Recent Advances in Understanding bacterial and archaeoeukaryotic Primases

Jan Bergsch[1,2], Frédéric H.-T. Allain[1], Georg Lipps[2]*,

[1]Department of Biology, Institute of Molecular Biology and Biophysics, ETH Zurich, 8093 Zurich, Switzerland

[2] Institute of Chemistry and Bioanalytics, University of Applied Sciences Northwestern Switzerland, Hofackerstrasses 30, 4132 Muttenz, Switzerland

*To whom correspondence and requests should be addressed. Tel: +41-612285452 e-mail: georg.lipps@fhnw.ch

short title: Structural Biology of Primases

keywords: replication, primosome, bacterial primases, archaeoeukaryotic primases, protein-nucleic acid interactions

Highlights

- Primases are part of the replisome, a flexible and highly dynamic multiprotein complex at the replication fork.
- Initiation of primer synthesis requires binding of three substrates that yield the dinucleotide/template hybrid further elongated by the primase.
- Substrate binding involves synergistic interactions on the non-catalytic domain as exemplified by an archaeal homolog.
- Beside replicative functions, primases such as PrimPol are a key factor for genome stability.

Abstract

DNA replication in all forms of life relies upon the initiation of synthesis on a single strand template by formation of a short oligonucleotide primer, which is subsequently elongated by DNA polymerases. Two structurally distinct classes of enzymes have evolved to perform this function, namely the bacterial DnaG-type primases and the Archaeal and Eukaryotic primases (AEP). Structural and mechanistic insights have provided a clear understanding of the role of the different domains of these enzymes in the context of the replisome and recent work sheds light upon primase-substrate interactions. We herein review the emerging picture of the

primase mechanism on the basis of the structural knowledge obtained to date and propose future directions of this essential aspect of DNA replication.

Introduction

Before a cell divides, its DNA has to be faithfully replicated. Considering its speed, accuracy and importance for integrity of the genomic information, replication is a fascinating biomolecular mechanism carried out by a highly complex and orchestrated machinery involving dozens of proteins commonly referred to as the replisome. Replication proceeds semi-conservatively: the double-stranded DNA is unwound and both ensuing single strands are replicated by specialized protein complexes. Although double-stranded DNA is *per se* symmetric, the symmetry is broken as soon as the replisome is assembled since DNA polymerases only proceed in 5' to 3' direction. Consequently, the leading strand can be replicated continuously by the action of a processive DNA polymerase whereas the lagging strand is replicated piecewise rendering the copying mechanism more complicated. Replicative DNA polymerases are not able to synthesize DNA *de novo*, instead requiring a 3'-end that they can elongate by incorporating nucleotides cognate to the template strand [1].

DNA primases, which are specialized RNA polymerases, can however initiate synthesis using a ssDNA template. Therefore, cooperation of the DNA primase and DNA polymerase is required. DNA primases synthesize with low fidelity a short RNA primer [2] that, after extension by the DNA polymerases, is specifically removed by additional enzymes acting on the lagging strand [3]. Cells use primases for the replication of the genetic material but priming can also be accomplished with proteins or tRNAs, for example in viral DNA replication [4].

Although the basic organisation of DNA replication is universally conserved, the proteins of the replication machinery are not [5]. In fact, DNA helicases, primases and replicative polymerases differ between the bacterial and the archaeoeukaryotic replisomes. Bacterial cellular primases (DnaG) are structurally different from the heterodimeric archaeoeukaryotic primases, which consist of a small catalytic subunit (PriS) and a large accessory subunit (PriL). Although both types of primases perform the same reaction and share similar acidic catalytic residues in their active sites, they are comprised of two unrelated protein folds, namely the toprim fold (bacteria) and the RRM fold (archaea and eukayotes) [6,7].

DNA primases thus represent a clear example of convergent evolution. It is possible that the replication machinery may have emerged twice during evolution or that the transition from an RNA world to DNA occurred independently at least twice [8].

The bacterial cellular primase DnaG

DnaG mainly acts on the lagging strand of the replication fork where it repeatedly initiates the synthesis of Okazaki fragments (Figure 1). The bacterial primase is closely associated with the bacterial replicative hexameric helicase DnaB. DnaB, a member of the helicase superfamily 4,

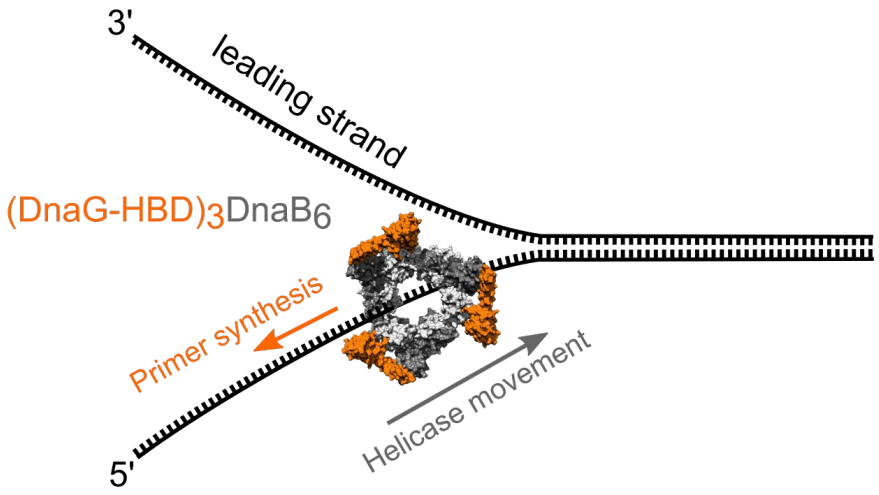
encircles the lagging strand and moves in 5’ to 3’ direction towards the double-stranded DNA fork [9]. The movement of the helicase and the ensuing unwinding is powered by ATP hydrolysis. Two subunits of the DnaB hexamer form the interface for one DnaG molecule, therefore three primase molecules are present at a bacterial replication fork (Figure 1A). DnaG is organized in three domains, the N-terminal zinc-binding domain (ZBD), the central RNA polymerase domain (RPD) and the C-terminal helicase-binding domain (HBD). The role of the ZBD is to recognize the priming site, which is 5’-C(A/T)G [9]. The RPD carries the active site with three catalytic acidic residues that are exposed at a cleft in the center of the protein. This part of the proteins has structural similarity with the toprim fold, a region of ~100 amino acids found in topoisomerases [6]. The HBD anchors the primase at the hexameric ring of the helicase. The latter interaction is particularly important as DnaG is activated about 5000 fold when bound to DnaB [10]. Within the replisome, two DnaG molecules cooperate, one molecule binding the template at the trinucleotide priming site and the other catalysing primer synthesis [11].

A full-length structure of a bacterial primase is currently not available. The difficulty may lie in the highly dynamic and complex molecular assembly of this replisome, which might render the structural investigation of physiologically relevant substructures difficult. Only partial structures of bacterial primases have been determined (Figure 1C). Nevertheless, in several of these structures from different organisms, DnaG was bound to ssDNA template (*M. tuberculosis*, pdb: 5W35), to primer (*M. tuberculosis*, pdb: 5W36) and to a ribonucleotide (*S. aureus*, pdb: 4EDG) [12–14]. Although the ZBD is thought to recognize the trinucleotide primase recognition site on the template strand, the template is mainly bound by the N-terminal part of the RPD. The Primer and the ribonucleotide are situated at the RPD active site cleft containing highly conserved acidic residues (Figure 1B). Helicase translocation and primer synthesis proceed in opposite directions (Figure 1A). Therefore, the helicase, which typically moves with a velocity of about 1000 nucleotides/s, would need to pause during primer synthesis or alternatively a priming loop would need to form between primase and helicase until primer synthesis is complete [9]. In either case, a flexible arrangement between the HBD and RPD is needed to accommodate the structural changes during primer synthesis since the primases remain anchored to the helicase.

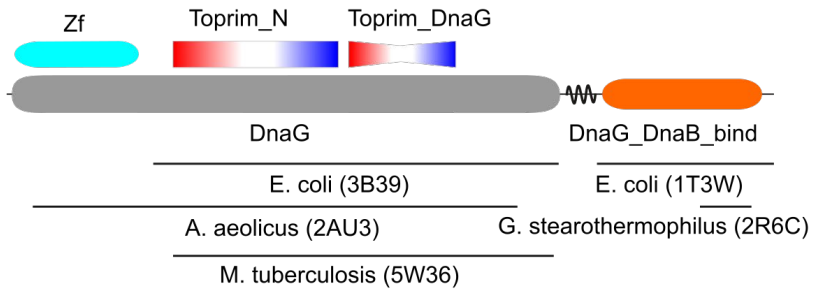
The replicative enzymes and the replisome of bacteriophages are similar to the bacterial replication system. The replication of the bacteriophage T7 has been thoroughly investigated in biochemical and structural studies. Recently, several cryo EM structures of the replisome revealed the architecture of the complete T7 replisome which encompass the leading strand DNA polymerase (gp5), the host processivity factor thioredoxin, the hexameric ring of gp4 (helicase-primase) and two lagging-strand DNA polymerases (gp5) assembled onto a synthetic DNA fork substrate [15]. The substructure of the lagging strand replisome depicted in figure 2 demonstrates how the replicative helicase, the primase and the DNA polymerase

cooperate to ensure replication of the lagging strand. In particular, the ZBD contacting the primer recognition site of the template is in close contact to the DNA polymerase facilitating extension of the freshly synthesized primer. It can be envisioned that if the DNA polymerase remains attached to the helicase hexameric ring, single stranded DNA unwound by the helicase would form a loop ahead of the DNA polymerase until the Okazaki fragment is fully synthesized and released by the DNA polymerase. Possibly, an additional priming event could occur at the single-stranded loop before the Okazaki fragment is released by the DNA polymerase. Additional structural and functional studies are required to understand the dynamic interplay within the replisome and in particular the dynamics and conformational changes required for lagging strand replication.

A


3'
leading strand
(DnaG-HBD)3DnaB6
Primer synthesis
Helicase movement
5'

B


Zf
Toprim_N
Toprim_DnaG
DnaG
DnaG_DnaB_bind
E. coli (3B39)
E. coli (1T3W)
A. aeolicus (2AU3)
G. stearothermophilus (2R6C)
M. tuberculosis (5W36)

C

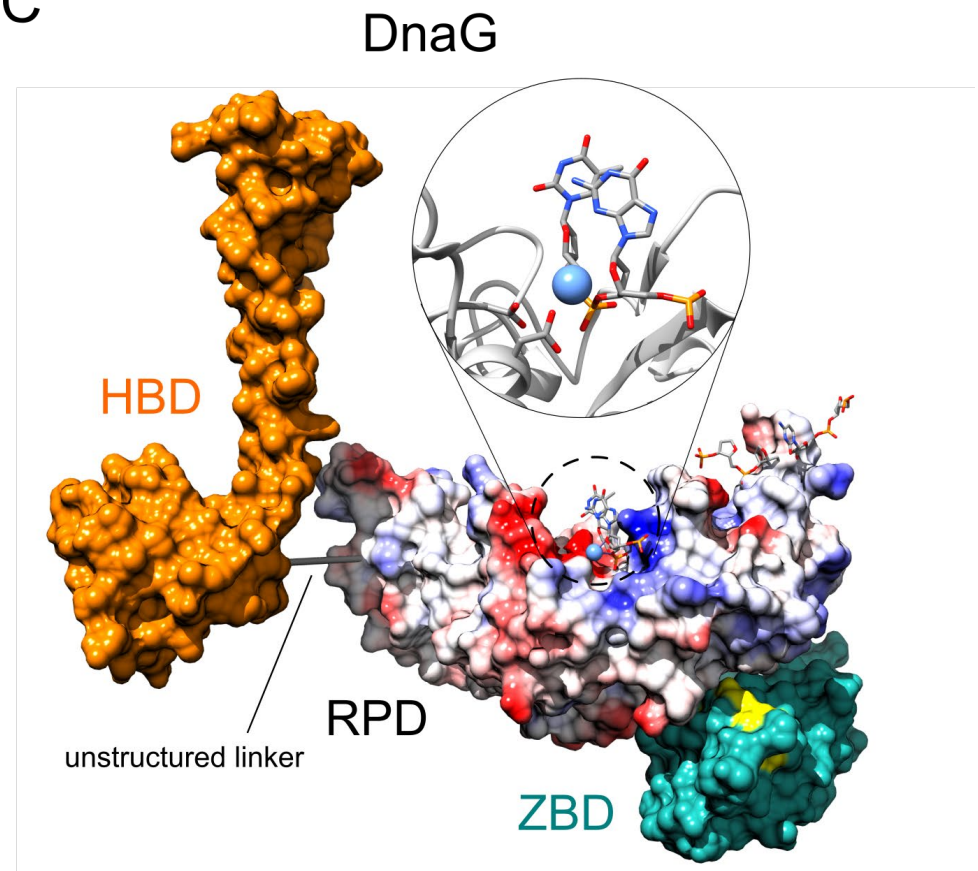

DnaG
HBD
RPD
unstructured linker
ZBD

Figure 1: Bacterial primase DnaG

A: The bacterial replicative helicase DnaB (gray, *G. stearothermophilus*, pdb: 2R6C [16]) encircles the lagging strand of the replication fork and delivers single-stranded DNA to the primase DnaG, whose helicase binding domain (HBD) is shown orange. B: The bacterial primase initiates primer synthesis preferentially at specific trinucleotide sequences, which are recognized by the ZBD (cyan, *A. aeolicus*, pdb: 2AU3 [11], the zinc-sulfur cluster highlighted in yellow). Primer synthesis is catalyzed by the RNA polymerase domain (RPD, *E. coli*, pdb: 3B39 [17] colored according to surface charge. The position of the primer and template DNA was derived from the structure of *M. tuberculosis* DnaG (pdb: 5W36 and 5W35 [14]).The inset shows the catalytic cleft of the *M. tuberculosis* DnaG with a dinucleotide primer and a strontium ion and two aspartate residues highlighted (pdb: 5W36). The primase HBD, colored orange (*E. coli*, pdb: 1T3W [18]), anchors the primase at the replicative helicase. The three domains of DnaG are flexibly tethered. Shown is the docking of ZBD to the RPD as found in the crystal structure of *A. aeolicus*. The position of HBD relative to RPD is undefined; the connecting flexible linker of ~20 amino acids is tentatively shown as a grey tube. C: Domain structure of *E. coli* DnaG. The wavy lines indicate regions of low sequence conservation and high flexibility. Below the domain organization, important partial structures of DnaG are depicted as horizontal lines.

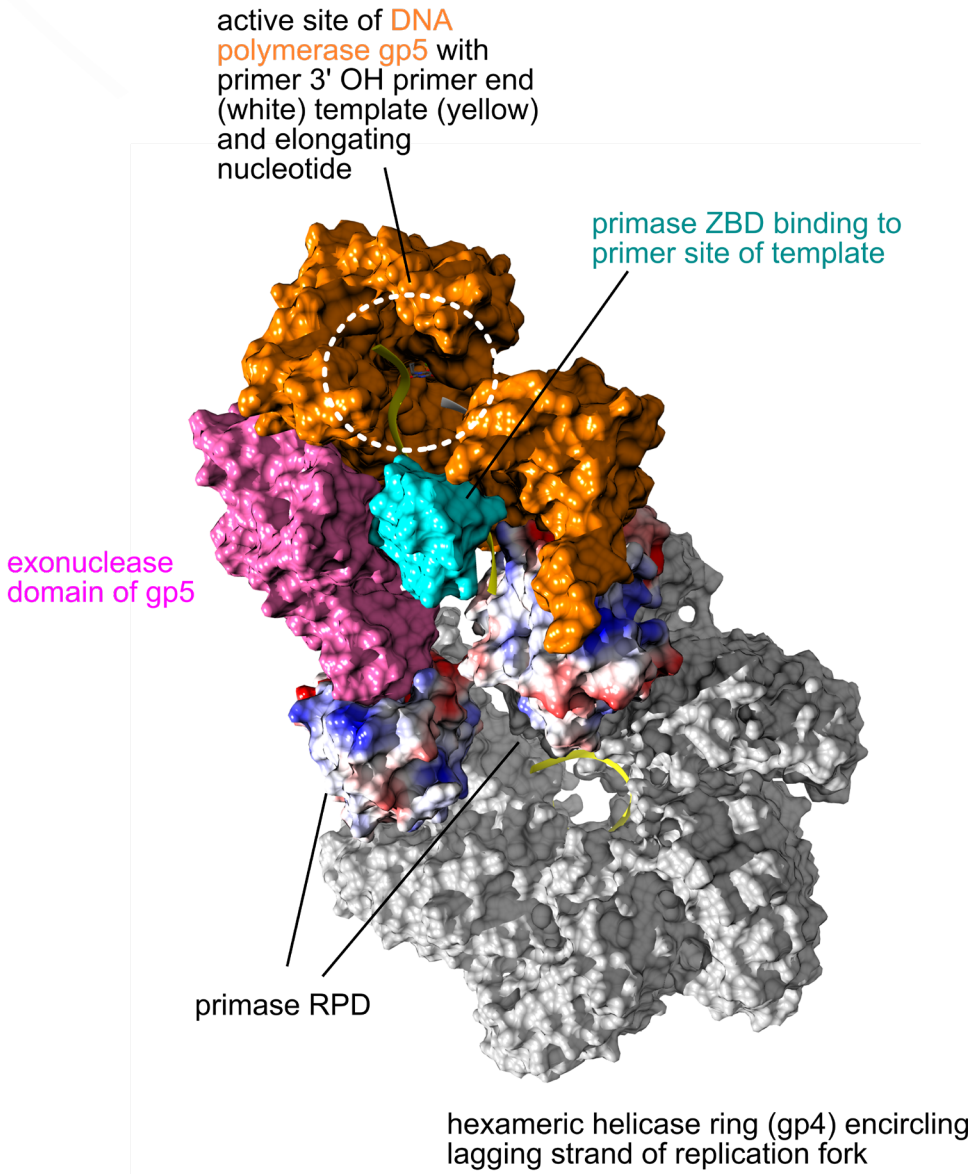


Figure 2: The lagging strand T7 replisome

Cryo-EM structure of the T7 replisome [15] bound to a short primer-template duplex mimicking the lagging strand of the replisome (white/yellow strands). The complex is composed of six copies of the helicase-primase gp4 and the DNA polymerase, representing a state following the hand-over of the primer from the primase active site to the DNA polymerase active site. Only two gp4 RPDs are visible in the structure; the remaining four RPDs and five ZBD of the gp4 hexamer as well as the complete template strand could not be resolved by the single-particle analysis.

The archaeoeukaryotic Primase PriSL

Archaea and Eukaryotes share the basic architecture of the replisome whose main components are a hexameric replicative helicase (MCM, Minichromosome maitenance), a primase (PriSL, primase small and large subunit) together with replicative DNA polymerases

and the sliding clamp (PCNA, proliferating cell nuclear antigen). The replicative helicase of archaeoeukaryotes has reversed directionality compared to the bacterial helicase, encircling the leading strand of the replication fork as it moves in the 3' – 5' direction. Accordingly, the archaeoeukaryotic primase is not associated with the helicase.

The DNA replication machinery in archaea is much less complex than in eukaryotes and therefore more amenable to detailed functional and structural studies, as exemplified by several key structures first determined in archaea [19,20]. The archaeoeukaryotic primase is a heterodimer composed of a small catalytic subunit (PriS) and a large accessory subunit (PriL). While it is long known that PriS carries the active site and is responsible for the chemical steps of nucleotide condensation, the contribution of PriL towards primer synthesis remained elusive until recent studies implicated it in initiation and termination of primer synthesis [21].

Whereas bacterial primases display sequence specificity, archaeoeukaryotic primases are thought to initiate primers regardless of the template sequence. There are exceptions, exemplified by an archaeal plasmid-encoded primase which strictly requires the trinucleotide 5'-GTG in the template before starting priming [22]. We recently performed structural work on this unusual archaeoeukaryotic primase using solution-state and solid-state NMR in order to functionally understand this sequence specificity [23]. This demonstrated that in the absence of ATP, the GTG-containing DNA template is bound unspecifically by a small helix bundle domain (HBD) of the primase independent of the catalytic domain (Figure 3). In the presence of ATP sequence-specific recognition of the trinucleotide GTG is achieved by the synergistic binding of the template and two molecules of ATP to the HBD [23]. Such sequence-specificity enhancement of a DNA binding protein triggered by ATP or other ligands is unprecedented. Furthermore, the structures also suggest that the two nucleotides substrates required for the initial step of the primer synthesis (di-nucleotide formation) might bind in the same nucleotide pockets. In this context, the HBD of the archaeoeukaryotic primases "prepares" priming by assembling the DNA templates and the two substrates in one complex [23]. The evolutionary conservation of this small HBD in archaeoeukaryotic primases (see Figure 4A) raises the question whether other archaeoeukaryotic primases really bind DNA template without sequence specificity

In most archaea, the active primase is probably the heterodimer PriSL. PriX, an additional subunit that was identified in *Sulfolobus solfataricus*, greatly stimulates the primase activity with both dNTPs and rNTPs, suggesting that the trimeric primase PriSLX may synthesize a mixed RNA/DNA primer [24]. Of note, homologs of PriX are present in other archaeal genomes, either as single proteins or fusion proteins with PriS.

In eukaryotes, the heterodimer PriS-PriL (in *Homo sapiens* commonly named p49-p58) forms associates with DNA polymerase α (Polα, ~180 kDa) and its accessory subunit p70 (~70 kDa) to form the primosome. Atomic resolution structures of the entire *Homo sapiens* primosome

[25] as well as primosome subunits bound to various substrates [26] have been resolved (Figure 3), revealing the conformations relevant to the reaction mechanism.

Similar to the bacterial primosome, the catalytic cycle of human primase demands a number of conformational changes for primer initiation, elongation, termination, and handover to Polα. The human primosome consists of a rather stable platform of four domains, namely PriS, the N-terminal domain (NTD) of PriL, the C-terminal domain (CTD) of Polα and the CTD of the p70 accessory domain of Polα (Figure 3). Flexibly tethered to this platform are the CTD of PriL and the core of Polα as well as the NTD of p70 [25]. The flexible arrangement of these domains permits PriL-CTD to approach the active site of PriS. In this way, PriL-CTD is able to supply the initiating nucleotide and the template to the active site to initiate primer synthesis. The structure of the PriL-CTD bound to a primer-template duplex (pdb: 5F0Q) [25] , together with structures of the Sulfolobus PriSLX (pdb: 5OF3) [27] and plasmid-encoded primase pRN1 from *Sulfolobus islandicus* (pdb: 6GVT) [23] suggest that the substrates are not only initially delivered to the active site for dinucleotide synthesis but also that the 5' triphosphorylated primer end (derived from the initiating nucleotide) remains bound to PriL-CTD during primer elongation. Accommodation of the growing primer, which forms a helical RNA-DNA heteroduplex with the template requires a rotational and translational movement between the active site of polymerization PriS (3' primer end) and PriL-CTD (5' primer end). Modeling this movement based on the structure of the human primosome suggest that a steric clash prevents further primer extension once the observed primer unit length of nine nucleotides is reached [25]. A similar model of primer termination has been proposed for the *Sulfolobus solfataricus* primase [27].

For the human primosome, the next step is the transfer of the primer-template duplex from the PriS active site to the Polα active site. Polα then extends the RNA primer by a stretch of about 20 deoxynucleotides. Modeling suggests that the primer-template duplex remains bound to PriL-CTD and the 3' primer end is transferred by the PriL-CTD between both active sites [25]. Clearly, the conformational changes during primer synthesis require a highly dynamic arrangement of the primosome, which is likely to be supported by the linkers between the flexible domains and the platform (Figure 3). As flexibility has hindered its crystallographic analysis, cryo-EM may be a more promising approach to define the conformational changes occurring during the primer formation. Complementary to this approach, molecular details of the enzyme-substrate interactions could be revealed by NMR, as demonstrated for pRN1 primase [23]. Further investigations are also needed to clarify the contribution of the 4Fe-4S cluster found in the PriL-CTD of eukaryotic primases and in many of the corresponding archaeal proteins. A switch of the iron oxidation state by charge transport along the DNA has been proposed to modulate the affinity of the PriL-CTD towards DNA [28,29]. Under reducing conditions, the affinity towards DNA is lowered, triggering primer handoff to Polα. However, this hypothesis has recently been challenged [30], warranting further investigation.

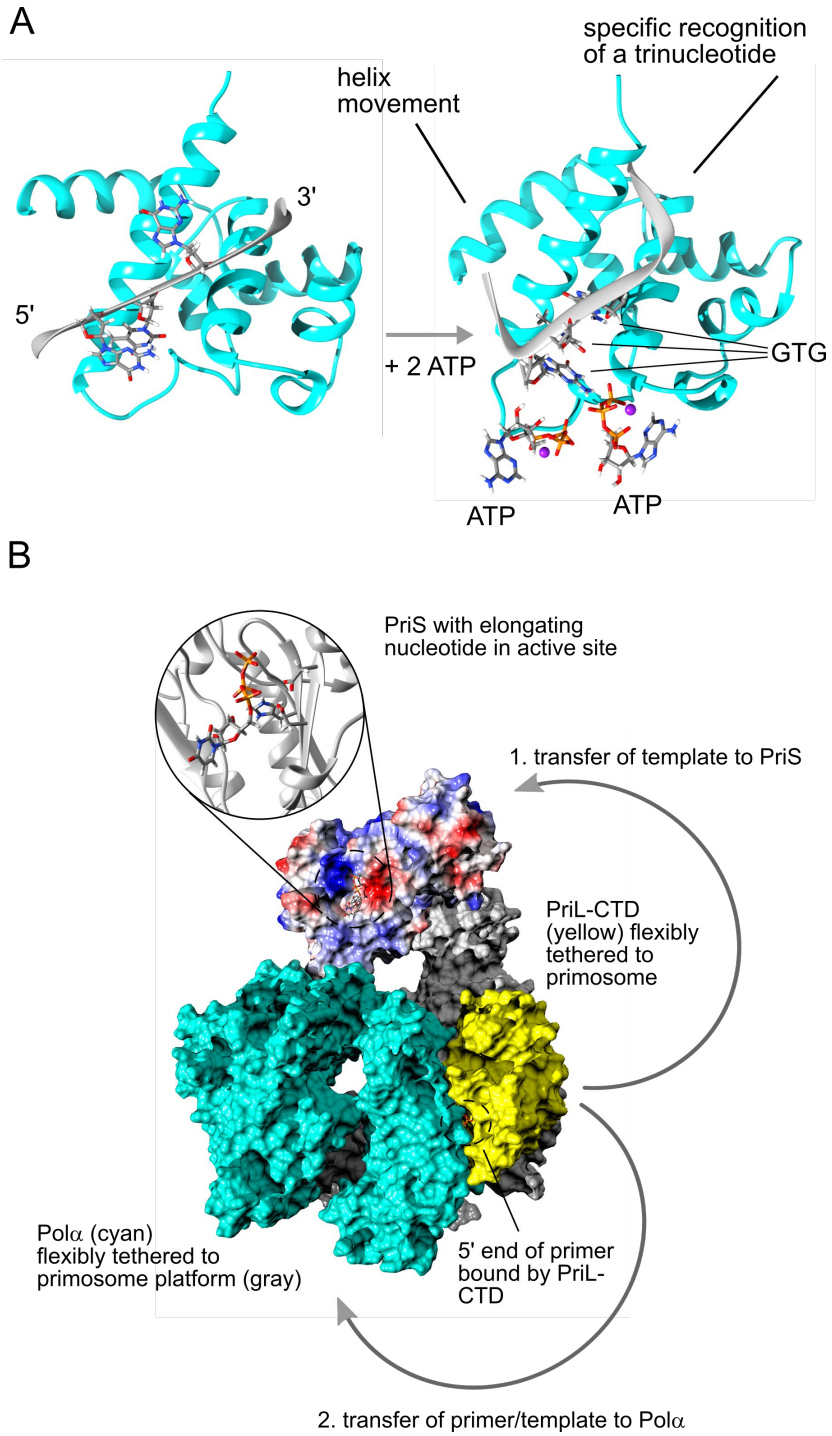


Figure 3: Conformational changes of the helical interface of the pRN1 primase upon nucleotide binding and of the human primosome during primer synthesis.

A: The accessory domain of the pRN1 primase is able to bind template DNA and two nucleotides (pdb: 6GVT [23]). Without nucleotides, the template DNA is bound in a non-sequence-specific manner (top). However, in the presence of ATP the nucleoprotein complex undergoes a major conformational change with the trinucleotide motif GTG (ball and stick representation) inserting in a grove (bottom). B: The human primosome (pdb: 5EXR [25], apoenzyme) is a heterotetramer composed of seven structural domains.[25]). Three domains

(gray) and PriS (colored according to surface charge) form a stable platform. Polα (cyan) and PriL-CTD (yellow) are flexibly tethered to the platform, allowing the PriL-CTD to transfer the template to the active site of PriS and the template/primer to Polα active site (not visible in the depicted orientation). The position of the elongating nucleotide (stick representation) in the active site of PriS and the position of triphosphorylated 5' end of the primer (sticks representation) bound to the interface of PriL-CTD and Polα have been modeled based on partial structures of the primosome (pdbs: 4BPW [26] & 5F0Q [25]). The inset shows the position of the elongating nucleotide in the active site of PriS relative to the two catalytically important aspartate residues as well as the catalytic histidine residue (pdb: 4BPW [26]).

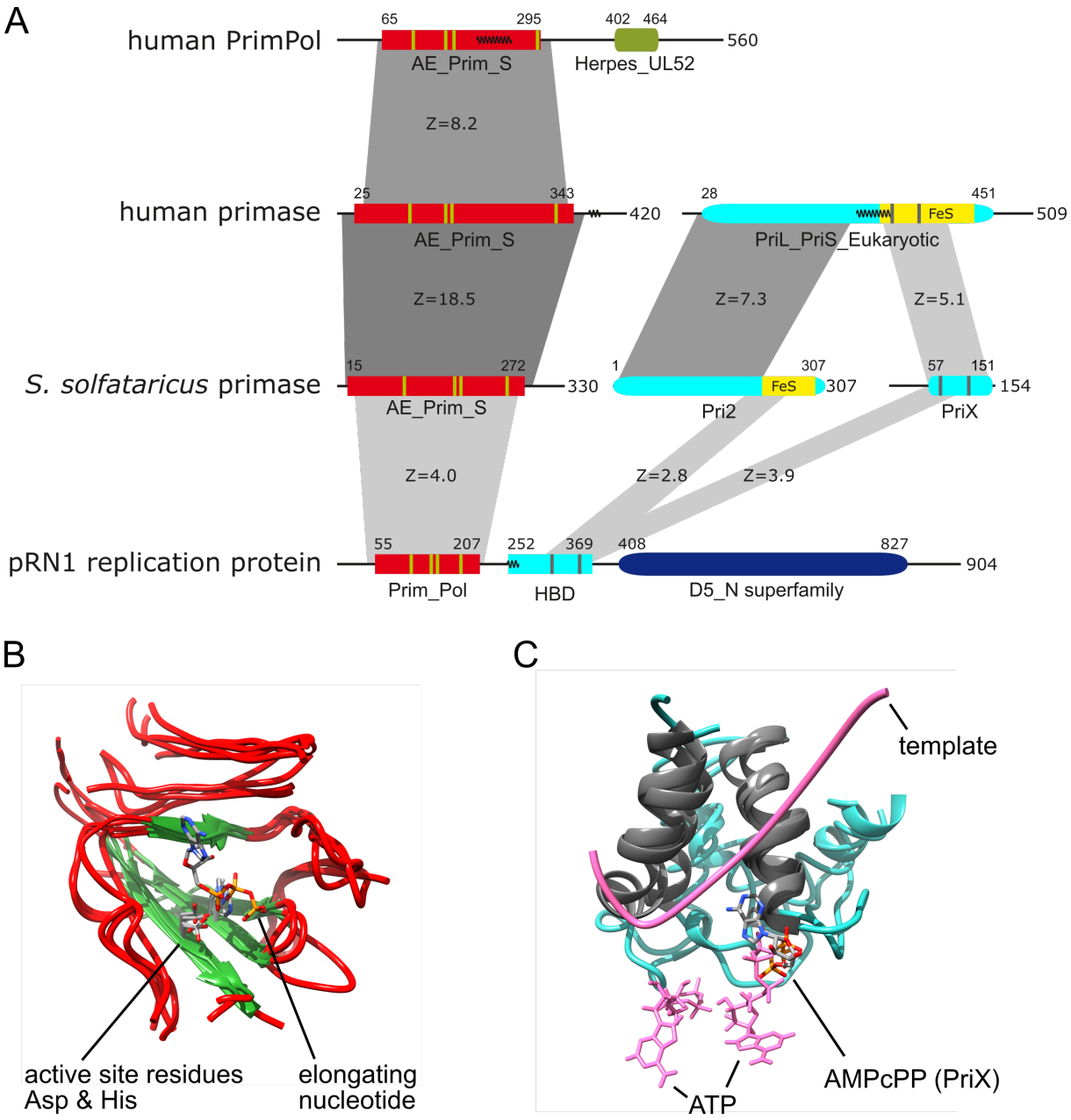


Figure 4: Comparison of the domain architectures of archaeoeukaryotic primases

A: Archaeoeukaryotic primases harbour a structurally conserved catalytic domain. The domain AE_Prim_S is found in human PrimPol (partial structures pdb 5L2X & 5N8A [31,32]), human primase (pdb: 5EXR [25]) and *Sulfolobus solfataricus* primase (pdb: 5OF3 [33]). In contrast the related domain Prim_Pol is present in the pRN1 replication protein (partial structure of the primase part, pdb: 3M1M [34]). Grey trapezoids indicate extent of structural similarity; the Z-value refers to the quality of the structural alignment with DALI. Wavy lines indicate unordered or flexible parts of the proteins. B: Structural superposition of the catalytic core of these primases. Four conserved strands of the RRM fold are highlighted in green and their positions in the domain are indicated as green bars in panel A. Active site histidine and aspartate residues are shown in ball and stick representation together with the elongating nucleotide (based on the human PrimPol structure). C: The cellular primases from eukaryotes and archaea and the pRN1 replication protein have a helical interface, that likely prepares primer synthesis by binding template DNA and initiating nucleotide. Shown here is the superposition of these helical interfaces from the primase accessory domains. Highlighted in gray are the two helices of the binding interface for template and nucleotides. Template and two nucleotides

bound via their triphosphate moiety to the pRN1 primase are shown in magenta and the non-hydrolyzable nucleotide cocrystallizing with PriX is coloured according to element. The positions of the two structurally conserved helices are also indicated as gray bars in the domain architecture.

PrimPol

Increasing understanding of the archaeoeukayotic primases (AEPs) reveals that homologues of these contribute to functions in nucleic acid metabolism beyond their classical role in priming the template strand for replication. An example of these functions are AEP related domains of bacterial LigD involved in non-homologous end joining (NHEJ) [35,36]. In 2013, a second eukaryotic primase in addition to the PriSL enzyme was discovered in *H. sapiens* and termed PrimPol as it possesses both polymerase and primase activity [37,38]. The PrimPol gene is present in all vertebrates but orthologues were also detected in worms and arthropods and even trypanosomatids [39]. Reflecting the functional diversity of AEPs, PrimPol plays a role in DNA damage tolerance rather than initiation of DNA replication. Due to its ability to carry out translesion synthesis and to bypass template lesions such as thymine dimers, PrimPol is important for re-priming and resolution of stalled replication forks [31,40,41]. Physiologically, PrimPol resolves stalled replication forks that occur if DNA lesions such as UV-induced cyclobutane thymine dimers and (6-4) thymine dimers obstruct the replisome function [42]. Unlike the PriSL primases, human PrimPol preferentially incorporates dNTPs. The enzyme comprises the N-terminal catalytic domain conserved with other AEPs and a C-terminal Herpes_UL52 domain which harbours a C-terminal CHC2 zinc finger motif (Figure 4A). A partial structure of the human enzyme bound to a primer-template duplex and an incoming dATP has been solved [31]. The structure reveals that priming and elongation in PrimPol follow the established catalytic mechanism involving two divalent cations [43]. Notably, the structure shows very few contacts between the protein and the primer, which may explain why de-novo synthesis can occur [31] but raises additional questions as to how denaturing of the nascent primer-template is prevented. The C-terminal zinc finger module has single-stranded DNA binding activity [44] and is required for the primase activity of PrimPol, but not for its polymerase activity, suggesting that the zinc finger may bind template and/or the 5'-terminus of the primer, analogous to the HBD (Figure 4C) in PriL and the pRN1 primase [31].

The minimal functional core of primases

Although bacterial and archaeoeukaryotic primase adopt different structural folds to shape the primase active site, they both appear to follow a similar reaction mechanism as described for DNA polymerase [45]. Appropriately positioned acidic residues (see insets of Figure 1B and Figure 3B) bind two divalent cations that activate the 3’-OH group of the primer end and position the elongating nucleotide.

The unique and critical step of primer synthesis is the initial formation of a dinucleotide intermediate from the initiating and elongating nucleotides. To accomplish this reaction, the primase has to bind and position three substrates simultaneously: the template and both nucleotides. The core machinery of primer synthesis appears to be established by two relatively compact subdomains providing on the one hand the active site and on the other hand a substrate binding platform. In bacterial cellular primases, the N-terminal ZBD contributes to template binding but the elongating nucleotide and the primer 3' OH are bound by the active site cleft of the RPD [12,14]. Likewise, the archaeoeukaryotic primase catalytic subunit (Figure 4B) binds the primer-template duplex and the elongating nucleotide [31,33,46]. Moreover a helical interface (HBD, Figure 4C) provided by a distinct subunit (or a flexibly tethered accessory domain) assists in dinucleotide formation by providing binding sites for the template and the initiating nucleotide [23,25,33,47], and potentially also for the first elongating nucleotide [23].

Clearly, further structural investigations are required to elucidate how dinucleotide formation occurs in bacterial and archaeoeukaryotic primases. Primases critically contribute to cellular DNA replication and their regulation and integration in this highly dynamic multiprotein machinery is mediated by additional domains. As structural knowledge of the conformational rearrangements within these protein-DNA complexes grows, a more detailed molecular picture of the process of DNA replication initiation will emerge.

Acknowledgements

Our research in replication is funded by the Swiss National Science Foundation (SNF grant numbers: 310030_163345 and 310030_141160).

Conflict of Interest

The authors declare no conflicting interest.

References

1. Masai H: *DNA replication: from old principles to new discoveries*. Springer Berlin Heidelberg; 2017.

2. Sheaff RJ, Kuchta RD: **Misincorporation of nucleotides by calf thymus DNA primase and elongation of primers containing multiple noncognate nucleotides by DNA polymerase alpha.** *J Biol Chem* 1994, **269**:19225–19231.

3. Kornberg A: *DNA replication*. Freeman; 1980.

4. Litvak S, Sarih-Cottin L, Fournier M, Andreola M, Tarrago-Litvak L: **Priming of HIV replication by tRNA(Lys3): role of reverse transcriptase**. *Trends Biochem Sci* 1994, **19**:114–118.

5. Yao NY, O'Donnell ME: **Evolution of replication machines**. *Critical Reviews in Biochemistry and Molecular Biology* 2016, **51**:135–149.

6. Podobnik M, McInerney P, O'Donnell M, Kuriyan J: **A TOPRIM domain in the crystal structure of the catalytic core of Escherichia coli primase confirms a structural link to DNA topoisomerases**. *JMolBiol* 2000, **300**:353–362.

7. Iyer LM, Koonin EV, Leipe DD, Aravind L: **Origin and evolution of the archaeo-eukaryotic primase superfamily and related palm-domain proteins: structural insights and new members**. *Nucleic Acids Res* 2005, **33**:3875–3896.

8. Leipe DD, Aravind L, Koonin EV: **Did DNA replication evolve twice independently?** *Nucleic Acids Res* 1999, **27**:3389–3401.

9. Lewis JS, Jergic S, Dixon NE: **Chapter Two - The E. coli DNA Replication Fork**. In *The Enzymes*. Edited by Kaguni LS, Oliveira MT. Academic Press; 2016:31–88.

10. Johnson SK, Bhattacharyya S, Griep MA: **DnaB Helicase Stimulates Primer Synthesis Activity on Short Oligonucleotide Templates**. *Biochemistry* 2000, **39**:736–744.

11. Corn JE, Pease PJ, Hura GL, Berger JM: **Crosstalk between Primase Subunits Can Act to Regulate Primer Synthesis in trans**. *Molecular Cell* 2005, **20**:391–401.

12. Rymer RU, Solorio FA, Tehranchi AK, Chu C, Corn JE, Keck JL, Wang JD, Berger JM: **Binding mechanism of metalNTP substrates and stringent-response alarmones to bacterial DnaG-type primases**. *Structure* 2012, **20**:1478–1489.

13. Zhou Y, Luo H, Liu Z, Yang M, Pang X, Sun F, Wang G: **Structural Insight into the Specific DNA Template Binding to DnaG primase in Bacteria**. *Scientific Reports* 2017, **7**.

14. Hou C, Biswas T, Tsodikov OV: **Structures of the Catalytic Domain of Bacterial Primase DnaG in Complexes with DNA Provide Insight into Key Priming Events**. *Biochemistry* 2018, **57**:2084–2093.

15. Gao Y, Cui Y, Fox T, Lin S, Wang H, Val N de, Zhou ZH, Yang W: **Structures and operating principles of the replisome**. *Science* 2019, doi:10.1126/science.aav7003.

16. Bailey S, Eliason WK, Steitz TA: **Structure of Hexameric DnaB Helicase and Its Complex with a Domain of DnaG Primase**. *Science* 2007, **318**:459–463.

17. Corn JE, Pelton JG, Berger JM: **Identification of a DNA primase template tracking site redefines the geometry of primer synthesis**. *Nature Structural & Molecular Biology* 2008, **15**:163–169.

18. Oakley AJ, Loscha KV, Schaeffer PM, Liepinsh E, Pintacuda G, Wilce MCJ, Otting G, Dixon NE: **Crystal and Solution Structures of the Helicase-binding Domain of Escherichia coli Primase o**. *J Biol Chem* 2005, **280**:11495–11504.

19. Augustin MA, Huber R, Kaiser JT: **Crystal structure of a DNA-dependent RNA polymerase (DNA primase)**. *Nat Struct Biol* 2001, **8**:57–61.

20. Fletcher RJ, Bishop BE, Leon RP, Sclafani RA, Ogata CM, Chen XS: **The structure and function of MCM from archaeal M. Thermoautotrophicum**. *Nat Struct Biol* 2003, **10**:160–167.

21. Baranovskiy AG, Tahirov TH: **Elaborated Action of the Human Primosome**. *Genes* 2017, **8**:62.

22. Beck K, Lipps G: **Properties of an unusual DNA primase from an archaeal plasmid**. *Nucleic Acids Res* 2007, **35**:5635–5645.

23. Boudet J, Devillier J-C, Wiegand T, Salmon L, Meier BH, Lipps G, Allain FH-T: **A Small Helical Bundle Prepares Primer Synthesis by Binding Two Nucleotides that Enhance Sequence-Specific Recognition of the DNA Template**. *Cell* 2019, **176**:154-166.e13.

24. Liu B, Ouyang S, Makarova KS, Xia Q, Zhu Y, Li Z, Guo L, Koonin EV, Liu ZJ, Huang L: **A primase subunit essential for efficient primer synthesis by an archaeal eukaryotic-type primase**. *NatCommun* 2015, **6**:7300-.

25. Baranovskiy AG, Babayeva ND, Zhang Y, Gu J, Suwa Y, Pavlov YI, Tahirov TH: **Mechanism of Concerted RNA-DNA Primer Synthesis by the Human Primosome**. *J Biol Chem* 2016, **291**:10006–10020.

26. Kilkenny ML, Longo MA, Perera RL, Pellegrini L: **Structures of human primase reveal design of nucleotide elongation site and mode of Pol α tethering**. *Proc Natl Acad Sci USA* 2013, **110**:15961–15966.

27. Yan J, Holzer S, Pellegrini L, Bell SD: **An archaeal primase functions as a nanoscale caliper to define primer length**. *Proc Natl Acad Sci U S A* 2018, **115**:6697–6702.

28. O'Brien E, Holt ME, Thompson MK, Salay LE, Ehlinger AC, Chazin WJ, Barton JK: **The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport**. *Science* 2017, **355**:eaag1789.

29. O'Brien E, Salay LE, Epum EA, Friedman KL, Chazin WJ, Barton JK: **Yeast require redox switching in DNA primase**. *PNAS* 2018, doi:10.1073/pnas.1810715115.

30. Pellegrini L: **Comment on "The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport."** *Science* 2017, **357**:eaan2954.

31. Rechkoblit O, Gupta YK, Malik R, Rajashankar KR, Johnson RE, Prakash L, Prakash S, Aggarwal AK: **Structure and mechanism of human PrimPol, a DNA polymerase with primase activity**. *Science Advances* 2016, **2**:e1601317.

32. Guilliam TA, Brissett NC, Ehlinger A, Keen BA, Kolesar P, Taylor EM, Bailey LJ, Lindsay HD, Chazin WJ, Doherty AJ: **Molecular basis for PrimPol recruitment to replication forks by RPA**. *Nature Communications* 2017, **8**:15222.

33. Holzer S, Yan J, Kilkenny ML, Bell SD, Pellegrini L: **Primer synthesis by a eukaryotic-like archaeal primase is independent of its Fe-S cluster**. *Nature Communications* 2017, **8**.

34. Beck K, Vannini A, Cramer P, Lipps G: **The archaeo-eukaryotic primase of plasmid pRN1 requires a helix bundle domain for faithful primer synthesis**. *Nucleic Acids Res* 2010, **38**:6707–6718.

35. Weller GR, Doherty AJ: **A family of DNA repair ligases in bacteria?** *FEBS Letters* 2001, **505**:340–342.

36. Koonin EV, Wolf YI, Kondrashov AS, Aravind L: **Bacterial homologs of the small subunit of eukaryotic DNA primase**. *J Mol Microbiol Biotechnol* 2000, **2**:509–512.

37. Wan L, Lou J, Xia Y, Su B, Liu T, Cui J, Sun Y, Lou H, Huang J: **hPrimpol1/CCDC111 is a human DNA primase-polymerase required for the maintenance of genome integrity**. *EMBO Rep* 2013, **14**:1104–1112.

38. Garcia-Gomez S, Reyes A, Martinez-Jimenez MI, Chocron ES, Mouron S, Terrados G, Powell C, Salido E, Mendez J, Holt IJ, et al.: **PrimPol, an Archaic Primase/Polymerase Operating in Human Cells**. *MolCell* 2013, **52**:541–553.

39. Rudd SG, Glover L, Jozwiakowski SK, Horn D, Doherty AJ: **PPL2 Translesion Polymerase Is Essential for the Completion of Chromosomal DNA Replication in the African Trypanosome**. *Mol Cell* 2013, **52**:554–565.

40. Helleday T: **PrimPol breaks replication barriers**. *Nature Structural & Molecular Biology* 2013, **20**:1348–1350.

41. Mouron S, Rodriguez-Acebes S, Martinez-Jimenez MI, Garcia-Gomez S, Chocron S, Blanco L, Mendez J: **Repriming of DNA synthesis at stalled replication forks by human PrimPol**. *NatStructMolBiol* 2013, **20**:1383–1389.

42. Bianchi J, Rudd SG, Jozwiakowski SK, Bailey LJ, Soura V, Taylor E, Stevanovic I, Green AJ, Stracker TH, Lindsay HD, et al.: **PrimPol Bypasses UV Photoproducts during Eukaryotic Chromosomal DNA Replication**. *Molecular Cell* 2013, **52**:566–573.

43. Yang W, Lee JY, Nowotny M: **Making and Breaking Nucleic Acids: Two-Mg2+-Ion Catalysis and Substrate Specificity**. *Molecular Cell* 2006, **22**:5–13.

44. Keen BA, Jozwiakowski SK, Bailey LJ, Bianchi J, Doherty AJ: **Molecular dissection of the domain architecture and catalytic activities of human PrimPol**. *Nucleic Acids Res* 2014, **42**:5830–5845.

45. Steitz TA, Smerdon SJ, Jager J, Joyce CM: **A unified polymerase mechanism for nonhomologous DNA and RNA polymerases**. *Science* 1994, **266**:2022–2025.

46. Kilkenny ML, Longo MA, Perera RL, Pellegrini L: **Structures of human primase reveal design of nucleotide elongation site and mode of Pol alpha tethering**. *ProcNatlAcadSciUSA* 2013, **110**:15961–15966.

47. Bell SD: **Initiating DNA replication: a matter of prime importance**. *Biochemical Society Transactions* 2019, doi:10.1042/BST20180627.

References to be highlighted

Of the references cited in our review "Structural Biology of Primase" the following are of outstanding (**) or special (*) interest to researchers in the field, representing in our view significant milestones in the mechanistic and structural understanding of these enzymes.

"Outstanding Interest" (**)

Gao Y, Cui Y, Fox T, Lin S, Wang H, Val N de, Zhou ZH, Yang W: **Structures and operating principles of the replisome**. *Science* 2019, doi:10.1126/science.aav7003.
Cryo EM structures of the complete bacteriophage T7 replisome including the helicase-primase on the lacking strand

Boudet J, Devillier J-C, Wiegand T, Salmon L, Meier BH, Lipps G, Allain FH-T: **A Small Helical Bundle Prepares Primer Synthesis by Binding Two Nucleotides that Enhance Sequence-Specific Recognition of the DNA Template**. *Cell* 2019, **176**:154-166.e13.
NMR structures of template and nucleotide interacting with the C-terminal domain of the pRN1 primase

Baranovskiy AG, Babayeva ND, Zhang Y, Gu J, Suwa Y, Pavlov YI, Tahirov TH: **Mechanism of Concerted RNA-DNA Primer Synthesis by the Human Primosome**. *J Biol Chem* 2016, **291**:10006–10020.
Structure of the human primosome assembly and mechanistic explanation of primer length determination and primer/template hand-over to Polα

Rechkoblit O, Gupta YK, Malik R, Rajashankar KR, Johnson RE, Prakash L, Prakash S, Aggarwal AK: **Structure and mechanism of human PrimPol, a DNA polymerase with primase activity**. *Science Advances* 2016, **2**:e1601317.
Crystal structure of human PrimPol with substrate primer-template and incoming dNTP.

Holzer S, Yan J, Kilkenny ML, Bell SD, Pellegrini L: **Primer synthesis by a eukaryotic-like archaeal primase is independent of its Fe-S cluster**. *Nature Communications* 2017, **8**.
Crystal structure of the heterotrimeric primase PriSLX from S. solfataricus, role of PriX in archaeal primase

"Special Interest" (*)

Hou C, Biswas T, Tsodikov OV: **Structures of the Catalytic Domain of Bacterial Primase DnaG in Complexes with DNA Provide Insight into Key Priming Events**. *Biochemistry* 2018, **57**:2084–2093.
First crystal structures of noncovalent complex of a DnaG type primase with template DNA

O'Brien E, Holt ME, Thompson MK, Salay LE, Ehlinger AC, Chazin WJ, Barton JK: **The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport**. *Science* 2017, **355**:eaag1789.
Controversial concept of primase iron-sulfur cluster acting as a redox switch.

Baranovskiy AG, Zhang Y, Suwa Y, Gu J, Babayeva ND, Pavlov YI, Tahirov TH: **Insight into the Human DNA Primase Interaction with Template-Primer**. *J Biol Chem* 2016, **291**:4793–4802.
Crystal structures of PriL-CTD liganded to a primer-template.

Garcia-Gomez S, Reyes A, Martinez-Jimenez MI, Chocron ES, Mouron S, Terrados G, Powell C, Salido E, Mendez J, Holt IJ, et al.: **PrimPol, an Archaic Primase/Polymerase Operating in Human Cells**. *MolCell* 2013, **52**:541–553.
Discovery of the second AEP type primase in H. sapiens.

Yan J, Holzer S, Pellegrini L, Bell SD: **An archaeal primase functions as a nanoscale caliper to define primer length**. *Proc Natl Acad Sci U S A* 2018, **115**:6697–6702.
Mechanistic concept of distance between PriX and PriL binding sites as basis for primer length determination.