PRIMASES: A POTENTIAL DRUG TARGET A BRIEF SUMMARY

Abstract

DNA primases are more than just RNA primer makers for DNA replication. Primase has been classified into two types, one in DnaG and the other one isthe archaeo-eukaryotic primase (AEP) superfamily, which has many functions in different domains of life. AEPs can act as primase-polymerases, which can synthesize both RNA and DNA, and participate in various DNA processes, such as damage tolerance and repair. AEPs are diverse and multifunctional enzymes that deserve a new classification and more attention. Here in this book chapter, a brief discussion about both families with an example of drug targets.

Keywords: RNA, DNA, DNAG.

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I. PRIMASES

Primase functions as a single-stranded DNA-dependent RNA polymerase responsible for generating RNA primers in the process of DNA replication. Like other DNA and RNA polymerases, primase exhibits structural and functional characteristics that play a role in the extension of polymers(Griep, 1995).It facilitates the creation of a brief RNA segment known as a primer, which aligns with a single-stranded DNA template. Following this elongation, a 5' to 3' exonuclease eliminates the RNA fragment, subsequently replenished with DNA(Bocquier *et al.*, 2001).

II. DNAG

The majority of bacteria contain the DnaG primase enzyme, which functions as a monomer unit in the priming process. DnaG interacts with DnaB (helicase), the single-stranded DNA binding protein (SSB), and DNA polymerase-III holoenzyme. While DnaG has the capability to synthesize primers up to sixty nucleotides long in vitro, during the initial stages of replication, it is limited to eleven nucleotides(Griep, 1995). In the *E. coli* genome, the DnaG-protein transcribes around 2000 to 3000 RNA primers at a rate of approximately one nucleotide per second in the lagging strand nucleotide synthesis(Keck *et al.*, 2000).Homologs of DnaG have been identified in both prokaryotes and many bacteriophages. These proteins exhibit structural differences from the primase responsible for replication in archaeal and eukaryotic systems, as deduced from sequence alignment analysis.

The proteolysis of DnaG has revealed the presence of three distinct structural domains: a 12-kD NH2-terminal Zn2+ binding domain (ZBD), a central polymerase area weighing 36 kD, and a 15-kD COOH-terminal domain responsible for interacting with DnaB (DnaB-ID). The core fragment of the recombinant DnaG, overexpressed and containing residues 111 to 433 (DnaG-RNAP), is capable of in vitro transcription of RNA, albeit with reduced RNA polymerase activity. However, its in vivo replication function is not anticipated due to the absence of both the DnaB-ID and ZBD domains(Keck *et al.*, 2000).

III. DNAG AS A DRUG TARGET

DnaG presents a compelling target for drug intervention, aiming to impede the replication process in various bacterial organisms, potentially culminating in organism fatality. Below are a few examples, accompanied by ongoing efforts in the continual development of novel drug compounds.

1. **NTP Analogs:** Several chemical compounds bear resemblance to NTP and actively participate in RNA priming, thereby impeding the function of DnaG. Notable examples include AraATP (Vidarabine), which serves as both a substrate and an inhibitor.

   For instance, 2′,3′-dideoxynucleoside 5′-triphosphates (ddNTPs) are employed as substrates by E. coli DnaG. Upon incorporation into the priming chain, they halt elongation due to the absence of a 3' hydroxyl group, necessary for the formation of phosphodiester bonds with adjacent nucleotides. Another compound of significance is 2-fluoro-AraATP, containing a modified sugar. This compound exhibits inhibitory effects on both eukaryotic cells and herpes viruses(Ilic *et al.*, 2018).

2. **Non-NTP Analog Inhibition:** Numerous chemical compounds have been identified as inhibitors of DnaG. Most of these inhibitors are discovered using high throughput screening (HTS) methods.

   For example, Inhibition of *M. tuberculosis* DnaG was achieved via High Throughput Screening (HTS) utilizing a DnaG inhibition assay. These compounds include doxorubicin, suramin, and ellagic acid. However, the mechanism of action for doxorubicin and suramin likely involves inhibition of nucleotide triphosphate binding, achieved by interacting with multiple sites on DnaG. This interaction is facilitated by the presence of aromatic rings along with polar functional groups in these compounds. Interestingly, suramin also exhibits inhibition of Eukaryotic Primase by competing with GTP, likely employing a similar mechanism as seen with DnaG(Ilic *et al.*, 2018). Apart from this, natural compound can also inhibit the bacterial primase enzyme effectively such as Sch642305(Chu *et al.*, 2003).

IV. ARCHAEO-EUKARYOTIC PRIMASE (AEP)

Archaeo-eukaryotic primases (AEPs) engaged in replication typically establish a heterodimeric complex, featuring a compact catalytic subunit (PriS / Prim1) and an extensive complement of accessory components (PriL / Prim2). In the context of eukaryotes, this heterodimer interfaces with DNA Pol subunits (PolA1 and PolA2), working in concert to initiate DNA replication(Frick & Richardson, 2001). The hallmark of the AEP superfamily resides in its distinct catalytic core, comprising two modules: an N-terminal (αβ)2 unit, unparalleled in structural equivalence to other proteins in the Structural Database (PDB), and a C-terminal unit harboring a notably derived RNA Recognition Motif (RRM), reminiscent of A-, B-, and Y-family DNA polymerases. Within this catalytic core, three motifs are conserved (motifs I, II, and III), encompassing a hhDhD/E pattern (where 'h' denotes a hydrophobic residue), an sxH pattern (where 's' signifies a small residue and 'x' represents any residue), and an hD/E pattern(Iyer *et al.*, 2005). Divalent metal ion coordination for catalytic activity occurs within the first and third motifs, while the sxH motif plays a role in genome binding(Lipps *et al.*, 2004). Multiple mutagenesis studies have underscored the indispensability of these features for catalysis. Additionally, certain AEPs encompass supplementary domains such as zinc-binding and helicase domains.

The highly conserved catalytic aspartate residues found in these enzymes are juxtaposed with the catalytic center of X-family DNA polymerases, such as Pol-β. This observation takes into account the distinct catalytic fold of AEPs(Frick & Richardson, 2001). Nonetheless, it is posited that this apparent similarity arises due to convergent evolution, given the differing secondary structural contexts surrounding these aspartate residues. This correlation, in conjunction with the catalytic necessity for divalent metal ions, leads to the inference of a catalytic system involving two metal ions, akin to the mechanism employed by DNA polymerases(Steitz *et al.*, 1994; Kirk & Kuchta, 1999).

V. EVOLUTION

The lack of resemblance in the AEP superfamily extends to other replication-associated domains such as DNA polymerase and helicase. Discrepancies between bacterial and eukaryotic replication mechanisms have sparked scientific discourse concerning the evolutionary origins of both enzyme groups. Interestingly, despite variations in replication

mechanisms between bacterial and AEP enzymes, core components of transcription and translation remain conserved(Leipe *et al.,* 1999). This foundational observation underscores the independent evolutionary paths taken by bacterial and AEP enzymes from their common ancestor, resulting in the emergence of reverse transcription and the subsequent evolution of RNA/DNA replication for genomic content(Sweetser *et al.,* 1987). These evolutionary trajectories led to the abandonment of reverse transcription, leading competent cells to adopt a more stable DNA replication mechanism.

Numerous lines of evidence substantiate the demonstration of reverse transcription activity by engineered enzymes, including primase and polymerase(Jozwiakowski *et al.,* 2015). An alternative model posits the presence of both AEP and TOPRIM primase enzymes in the last universal common ancestor (LUCA). As a result of evolutionary pressures, bacteria relinquished replicative primase akin to AEP, while archaea adopted TOPRIM as their replicative primase, presenting an intricate interplay of evolutionary forces shaping these enzyme systems(Hu *et al.,* 2012).

Conversely, numerous bacteria and archaea continue to retain the TOPRIM and AEP elements, respectively. However, their functions have evolved and now encompass roles in DNA repair. For instance, AEP takes part in Non-Homologous End Joining (NHEJ) within bacteria, while in archaea, TOPRIM assumes a pivotal function in RNA degradation(Della *et al.,* 2004). This conceptual framework also posits the eventual loss of DnaG in eukaryotes, with another protein assuming its primase role. Alternatively, these models suggest that the Last Universal Common Ancestor (LUCA) harbored either the AEP or TOPRIM primase family. Owing to heightened selective pressures, LUCA opted for one of these primase families while relinquishing the other, leading to the emergence of the second primase family in bacteria, or the AEP family(Iyer *et al.,* 2005). Under such circumstances, bacteria and viruses might have acquired AEPs subsequently through horizontal gene transfer, augmenting their capabilities in DNA replication, repair, and damage tolerance to fulfill alternative functions. These models present a plausible and substantiated scenario.

In conjunction with prokaryotic DnaG primases, the initiation of DNA replication in archaea and eukaryotes is indispensably reliant on Archaeo-eukaryotic primases (AEPs). Intriguingly, archaeal genomes have revealed the presence of DnaG-like primases, and AEPs have been identified across diverse life forms. Notably, despite the lack of homology in the primase superfamily between bacterial and archaeal/eukaryotic protein domains, other replicative proteins containing DNA polymerase and helicase functionalities also demonstrate a parallel evolutionary trajectory with DnaG TOPRIM primases.

An *insilico* investigation by Iyer *et al.* extensively focused on the AEP superfamily, revealing that the closest relatives of the AEP-fold family are the endonucleases associated with rolling-circle replication (RCRE) and the origin-binding domain proteins (OBDs) found in papillomaviruses. The pronounced evolutionary connection between AEPs and RCRE underscores their relationship with the topoisomerases of DnaG TOPRIM primases. Significantly, this linkage highlights that the two primase superfamilies share evolutionary ties with nucleases, providing an alternative solution to the complexities of DNA replication(Iyer *et al.,* 2005).

In a specific context, the transfer of a 5' end of a nicked DNA strand to a tyrosine residue within the nuclease allows DNA polymerase to extend the free 3' OH group essential

for synthesizing a new strand. This mechanism is harnessed in rolling circle replication observed in various DNA viruses and phages. Iyer et al. suggest that RCRE and OBD share a common ancestor with polymerase-active AEPs. Subsequently, RCRE evolved from this ancestor by incorporating nuclease activity while omitting polymerase functionality. Nevertheless, it is postulated that the shared ancestor of AEP-RCRE-OBD originally served as a nucleic acid binding enzyme, employing divalent cations to coordinate its acid residues and facilitate DNA binding. This ancient protein might have later acquired nuclease capabilities, while various descendant lineages independently acquired polymerase activity. Currently, the AEP superfamily comprises 13 primary families, 12 of which can be further classified into three broader clades: AEP proper, NCLDV herpes, and PrimPol clade(Iyer *et al.*, 2005; Guilliam *et al.*, 2015).

VI. AEP AS A POLYMERASE

Beyond PriS, several archaeal organisms host additional Archaeo-eukaryotic primases (AEPs) that are encoded by extrachromosomal plasmids. It is postulated that these primases play a pivotal role in the initiation and replication of these plasmids. The archetype of this particular type of AEP is ORF904 derived from the *Sulfolobus islandicus* plasmid pRN1, measuring around 5 kb in size. ORF904 belongs to a newly characterized primase family termed the Prim-Pol family. This distinctive AEP periodically emerges in crenarchaeal and Gram-positive bacterial plasmids. In this family, the N-terminal region encompasses AEP functionality, while the C-terminal domain features a helicase/translocase domain. The AEP segment exhibits DNA-dependent RNA/DNA primase and DNA polymerase activities, while the DNA helicase domain undertakes DNA-dependent ATPase activity(Lipps *et al.*, 2003; Guilliam *et al.*, 2015).

ORF904 exhibits a pronounced affinity for synthesizing DNA primers, which can undergo substantial expansion by several kilobases in the presence of dNTPs. The crystallographic depiction of its Archaeo-eukaryotic primase (AEP) domain reveals a remarkable structural resemblance to the archaeal primase found in *Pyrococcus*. Notably, this resemblance becomes more evident when analyzing the spatial arrangement of amino acid residues responsible for metal interactions, which are securely ensconced within the confines of the beta-sheet region. It's noteworthy that both enzymes harbor zinc-binding motifs proximal to their respective catalytic centers. Interestingly, the nature of these motifs diverges considerably between the two enzymes(Beck & Lipps, 2007).

This finding significantly implies that the shared predecessor of the two enzymes lacked a zinc-binding domain. This domain's emergence appears to be the result of two distinct insertion events, each occurring during the evolution of their respective families. An intricately related protein, termed Rep, was identified within the context of the *Sulfolobus solfataricus* pIT3 plasmid. This protein, denoted as Rep, in conjunction with ORF904, features an AEP domain that is fused with a putative helicase. The Rep245 domain located at the N-terminus of this protein, which is associated with replication, exhibits both DNA polymerase activity and primer synthesis capabilities mediated by dNTP/rNTP substrates(Prato *et al.*, 2008).

The fascinating enzyme function of an AEP named PolpTN2, coded by the *Thermococcoccus nautilus* pTN2 plasmid, has been addressed recently. PolpTN2 seems to be a specific combination of a PriS-like N-terminal domain and a PriL-like C-terminal domain. The confirmation of this domain is opposite to other plasmid coded primases usually fused

into helicases. However, PolpTN2 shows primase and DNA polymerase activities, which are analogous to other archaeal plasmid-translated primases. PolpTN2 primase activity is restricted to the use of dNTPs only(Gill *et al.*, 2014).

Furthermore, the enzyme also exercises terminal transferase function, which is improved significantly by removing the PriL-like protein region.This deletion also gives the primase to possess reverse transcriptase activity. Of particular note is that there is a lack of zinc-binding motif in PolpTN2 and Rep(pIT3), i.e., present most other AEPs. The discovery that each AEP family has its zinc-binding motifs indicates that those AEPs might be evolutionarily ancestral(Prato *et al.*, 2008; Gill *et al.*, 2014).

Bacteria often carry extrachromosomal plasmid DNA like archaea. Two decades ago, DNA-primase activity was identified in Rep protein of the colicin E2 (ColE2) plasmid. A decade later, this primase was also seen as a member of the AEP family, far from archaeal AEPs ORF904 (pRN1) and Rep (pIT3). However, it seems that Rep (ColE2) acts as an RNA primase rather than as a DNA primase polymerase, as opposed to the archaeal plasmid AEPs. This enzyme is essential to replicate ColE2 DNA in vitro, in addition to DNA Polymerase I. Rep (ColE2) precisely attaches to the origin of replication of the plasmid in which it initiates transcription by producing a short RNA primer that enables DNA polymerase I to duplicate DNA progressively. Therefore, Rep (ColE2) seems to be a bacterial plasmid-specific primase(Beck & Lipps, 2007).

RSF1010, an additional bacterial plasmid, also contains 3 Rep proteins present in a broad range of hosts over Gram-negative and certain Gram-positive bacteria(Scherzinger *et al.*, 1984).This includes RepA, a helicase, RepB, an AEP primase, and RepC, a replication initiator protein. The RSF1010 includes two sites, ssiA, and ssiB, for primase recognition, where both are recognized in RepB's such that two primers can be independently synthesized and then expanded by DNA polymerase III. RepB's crystal structure showed two different domains: a large N-terminal domain with two antiparallel beta sheets flanked by six-helices and a smaller C-terminal region with a five-helices bundle. In fact, there is no zinc-binding motif in the enzyme(Scherzinger *et al.*, 1991; Geibel *et al.*, 2009).

This structure shows that the N-terminal domains of RepB are closely linked to the catalytic domain of *P. furiosus* PriS. They also have a minimal sequence homology of between each other. Apart from this,functional differences in ssDNA template identification and in their criteria for priming. In order to identify DNA during replication by these primases, the architecture of the RepB catalytic coreis attached to a ssiA recognition site and provides a comprehensive mechanism for initiating thereplication of plasmid DNA. Interestingly, the higher temperature stability of RepB', possibly because of its structural resemblance to the thermophilic archaeal primases, poses important questions about the evolutions of the plasmid RSF1010(Geibel *et al.*, 2009).

Therefore, the two plasmids bacterial AEPs discussed here in contrast to archaea. The enzymes of bacterial Rep (ColE2) and RepB constitute prototype of AEPs, which are used primarily to initiate replication by synthesizing a short RNA primer. In comparing archaeal plasmids, AEPs primase is adept primase-polymerase, which has adequate capacity to initiate and carry out prevalent replication of their host plasmid DNA. The prudent capacity and absence of polymerase activity of the bacterial primases should not be considered as characteristic of all bacterial AEPs. An integrated prophage primase/helicase AEP/MCM is

translated by the *Bacillus cereus* genome used as a BcMCM (mini-chromosome maintenance)(McGeoch & Bell, 2005).

Initially, BcMCM was identified with an N-terminal field of weak AEP homology by BLAST analyses as an MCM homolog. Initial biochemical researches showed that ATPase activity was performed by helicaseat 3’-5' direction, and activity was stimulated via ssDNA, but on the other hand, primase activity was absent as well. However, helicase activity and DNA-dependent DNA polymerase and primase activity have been observed through a more recent structure/function analysis. Interestingly, much like many archaeal AEPs, BcMCM has a unique robust predilections for dNTPs during primer synthesis and extension. In accordance with these results, BcMCM may serve as an essential multifunctional enzyme that is potentially implemented during *B. cereal* DNA replication, e.g., leading strand replication re-initiation to go along with the fork stalling. Most notably, BcMCM is not the only alternative bacterial AEP. For DNA DSB and other repair processes in most bacterial organisms, multifunctional AEPs are also needed(Samuels *et al.*, 2009; Sanchez-Berrondo *et al.*, 2012).

VII. VIRAL AEPS INVOLVED IN DNA REPLICATION

Viral origin Archaeo-eukaryotic primases (AEPs) are commonly distributed across diverse species encompassing bacterial, archaeal, and eukaryotic genomes. Notably, numerous viruses also encode their own AEPs, exemplified by UL52-like primases in herpes simplex viruses, D5-like primases in Nucleocytoplasmic Large DNA Viruses (NCLDVs), and Lef-1 primases in phages and baculoviruses (Iyer *et al.*, 2005). Similar to cellular AEPs, viral DNA AEPs play pivotal roles in various facets of replication processes. Among these viral AEPs, the heterotrimeric (UL5-UL8-UL52) primase-helicase complex within the Herpes Simplex Virus (HSV) family has received considerable research attention (Crute & Lehman, 1991). This complex was initially identified within Herpes Simplex Virus type 1 (HSV-1), a large double-stranded DNA virus. Notably, three out of the seven genes crucial for HSV-1 replication are encoded as the UL5-UL8-UL52 complex. UL52 serves as the AEP responsible for DNA replication initiation, UL5 possesses helicase activity, while UL8 is integral for interaction with UL30/UL42 primers. Remarkably, UL8 not only contributes to helicase activity but also influences UL5/UL52 primase activity. Typically, primases feature a zinc-binding motif within their catalytic domains; however, UL52 diverges with a strand-rich zinc finger domain positioned at the C-terminus of the Primase subunit. This zinc finger domain is indispensable for in-vitro activities. Functionally, the UL52 primase can synthesize ribonucleotide primers spanning approximately 8 to 12 nucleotides, a process crucial for initiating replication across the 153-kilobase viral genome (Crute *et al.*, 1989; Biswas & Weller, 1999).

Poxviruses include smallpox that performs DNA replication in the infected cell cytoplasm, as another category of large viruses encoding AEPs. The vaccinia virus (VACV) with D5 and AEP-helicase-fusion protein has been the subject of most poxvirus studies. This enzyme's C-terminal region is categorized into the helicase superfamily III, and the structural and sequence similarity to AEPs lies in the N-terminal region of this enzyme. For viral replication in VACV-infected cells, the N-terminal AEP domain, i.e., D5, is necessary. This enzyme also has an in-vitro primase function and strict template specificity, highlighting a primary role in VACV replication of DNA in that enzyme. D5-like primase is composed of poxviruses, irdoviruses, mimiviruses, African swine fever viruses, the herpes simplex virus primases, and Eukaryotic homolog PrimPol has been detailed studied by Iyer et al. using *in*

silico analysis methods. In addition to the phycodnaviruses, A468R-like proteins create the NCLDV Herpesvirus clade of AEP. However, not all viral AEPs are part of this primase clade(Iyer *et al.*, 2005; De Silva *et al.*, 2007; De Silva *et al.*, 2009; Guilliam *et al.*, 2015).

In contradistinction to the UL52 herpesvirus and D5 poxvirus AEPs, the Lef-1-like baculovirus primases constitute a subset within the AEP family. These primases are associated with both replication and non-homologous end joining (NHEJ) AEPs, collectively constituting the AEP-proper clade. The Lef-1-like baculovirus primases exhibit the capability to synthesize RNA primers, which can extend over multiple kilobases. This primer extension activity aligns with the stable capacity observed in Pyrococcus primase PriS, an archaeal replicative primase, confirming their inclusion within the same AEP clade. However, it has been suggested that in vivo, additional replication factors might modulate the extension potential of Lef-1-like primases. Nevertheless, this ability may confer distinct functions to these enzymes in the context of primer expansion(Mikhailov & Rohrmann, 2002).

In contrast to the RNA-directed primase activities demonstrated by the aforementioned viral AEPs, the gp43-like proteins encoded by the corynephageBFK20 strain do not exhibit a preference for rNTP integration. Instead, these proteins, which belong to the AEP Prim-Pol clade along with ORF904 and Rep(pIT3), exclusively incorporate dNTPs. Notably, the gp43-like proteins display dual functionality encompassing both primase and DNA polymerase activities, paralleling the characteristics of archaeal AEPs. Consequently, AEPs constitute a distinct enzyme category that maintains its uniqueness across viruses, characterized by discrete catalytic functions and potentially divergent roles(Halgasova *et al.*, 2012).

VIII. PRIMASES INVOLVED IN DNA DOUBLE-STRAND BREAK REPAIR

In the realm of prokaryotic genomes, the identification of Archaeo-eukaryotic primase (AEP) orthologs initially defied expectations, given the initial categorization of archaeal primases as template-dependent polymerases. Frequently, these AEP genes collaborate with the Ku protein, an entity binding to the termini of DNA double-strand breaks (DSBs) during the non-homologous end joining (NHEJ) process in eukaryotes(Della *et al.*, 2004). These early observations implied that prokaryotes retain NHEJ pathways and that AEPs might play an active role in DSB repair mechanisms. Subsequent investigations have validated the existence of the NHEJ DSB repair mechanism in bacteria, an element of a multifaceted repair system denoted as ligase D (LigD)(Bartlett *et al.*, 2013).

Recent advancements have uncovered analogous NHEJ mechanisms in several archaeal species. In Mycobacteria, LigD encompasses AEP, nuclease, and ligase domains. Nonetheless, these domains are present as discrete proteins in numerous species, their cooperative expression culminating in the assembly of a functional NHEJ complex. The prokaryotic NHEJ process is believed to be facilitated by the Ku-LigD complex, which effectively undertakes all requisite activities for terminal DSB binding and catalyzes the rejoining process(Della *et al.*, 2004; Pitcher *et al.*, 2007; Bartlett *et al.*, 2013).

Non-Homologous End Joining (NHEJ) Archaeo-Eukaryotic Primases (AEPs) display a remarkably diverse repertoire of nucleotide transferase activities. This adaptability is likely an outcome of their capacity to accommodate the myriad end configurations that arise during the formation of DNA double-strand breaks (DSBs). Notably, these enzymes demonstrate

proficiency in various enzymatic tasks, including template-guided RNA/DNA polymerase activity, strand displacement, terminal transfers, and gap-filling. Particularly noteworthy is their propensity for integrating ribonucleotides.

Furthermore, these AEPs exhibit the ability to expediently extend primers along incompatible templates, effectively bypassing lesions such as 8-oxo-dG and abasic (Ap) sites through Translesion Synthesis (TLS)(Della *et al.*, 2004; Pitcher *et al.*, 2007).The contemporary revelation of AEPs' involvement in DSB repair mechanisms among bacteria and archaea raises intriguing questions regarding the evolutionary rationale behind primase families serving as primary NHEJ polymerases. Specifically, primases that have evolved into repair enzymes within the AEP-proper clade, including those possessing replicative primase functions, point to a compelling narrative. These primases, stemming from ancestral AEPs with an inherent capability to synthesize concise RNA primers, have given rise to a novel family of polymerases. This polymerase family is tasked with end-joining responsibilities, thereby potentially playing a pivotal role in processing DNA ends during break repair events. In a comparative analysis between NHEJ-associated AEPs and replicative enzymes (PriS), both sequence and structural considerations uncover similarities and distinctive adaptations. Common catalytic domains are present, as are unique binding strategies that set NHEJ AEP polymerases apart from related enzymes, enabling functionality even at DNA termini. These AEPs feature a positively charged catalytic pocket, facilitating binding to 5' phosphates at or near the DNA double-strand break (DSB) end. This stable attachment allows for DNA end-repair at the DSB site.

Significantly, distinctive catalytic surface loops, referred to as Loops 1 and 2, have evolved. These loops enhance the capacity to facilitate DNA break synapses, a process through which DNA breaks are aligned and annealed using microhomology-mediated end joining (MMEJ). Here, DNA polymerase binds to each side of the break, forming a pre-ternary complex with the intention of connecting to the opposite end of the break. These surface loops are highly conserved in these AEPs and play a crucial role in joining the DSB to the other end, thereby expediting break repair.

At the 3' overhang site, this approach strategically positions the break, allowing for the assembly of synthesis necessary for gap filling, which occurs in a trans manner. This process also underpins the molecular foundation of the template-dependent enzymatic reaction catalyzed at the 3' end by terminal transferase. While this exceptional MMEJ process was initially considered unique to these polymerases, similar polymerases involved in MMEJ mechanisms have since been identified in mammalian Polq, archaeal PriS, and terminal transferase (TdT). This suggests the conservation of this functional mechanism across different polymerases(Brissett *et al.*, 2007; Brissett *et al.*, 2011; Brissett *et al.*, 2013).

While NHEJ and replicative AEPs have distinct biological functions, they are closely linked and These enzymes share a clade affiliation, implying a commonality despite their distinct evolutionary origins. The crystal structures of mycobacterial non-homologous end joining (NHEJ) archaeo-eukaryotic primases (AEPs) bound to DNA elucidate the prevalent catalytic mechanism characteristic of AEPs. This insight also underscores the rationale behind these enzymes' suitability for DNA break repair.

The pre-ternary complex structure of NHEJ AEP-DNA underscores the resemblance of these enzymes to polymerases, as both employ a catalytic mechanism involving two metal

ions. The binding of incoming nucleotides prompts the association of the second metal ion with both the enzyme's active site and the DNA template strand. As indicated, these AEPs have the ability to recognize the approaching primer-template configuration through a neighboring pre-ternary complex of AEP, facilitating primer-template binding and extension, which occurs over a shorter length than a dinucleotide.This mode of action closely mirrors the initiation steps executed by replicative primases. A binary complex forms initially between the enzyme, ssDNA, and the 3' nucleotide, establishing a pre-ternary complex. Subsequent recruitment of the 5' nucleotide as a primer-template results in a ternary complex formation. Notably, a distinctive ribonucleotide addition in the 3'-5' direction follows, succeeded by a 5'–3' extension catalyzed by both NHEJ and AEPs(Brissett *et al.*, 2011; Guilliam *et al.*, 2015).

IX. PRIMASES INVOLVED IN DNA REPAIR MECHANISMS

Besides the absence of homologs polymerase, several archaeal species are often absent of TLS polymerase, which only present in few species with Y-family polymerases. Many archaea do not synthesize photolyase or NER pathways responsible for removing hindered replication fork generated after the DNA damage occurs by UV(Kelman & White, 2005). This raises the question of how archaeal species survive with DNA damage because they lack such damage repair pathways, which is exceptionally essential for the archaea to survive in the extreme environment. The recent report stated that replicative primase, PriS, responsible for the DNA damage forbearance in the Y-family polymerase lacking archaeal species(Jozwiakowski *et al.*, 2015).

Remarkably, PriS function was demonstrated in these types of organisms, and they are capable of bypassing the extreme DNA-misleading CPDs induced by the 8-oxo-dG accurately. The extremophilic archaea (thermophilic archaea) produces enormous amounts of cytosine deamination and is accountable for developing uracil base adducts that cause an intense fork stall encountered by the archaeal replicative primase B- and D-family polymerases during replication. Even though stalled replicative polymerase has been bound to the template strand, PriS often replicates uracil bases that help the replisome sustain successful fork progression during the replication. These results suggest that, in addition to primer synthesis, archaeal replicative primases also play an essential role in DNA replication(Iyer *et al.*, 2005; Guilliam *et al.*, 2015).

1. Primase as a Drug Target:

- **NTP Analogs:** A few chemical compounds have similarity to NTP and actively participate in RNA priming; Such compound are AraATP (Vidarabine) act as a substrate as well as an inhibitor(Ilic *et al.*, 2018).
  e.g., 2-fluoro-AraATP, containing sugar inhibits both Eukaryotic as well as herpes virus.

- BAY 57-1293 (N-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-N-methyl-2-[4-(2-pyridinyl)phenyl]acetamide) compound shows the strong anti-herpes efficacy by inhibiting the HSV helicase-primase(Biswas *et al.*, 2007).

REFERENCES

[1] Bartlett EJ, Brissett NC, Doherty AJ. 2013. Ribonucleolytic resection is required for repair of strand displaced nonhomologous end-joining intermediates. *Proc Natl Acad Sci U S A*110(22): E1984-1991.
[2] Beck K, Lipps G. 2007. Properties of an unusual DNA primase from an archaeal plasmid. *Nucleic Acids Res*35(17): 5635-5645.
[3] Biswas N, Weller SK. 1999. A mutation in the C-terminal putative Zn2+ finger motif of UL52 severely affects the biochemical activities of the HSV-1 helicase-primase subcomplex. *J Biol Chem*274(12): 8068-8076.
[4] Biswas S, Jennens L, Field HJ. 2007. The helicase primase inhibitor, BAY 57-1293 shows potent therapeutic antiviral activity superior to famciclovir in BALB/c mice infected with herpes simplex virus type 1. *Antiviral Res*75(1): 30-35.
[5] Bocquier AA, Liu L, Cann IK, Komori K, Kohda D, Ishino Y. 2001. Archaeal primase: bridging the gap between RNA and DNA polymerases. *Curr Biol*11(6): 452-456.
[6] Brissett NC, Martin MJ, Bartlett EJ, Bianchi J, Blanco L, Doherty AJ. 2013. Molecular basis for DNA double-strand break annealing and primer extension by an NHEJ DNA polymerase. *Cell Rep*5(4): 1108-1120.
[7] Brissett NC, Martin MJ, Pitcher RS, Bianchi J, Juarez R, Green AJ, Fox GC, Blanco L, Doherty AJ. 2011. Structure of a preternary complex involving a prokaryotic NHEJ DNA polymerase. *Mol Cell*41(2): 221-231.
[8] Brissett NC, Pitcher RS, Juarez R, Picher AJ, Green AJ, Dafforn TR, Fox GC, Blanco L, Doherty AJ. 2007. Structure of a NHEJ polymerase-mediated DNA synaptic complex. *Science*318(5849): 456-459.
[9] Chu M, Mierzwa R, Xu L, He L, Terracciano J, Patel M, Gullo V, Black T, Zhao W, Chan TM, et al. 2003. Isolation and structure elucidation of Sch 642305, a novel bacterial DNA primase inhibitor produced by Penicillium verrucosum. *J Nat Prod*66(12): 1527-1530.
[10] Crute JJ, Lehman IR. 1991. Herpes simplex virus-1 helicase-primase. Physical and catalytic properties. *J Biol Chem*266(7): 4484-4488.
[11] Crute JJ, Tsurumi T, Zhu LA, Weller SK, Olivo PD, Challberg MD, Mocarski ES, Lehman IR. 1989. Herpes simplex virus 1 helicase-primase: a complex of three herpes-encoded gene products. *Proc Natl Acad Sci U S A*86(7): 2186-2189.
[12] De Silva FS, Lewis W, Berglund P, Koonin EV, Moss B. 2007. Poxvirus DNA primase. *Proc Natl Acad Sci U S A*104(47): 18724-18729.
[13] De Silva FS, Paran N, Moss B. 2009. Products and substrate/template usage of vaccinia virus DNA primase. *Virology*383(1): 136-141.
[14] Della M, Palmbos PL, Tseng HM, Tonkin LM, Daley JM, Topper LM, Pitcher RS, Tomkinson AE, Wilson TE, Doherty AJ. 2004. Mycobacterial Ku and ligase proteins constitute a two-component NHEJ repair machine. *Science*306(5696): 683-685.
[15] Frick DN, Richardson CC. 2001. DNA primases. *Annu Rev Biochem*70: 39-80.
[16] Geibel S, Banchenko S, Engel M, Lanka E, Saenger W. 2009. Structure and function of primase RepB' encoded by broad-host-range plasmid RSF1010 that replicates exclusively in leading-strand mode. *Proc Natl Acad Sci U S A*106(19): 7810-7815.
[17] Gill S, Krupovic M, Desnoues N, Béguin P, Sezonov G, Forterre P. 2014. A highly divergent archaeo-eukaryotic primase from the Thermococcus nautilus plasmid, pTN2. *Nucleic Acids Res*42(6): 3707-3719.
[18] Griep MA. 1995. Primase structure and function. *Indian J Biochem Biophys*32(4): 171-178.
[19] Guilliam TA, Keen BA, Brissett NC, Doherty AJ. 2015. Primase-polymerases are a functionally diverse superfamily of replication and repair enzymes. *Nucleic Acids Res*43(14): 6651-6664.
[20] Halgasova N, Mesarosova I, Bukovska G. 2012. Identification of a bifunctional primase-polymerase domain of corynephage BFK20 replication protein gp43. *Virus Res*163(2): 454-460.
[21] Hu J, Guo L, Wu K, Liu B, Lang S, Huang L. 2012. Template-dependent polymerization across discontinuous templates by the heterodimeric primase from the hyperthermophilic archaeon Sulfolobus solfataricus. *Nucleic Acids Res*40(8): 3470-3483.
[22] Ilic S, Cohen S, Singh M, Tam B, Dayan A, Akabayov B. 2018. DnaG Primase-A Target for the Development of Novel Antibacterial Agents. *Antibiotics (Basel)*7(3).

[23] Iyer LM, Koonin EV, Leipe DD, Aravind L. 2005. Origin and evolution of the archaeo-eukaryotic primase superfamily and related palm-domain proteins: structural insights and new members. *Nucleic Acids Res*33(12): 3875-3896.
[24] Jozwiakowski SK, Borazjani Gholami F, Doherty AJ. 2015. Archaeal replicative primases can perform translesion DNA synthesis. *Proc Natl Acad Sci U S A*112(7): E633-638.
[25] Keck JL, Roche DD, Lynch AS, Berger JM. 2000. Structure of the RNA polymerase domain of E. coli primase. *Science*287(5462): 2482-2486.
[26] Kelman Z, White MF. 2005. Archaeal DNA replication and repair. *Curr Opin Microbiol*8(6): 669-676.
[27] Kirk BW, Kuchta RD. 1999. Arg304 of human DNA primase is a key contributor to catalysis and NTP binding: primase and the family X polymerases share significant sequence homology. *Biochemistry*38(24): 7727-7736.
[28] Leipe DD, Aravind L, Koonin EV. 1999. Did DNA replication evolve twice independently? *Nucleic Acids Res*27(17): 3389-3401.
[29] Lipps G, Röther S, Hart C, Krauss G. 2003. A novel type of replicative enzyme harbouring ATPase, primase and DNA polymerase activity. *Embo j*22(10): 2516-2525.
[30] Lipps G, Weinzierl AO, von Scheven G, Buchen C, Cramer P. 2004. Structure of a bifunctional DNA primase-polymerase. *Nat Struct Mol Biol*11(2): 157-162.
[31] McGeoch AT, Bell SD. 2005. Eukaryotic/archaeal primase and MCM proteins encoded in a bacteriophage genome. *Cell*120(2): 167-168.
[32] Mikhailov VS, Rohrmann GF. 2002. Baculovirus replication factor LEF-1 is a DNA primase. *J Virol*76(5): 2287-2297.
[33] Pitcher RS, Brissett NC, Picher AJ, Andrade P, Juarez R, Thompson D, Fox GC, Blanco L, Doherty AJ. 2007. Structure and function of a mycobacterial NHEJ DNA repair polymerase. *J Mol Biol*366(2): 391-405.
[34] Prato S, Vitale RM, Contursi P, Lipps G, Saviano M, Rossi M, Bartolucci S. 2008. Molecular modeling and functional characterization of the monomeric primase-polymerase domain from the Sulfolobus solfataricus plasmid pIT3. *Febs j*275(17): 4389-4402.
[35] Samuels M, Gulati G, Shin JH, Opara R, McSweeney E, Sekedat M, Long S, Kelman Z, Jeruzalmi D. 2009. A biochemically active MCM-like helicase in Bacillus cereus. *Nucleic Acids Res*37(13): 4441-4452.
[36] Sanchez-Berrondo J, Mesa P, Ibarra A, Martínez-Jiménez MI, Blanco L, Méndez J, Boskovic J, Montoya G. 2012. Molecular architecture of a multifunctional MCM complex. *Nucleic Acids Res*40(3): 1366-1380.
[37] Scherzinger E, Bagdasarian MM, Scholz P, Lurz R, Rückert B, Bagdasarian M. 1984. Replication of the broad host range plasmid RSF1010: requirement for three plasmid-encoded proteins. *Proc Natl Acad Sci U S A*81(3): 654-658.
[38] Scherzinger E, Haring V, Lurz R, Otto S. 1991. Plasmid RSF1010 DNA replication in vitro promoted by purified RSF1010 RepA, RepB and RepC proteins. *Nucleic Acids Res*19(6): 1203-1211.
[39] Steitz TA, Smerdon SJ, Jäger J, Joyce CM. 1994. A unified polymerase mechanism for nonhomologous DNA and RNA polymerases. *Science*266(5193): 2022-2025.
[40] Sweetser D, Nonet M, Young RA. 1987. Prokaryotic and eukaryotic RNA polymerases have homologous core subunits. *Proc Natl Acad Sci U S A*84(5): 1192-1196.

