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Mechanism and Evolution of DNA Primases

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A hallmark of DNA polymerases is their inability to synthesize new DNA strands *de novo* – i.e., starting with the polymerization of 2 dNTPs. Rather, DNA polymerases require a stable primer already bound to the template strand in order to synthesize DNA. As discussed below, the primer can be as short as one nucleotide, but more commonly is around 10 nucleotides long. Evolutionarily, this absolute requirement for a primer necessitated the development of cellular machinery to generate these primers.

Why can't DNA polymerases initiate new DNA strands *de novo*?

DNA polymerase's requirement for a pre-existing primer appears universal, but why? Potentially, this could result from replication specific issues. For example, a very short primer might increase the number of errors due to difficulties in effectively binding the primer-template and consequent slippage of the primer in the polymerase active site [1,2]. However, data from RNA synthesis suggest that the requirement is not a replication specific problem. RNA polymerases involved in transcription generate new mRNA strands starting from 2 NTPs. More importantly, RNA replicases, the RNA-dependent RNA polymerases that replicate viral RNAs, also do not require a primer. Since the requirement for a primer extends to replication of viral DNA (Ex., adenovirus and herpes) but not to replication of viral RNA (Ex., influenza and polio), the primer requirement is probably not purely a replication-specific requirement. Alternatively, the requirement could reflect the much higher intracellular concentration of NTPs than of dNTPs [3]. Whereas NTP concentrations vary from around 500 μ M for CTP to 4 mM for ATP, dNTP concentrations are only typically 10–100 μ M. Since the initiation reaction involves binding of 2 NTPs, using NTPs rather than dNTPs provides a significant catalytic advantage, especially since initiation usually involves 2 purine NTPs whose concentrations are generally higher than those of the pyrimidine NTPs. However, some archaeal primases have the unique ability to synthesize primers using only dNTPs, indicating that the “mass action” effects of higher NTP concentrations cannot be the complete explanation for why primase exists [4,5].

Primer removal and its consequences

After completion of DNA replication, the cell must remove the RNA primer and replace it with DNA. This replacement will minimally require a protein to remove the RNA, a DNA polymerase to fill the resulting gap, and a ligase to seal the nick and generate a continuous DNA strand. The transient nature of the RNA primase has two other important consequences – mistakes during primer synthesis will have no effect on chromosomal integrity, and it required the evolution of new proteins for the evolution of linear chromosomes.

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The inability of DNA polymerases to initiate DNA synthesis *de novo* and the consequent evolution of an enzyme that made RNA primers was a real trouble maker during chromosome evolution. On circular chromosomes, the involvement of primase poses no problems since after RNA primer removal, a DNA polymerase can easily fill the resulting gap and complete the circle. However, the requirement for primase and the subsequent removal of the RNA primer precludes the complete replication of a linear chromosome in the absence of additional activities. On a linear chromosome, leading strand synthesis can proceed smoothly to the very end of the chromosome. However, since DNA polymerases only copy the template 3'–5' and require a primer, the 3'-end of a linear chromosome cannot be directly converted into DNA. Even if primer synthesis begins at the very end of the chromosome, the cell will lose a small amount of DNA at the end of the chromosome after each replication cycle. Thus, the evolutionary development of linear chromosome necessitated the parallel evolution of telomerase and the telomere maintenance machinery to restore the ends of chromosomes [6].

Nature has evolved at least 4 different mechanisms for generating primers suitable for elongation by a DNA polymerase. (1) Some viruses use a protein primer to initiate replication [7]. In the case of adenovirus, Ser580 of the terminal protein reacts with dCTP resulting in a phosphodiester bond between dCMP and the protein, and leaving a free 3'-hydroxyl onto which the adenovirus DNA polymerase can polymerize dNTPs. In this case, all of the product DNAs contain a protein at the 5'-end. (2) Rather than use a specialized primase for initiating new DNA strands, mitochondrial DNA replication employs the mitochondrial RNA polymerase [8,9]. The RNA polymerase synthesizes abortive transcripts on both the leading and lagging strands that pol γ then elongates to complete replication of both the H and L strands. (3) HIV uses an endogenous cellular tRNA as a primer for converting its RNA genome into DNA [10]. (4) The most common solution to the primer requirement problem, however, is the use of a specialized RNA polymerase, primase. For those organisms that code for a primase, both eliminating expression of the enzyme and generating a catalytically inactive form is fatal, indicating that primer synthesis is essential [11]. With the exception of the few viruses known to use a protein primer and mitochondrial replication, all other systems tested require primase.

In this review, we will examine the primase solution to priming and the mechanism of these enzymes. A large number of primases have been identified, although in many cases limited mechanistic information exists. Thus, we will primarily focus on those systems where the most mechanistic detail exists – T7, eukaryotic, archaeal, and herpes.

Primase evolution and structure

Primases from a large and disparate number of organisms have been sequenced and their evolutionary relationships analyzed [12,13]. Several distinct primase families exist with no obvious sequence relationship between the families, consistent with primase evolving more than once. Curiously, however, even primases that bear no obvious relationship based on sequence analysis often share common catalytic features (see below).

Structurally, primases can be differentiated in two general ways – does activity require one versus two subunits, and for those whose high resolution structure exists, do they have an archaeal/eukaryotic fold or the toprim architecture found in bacterial and phage primases. In those cases where structures do not exist, sequence similarity often provides insight into the structural class.

The single subunit primases include those from many phages, eubacteria, and some specialized primases involved in plasmid replication [11]. Since these enzymes can bind single-stranded DNA and then polymerize NTPs to generate a RNA primer, they must contain a single-stranded DNA binding site as well as two NTP binding sites. While these enzymes can synthesize a primer, the rates of the isolated primase are often remarkably slow [14–17]. However,

association of these primases with additional enzymes, especially helicases, often greatly stimulates activity (see below). Some primases such as that from T7 have taken this helicase stimulation to the next stage and generated a single protein that contains both helicase and primase activity [11].

Recently, the structure of RepB', a single subunit primase involved in replication of a broad host range plasmid, was determined [18]. Unlike the bacterial and phage single subunit plasmids, RepB' contains a fold similar to the archaeal primases (see below). This enzyme also lacks a Zn^{+2} binding domain, distinguishing it from other primases.

The two subunit primases include those from all eukaryotes, herpes viruses, and archae. In the case of the archaeal and eukaryotic primases, the two subunits are typically referred to as the small, catalytic subunit and the large, accessory subunit. The herpes primases consist of a "primase" subunit and a "helicase" subunit. Consistent with their similar construction, the archaeal and eukaryotic primase share varying amounts of sequence similarity between both the large and small subunits, while herpes primase only exhibits significant sequence similarity between its primase subunit and the small, catalytic subunit of eukaryotes and archaea [13]. For human primase and herpes primase, the absence of the large subunit and "helicase" subunit, respectively, precludes detectable primase activity on a single stranded template [19–23]. However, both enzymes can elongate a RNA primer:template via NTP polymerization [21–24]. Thus, the small subunit must contain the entire active site for NTP polymerization, while the large subunit contributes key components needed for the initiation reaction. The isolated small subunit of different archaeal primases show either no activity or minimal activity on single stranded DNA. In the latter case, adding the large subunit dramatically increases primase activity.

The eukaryotic and archaeal primase are somewhat enigmatic with respect to their relationship. Both types of primase have two subunits, they contain a Fe-S cluster [25,26], and sequence alignments between these enzymes reveal that some regions of both the small and large subunit can be aligned (Table 1 [13]). The similarity, while limited, occurs in catalytically critical residues, thereby suggesting that it is biologically significant. However, regions of both the small and large subunits of eukaryotic primase can also be aligned with pol β , a member of the X family of polymerases (Table 1 [27]). In the small subunit, the region near the catalytic aspartates of eukaryotic primase more closely resembles the archaeal primase. In contrast, the large subunits of eukaryotic primase and pol β are surprisingly similar: eukaryotic primase contains approximately half of the 8 kDa domain of pol β , with all of the hydrophobic core residues this domain conserved in primase. Helices C and D of pol β stack onto each other, hence the increased length of these two regions in primase are consistent with a maintained interaction. The archaeal primases, however, do not share this similarity [13]. Additionally, the archaeal primases have a number of mechanistic highlights distinct from the eukaryotic, and in fact all other primases (see below). Unfortunately, no high resolution structures of eukaryotic primases exist to show how closely they resemble archaeal primases versus pol β . Alternatively, eukaryotic primase may be a hybrid of archaeal primase and pol β domains (See below).

High resolution structures of a number of primases exist, including those from *E. coli*, T7, *Pyrococcus horikoshii*, *Pyrococcus furiosus*, and *Sulfolobus solfataricus* [28–33]. The structures fall into two distinct classes. The polymerase cores of *E. coli* and T7 primase have a toprim fold, similar to topoisomerases (Figure 1 [29,31,32]). In this almond-like structure, the active site residues involved in phosphodiester bond formation reside within a shallow cleft flanked on opposite sides by two subdomains. Berger et al. solved the structure of *E. coli* primase with bound single-stranded DNA and found single-stranded DNA bound in two distinct conformations. In contrast, the archaeal primases adopt a very different fold that

consists of two sets of antiparallel- β sheets stacked on top of each other and flanked by two walls of α -helices (Figure 2 [28,30,33]) The active site resides within the β -sheets. As noted earlier, no structures of eukaryotic primases exist.

Primases frequently contain a Zn^{+2} -binding domain in addition to the NTP polymerizing domain [34–36]. Mutating this domain significantly impairs activity, demonstrating that it plays a critical role in primer synthesis. As described in greater detail below, these functions include DNA binding, initiation site recognition, and counting. The large subunits of the archaeal and eukaryotic primases contain a Fe:S cluster that likely plays an important, but uncharacterized role in primase activity [25,26]. Mutation of one of the cysteines that likely bind the cluster or deletion of the p58 domain that binds the Fe:S cluster greatly inhibited primase activity of the two subunit primase complex [26].

Primases and helicases

DNA primases frequently interact with replicative helicases. The gene 4 protein from T7 contains both primase activity and helicase activity, albeit on separate domains [31]. The protein forms hexamers, similar to many replicative helicases, such that the T7 replisome actually contains 6 primase active sites [37,38]. Deletion of 63 amino acids from the N-terminus of the protein generates a monomeric form of the T7 primase-helicase that cannot form hexamers and, therefore, lacks helicase activity. However, the monomeric form retains primase activity, demonstrating that primase activity does not require helicase activity.

T4 primase (gp61) binds tightly to the hexameric T4 helicase (gp41) to form a primosome complex. While the primase alone is active, interaction with the hexameric helicase greatly stimulates activity ([39,40]). Conflicting data exist with respect to the oligomeric nature of the gp61 primase exist [40–42]. Early studies using gel shift analysis found that 1 primase associated with each hexameric helicase ([41]). However, more recent crosslinking, electron microscopy, fluorescence anisotropy and kinetic studies indicate that the primase hexamerizes [40,42]. From a symmetry perspective, such a structure would fit very nicely with the hexameric helicase structure.

E. coli primase (DnaG) likewise binds to the replicative helicase (DnaB) with dramatic consequences for catalytic properties of the primase. Fluorescence anisotropy studies showed that each hexameric DnaB helicase can bind 3 molecules of primase [43]. Binding of the helicase to primase greatly increases the affinity of the primase for single-stranded DNA and the primer initiation sites [43,44]. This interaction also increases the rate of primer synthesis, such that the helicase-primase complex is >1000-fold more active than the isolated primase [44]. Additionally, binding of helicase to primase limits the length of primers synthesized to around 10–14 nucleotides long [44,45].

Herpes primase has evolved a rather unusual relationship with the associated helicase. The core helicase-primase complex contains 3 proteins, UL5, UL8 and UL52 [46,47]. UL5 contains conserved helicase motifs, while UL52 contains motifs consistent with a primase [35,48–51]. Helicase and primase activity require both UL5 and UL52, while removing UL8 only reduces activity [19,20,22]. Unlike most replicative helicases, there is no evidence that the herpes helicase forms a hexameric structure. More surprising, however, is the relationship between the two activities. Mutating UL5 to eliminate helicase activity stimulates primase activity, indicating a complex interplay between these two proteins [49,52]. This result also contrasts with other replication systems where the helicase stimulates primase activity.

Initiation specificity

For all primases analyzed to date, primer synthesis does not occur randomly on a template. Some primases initiate synthesis at specific template trinucleotides, most commonly a trinucleotide 3'-XN₁N₂-5' (Table 2, [11,53–55]). Enzymes that employ this initiation strategy include both single subunit primases such as *E. coli*, T4 and T7 primase, as well as two subunit primases such as herpes primase. The two nucleotides denoted N code for the first two nucleotides of the primer such that the X is not replicated and only serves to direct the enzyme where to initiate synthesis. Except for archaeal and occasionally *Aquifex aeolicus* primase, primase always employs a purine as the 5'-terminal nucleotide, hence N₁ is always a pyrimidine. Primase from the archaeon *Sulfolobus solfataricus* synthesized primers on oligo(dC) and oligo(dG) more rapidly than on oligo(T) or oligo(A), suggesting that the base-pair strength defines primer initiation sites [5].

Others, most notably the eukaryotic primases, have minimal template specificity and only require a template pyrimidine to code for the 5'-terminal nucleotide of the primer [56–59]. In vitro, the purified enzymes greatly prefer pyrimidine rich templates. However, whole cells studies that examined where primase initiated the synthesis of new Okazaki fragments showed no preference for pyrimidine rich templates [59–63]. The large difference in priming site selection suggests that factors missing from the purified studies help direct primer synthesis. The identity of these factor(s), probably other replication proteins, remains unknown. Eukaryotic primase binds more tightly to pyrimidine rich DNA than to purine rich DNA [64], suggesting that a likely mechanism by which these factors alter initiation site selection is recruiting primase to DNA sequences to which it would not normally bind.

Herpes primase makes a rather unusual use of the 3'-G in the G-Py-Py triplet, where Py is either T or C [15,65]. The enzyme will actually initiate primer synthesis opposite any pair of template pyrimidines at similar rates. However, it does not elongate the growing primer past 2 or 3 nucleotides long in the absence of the 3'-G. Mechanistically, the increased processivity in the presence of G could result from more efficient polymerization of the third and latter nucleotides, a decreased rate of primer dissociation from the primase-ssDNA-(short primer) complex, or a decreased rate of dissociation of the enzyme away from the site of primer synthesis.

T7 primase exhibits significant template specificity besides the template 3'-CTG [11,66,67]. Studies by Richardson and coworkers showed that the preferred sequences actually encompassed 5 template nucleotides (3'-CTGTG, 3'-CTGGG, and 3'-CTGGT). At other nucleotide sequences that began 3'-CTG..., the T7 enzyme rapidly synthesized dinucleotide, but then dissociated along the template before adding additional NTPs.

Besides the inherent sequence specificity of different primases, other factors can influence where on the template primase synthesizes a primer. For human and herpes primase, the relative concentration of NTPs can significantly alter what initiation sites the enzyme uses [16,65]. When synthesizing a pppNpN' dinucleotide, N'TP binds first followed by NTP. Thus, if given a choice of initiation sites that direct the synthesis of pppApG and pppGpA dinucleotides, high ATP concentrations will drive primase to synthesize pppGpA while high GTP concentrations will drive primase to synthesize pppApG. Sequences surrounding a potential initiation site can also affect initiation. For example, herpes primase synthesizes primers at very different rates depending upon the sequence surrounding a potential initiation site, although how this occurs remains unknown [65]. As noted earlier, interactions with other proteins also likely influence where primase synthesizes primers in vivo.

Mechanism of primer synthesis

After binding DNA, primase must bind two NTPs to generate the E-DNA-NTP-NTP quaternary complex. The K_{MS} for NTPs varies substantially among different primases and templates, typically ranging from around $20\ \mu\text{M}$ – $1\ \text{mM}$ [15–17,65,68]. For primases from *E. coli*, herpes, and two eukaryotes (bovine and human), the rate limiting step occurs after NTP binding but before or during dinucleotide synthesis [15–17]. In each case, dinucleotides do not accumulate prior to formation of longer products, as would occur if the rate limiting step occurred after dinucleotide synthesis.

As with many RNA polymerases, primases can also undergo abortive initiation. T7 primase can initiate synthesis at any 3'-CTG, however the level of abortive initiation by T7 primase varied greatly depending upon the identity of the third template base to be replicated. At preferred initiation sites, the enzyme occasionally aborted synthesis but at non-preferred sites, it primarily aborted synthesis and generated copious quantities of dinucleotides [11,66,67]. Eukaryotic primase also generates large amount of di- and tri-nucleotides, even though the associated pol α only uses primers at least 7 nucleotides long [16,69]. Abortive synthesis appears to result from the instability of the di- and tri-nucleotide since eukaryotic primase synthesizes more short products at higher temperature and increasing the number of G:C base-pairs in the short products decreases the amount of them. Herpes primase also synthesizes large amounts of di- and tri-nucleotide [15,65]. Unlike other primases, however, the relative amount of abortive products appears independent of either the stability of the abortive products or the initiation sequence, suggesting that sequences surrounding the initiation site affect the efficiency with which herpes primase converts very short products into longer ones.

For most nucleotide polymerases, signals present in the template (Eg., transcriptional RNA polymerases) or a competition between dissociation from the template versus addition of the next (d)NTP dictate product length (Eg. DNA polymerases) [70]. In contrast, with the exception of the archaeal enzymes, primases have an inherent ability to count and thereby generate products of defined length [11]. Of those enzymes that count, most generate products around 10 nucleotides long, at which time they either dissociate from the newly generated primer or at least pause synthesis. T7 and T4 primase provide intriguing exceptions to this length, as they synthesize primers only 4 or 5 nucleotides long [11,39,66]! The eukaryotic primases also exhibit counting in another very unusual reaction, the continued elongation of a newly synthesized primer. After eukaryotic primase generates a primer of around 10 nucleotides long, it will polymerize additional “bursts” of NTPs in multiples of around 10, thus generating a series of longer primers centered around 20, 30, etc. nucleotides long [71,72].

Mechanistically, how might primase count? Ultimately, counting requires either that the enzyme remember where it began, that it keeps track of the number of phosphodiester bonds that it has formed, or that primase initially binds DNA in a manner that inherently limits the length of the product. For the hexameric T7 primase, Richardson et al. have proposed an elegant model based on structural studies and mutagenesis (Figure 3 [38]). In this model, the Zn^{+2} finger domain of one primase molecule interacts with the polymerase domain of the neighboring primase molecule to provide this polymerase domain with a limited amount of template.

Counting by eukaryotic primase involves the “pol β -like” domain in the p58 subunit since mutating this domain strongly interferes with the ability of human primase to count [23]. The pol β -like domain is extremely closely related to the 8 kDa domain in pol β . Pol β does not processively elongate a simple primer-template [73–75]. However, modification of the primer-template to resemble a gapped DNA by adding a second DNA molecule to the template results in a substrate that pol β processively replicates. Importantly, the 8 kDa domain binds to this

downstream DNA, thereby keeping the pol β catalytic domain bound to the gapped substrate and resulting in processive synthesis (Figure 4). This type of binding by the pol β -like domain of p58 could also account for counting by eukaryotic primase. p49/p58 would initially bind to single-stranded DNA in an open complex, with p58 in front of p49 and the pol β -like domain bound to the DNA. p49 could smoothly initiate synthesis, but after polymerizing 10 nucleotides it would run into p58 as the complex converted into a closed complex, thereby limiting further primer synthesis. In this “hinge-closing” model, synthesis of an additional burst of around 10 nucleotides onto the just completed primer would require primase resetting to the open complex. Alternatively, one could imagine the complex binding as a “closed” hinge, with polymerization proceeding until the primase reached its maximum opening (Figure 4).

Similar to T7 primase, *E. coli* primase may also use the Zn^{+2} binding domain to enforce counting by the polymerase active site, and this interaction likely occurs via a trans interaction between two primase molecules (Figure 5 [45]). Berger and colleagues showed that mutating the Zn^{+2} binding domain eliminated the ability of primase to count, as well as dramatically decreased primase activity [45]. The DnaB would be a key player in counting since it acts as a scaffold for 3 primases to bind and allow the Zn^{+2} binding domain of one primase monomer to interact with the polymerase domain of a second monomer [43].

Unlike all other primases, those from archaea do not have a strictly enforced counting mechanism [4,76]. They can synthesize products >1000 nucleotides long, and in some regards act as a hybrid between a primase and a polymerase. Archaeal primases efficiently incorporate multiple consecutive dNTPs, unlike other primases, and can initiate “primer” synthesis using dNTPs, a reaction that classical DNA polymerases cannot do [4].

This inability to count may also suggest a hybrid solution to understanding the evolutionary relationship between eukaryotic primase, archaeal primase, and pol β . Eukaryotic primase maintained the catalytic core of the archaeal primase, but then further acquired the 8 kDa domain of pol β in order to count, thereby shortening the length of primers (or, of course, that primase first developed this counting domain and pol β then acquired it from primase in order to efficiently fill short gaps).

Transfer of the primer from primase to a DNA polymerase

Since the biological purpose of primase is to synthesize primers that a replicative DNA polymerase can further elongate, primase must first synthesize a primer the polymerase can elongate and then transfer the primer to the polymerase. Furthermore, since primase and DNA polymerase often coexist in a complex, once the polymerase begins elongating the primer, primase should not elongate it. The ability of primase to count helps ensure efficient and timely transfer of primers to the polymerase. First, counting likely ensures that the primer will be long enough for the polymerase to elongate it. T7 polymerase will use RNA primers as short as 4 nucleotides, and T7 primase accommodately synthesizes primers 4 nucleotides long [77]. In contrast, pol α , the polymerase that elongates primers synthesized by eukaryotic primase, requires primers of at least 7 nucleotides [69]. Thus, the counting mechanism of eukaryotic primase results in the synthesis of primers around 10 nucleotides long. Second, counting ensures a timely switch from primer synthesis to DNA synthesis by limiting how long primase will remain active. Human and herpes primase avoid elongating primers onto which the polymerase has added dNTPs by requiring the presence of a 2'-hydroxyl at the 3'-terminal nucleotide of the primer in order to polymerize a NTP [78]. *Staphylococcus aureus* and *E. coli* primase can incorporate dNTPs, but elongate primers containing a deoxynucleotide at the 3' terminus less efficiently than a ribonucleotide at the 3' terminus [17,53]. As noted earlier, however, the archaeal primases appear unique in terms of counting and dNTP polymerization.

T7 primase synthesizes primers only 4 nucleotides long, and thus faces the unique challenge of an extremely unstable primer-template. The primase and polymerase solve this issue via a direct hand-off of primers between the enzymes [79]. T7 primase and polymerase bind to each other and after primer synthesis, form a remarkably stable complex with the newly generated primer-template that is stable for >60 minutes [77]. At least part of this stability results from interactions with the 5'-terminal triphosphate of the primer.

The eukaryotic primase forms a tight complex with pol α . Primase passes newly generated primer-templates to pol α via a direct handoff [80], similar to the T7 system. The large, p58 subunit of primase passes the newly generated primer-template from primase to pol α (Figure 6). The small p49 primase subunit contains the active site but does not contact pol α , whereas the p58 subunit contacts both pol α and p49 [24]. Transfer of the primer to pol α has two significant effects on primase activity [80]. First, it prevents primase from further elongating the newly generated primer into a double-length “primer-dimer”. Second, if pol α can also elongate the newly generated primer, it reactivates primase so that it can synthesize another primer. In the absence of pol α -catalyzed primer elongation, synthesis of a primer at least 7 nucleotides long results in primase entering a refractory mode where it only slowly initiates further primer synthesis.

E. coli primase also passes the primer directly to the associated replicative DNA pol III, but this is probably one of the few instances where the prokaryotic process is more complicated than the eukaryotic process (Figure 7). Productive transfer of the primer-template to pol III requires the assembly of pol III's processivity factor (β) on the RNA primer-template and displacement of single-stranded DNA binding protein [81]. As suggested by the assembly of the processivity factor on the RNA primer-template, the γ clamp-loading complex plays a key role. After primase synthesizes a primer, it remains tightly bound to the resulting primer-template and protected from other proteins in solution (Eg., polymerases and nucleases). The χ subunit of the γ complex helps release single-stranded DNA binding protein from primase, reducing the affinity of primase for the newly generated primer-template. Now, the γ complex can load the β sliding clamp onto the primer-template and pol III can bind to complete the primer-template switch. This multipoint transfer in bacteria contrasts with the much simpler “primase, meet polymerase” switch in eukaryotes.

Perhaps the most unusual switch is that of the herpes replication apparatus. Herpes viruses synthesize seven proteins essential for DNA replication, including a 3 subunit primase-helicase complex and DNA polymerase-processivity factor complex [46]. Additionally, these two complexes bind to each other [82]. However, herpes polymerase uses the primase-synthesized primers extremely inefficiently – in the presence of equimolar amounts of primase and polymerase, the polymerase elongates at most 1–2 % of the primers [83]. This occurs even though herpes primase polymerizes NTPs several orders of magnitude slower than herpes polymerase incorporates dNTPs. Herpes polymerase also uses chemically synthesized RNA primers extremely inefficiently, indicating that the chemical structure of the primase-synthesized primer interferes with elongation. Potential explanations for this rather unusual result include missing proteins from the *in vitro* assays, or that a host cell polymerase actually elongates the primer.

Primase fidelity, or lack thereof

Primases tend to have extremely low fidelity, consistent with the ultimate removal of the RNA primer from the completed genome. Indeed, Lehman described primase as “An enzyme that can count but can't read” [84]. Human primase misincorporates NTPs at a frequency of around 0.01 errors per nucleotide polymerized, while herpes primase misincorporates NTPs at a frequency around 0.03 errors per nucleotide polymerized [2,85]. These enzymes also have the

capacity to misincorporate consecutive nucleotides. Only the Y family, “lesion bypass” DNA polymerases exhibit similarly low fidelity.

Surprisingly, while primases will misincorporate natural NTPs, they discriminate remarkably effectively against NTPs bearing unnatural bases [86–88]. Both human and herpes primase discriminate very strongly against NTPs containing bases such as benzimidazole, 6-methyl-1-deazpurine (an adenine isostere), and even purine (Chart 1)! Indeed, the only NTPs that these enzymes efficiently polymerize are those that can form Watson-Crick hydrogen bonds with the template base using either a major or minor tautomer of the bases (Chart 2). Presently, the mechanistic basis for this extreme inaccuracy yet high selectivity remains unclear.

Inhibition

Primase is a particularly intriguing chemotherapeutic target due to its essential role in DNA replication, and number of inhibitors have been synthesized (Chart 3). Some nucleotide analogues will inhibit different DNA primases. NTP analogues containing arabinofuranosyl sugars (araATP, 2-Fluoro-araATP) inhibit both eukaryotic and herpes primase [89,90]. Primase readily incorporates them into the growing DNA strand where they now act as very strong chain terminators. It is unclear if any of the cytotoxicity associated with treating cells or patients with the corresponding nucleoside results from primase inhibition, not surprisingly since the mechanism by which these analogues kills cells remains unknown. Several very potent inhibitors of herpes primase have recently been synthesized, and at least one is undergoing animal studies [91–93]. The natural product (+)-Sch642305 potently inhibits bacterial primase [94].

The relatively limited number of inhibitors discovered for primase probably reflects the inherent difficulty of primase assays, rather than a lack of potential binding sites on the enzyme. The short length of products synthesized and the generally slow rate of the enzyme as compared to other replication enzymes complicate developing high throughput screening approaches to discover new inhibitors. Thus, more sensitive assays would likely greatly facilitate the discovery of new inhibitors of this medically attractive target.

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References

1. Kornberg, A.; Baker, T. DNA Replication. 2. W. H. Freeman & Co; San Francisco: 1992.
2. Zhang SS, Grosse F. Accuracy of DNA Primase. Journal of Molecular Biology 1990;216:475–479. [PubMed: 2258921]
3. Hauschka PV. Analysis of Nucleotide Pools in Animal Cells. Methods in Cell Biology 1973;3:362–462.
4. LeBreton M, Henneke G, Norais C, Flament D, Myllykallio H, Querellou J, Raffin JP. The Heterodimeric Primase from the Euryarchaeon *Pyrococcus abyssi*: a Multifunctional Enzyme for Initiation and Repair? J Mol Biol 2007;374:1172–1185. [PubMed: 17991487]
5. Lao-Sireix SH, Bell SD. The Heterodimeric Primase of the Hyperthermophilic Archaeon *Sulfolobus solfataricus* Possesses DNA and RNA Primase, Polymerase, and 3'-terminal Nucleotidyl Transferase Activities. J Mol Biol 2004;344:1251–1263. [PubMed: 15561142]
6. Lingner J, Cooper JP, Cech TR. Telomerase and DNA End Replication: No Longer a Lagging Strand Problem. Science 1995;269:1533–1534. [PubMed: 7545310]
7. Pronk R, van del Vliet PC. The Adenovirus Terminal Protein Influences Binding of Replication Proteins and Changes the Origin Structure. Nucleic Ac Res 1993;21:2293–2300.

8. Pham XH, Farge G, Shi Y, Gaspari M, Gustafsson CM, Falkenberg M. Conserved Sequence Box II Directs Transcription Termination and Primer Formation in Mitochondria. *J Biol Chem* 2006;281:24057–24062.
9. Wanrooij S, Fuste JM, Farge G, Shi Y, Gustafsson CM, Falkenberg M. Human Mitochondrial RNA Polymerase Primers Lagging-Strand DNA Synthesis *in vitro*. *Proc Natl Acad Sci USA* 2008;105:11123–11127.
10. Mak J, Kleiman L. Primer tRNAs for Reverse Transcription. *J Virol* 1997;71:8087–8095. [PubMed: 9343157]
11. Frick DN, Richardson CC. DNA Primases. *Ann Rev Biochem* 2001;70:39–80. [PubMed: 11395402]
12. Aravind L, Leipe DD, Koonin EV. Toprim - A Conserved Catalytic Domain in Type IA and II Topoisomerases, DnaG-type Primases, OLD Family Nucleases and RecR Proteins. *Nucleic Acids Res* 1998;26:4205–4213. [PubMed: 9722641]
13. Lakshminarayan MI, 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 Research* 2005;33:3875–3896w. [PubMed: 16027112]
14. Arezi B, Kirk BW, Copeland WC, Kuchta RD. Interactions of DNA with Human DNA Primase Monitored with Photoactivatable Cross-linking Agents: Implications for the Role of the p58 Subunit. *Biochemistry* 1999;38:12899–12907. [PubMed: 10504261]
15. Ramirez-Aguilar K, Kuchta RD. Mechanism of Primer Synthesis by the Herpes Simplex Virus 1 Helicase-Primase. *Biochemistry* 2004;43:1103–1112.
16. Sheaff R, Kuchta RD. The Mechanism of Calf Thymus DNA Primase: Slow Initiation, Rapid Polymerization and Intelligent Termination. *Biochemistry* 1993;32:3027–3037. [PubMed: 7681326]
17. Swart JR, Griep MA. Primer Synthesis Kinetics by Escherichia coli Primase on Single-Stranded DNA Templates. *Biochemistry* 1995;34:16097–16106. [PubMed: 8519767]
18. Geibel S, Banchenko S, engel M, Lanka, Saenger W. Structure and Function of Primase RepB' Encoded by Broad-Host-Range Plasmid RSF1010 that Replicates Exclusively in Leading-Strand Mode. *Proc Natl Acad Sci USA* 2009;106 In press. 10.1073/pnas.0902910106
19. Calder JM, Stow ND. Herpes simplex Virus Helicase-Primase: the UL8 Protein Is not Required for DNA-Dependent ATPase and DNA Helicase Activities. *Nucleic Acids Research* 1990;18:3573–3578. [PubMed: 2163521]
20. Dodson MS, Lehman IR. Association of DNA helicase and primase activities with a subassembly of the herpes simplex virus 1 helicase-primase composed of the UL5 and UL52 gene products. *Proceedings of the National Academy of Science* 1991;88:1105–1109.
21. Kirk BW, Kuchta RD. Human DNA Primase: Anion Inhibition, Manganese Stimulation, and Their Effects on *in vitro* Start Site Selection. *Biochemistry* 1999;38:10126–10134. [PubMed: 10433721]
22. Sherman G, Gottlieb J, Challberg MD. The UL8 Subunit of the Herpes Simplex Virus Helicase-primase Complex Is Required for Efficient Primer Utilization. *Journal of Virology* 1992;66:4884–4889. [PubMed: 1321275]
23. Zerbe L, Kuchta RD. The p58 Subunit of Human DNA Primase Is Important for Primer Initiation, Elongation, and Counting. *Biochemistry* 2002;41:4891–4900. [PubMed: 11939784]
24. Copeland WC, Wang TSF. Enzymatic Characterization of the Individual Mammalian Primase Subunits Reveals a Biphasic Mechanism for Initiation of DNA Replication. *J Biol Chem* 1993;268:26179–26189. [PubMed: 8253737]
25. Klinge S, Hirst J, Maman JD, Krude T, Pellegrini L. An Iron-Sulfur Domain of the Eukaryotic Primase Is Essential for RNA Primer Synthesis. *Nature Struct Molec Biol* 2007;14:875–877. [PubMed: 17704817]
26. Weiner BE, Huang H, Dattilo BM, Nilges MJ, Fanning EC, Chazin WJ. An Iron-Sulfur Cluster in the C-Terminal Domain of the p58 Subunit of Human DNA Primase. *J Biol Chem* 2007;282:33444–33451. [PubMed: 17893144]
27. Kirk BW, Kuchta RD. 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* 1999;38:7727–7737. [PubMed: 10387012]
28. Augustin MA, Huber R, Kaiser JT. Crystal Structure of a DNA-Dependent RNA Polymerase (DNA Primase). *Nature Struct Molec Biol* 2001;8:57–61.

29. Corn JE, Pelton JG, Berger JM. Identification of a DNA Primase Template Tracking Site Redefines the Geometry of Primer Synthesis. *Nature Struct Molec Biol* 2006;15:163–169. [PubMed: 18193061]
30. Ito N, Nureki O, Shirouzu M, Yokoyama S, Hanaoka F. Crystal Structure of the *Pyrococcus horikoshii* DNA Primase-UTP Complex: Implications for the Mechanism of Primer Synthesis. *Genes Cells* 2003;8:913–923. [PubMed: 14750947]
31. Kato M, Ito T, Wagner G, Richardson CC, Ellenberger T. Modular Architecture of the Bacteriophage T7 Primase Couples RNA Primer Synthesis to DNA Synthesis. *Molec Cell* 2003;11:1349–1360. [PubMed: 12769857]
32. Keck JL, Roche DD, Lynch AS, Berger JM. Structure of the RNA Polymerase Domain of *E. coli* Primase. *Science* 2000;287:2482–2486. [PubMed: 10741967]
33. Lao-Sireix SH, Nookala RK, Roversi P, Bell SD, Pelligrini L. Structure of the Heterodimeric Core Primase. *Nature Struct Molec Biol* 2005;12:1137–1144. [PubMed: 16273105]
34. Mendelman LV, Beauchamp BB, Richardson CC. Requirement for a Zinc Motif for Template Recognition by the Bacteriophage T7 Primase. *EMBO J* 1994;13:3909–3916. [PubMed: 8070418]
35. Biswas N, Weller SK. A Mutation in the C-terminal Putative Zn²⁺ Finger Motif of UL52 Severely Affects the Biochemical Activities of the HSV-1 Helicase-Primase Subcomplex. *Journal of Biological Chemistry* 1999;274:8068–8076. [PubMed: 10075707]
36. Griep MA, Lokey ER. The Role of Zinc and the Reactivity of Cysteines in *E. coli* Primase. *Biochemistry* 1996;35:8260–8267. [PubMed: 8679581]
37. Guo S, Tabor S, Richardson CC. The Linker Region between the Helicase and Primase Domains of the Bacteriophage T7 Gene 4 Protein Is Critical for Hexamer Formation. *J Biol Chem* 1999;274:30303–30309. [PubMed: 10514525]
38. Qimron U, Lee SJ, Hamdan SM, Richardson CC. Primer Initiation and Extension by T7 DNA Primase. *EMBO J* 2006;25:2198–2208.
39. Hinton DM, Nossal NG. Bacteriophage T4 DNA Primase-Helicase. Characterization of Oligomer Synthesis by T4 61 Protein Alone and in Conjunction with T4 41 Protein. *J Biol Chem* 1987;262:10873–10878. [PubMed: 3038900]
40. Yang J, Xi J, Zhuang Z, Benkovic SJ. The Oligomeric T4 Primase Is the Functional Form during Replication. *J Biol Chem* 2005;280:25416–25423. [PubMed: 15897200]
41. Dong F, von Hippel PH. The ATP-Activated Hexameric Helicase of Bacteriophage T4 (gp41) Forms a Stable Primosome with a Single Subunit of T4-Coded Primase (gp61). *J Biol Chem* 1996;271:19625–19631. [PubMed: 8702659]
42. Valentine AM, Ishmael FT, Shier BK, Benkovic SJ. A Zinc Ribbon Protein in DNA Replication: Primer Synthesis and Macromolecular Interactions by the Bacteriophage T4 Primase. *Biochemistry* 2001;40:15074–15085. [PubMed: 11735390]
43. Mitkova AV, Khopde SJ, Biswas SB. Mechanisms and Stoichiometry of Interaction of DnaG Primase with DnaB Helicase of *E. coli* in RNA Primer Synthesis. *J Biol Chem* 2003;278:52253–52261. [PubMed: 14557266]
44. Johnson SK, Bhattacharyya S, Griep MA. DnaB Helicase Stimulates Primer Synthesis Activity on Short Oligonucleotide Templates. *Biochemistry* 2000;39:736–744. [PubMed: 10651639]
45. Corn JE, Pease PJ, Hura GL, Berger JM. Crosstalk between Primase Subunits Can Act to Regulate Primer Synthesis in *trans*. *Molec Cell* 2005;20:391–401. [PubMed: 16285921]
46. Boehmer PE, Lehman IR. Herpes Simplex Virus DNA Replication. *Annual Reviews of Biochemistry* 1997;66:347–384.
47. Crute JJ, Lehman IR. Herpes Simplex Virus-1 Helicase Primase. Physical and Catalytic Properties. *J Biol Chem* 1991;266:4484–4488. [PubMed: 1847923]
48. Dracheva S, Koonin EV, Crute JJ. Identification of the Primase Active Site of the Herpes Simplex Virus Type I Helicase-primase. *Journal of Biological Chemistry* 1995;270:14148–14153. [PubMed: 7775476]
49. Graves-Woodward KL, Gottlieb J, Challberg MD, Weller SK. Biochemical Analyses of Mutations in the HSV-1 Helicase-Primase That Alter ATP Hydrolysis, DNA Unwinding, and Coupling Between Hydrolysis and Unwinding. *Journal of Biological Chemistry* 1997;272:4623–4630. [PubMed: 9020191]

50. Graves-Woodward KL, Weller SK. Replacement of Gly815 in Helicase Motif V Alters the Single-stranded DNA-dependent ATPase Activity of the Herpes Simplex Virus Type 1 Helicase-Primase. *Journal of Biological Chemistry* 1996;13629–13635. [PubMed: 8662872]
51. Klinedinst DK, Challberg MD. Helicase Primase Complex of Herpes Simplex Virus Type 1: A Mutation in the UL52 Subunit Abolishes Primase Activity. *J Virol* 1994;68:3693–3701. [PubMed: 8189507]
52. Biswas N, Weller SK. The UL5 and UL52 Subunits of the Herpes Simplex Virus Type 1 Helicase-Primase Subcomplex Exhibit a Complex Interdependence for DNA Binding. *Journal of Biological Chemistry* 2001;276:17610–17619. [PubMed: 11278618]
53. Koepsell SA, Larson MA, Frey CA, Hinrichs SH, Griep MA. Staphylococcus aureus Primase Has Higher Initiation Specificity, Interacts with Single-stranded DNA Stronger, but Is Less Stimulated by Its Helicase than E. coli Primase. *Molec Microbiol* 2008;68:1570–1582. [PubMed: 18452509]
54. Larson MA, Bressani R, Sayood K, Corn JE, Berger JM, Griep MA, Hinrichs SH. Hyperthermophilic Aquifex aeolicus Initiates Primer Synthesis on a Limited Set of Trinucleotides Comprised of Cytosines and Guanines. *Nucleic Acids Res* 2008;36:5260–5269. [PubMed: 18684998]
55. Thirlway J, Soutanas P. In the Bacillus stearothermophilus DnaB-DnaG Complex, the Activities of the Two Proteins Are Modulated by Distinct but Overlapping Networks of Residues. *J Bacteriol* 2006;188:1534–1539. [PubMed: 16452437]
56. Davey SK, Faust EA. Murine DNA Polymerase Alpha-Primase Initiates RNA-primed DNA Synthesis Preferentially Upstream of a 3'-CC(C/A)-5' Motif. *Journal of Biological Chemistry* 1990;265:3611–3614. [PubMed: 2303465]
57. Grosse F, Krauss G. The Primase Activity of DNA Polymerase alpha from Calf Thymus. *Journal of Biological Chemistry* 1988;260:1881–1888. [PubMed: 2578465]
58. Tseng BY, Ahlem CN. Mouse Primase Initiation Sites in the Origin Region of SV40. *Proc Natl Acad Sci* 1984;81:2342–2346. [PubMed: 6326122]
59. Yamaguchi M, Hendrickson EA, DePamphilis ML. DNA Primase-DNA Polymerase alpha from Simian Cells: Sequence Specificity of Initiation Sites on Simian Virus 40 DNA. *Mol Cell Biol* 1985;5:1170–1183. [PubMed: 2582240]
60. Bullock PA, Tevosian S, Jones C, Denis D. Mapping Initiation Sites for SV40 DNA Synthesis Events in Vitro. *Mol Cell Biol* 1994;14:5043–5055. [PubMed: 8035788]
61. Hay RT, DePamphilis ML. Initiation of SV40 DNA Replication in vivo: Location and Structure of 5' Ends of DNA Synthesized in the ori Region. *Cell* 1982;28:767–779. [PubMed: 6178514]
62. Hay RT, Hendrickson EA, DePamphilis ML. Sequence Specificity for the Initiation of RNA-Primed SV40 DNA Synthesis in Vivo. *Journal of Molecular Biology* 1984;175:131–157. [PubMed: 6202875]
63. Suzuki M, Savoyesky E, Izuta S, Tatebe M, Okajima T, Yoshida S. RNA Priming Coupled with DNA Synthesis on Natural Template by Calf Thymus DNA Polymerase alpha-Primase. *Biochemistry* 1993;32:12782–12792. [PubMed: 7504526]
64. Holmes AM, Cheriathundam E, Bollum FJ, Chang LMS. Initiation of DNA Synthesis by the Calf Thymus DNA Polymerase-Primase Complex. *J Biol Chem* 1985;260:10840–10846. [PubMed: 3161883]
65. Ramirez-Aguilar KA, Low-Nam NA, Kuchta RD. Key Role of Template Sequence for Primer Synthesis by the Herpes Simplex Virus 1 Helicase-Primase. *Biochemistry* 2002;41:4569–4579.
66. Frick DN, Richardson CC. Interaction of Bacteriophage T7 Gene 4 Primase with Its Template Recognition Site. *J Biol Chem* 1999;274:35889–35898. [PubMed: 10585474]
67. Kusakabe T, Richardson CC. Template Recognition and Ribonucleotide Specificity of the DNA Primase of Bacteriophage T7. *J Biol Chem* 1997;272:5943–5951. [PubMed: 9038214]
68. Tseng BY, Ahlem CN. A DNA Primase from Mouse Cells. Purification and Partial Characterization. *J Biol Chem* 1983;258:9845–9849. [PubMed: 6885774]
69. Sheaff R, Kuchta RD, Ilsley D. Calf Thymus DNA Polymerase Alpha-Primase: "Communication" and Primer:Template Movement between the Two Active Sites. *Biochemistry* 1994;33:2247–2254. [PubMed: 8117681]
70. Arezi B, Kuchta RD. Eucaryotic DNA Primase. *Trends in Biochemical Science* 2000;25:572–576.

71. Kuchta RD, Reid B, Chang LMS. DNA Primase: Processivity and the Primase to Polymerase Alpha Activity Switch. *Journal of Biological Chemistry* 1990;265:16158–16165. [PubMed: 2398049]
72. Singh H, Brooke RG, Pausch MH, Williams GT, Trainor C, Dumas LB. Yeast DNA Primase and DNA Polymerase Activities: An Analysis of RNA Priming and Its Coupling to DNA Synthesis. *Journal of Biological Chemistry* 1986;261:8564–8569. [PubMed: 2424899]
73. Chagovetz AM, Sweasy JB, Preston BD. Increased Activity and Fidelity of DNA Polymerase Beta on Single-nucleotide Gapped DNA. *Journal of Biological Chemistry* 1997;272:27501–27504. [PubMed: 9346877]
74. Pelletier H, Sawaya MR, Wolfe W, Wilson SH, Kraut J. Crystal Structures of Human DNA Polymerase Beta Complexed with DNA: Implications for Catalytic Mechanism, Processivity, and Fidelity. *Biochemistry* 1996;35:12742–12761. [PubMed: 8841118]
75. Singhal RK, Wilson SH. Short Gap-filling Synthesis by DNA Polymerase beta Is Processive. *Journal of Biological Chemistry* 1993;268:15906–15911. [PubMed: 8340415]
76. Liu L, Komori K, Ishino S, Bocquier AA, Cann IKO, Kohda D, Ishino Y. The Archaeal DNA Primase. *J Biol Chem* 2001;276:45484–45490. [PubMed: 11584001]
77. Kato M, Frick DN, Lee J, Tabor S, Richardson CC, Ellenberger T. A Complex of the Bacteriophage T7 Primase-Helicase and DNA Polymerase Directs Primer Utilization. *J Biol Chem* 2001;276:21809–21820. [PubMed: 11279245]
78. Keller KE, Cavanaugh NA, Kuchta RD. Interaction of Herpes Primase with the Sugar of a NTP. *Biochemistry* 2008;47:8977–8984. [PubMed: 18672908]
79. Kato M, Ito T, Wagner G, Ellenberger T. A Molecular Handoff between Bacteriophage T7 DNA Primase and T7 DNA Polymerase Initiates DNA Synthesis. *J Biol Chem* 2004;279:30554–30562. [PubMed: 15133047]
80. Sheaff, R. The DNA Polymerase alpha-primase Catalytic Duet. University of Colorado; 1994.
81. Yuzhakov A, Kelman Z, O'Donnell M. Trading Places on DNA - A Three-point Switch Underlies Primer Handoff from Primase to the Replicative DNA Polymerase. *Cell* 1999;96:153–163. [PubMed: 9989506]
82. Marsden HS, Cross AM, Francis GJ, Patel AJ, MacEachran K, Murphy M, McVey G, Haydon D, Abbotts A, Stow ND. The Herpes Simplex Virus Type 1 UL8 Protein Influences the Intracellular Localization of the UL52 but not the ICP8 or POL Replication Proteins in Virus-Infected Cells. *J Gen Virol* 1996;77:2241–2249. [PubMed: 8811024]
83. Cavanaugh NA, Kuchta RD. Initiation of New DNA Strands by the Herpes Primase-Helicase Complex and either Herpes DNA Polymerase or Human DNA Polymerase alpha. *J Biol Chem* 2009;284:1523–1532. [PubMed: 19028696]
84. Cotterill S, Chui G, Lehman IR. DNA Polymerase-Primase from Embryos of *Drosophila melanogaster*. *Journal of Biological Chemistry* 1987;262:16105–16108. [PubMed: 3119584]
85. Sheaff R, Kuchta RD. Misincorporation of Nucleotides by Calf Thymus DNA Primase and Elongation of Primers Containing Multiple Non-cognate Nucleotides by DNA Polymerase Alpha. *Journal of Biological Chemistry* 1994;269:19225–19231. [PubMed: 8034683]
86. Moore CL, Chiaramonte M, Higgins T, Kuchta RD. Synthesis of Nucleotide Analogs That Potently and Selectively Inhibit Human DNA Primase. *Biochemistry* 2002;41:14066–14075. [PubMed: 12437364]
87. Moore CL, Zivkovic A, Engels J, Kuchta RD. Human DNA Primase Uses Watson-Crick Hydrogen Bonding Groups to Distinguish between Correct and Incorrect NTPs. *Biochemistry* 2004;43:12367–12374. [PubMed: 15379576]
88. Ramirez-Aguilar KA, Moore CL, Kuchta RD. Herpes Simplex Virus I Primase Employs Watson-Crick Hydrogen Bonding to Identify Cognate NTPs. *Biochemistry* 2005;44:15585–15593. [PubMed: 16300408]
89. Kuchta RD, Ilesley D, Kravig KD, Schubert S, Harris B. Inhibition of DNA Primase and Polymerase Alpha by Arabinofuranosyl nucleoside Triphosphates and Related Compounds. *Biochemistry* 1992;31:4720–4728. [PubMed: 1581321]
90. Parker WB, Cheng YC. Inhibition of DNA Primase by Nucleoside Triphosphates and Their Arabinofuranosyl Analogs. *Mol Pharmacol* 1987;31:146–151. [PubMed: 3807892]

91. Crute JJ, Ctygon CA, Hargrave KD, Simoneau B, Faucher AM, Bolger G, Kibler P, Liuzzi M, Cordingley MG. Herpes Simplex Virus Helicase-primase Inhibitors Are Active in Animal Models of Human Disease. *Nature Medicine* 2002;8:386–391.
92. Kleymann G, Fischer R, Betz UA, Hendrix M, Bender W, Schneider U, Handke G, Eckenberg P, Hewlett G, Pevzner V, Baumeister J, Weber O, Henninger K, Keldenich J, Jensen A, Kolb J, Bach U, Popp A, Maben J, Frappa I, Haebich D, Lockhoff O, Rubsamen-Waigmann H. New Helicase-primase Inhibitors as Drug Candidates for the Treatment of Herpes Simplex Disease. *Nature Medicine* 2002;9:392–398.
93. Baumeister J, Fischer R, Eckenberg P, Henninger K, Rubsamen-Waigmann H, Kleymann G. Superior Efficacy of Helicase-Primase Inhibitor BAY57-1293 for Herpes Infection and Latency in the Guinea Pig Model of Human Genital Herpes Disease. *Antivir Chem Chemother* 2007;18:35–48. [PubMed: 17354650]
94. Wilson EM, Trauner D. Concise Synthesis of the Bacterial DNA Primase Inhibitor (+)-Sch642305. *Org Lett* 2007;9:1327–1329. [PubMed: 17338537]

Figure 1.

Structures of the RNA polymerase domains (RPD) of bacterial and phage primases. **A.** Solvent accessible surface of the *E. coli* DnaG RPD, emphasizing the overall almond-like shape of these enzymes. A short piece of single-stranded DNA (shown as stick model) occupies the putative template binding groove. The RPD's of *E. coli* DnaG primase (**B**; 3B39.pdb), *A. aeolicus* DnaG primase (**C**; 2AU3.pdb) and bacteriophage T7 primase (**D**; 1NUI.pdb) are displayed as ribbon models using identical colors for structurally homologous domains. The toprim fold and the N-terminal RPD subdomain 1 are presented in grey and green, respectively. The C-terminal RPD subdomain 3 is highlighted in orange. The oxygens of putative active site residues are shown as red sticks. The structures of *A. aeolicus* and T7 feature a zinc binding domain (ZBD; purple) that is also present in *E. coli* but so far has not been resolved by X

crystallography. Interestingly, the ZBD of *A. aeolicus* binds tightly to the rear of the associated polymerase domain, whereas the ZBD of T7 adopts an uncoupled and extended conformation.

Figure 2.

Archaeal primase fold. Ribbon presentations of the small primase subunits of *Sulfolobus solfataricus* (*Sso*, pdb entry: 1ZTZ) and *Pyrococcus horikoshii* (*Pho*, pdb entry: 1V34). The β sheets are colored blue, the helical regions grey. *Pho* has a helical domain (magenta) that *Sso* is mostly lacking. The *Pho* structure contains a UTP (orange sticks), Zn^{2+} ions are shown in green. The expected catalytic triads are depicted as grey sticks with oxygens in red (*Sso*: D101, D103 and D235; *Pho*: D95, D97 and D280).

Figure 3. Model of the T7 primase-helicase hexamer with a Zn²⁺ binding domain of primase-helicase interacting with the neighboring primase-helicase at a 3'-CTG initiation site.

Figure 4. Hinge model for counting by primase where the two subunits either begin in a “closed” complex and transition to an open complex (Top) or vice versa (Bottom).

Figure 5. Model of one subunit of the hexameric *E. coli* helicase and one subunit of the trimeric primase bound to DNA at an initiation site via a *trans* interaction between the Zn²⁺ binding domain of one primase molecule and the polymerase domain of a neighboring primase monomer.

Figure 6.
The subunit contacts in pol α -primase.

Figure 7.

The three-point hand off in transferring primers from *E. coli* primase to the processivity factor and polymerase. Primase binds and then synthesizes a primer on ssb coated DNA (black line). The γ -complex then loads the β clamp, followed by DNA polymerase binding.

Chart 1.
Examples of bases that human and herpes primase do not efficiently polymerize.

Chart 2.
Examples of bases that herpes and human primase efficiently polymerize.

Chart 3.
Structures of human primase inhibitors (Top) and herpes primase inhibitors (Bottom).

Table 1

Sequence alignments of pol β and primase are from [13], while the alignments of the archaeal primases are from [27]. Small subunit. The amino acids in yellow show the conserved amino acids in the catalytic core. Green show additional highly conserved amino acids in the archaeal and eukaryotic primases, while blue shows highly conserved amino acids in X-family polymerases. Large subunit. Amino acids in yellow form the hydrophobic core of the 8 kDa domain of pol β , while amino acids in blue show other highly conserved amino acids. The secondary structural units of pol β 's 8 kDa domain are shown above the large subunit. FL = flexible linker.

Small subunit

Pol β	Rat	(178)	CGSFRR-GAESSGDVDMLL	(251)	PHRRIDIRLIP
	Human	(178)	CGSFRR-GAESSGDVDMLL	(251)	PHRRIDIRLIP
	X. laev.	(178)	CGSFRR-GAESSGDVDMLL	(251)	PYRRIDIRLIP
Primase (small subunit)	Human	(96)	LGAFQAQEKELVFDIDMTD	(301)	CFPRLDINVSK
	Mouse	(96)	LGAFQAQEKELVFDIDMTD	(301)	CFPRLDINVSK
	S. cere.	(98)	KSELKALEKELVFDIDMDD	(309)	LYPKLDVEVTK
	D. mela.	(114)	PGGLTPVQRELVFDIDMTD	(329)	LYPRLDINVTR
	M. jann.	(87)	KGIFRR---ELAFDIDVHK	(246)	NKIELDEKVM
	A. fulg.	(114)	WGCENFLGQELAFDIDPEN	(215)	SHYAIDEWVTN
	P. hori.	(85)	EGWLGTGTELVDIDAKD	(271)	SY--FDGRVTV

Large subunit

		α Helix-B	Turn/Loop	α Helix-C	Loop 3	α Helix-D	FL	α Helix-E
Rat	(39)	YRKAASVIAKYPHKIKSG---	AEAKKLPGVGT	KIAEKI----	DEFLATGKLR--	KLEKIR		
Human	(39)	YRKAASVIAKYPHKIKSG---	AEAKKLPGVGT	KIAEKI----	DEFLATGKLR--	KLEKIR		
X. Laev	(39)	YRKAASVIAKYPTKIKSG---	TEAKKLPGVGT	KIAEKI----	DEFLATGKLR--	KLEKIR		
Mouse	(288)	MRQLHKAL-RENHHLRHGGRMQYGLFLKGIGLTLEQALQFWKQEFI-	KGKMDPDKFDKGY					
Human	(288)	MRQLHKAL-RENHHLRHGGRMQYGLFLKGIGLTLEQALQFWKQEFI-	KGKMDPDKFDKGY					
S. cere.	(337)	IKNLMEGL-KKNHHLRYYGRQQLSLFLKGIGLSADEALKFWSEAFTRNGNMTMEKFNKEY						
M. Jann.	(252)	IRGILNDILSGGSPSHYARRSFVVYWF	CRFIDELIEMLFKNVEDFDEKKTRY	YYIMHNIGY				
P. hori	(232)	IKNALKGVPQGIRNYAITVLLTSF	LSYVITEEILPIII	IEAANRCSPL	FEDQPNEIKNIW			

Table 2

Initiation sites used by primase from different sources. Pu = purine, Py = pyrimidine, N = any base ppp = the triphosphate on the 5'-end of the primer. Those bases that are required but do not code for a base in the primer ("cryptic" nucleotides) are underlined and in bold.

Organism	Template Sequence	Primer Synthesized
Herpes simplex-1	3'- <u>G</u> PyPy	pppPuPu
T4	3'- <u>T</u> TG	pppAC
T7	3'- <u>C</u> TG	pppAC
<i>E. coli</i>	3'- <u>G</u> TC	pppAG
<i>S. aureus</i>	3'- <u>A</u> TPy	pppAPu
<i>S. aureus</i>	3'- <u>A</u> TPy	pppAPu
<i>A. aeolicus</i>	3'- <u>C</u> CC, <u>C</u> CG, <u>C</u> GC	pppGG, pppGC, pppCG
<i>B. stearothermophilus</i>	3'- <u>A</u> TPy	pppAPu
Human	3'-PyNN	pppPuNN
Calf	3'-PyNN	pppPuNN