Elongation of replication

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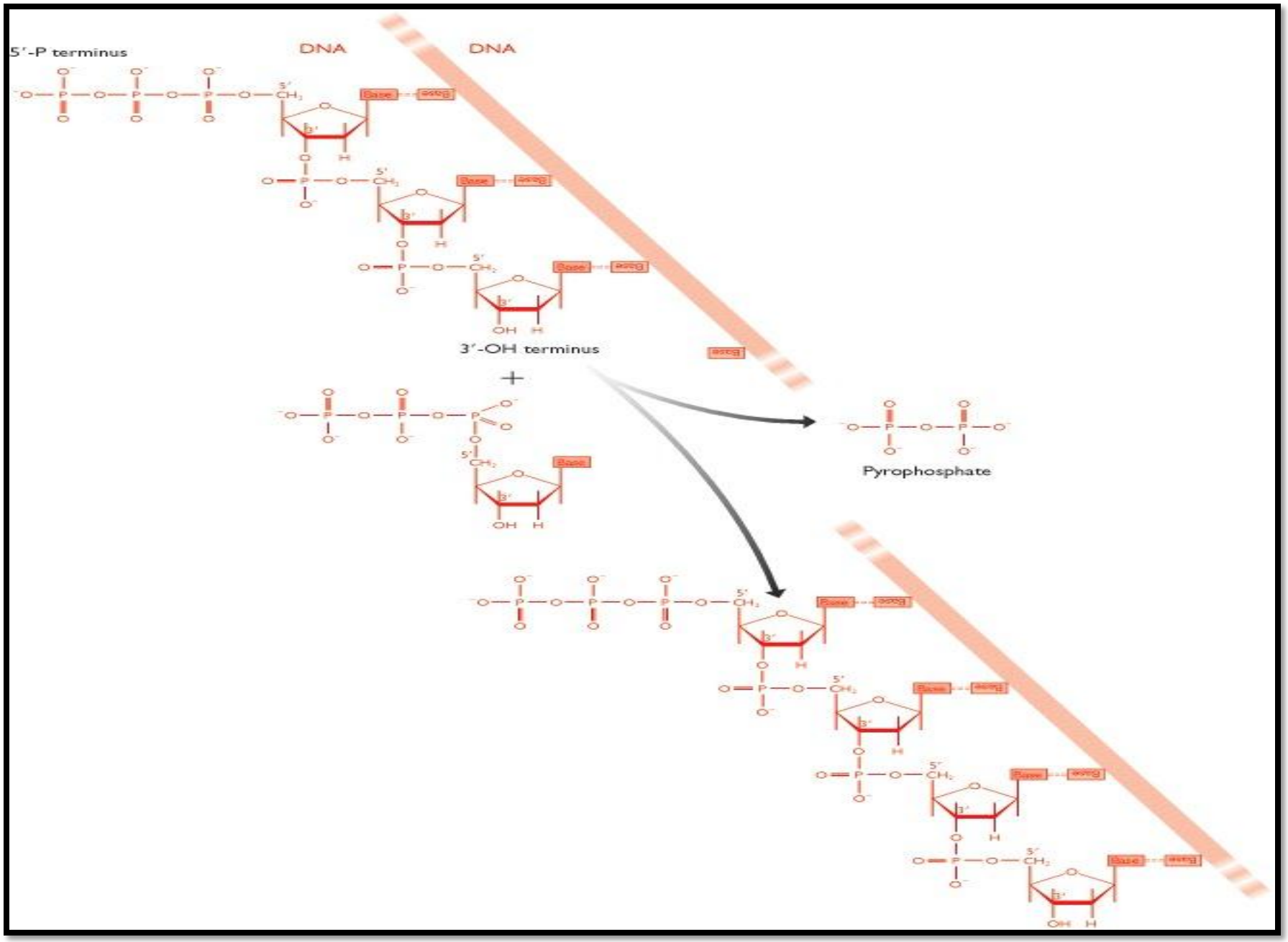
Template dependent synthesis of DNA

- Once replication has been initiated, the replication forks progress along the DNA and participate in the central activity of genome replication - the synthesis of new strands of DNA that are complementary to the parent polynucleotides.
- A template-dependent DNA polymerase makes a new DNA polynucleotide whose sequence is dictated, via the base-pairing rules, by the sequence of nucleotides in the DNA molecule that is being copied.
- At the chemical level the template-dependent synthesis of DNA is very similar to the template-dependent synthesis of RNA that occurs during transcription.


5′-P terminus
DNA
DNA
3′-OH terminus
Pyrophosphate

DNA replication vs Transcription

- During DNA replication both strands of the double helix must be copied. This is an important complication because DNA polymerase enzymes are only able to synthesize DNA in the 5′→3′ direction. This means that one strand of the parent double helix, called the leading strand, can be copied in a continuous manner, but replication of the lagging strand has to be carried out in a discontinuous fashion, resulting in a series of short segments that must be ligated together to produce the intact daughter strand.
- The second complication arises because template-dependent DNA polymerases cannot initiate DNA synthesis on a molecule that is entirely single-stranded: there must be a short double-stranded region to provide a 3′ end onto which the enzyme can add new nucleotides. This means that primers are needed, one to initiate complementary strand synthesis on the leading polynucleotide, and one for every segment of discontinuous DNA synthesized on the lagging strand.

DNA Polymerase

- An enzyme that synthesizes DNA is called a DNA polymerase and one that copies an existing DNA or RNA molecule is called a template-dependent DNA polymerase.
  - A DNA polymerase is unable to use an entirely single-stranded molecule as the template. In order to initiate DNA synthesis there must be a short double-stranded region to provide a 3’ end onto which the enzyme will add new nucleotides.

DNA Polymerase

- A second general feature of template-dependent DNA polymerases is that many of these enzymes are multifunctional, being able to degrade DNA molecules as well as synthesize them. As well as their 5′→3′ DNA synthesis capability, DNA polymerases can also have one or both of the following exonuclease activities:
  - A 3′→5′ exonuclease is possessed by many bacterial and eukaryotic template-dependent DNA polymerases.This activity enables the enzyme to remove nucleotides from the 3′ end of the strand that it has just synthesized. It is looked on as a proofreading activity whose function is to correct the occasional base-pairing error that might occur during strand synthesis .
  - A 5′→3′ exonuclease activity is less common but is possessed by some polymerases whose function in replication requires that they must be able to remove at least part of a polynucleotide that is already attached to the template strand that the polymerase is copying. This activity is utilized during the process that joins together the discontinuous DNA fragments synthesized on the lagging strand during bacterial DNA replication.

DNA polymerase of Bacteria

- DNA Polymerase I
- DNA Polymerase II
- DNA Polymerase III
- DNA Polymerase IV
- DNA Polymerase V

DNA Polymerase I

- DNA Polymerase I (or Pol I) is an enzyme that participates in the process of DNA replication. Discovered by Arthur Kornberg in 1956, it was the first known DNA polymerase (and, indeed, the first known of any kind of polymerase). It was initially characterized in E. coli and is ubiquitous in prokaryotes.
- In E. coli and many other bacteria, the gene that encodes Pol I is known as polA. The E. coli form of the enzyme is composed of 928 amino acids, and is an example of a processive enzyme—it can sequentially catalyze multiple polymerisations.
- Pol I possesses four enzymatic activities:
  - A 5'→3' (forward) DNA-Dependent DNA polymerase activity, requiring a 3' primer site and a template strand
  - A 3'→5' (reverse) exonuclease activity that mediates proofreading
  - A 5'→3' (forward) exonuclease activity mediating nick translation during DNA repair.
  - A 5'→3' (forward) RNA-Dependent DNA polymerase activity. Pol I operates on RNA templates with considerably lower efficiency (0.1–0.4%) than it does DNA templates, and this activity is probably of only limited biological significance.

DNA Polymerase II

- DNA polymerase II (also known as DNA Pol II or Pol II) is a prokaryotic DNA-Dependent DNA polymerase encoded by the PolB gene.

- DNA Polymerase II is an 89.9-kDa protein and is a member of the B family of DNA polymerases. It was originally isolated by Thomas Kornberg in 1970, and characterized over the next few years.

- The in vivo functionality of Pol II is under debate, yet consensus shows that Pol II is primarily involved as a backup enzyme in prokaryotic DNA replication. The enzyme has 5'->3' DNA synthesis capability as well as 3'->5' exonuclease proofreading activity. DNA Pol II interacts with multiple binding partners common with DNA Pol III in order to enhance its fidelity and processivity.

- DNA Pol II does participate in DNA replication. While it might not be as fast as DNA Pol III, it has some abilities that make it an effective enzyme. DNA Pol II is a high fidelity enzyme with an error rate of substitution: ≤0.2*10-5 and -1 deletions: ≤ 0.1*10-5. DNA Pol II can proofread and process mismatches caused by the Pol III.

- **DNA Pol II is involved with replication but it is strand dependent and preferentially replicates the lagging strand.** A proposed mechanism suggests that when DNA Pol III stalls or becomes non-functional, then DNA Pol II is able to be specifically recruited to the replication point and continue replication.

DNA Polymerase III

- DNA polymerase III holoenzyme is the primary enzyme complex involved in prokaryotic DNA replication. It was discovered by Thomas Kornberg (son of Arthur Kornberg) and Malcolm Gefter in 1970.
- The complex has high processivity (i.e. the number of nucleotides added per binding event) and, specifically referring to the replication of the E.coli genome, works in conjunction with four other DNA polymerases (Pol I, Pol II, Pol IV, and Pol V).
- Being the primary holoenzyme involved in replication activity, the DNA Pol III holoenzyme also has proofreading capabilities that correct replication mistakes by means of exonuclease activity working 3'→5'. DNA Pol III is a component of the replisome, which is located at the replication fork.

DNA Polymerase IV

- DNA polymerase IV is a prokaryotic polymerase that is involved in mutagenesis. It exhibits no 3'-5' exonuclease (proofreading) activity and hence is error prone. In E. coli, DNA polymerase IV (Pol 4) is involved in non-targeted mutagenesis.
- Pol IV is a Family Y polymerase expressed by the dinB gene that is switched on via SOS induction caused by stalled polymerases at the replication fork. During SOS induction, Pol IV production is increased tenfold and one of the functions during this time is to interfere with Pol III holoenzyme processivity. This creates a checkpoint, stops replication, and allows time to repair DNA lesions via the appropriate repair pathway.
- Another function of Pol IV is to perform **translesion synthesis** at the stalled replication fork like, for example, bypassing N2-deoxyguanine adducts at a faster rate than transversing undamaged DNA. Cells lacking dinB gene have a higher rate of mutagenesis caused by DNA damaging agents.

DNA Polymerase V

- DNA Polymerase V (Pol V) is a polymerase enzyme involved in **DNA repair mechanisms in the bacteria *Escherichia coli*.** It is composed of a UmuD' homodimer and a UmuC monomer, forming the UmuD'2C protein complex.It is part of the Y-family of DNA Polymerases, which are capable of performing DNA translesion synthesis (TLS).
- Translesion polymerases bypass DNA damage lesions during DNA replication, if a lesion is not repaired or bypassed the replication fork can stall and lead to cell death. However, Y polymerases have low sequence fidelity during replication (prone to add wrong nucleotides).
- When the UmuC and UmuD' proteins were initially discovered in E. coli, they were thought to be agents that inhibit faithful DNA replication and caused DNA synthesis to have high mutation rates after exposure to UV-light.The polymerase function of Pol V was not discovered until the late 1990s when UmuC was successfully extracted, consequent experiments unequivocally proved UmuD'2C is a polymerase. This finding lead to the detection of many Pol V orthologs and the discovery of the Y-family of polymerases.

DNA polymerase of eukaryotes

- **Family A polymerases: Polymerases γ and θ (gamma and theta)**
- Family B Polymerases: Polymerases α, δ, ε, Rev1 and ζ (alpha, delta, epsilon and zeta)
- Family X polymerases: Polymerases β, λ, σ, μ and Terminal deoxynucleotidyl transferase(beta, lambda, sigma, mu and Tdt)
- Family Y DNA polymerases: Polymerases η, ι and κ (eta, iota, and kappa)
- Telomerase

Polymerases γ and θ

- Pol γ (gamma) and Pol θ (theta) are Family A polymerases. Pol γ, encoded by the POLG gene, is the only mtDNA polymerase and therefore replicates, repairs, and has proofreading 3'-5' exonuclease and 5' dRP lyase activities. Any mutation that leads to limited or non-functioning Pol γ has a significant effect on mtDNA and is the most common cause of autosomal inherited mitochondrial disorders. Pol γ contains a C-terminus polymerase domain and an N-terminus 3'-5' exonuclease domain that are connected via the linker region, which binds the accessory subunit. The accessory subunit binds DNA and is required for processivity of Pol γ. Point mutation A467T in the linker region is responsible for more than one-third of all Pol γ-associated mitochondrial disorders.
- While many homologs of Pol θ, encoded by the POLQ gene, are found in eukaryotes, its function is not clearly understood. The sequence of amino acids in the C-terminus is what classifies Pol θ as Family A polymerase, although the error rate for Pol θ is more closely related to Family Y polymerases. Pol θ extends mismatched primer termini and can bypass abasic sites by adding a nucleotide. It also has Deoxyribophosphodiesterase (dRPase) activity in the polymerase domain and can show ATPase activity in close proximity to ssDNA

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Polymerase α (alpha)

- DNA polymerase alpha also known as Pol α is an enzyme complex found in eukaryotes that is involved in initiation of DNA replication.
- **Pol α has limited processivity and lacks 3′ exonuclease activity for proofreading errors.** Thus it is not well suited to efficiently and accurately copy long templates (unlike Pol Delta and Epsilon). Instead it plays a more limited role in replication.
- **Pol α is responsible for the initiation of DNA replication at origins of replication** (on both the leading and lagging strands) and during synthesis of Okazaki fragments on the lagging strand.
- The Pol α complex (pol α-DNA primase complex) consists of four subunits: the catalytic subunit POLA1, the regulatory subunit POLA2, and the small and the large primase subunits PRIM1 and PRIM2 respectively.
- Once primase has created the RNA primer, Pol α starts replication elongating the primer with ~20 nucleotides.

Polymerase δ (delta)

- DNA polymerase delta is an enzyme complex found in eukaryotes that is involved in DNA replication and repair. The DNA polymerase delta complex consists of 4 subunits: POLD1, POLD2, POLD3, and POLD4.
- **DNA Pol δ is the enzyme used primarily for leading and lagging strand synthesis.** It exhibits increased processivity when interacting with the proliferating cell nuclear antigen (PCNA).
- The multisubunit protein replication factor C, through its role as the clamp loader for PCNA (which involves catalysing the loading of PCNA on to DNA) is important for DNA Pol δ function.

Polymerase ε (epsilon)

- DNA polymerase epsilon is a member of the DNA polymerase family of enzymes. It is composed of the following four subunits: POLE (central catalytic unit), POLE2 (subunit 2), POLE3 (subunit 3), and POLE4 (subunit 4).
- Recent evidence suggests that it **plays a minor role in DNA replication,** and is mostly involved in correcting errors made by DNA polymerase delta during leading strand replication.

Polymerases Rev1 and ζ (zeta)

- Pol ζ another B family polymerase, is made of two subunits Rev3, the catalytic subunit, and Rev7, which increases the catalytic function of the polymerase, and is involved in translesion synthesis. Pol ζ lacks 3' to 5' exonuclease activity, is unique in that it can extend primers with terminal mismatches.
- Rev1 has three regions of interest in the BRCT domain, ubiquitin-binding domain, and C-terminal domain and has dCMP transferase ability, which adds deoxycytidine opposite lesions that would stall replicative polymerases Pol δ and Pol ε. These stalled polymerases activate ubiquitin complexes that in turn disassociate replication polymerases and recruit Pol ζ and Rev1.
- Together Pol ζ and Rev1 add deoxycytidine and Pol ζ extends past the lesion. Through a yet undetermined process, Pol ζ disassociates and replication polymerases reassociate and continue replication. Pol ζ and Rev1 are not required for replication, but loss of REV3 gene in budding yeast can cause increased sensitivity to DNA-damaging agents due to collapse of replication forks where replication polymerases have stalled.

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Polymerases β(beta)

- Polymerase (DNA directed), beta, also known as POLB, is an enzyme that, in humans, is encoded by the POLB gene.
- In eukaryotic cells, DNA polymerase beta (POLB) performs base excision repair (BER) required for DNA maintenance, replication, recombination, and drug resistance.

Polymerases λ (lambda)

- DNA polymerase lambda, also known as POLL, is a protein that, in humans, is encoded by the POLLA gene.
- Pol λ is a member of the X family of DNA polymerases. It is thought to resynthesize missing nucleotides during non-homologous end joining, a pathway of DNA double-strand break repair.
- The crystal structure of pol λ shows that, unlike the DNA polymerases that catalyze DNA replication, pol λ makes extensive contacts with the 5' phosphate of the downstream DNA strand. This allows the polymerase to stabilize the two ends of a double-strand break and explains how pol λ is uniquely suited for a role in non-homologous end joining.
- In addition to NHEJ, pol λ can also participate in base excision repair, where it provides backup activity in the absence of Pol β

Polymerases μ (mu)

- DNA polymerase mu is a human protein encoded by the POLM gene.
- Pol μ is a member of the X family of DNA polymerases. It participates in resynthesis of damaged or missing nucleotides during the non-homologous end joining (NHEJ) pathway of DNA repair.
- Pol μ interacts with Ku and DNA ligase IV, which also participate in NHEJ. It is structurally and functionally related to pol λ, and, like pol λ, pol μ has a BRCT domain that is thought to mediate interactions with other DNA repair proteins.
- Unlike pol λ, however, pol μ has the unique ability to add a base to a blunt end that is templated by the overhang on the opposite end of the double-strand break.
- Pol μ is also closely related to terminal deoxynucleotidyl transferase (TdT), a specialized DNA polymerase that adds random nucleotides to DNA ends during V(D)J recombination, the process by which B-cell and T-cell receptor diversity is generated in the vertebrate immune system. Like TdT, pol μ participates in V(D)J recombination, but only during heavy chain rearrangements.This is distinct from pol λ, which is involved in light chain rearrangements.

Polymerases Terminal deoxynucleotidyl transferase(Tdt)

- Terminal deoxynucleotidyl transferase (TdT), also known as DNA nucleotidylexotransferase (DNTT) or terminal transferase, is a specialized DNA polymerase expressed in immature, pre-B, pre-T lymphoid cells, and acute lymphoblastic leukemia/lymphoma cells.
- TdT adds N-nucleotides to the V,D, and J exons of the TCR and BCR genes during antibody gene recombination, enabling the phenomenon of junctional diversity. In humans, terminal transferase is encoded by the DNTT gene.
- As a member of the X family of DNA polymerase enzymes, it works in conjunction with polymerase λ and polymerase μ, both of which belong to the same X family of polymerase enzymes. The diversity introduced by TdT has played an important role in the evolution of the vertebrate immune system, significantly increasing the variety of antigen receptors that a cell is equipped with to fight pathogens.
- Studies using TdT knockout mice have found drastic reductions (10-fold) in T-Cell receptor (TCR) diversity compared with that of normal, or wild-type, systems. The greater diversity of TCRs that an organism is equipped with leads to greater resistance to infection.
- TdT catalyses the addition of nucleotides to the 3' terminus of a DNA molecule. **Unlike most DNA polymerases, it does not require a template.** The preferred substrate of this enzyme is a 3'-overhang, but it can also add nucleotides to blunt or recessed 3' ends. Cobalt is a necessary cofactor, however the enzyme catalyzes reaction upon Mg and Mn administration in vitro.

DNA polymerase of eukaryotes

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- Telomerase

Polymerases η(eta)

- DNA polymerase eta (Pol η), is a protein that in humans is encoded by the POLH gene.

- DNA polymerase eta is a eukaryotic DNA polymerase involved in the DNA repair by translesion synthesis. The gene encoding DNA polymerase eta is POLH, also known as XPV, because loss of this gene results in the disease xeroderma pigmentosum. Polymerase eta is particularly important for allowing accurate translesion synthesis of DNA damage resulting from ultraviolet radiation or UV.

- POLH gene encodes a member of the Y family of specialized DNA polymerases. It copies undamaged DNA with a lower fidelity than other DNA-directed polymerases. However, it accurately replicates UV-damaged DNA; when thymine dimers are present, this polymerase inserts the complementary nucleotides in the newly synthesized DNA, thereby bypassing the lesion and suppressing the mutagenic effect of UV-induced DNA damage. This polymerase is thought to be involved in hypermutation during immunoglobulin class switch recombination. Mutations in this gene result in XPV, a variant type of xeroderma pigmentosum.

Polymerases ι (iota)

- DNA polymerase iota is an enzyme that in humans is encoded by the POLI gene. It is found in higher eukaryotes, and is believed to have arisen from a gene duplication from Pol η. Pol ι, is a Y family polymerase that is involved in translesion synthesis.
- It can bypass 6-4 pyrimidine adducts and abasic sites and has a high frequency of wrong base incorporation. Like many other Y family polymerases Pol ι, has low processivity, a large DNA binding pocket and doesn't undergo conformational changes when DNA binds.
- These attributes are what allow Pol ι to carry out its task as a translesion polymerase. Pol ι only uses Hoogsteen base pairing, during DNA synthesis, it will add adenine opposite to thymine in the syn conformation and can add both cytosine and thymine in the anti conformation across guanine, which it flips to the syn conformation.

Polymerases κ (kappa)

- DNA polymerase kappa is an enzyme that in humans is encoded by the POLK gene.

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- **Telomerase**

Telomerase

- Telomerase is a ribonucleoprotein recruited to replicate ends of linear chromosomes because normal DNA polymerase cannot replicate the ends, or telomere. The single-strand 3’ overhang of the double-strand chromosome with the sequence 5’-TTAGGG-3’ recruits telomerase.
- Telomerase acts like other DNA polymerases by extending the 3’ end, but, unlike other DNA polymerases, **telomerase does not require a template.**
- The TERT subunit, an example of a reverse transcriptase, uses the RNA subunit to form the primer–template junction that allows telomerase to extend the 3’ end of chromosome ends. The gradual decrease in size of telomeres as the result of many replications over a lifetime are thought to be associated with the effects of aging.
- Telomerase is a reverse transcriptase enzyme that carries its own RNA molecule (e.g., with the sequence "**CCCAAUCCC**" in vertebrates) which is used as a template when it elongates telomeres.

Reverse transcriptase

- Retroviruses encode an unusual DNA polymerase called reverse transcriptase, which is an RNA-dependent DNA polymerase (RdDp) that synthesizes DNA from a template of RNA. The reverse transcriptase family contain both DNA polymerase functionality and RNase H functionality, which degrades RNA base-paired to DNA.
- It is mainly associated with retroviruses. However, non-retroviruses also use RT (for example, the hepatitis B virus, a member of the Hepadnaviridae, which are dsDNA-RT viruses, while retroviruses are ssRNA viruses).
- Retroviral RT has three sequential biochemical activities:
  - RNA-dependent DNA polymerase activity,
  - Ribonuclease H, and
  - DNA-dependent DNA polymerase activity.

Priming of DNA synthesis

- DNA polymerases cannot begin synthesis on an entirely single-stranded template, but it may relate to the proofreading activity of these enzymes, which is essential for the accuracy of replication.
- Priming is a necessity in DNA replication but does not present too much of a problem. Although DNA polymerases cannot deal with an entirely single-stranded template, RNA polymerases have no difficulty in this respect, so the primers for DNA replication are made of RNA.

Priming of DNA synthesis

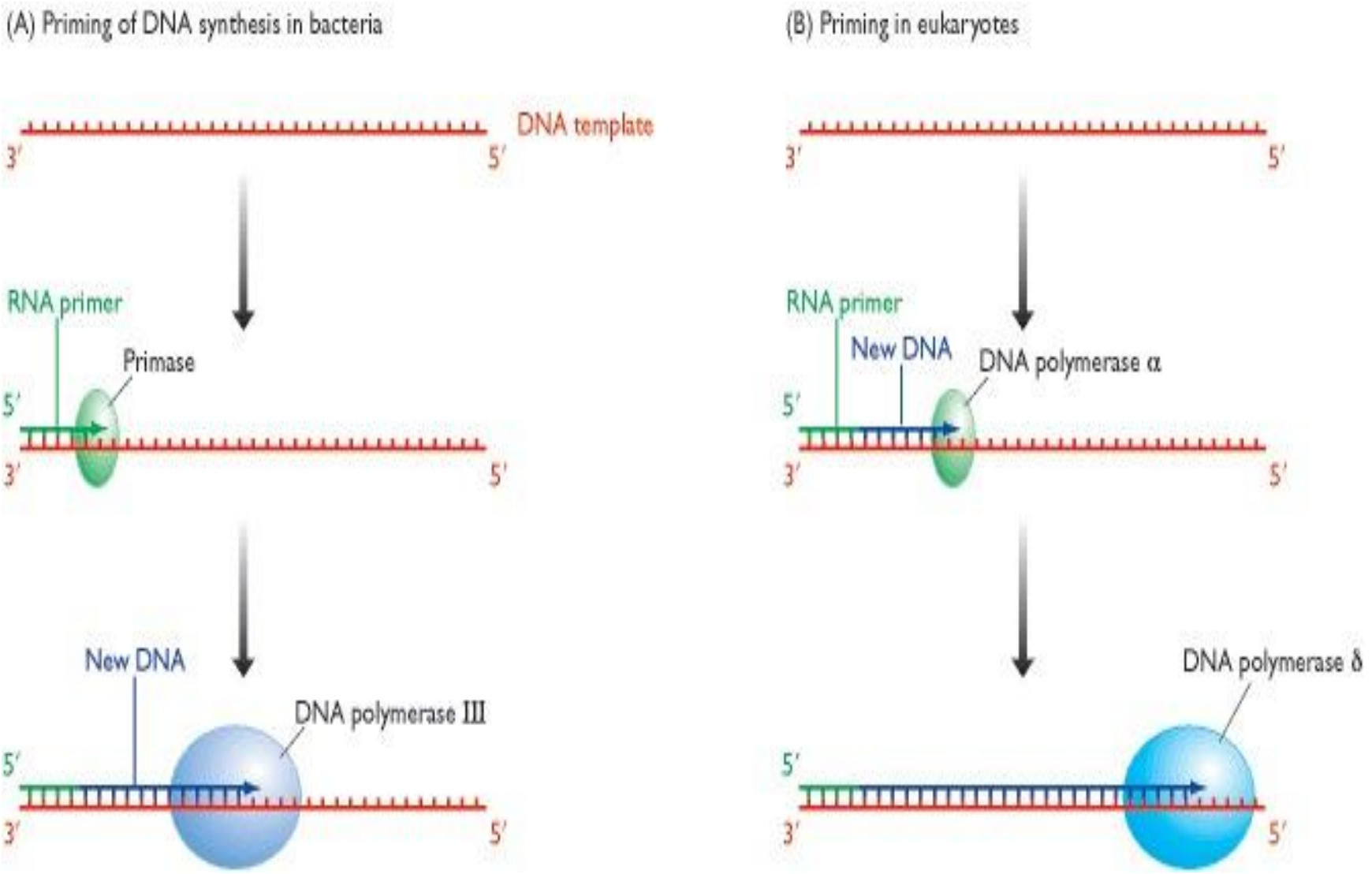
- Priming needs to occur just once on the leading strand, within the replication origin, because once primed, the leading-strand copy is synthesized continuously until replication is completed. On the lagging strand, priming is a repeated process that must occur every time a new Okazaki fragment is initiated.
- In E. coli, which makes Okazaki fragments of 1000–2000 nucleotides in length, approximately 4000 priming events are needed every time the genome is replicated. In eukaryotes the Okazaki fragments are much shorter and priming is a highly repetitive event.

Priming of DNA synthesis in bacteria

- In bacteria, primers are synthesized by primase, a special RNA polymerase unrelated to the transcribing enzyme, with each primer 4–15 nucleotides in length. Once the primer has been completed, strand synthesis is continued by DNA polymerase III.

Priming of DNA synthesis in eukaryotes

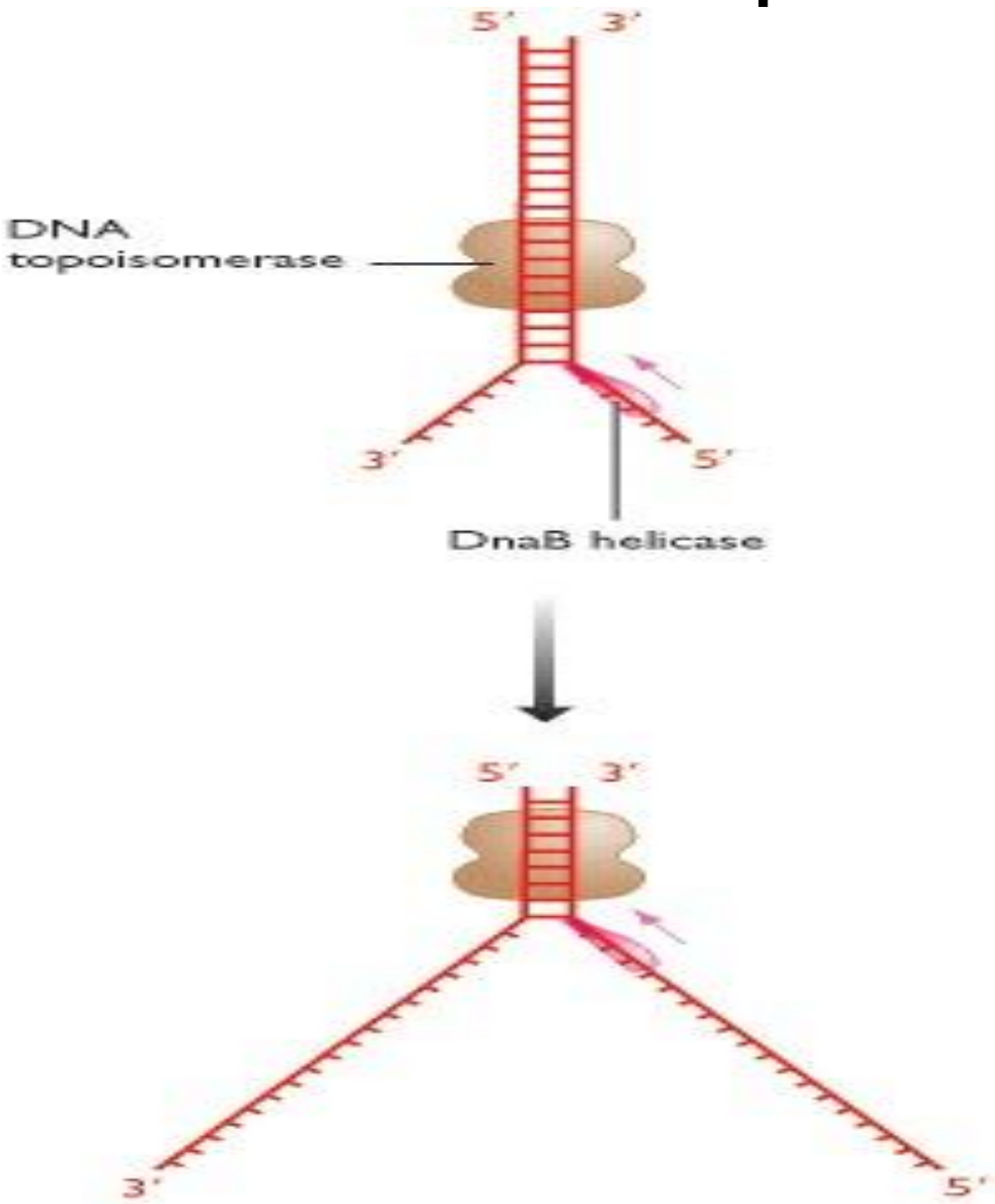
- In eukaryotes the situation is slightly more complex because the primase is tightly bound to DNA polymerase α, and cooperates with this enzyme in synthesis of the first few nucleotides of a new polynucleotide.
- This primase synthesizes an RNA primer of 8–12 nucleotides, and then hands over to DNA polymerase α, which extends the RNA primer by adding about 20 nucleotides of DNA.
- This DNA stretch often has a few ribonucleotides mixed in, but it is not clear if these are incorporated by DNA polymerase α or by intermittent activity of the primase. After completion of the RNA-DNA primer, DNA synthesis is continued by the main replicative enzyme, DNA polymerase δ.


(A) Priming of DNA synthesis in bacteria
DNA template
3′
5′
RNA primer
Primase
5′
3′
5′
New DNA
DNA polymerase III
5′
3′
5′
(B) Priming in eukaryotes
3′
5′
RNA primer
New DNA
DNA polymerase α
5′
3′
5′
DNA polymerase δ
5′
3′
5′

Events at the bacterial replication fork

- To a large extent, the division between initiation and elongation is artificial, the two processes running seamlessly one into the other.
- After the DnaB helicase has bound to the origin to form the prepriming complex, the primase is recruited, resulting in the primosome, which initiates replication of the leading strand. It does this by synthesizing the RNA primer that DNA polymerase III needs in order to begin copying the template

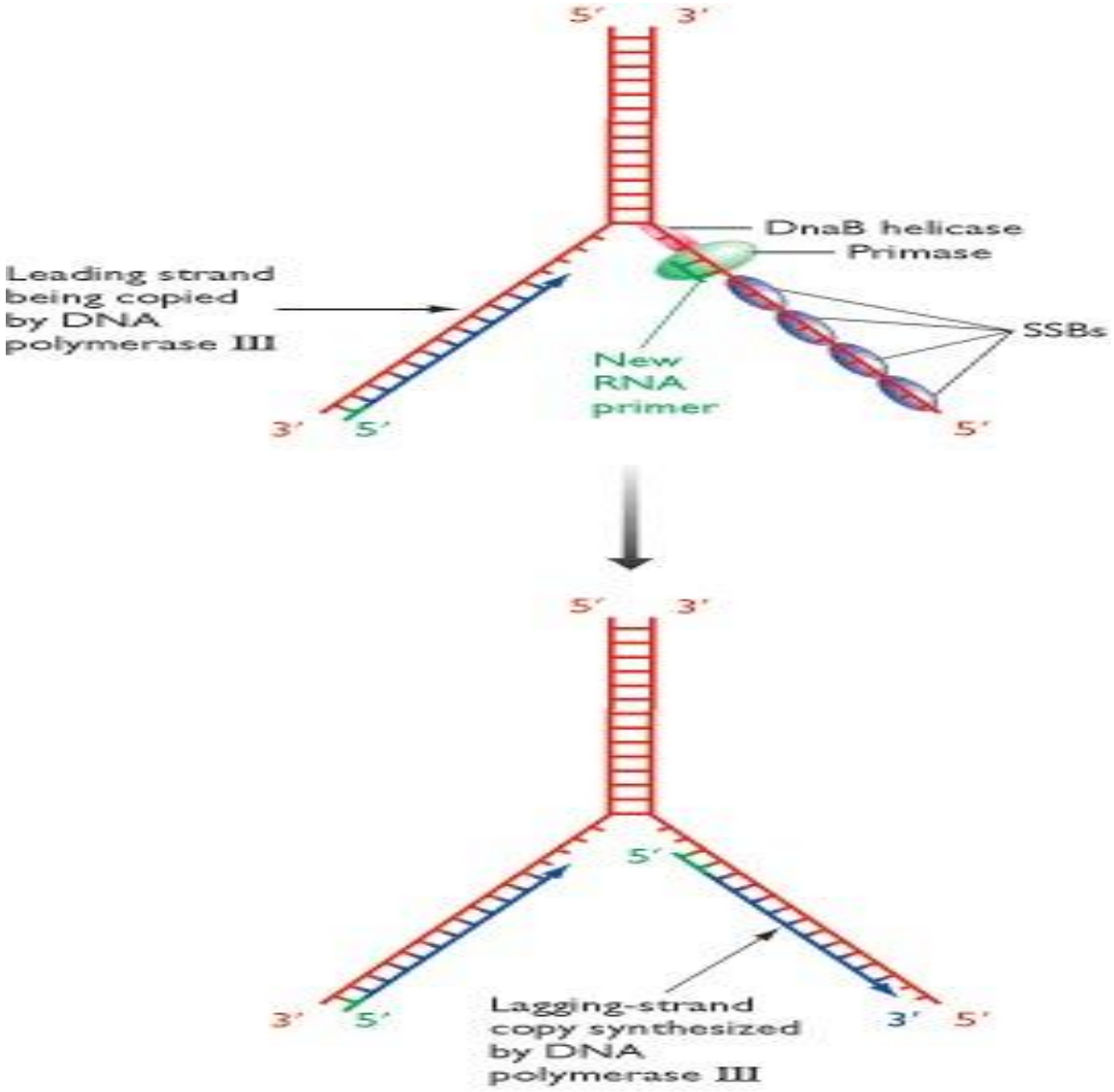
Events at the bacterial replication fork



Events at the bacterial replication fork

- Single-stranded DNA is naturally ‘sticky’ and the two separated polynucleotides produced by helicase action would immediately reform base pairs after the enzyme has passed, if allowed to. The single strands are also highly susceptible to nuclease attack and are likely to be degraded if not protected in some way.
- To avoid these unwanted outcomes, single-strand binding proteins (SSBs) attach to the polynucleotides and prevent them from reassociating or being degraded.
- The E. coli SSB is made up of four identical subunits and probably works in a similar way to the major eukaryotic SSB, called replication protein A (RPA), by enclosing the polynucleotide in a channel formed by a series of SSBs attached side by side on the strand Detachment of the SSBs, which must occur when the replication complex arrives to copy the single strands, is brought about by a second set of proteins called replication mediator proteins. As with helicases, SSBs have diverse roles in different processes involving DNA unwinding.

Events at the bacterial replication fork



Events at the bacterial replication fork

- The combination of the DNA polymerase III dimer and the primosome, migrating along the parent DNA and carrying out most of the replicative functions, is called the replisome.
- After its passage, the replication process must be completed by joining up the individual Okazaki fragments.
- DNA polymerase III releases the lagging strand and its place is taken by DNA polymerase I, which does have a 5′→3′ exonuclease and so removes the primer, and usually the start of the DNA component of the Okazaki fragment as well, extending the 3′ end of the adjacent fragment into the region of the template that is exposed.
- The two Okazaki fragments now abut, with the terminal regions of both composed entirely of DNA. All that remains is for the missing phosphodiester bond to be put in place by a DNA ligase, linking the two fragments and completing replication of this region of the lagging strand.

The eukaryotic replication fork: variations on the bacterial theme

- The progress of the replication fork in eukaryotes is maintained by helicase activity, although which of the several eukaryotic helicases that have been identified are primarily responsible for DNA unwinding during replication has not been established.
- The separated polynucleotides are prevented from reattaching by single-strand binding proteins, the main one of these in eukaryotes being Replication Protein A (RPA).

The eukaryotic replication fork: variations on the bacterial theme

- The eukaryotic DNA polymerase α cooperates with the primase enzyme to put in place the RNA-DNA primers at the start of the leading-strand copy and at the beginning of each Okazaki fragment.
- However, DNA polymerase α is not capable of lengthy DNA synthesis, presumably because it lacks the stabilizing effect of a sliding clamp equivalent to the β subunit of E. coli DNA polymerase III or the PCNA accessory protein that aids the eukaryotic DNA polymerase δ.
- This means that although DNA polymerase α can extend the initial RNA primer with about 20 nucleotides of DNA, it must then be replaced by the main replicative enzyme, DNA polymerase δ

The eukaryotic replication fork: variations on the bacterial theme

- The DNA polymerase enzymes that copy the leading and lagging strands in eukaryotes do not associate into a dimeric complex equivalent to the one formed by DNA polymerase III during replication in E. coli. Instead, the two copies of the polymerase remain separate.
- The function performed by the γ complex of the E. coli polymerase - controlling attachment and detachment of the enzyme from the lagging strand - appears to be carried out by a multi-subunit accessory protein called replication factor C (RFC).

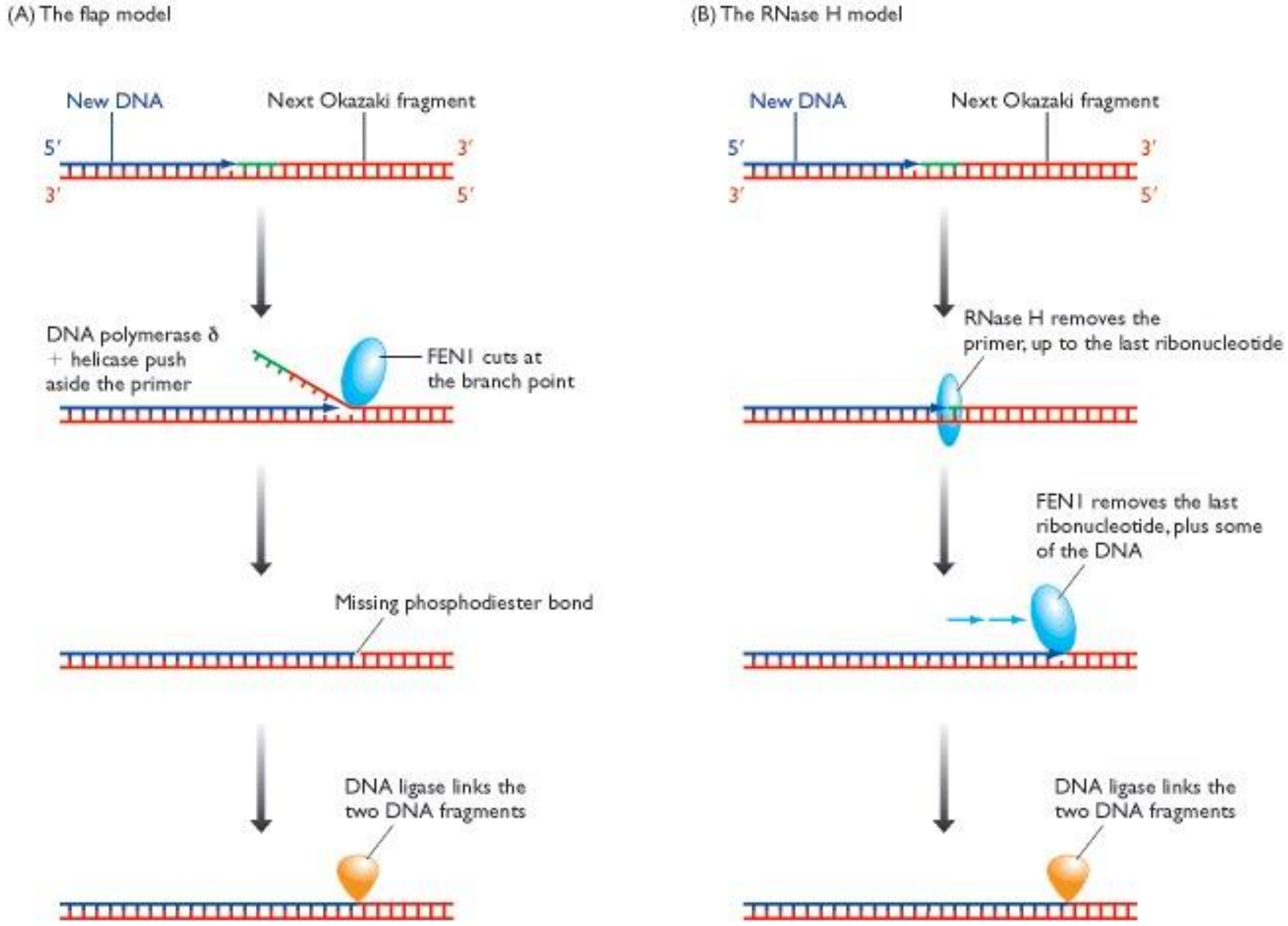
The eukaryotic replication fork: variations on the bacterial theme

- As in E. coli, completion of lagging-strand synthesis requires removal of the RNA primer from each Okazaki fragment. There appears to be no eukaryotic DNA polymerase with the 5′→3′ exonuclease needed for this purpose and the process is therefore very different to that described for bacterial cells.
- The central player is the ‘flap endonuclease’, FEN1 (previously called MF1), which associates with the DNA polymerase δ complex at the 3′ end of an Okazaki fragment, in order to degrade the primer from the 5′ end of the adjacent fragment. Understanding exactly how this occurs is complicated by the inability of FEN1 to initiate primer degradation because it is unable to remove the ribonucleotide at the extreme 5′ end of the primer, because this ribonucleotide carries a 5′-triphosphate group which blocks FEN1 activity.

The eukaryotic replication fork: variations on the bacterial theme

- Two alternative models to circumvent this problem have been proposed:
  - The first possibility is that a helicase breaks the base pairs holding the primer to the template strand, enabling the primer to be pushed aside by DNA polymerase δ as it extends the adjacent Okazaki fragment into the region thus exposed .
  - The flap that results can be cut off by FEN1, whose endonuclease activity can cleave the phosphodiester bond at the branch point where the displaced region attaches to the part of the fragment that is still base-paired.
  - Alternatively, most of the RNA component of the primer could be removed by RNase H, which can degrade the RNA part of a base-paired RNA-DNA hybrid, but cannot cleave the phosphodiester bond between the last ribonucleotide and the first deoxyribonucleotide. However, this ribonucleotide will carry a 5’-monophosphate rather than triphosphate and so can be removed by FEN1

The eukaryotic replication fork: variations on the bacterial theme



Termination of replication

- Replication forks proceed along linear genomes, or around circular ones, generally unimpeded except when a region that is being transcribed is encountered. DNA synthesis occurs at approximately five times the rate of RNA synthesis, so the replication complex can easily overtake an RNA polymerase, but this probably does not happen: instead it is thought that the replication fork pauses behind the RNA polymerase, proceeding only when the transcript has been completed.
- Eventually the replication fork reaches the end of the molecule or meets a second replication fork moving in the opposite direction. What happens next is one of the least understood aspects of genome replication.

Termination of *E. coli* genome

- Bacterial genomes are replicated bidirectionally from a single point, which means that the two replication forks should meet at a position diametrically opposite the origin of replication on the genome map. However, if one fork is delayed, possibly because it has to replicate extensive regions where transcription is occurring, then it might be possible for the other fork to overshoot the halfway point and continue replication on the 'other side' of the genome.
- It is not immediately apparent why this should be undesirable, the daughter molecules presumably being unaffected, but it is not allowed to happen because of the presence of terminator sequences. Seven of these have been identified in the E. coli genome, each one acting as the recognition site for a sequence-specific DNA-binding protein called Tus.

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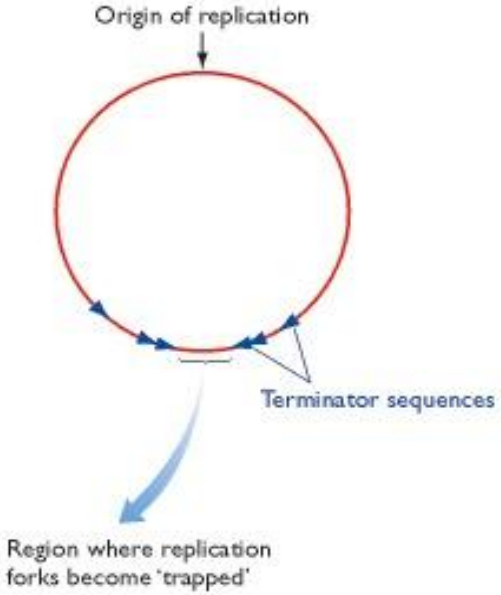
Termination of *E. coli* genome

- The mode of action of Tus is quite unusual. When bound to a terminator sequence, a Tus protein allows a replication fork to pass if the fork is moving in one direction, but blocks progress if the fork is moving in the opposite direction around the genome. The directionality is set by the orientation of the Tus protein on the double helix.
- When approached from one direction, Tus blocks the passage of the DnaB helicase, which is responsible for progression of the replication fork, because the helicase is faced with a 'wall' of β-strands which it is unable to penetrate. But when approaching from the other direction, DnaB is able to disrupt the structure of the Tus protein, probably because of the effect that unwinding of the double helix has on Tus, and so is able to pass by

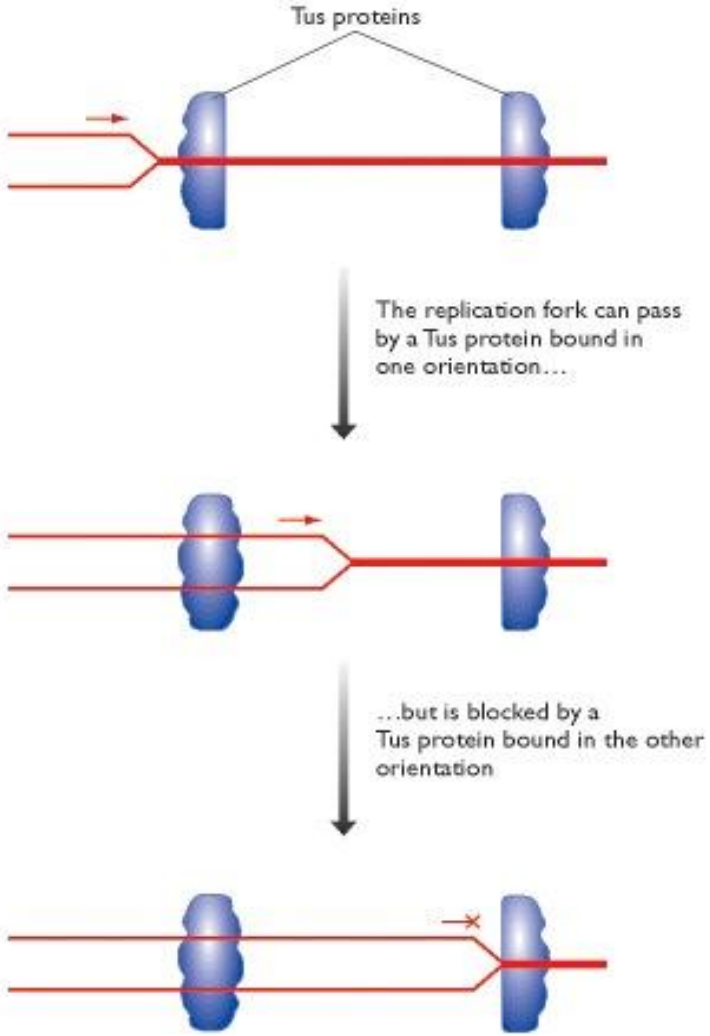
Termination of *E. coli* genome

- The orientation of the termination sequences, and hence of the bound Tus proteins, in the E. coli genome is such that both replication forks become trapped within a relatively short region on the opposite side of the genome to the origin .
- This ensures that termination always occurs at or near the same position. Exactly what happens when the two replication forks meet is unknown, but the event is followed by disassembly of the replisomes, either spontaneously or in a controlled fashion. The result is two interlinked daughter molecules, which are separated by topoisomerase IV.

(A) Terminator sequences in the *E. coli* genome



(B) The role of Tus



Termination of replication in eukaryotes

- No sequences equivalent to bacterial terminators are known in eukaryotes, and proteins similar to Tus have not been identified.
- Quite possibly, replication forks meet at random positions and termination simply involves ligation of the ends of the new polynucleotides.
- We do know that the replication complexes do not break down, because these factories are permanent features of the nucleus

Assignment

- Why DNA polymerase extends only from 5’ to 3’ direction ?[2.5]
- How is primer dependency of DNA polymerase helpful in Recombinant DNA technology ? [1]
- Why DNA polymerase require primer but RNA polymerase doesn’t ? [2.5]
- Write about different families and types of DNA polymerase.[7.5]
- Differentiate between DNA polymerase I and III. [2.5]
- How is DNA polymerase I helpful in prokaryotic DNA replication ?[1]
- Write about Translesion Polymerases. [2.5]
- What do you mean by processivity of DNA polymerase.[1]
- Write difference between prokaryotic and eukaryotic mode for elongation during replication. [2.5]

Upcoming Lecture

- Maintaining the ends of a linear DNA:
  - Okazaki fragments,
  - Synthesis of telomeric DNA,
  - Senescence of telomere length,
  - Extension of human chromosome by telomerase,
  - Shorting chromosomes leading to cancer and aging.