaction (the primer site); conversely, a pre-existing primer (the 3′-protruding end of a double-strand break) binds the 5′-site, and it can be extended with a NTP selected by the 3′-protrusion (acting as template) of a second DNA end, allowing its function in bacterial NHEJ [4,18,19]. Accordingly, MtPolDom is not fused to a helicase, but to both nuclease and ligase domains.

Based on *in silico* analysis, a second AEP was predicted to exist in human cells [7]. A detailed biochemical analysis of this putative new enzyme [20] allowed us to demonstrate two specific features: (1) its ability to start DNA chains with deoxynucleotides, unlike regular primases that use exclusively ribonucleotides; (2) its intrinsic DNA polymerase activity (the 17th discovered in human cells), tailored to bypass certain lesions, specially 8-oxoguanine (8oxoG), the most common oxidative lesion in DNA. Based on these features, the HUGO committee of human gene nomenclature accepted our proposal of naming this enzyme PrimPol (PRIMPOL for the gene). Fortunately, other reports describing the identification of the same enzyme used the same nomenclature [21,22].

Subcellular fractionation and immunodetection studies indicated that PrimPol is present in both nuclear and mitochondrial DNA compartments [20]. In these two compartments, the complement of polymerases involved in DNA synthesis significantly differs (see Fig. 1A). In mitochondria, initiation of DNA replication at both OriH and OriL is primed by the mitochondrial RNA polymerase (POLMRT), and these two single primers (one for each strand) are supposed to be processively elongated by Polγ, the mitochondrial replicase. Nuclear DNA replication, a much larger task, employs a dedicated two-subunits primase (Pri1 + Pri2) to make RNA primers, that will be "transformed" in DNA primers by their extension with dNTPs by a specific, proofreading-deficient DNA polymerase (Polα), being further transferred to the distinct replicases Polε and Polδ, operating either at the leading or the lagging strand, respectively. On top of that, PrimPol offers a third choice for priming (see Fig. 1A), as PrimPol is able to start synthesis with dNTPs, thus producing mostly DNA primers, although it is quite likely that *in vivo* a purine ribonucleotide occupies the 5′-position of the synthesized primer [20]. Not surprisingly, both primase and polymerase activities of PrimPol share the same catalytic active site (conserved in the AEP family). Fig. 1B shows a comparative scheme of the human PrimPol synthesis reactions, outlining the essential difference between

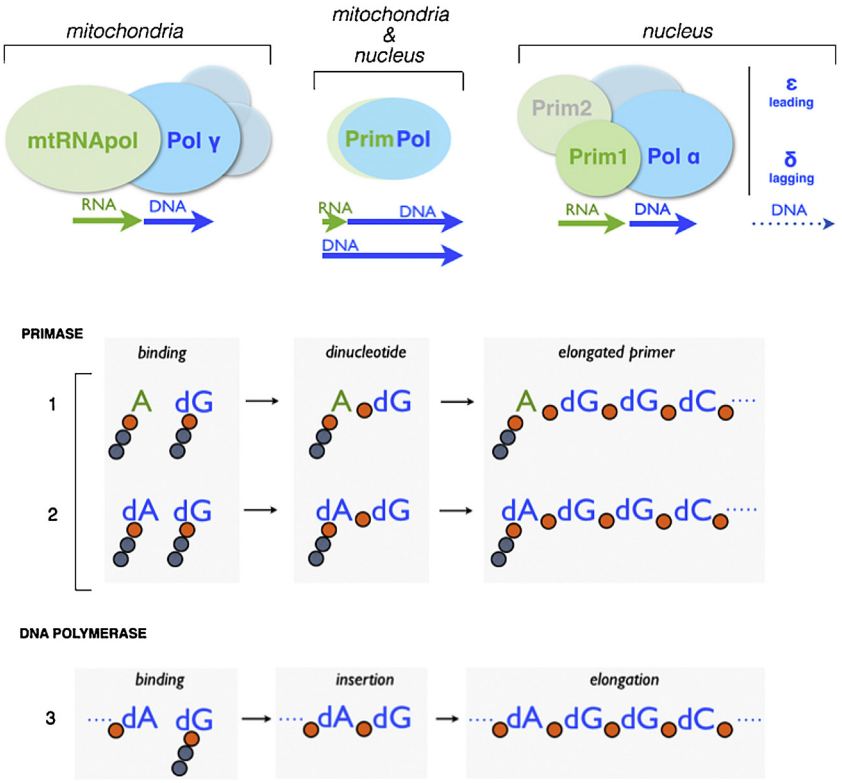

Fig. 1. (A) Primase/polymerase associations for DNA replication. Different combinations of primase (green) and DNA polymerase (blue) activities operating in nucleus and mitochondria (see main text for details). (B) Schematic representation of the favorite reactions performed by human PrimPol; PrimPol binds with the same efficiency a ribo (green) or a deoxi (blue) at the 5′ site, but prefers deoxis at the 3′ site. PrimPol behaves as a conventional DNA polymerase, consecutively adding dNTP units. As described in this paper, PrimPol behaves also as RNA polymerase (not shown in the scheme).