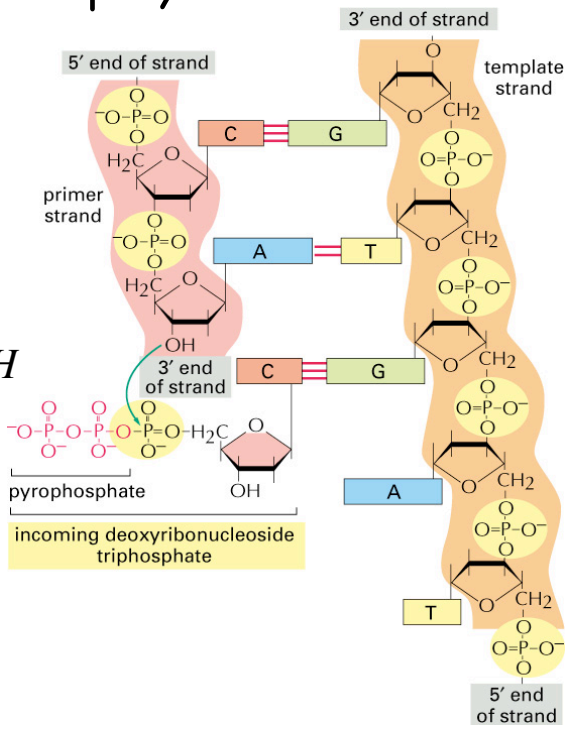
Arthur Kornberg discovered (the first) DNA polymerase

Using an "*in vitro*" system for DNA polymerase activity:

1. **Grow *E. coli***
2. **Break open cells**
3. **Prepare soluble extract**
4. **Fractionate extract to resolve different proteins from each other; repeat; repeat**
5. **Search for DNA polymerase activity using an biochemical assay: incorporate radioactive building blocks into DNA chains**

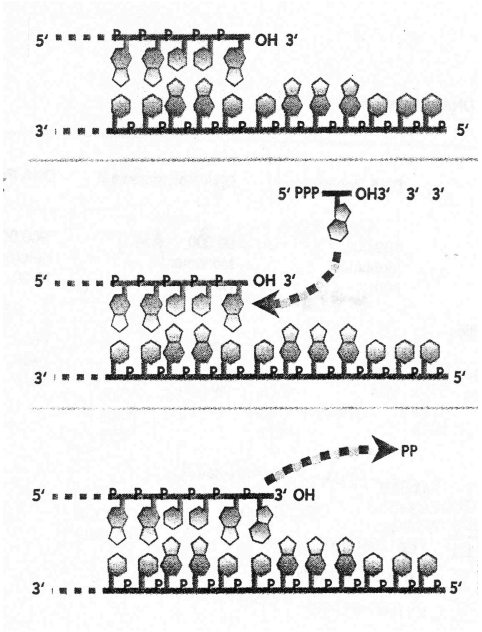
Four requirements of DNA-templated (DNA-dependent) DNA polymerases

- single-stranded template
- deoxyribonucleotides with *5' triphosphate* (dNTPs)
- magnesium ions
- annealed primer with *3' OH*



Synthesis ONLY occurs in the 5'-3' direction

E. coli DNA polymerase I
5′-3′ polymerase activity

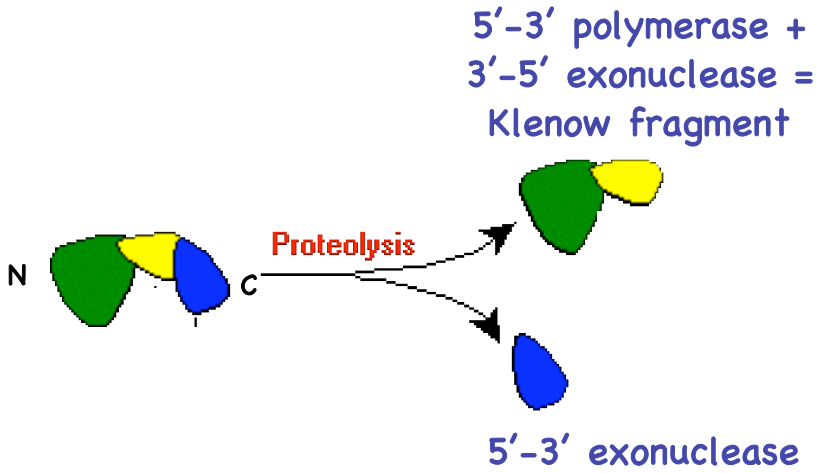


Primer has a 3′-OH

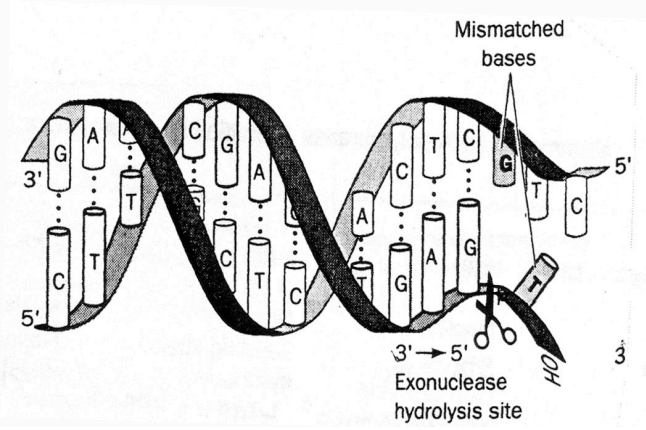
Incoming dNTP has a 5′ triphosphate

Pyrophosphate (PP) is lost when dNMP adds to the chain

E. coli DNA polymerase I: 3 separable enzyme activities in 3 protein domains



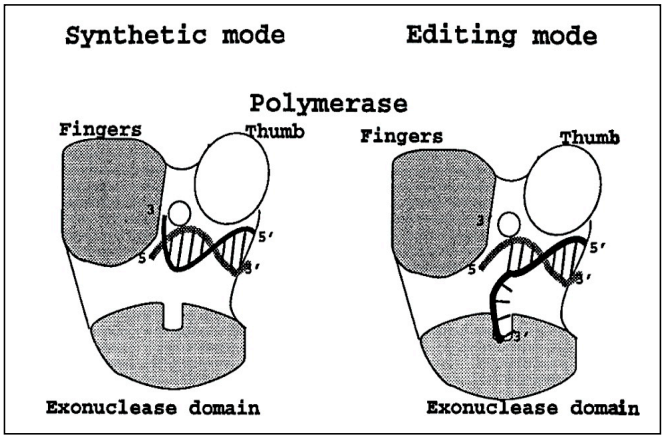
E. coli DNA polymerase I
3′-5′ exonuclease



Opposite polarity compared to polymerase: polymerase activity must stop to allow 3′-5′ exonuclease activity

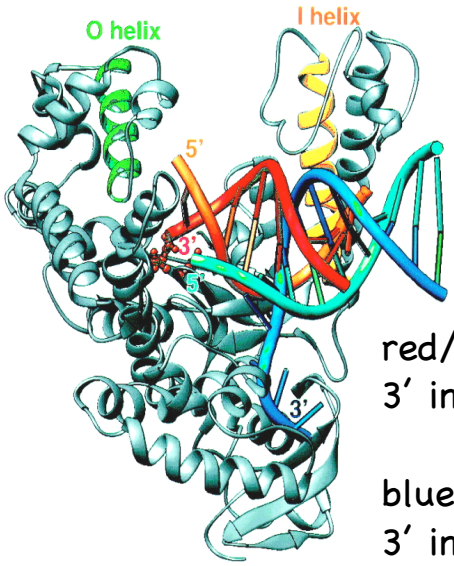
No dNTP can be re-made in reversed 3′-5′ direction: dNMP released by hydrolysis of phosphodiester backbone

Proof-reading (editing) of misincorporated 3′ dNMP by the 3′-5′ exonuclease



Fidelity is accuracy of template-cognate dNTP selection.
It depends on the polymerase active site structure and the balance of competing polymerase and exonuclease activities. A mismatch disfavors extension and favors the exonuclease.

Superimposed structure of the Klenow fragment of DNA pol I with two different DNAs



"Fingers"

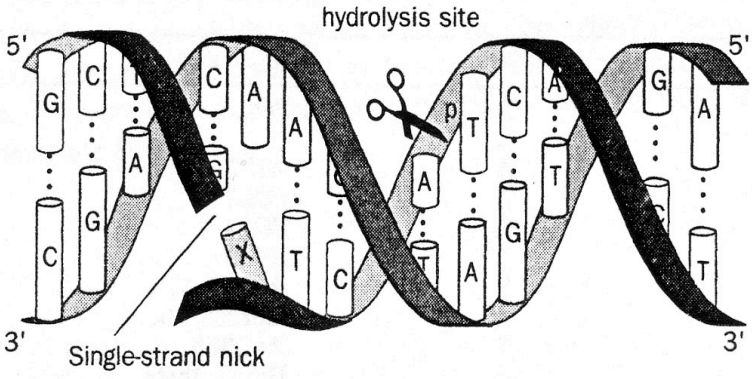
"Thumb"

"Palm"

red/orange helix:
3′ in red is elongating

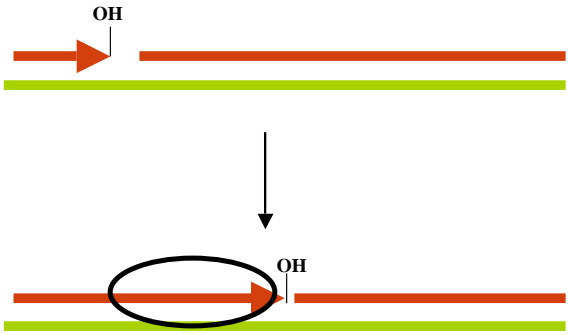
blue/cyan helix:
3′ in blue is getting edited

E. coli DNA polymerase I
5′-3′ exonuclease



Creates single-stranded template from dsDNA at the site of a nick, moving 5′-3′ on the nicked strand

E. coli DNA polymerase I 5′-3′ exo and 5′-3′ pol activities allow "nick translation"



Replacing base-pairs can be useful as DNA repair or in removing RNA primers of DNA replication

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

Polymerase active site mutant viable? Yes! Yes! No

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

Function	repair	repair	replication
Abundance	high		low

(also DNA pol IV and pol V, involved in DNA repair)

Examples of eukaryotic DNA polymerases

Greek name	HUGO name	Class	Other names	Proposed main function
α (alpha)	POLA	B	*POL1*	DNA replication
β (beta)	POLB	X		Base excision repair
γ (gamma)	POLG	A	*MIP1*	Mitochondrial replication
δ (delta)	POLD1	B	*POL3*	DNA replication
ε (epsilon)	POLE	B	*POL2*	DNA replication
ζ (zeta)	POLZ	B	*REV3*	Bypass synthesis
η (eta)	POLH	Y	*RAD30*, XPV	Bypass synthesis
θ (theta)	POLQ	A	mus308, eta	DNA repair
ι (iota)	POLI	Y	RAD30B	Bypass synthesis
κ (kappa)	POLK	Y	DinB1, theta	Bypass synthesis
λ (lambda)	POLL	X	*POL4*, beta2	Base excision repair
μ (mu)	POLM	X		Non-homologous end joining
σ (sigma)	POLS	X	*TRF4*, kappa	Sister chromatid cohesion
	REV1L	Y	*REV1*	Bypass synthesis
	TDT	X		Antigen receptor diversity

None have 5′-3′ DNA repair exonuclease.
Some have 3′-5′ proofreading exonuclease.

Eukaryotic DNA polymerases for MCB 110

alpha α — no 3′-5′ exonuclease — Functions in priming DNA synthesis for pol delta and episilon

delta δ

epsilon ϵ

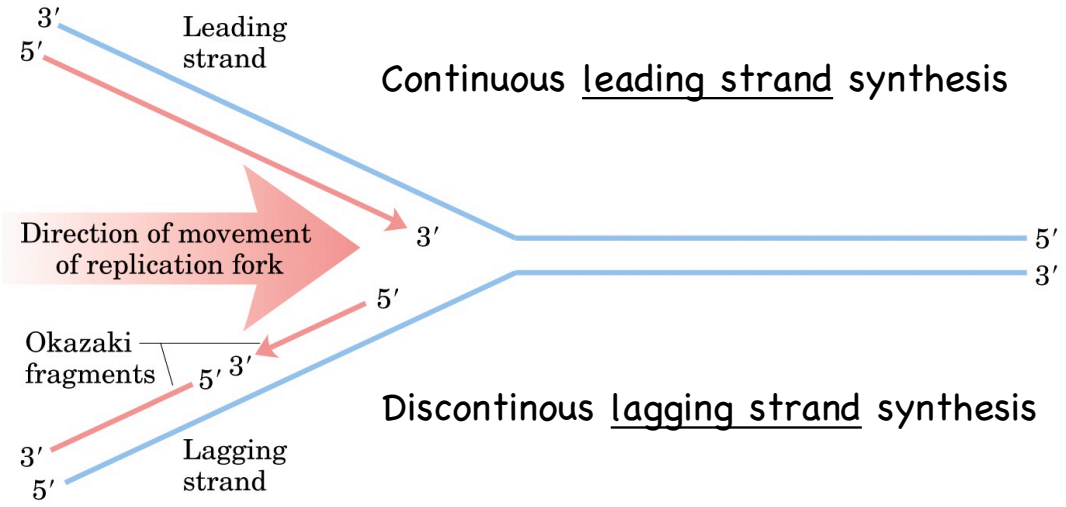
have 3′-5′ exonuclease — Genome replication

REQUIRE accessory factors: highly coordinated with other DNA replication machinery (like E. coli DNA pol III)

General DNA polymerase summary

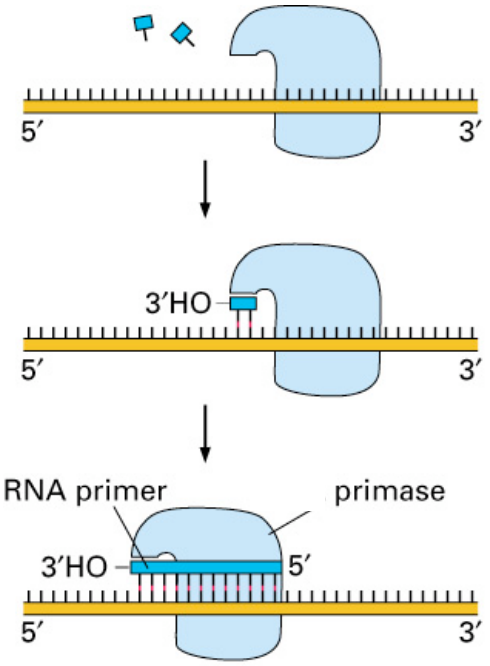
1. Each cell has many DNA polymerase enzymes that are specialized for different functions.
2. All DNA polymerases share 4 requirements for function: template, primer, dNTP and magnesium.
3. All DNA polymerases synthesizes DNA 5′-3′.
4. Editing/proofreading by 3′-5′ exo reduces errors. Mismatches in the primer-template duplex near the 3′ OH disfavor polymerase activity and favor 3′-5′ exonuclease activity. The lowest error rate is ~$1/10^9$.
5. DNA pol I has a unique additional activity: a 5′-3′ exonuclease that converts dsDNA to ssDNA template.
6. Different polymerases have different processivity: some fall off product after adding few dNTP (DNA pol I), others are much more processive (DNA pol III).

Coupled separation of parental strands and new strand synthesis, anti-parallel parental DNA strands, and 5′-3′ polarity of new DNA synthesis:



RNA polymerases can start chains without a primer

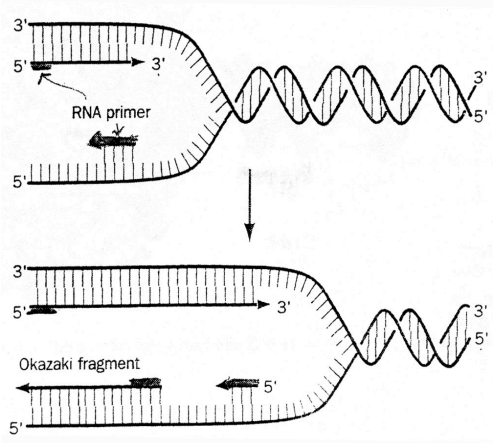
An RNA primer for DNA replication is 5-10 nt length: long enough to stay paired, but not longer than necessary (needs to be removed later).



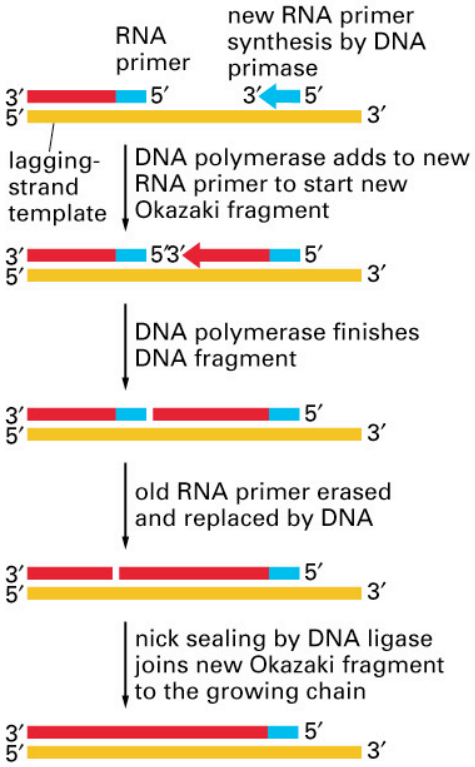
DNA replication uses RNA primers

Short RNA primers are synthesized by a specialized DNA-templated RNA polymerase called primase

The product is an RNA/DNA hybrid, which generally has A form helix geometry and has a free 3'OH



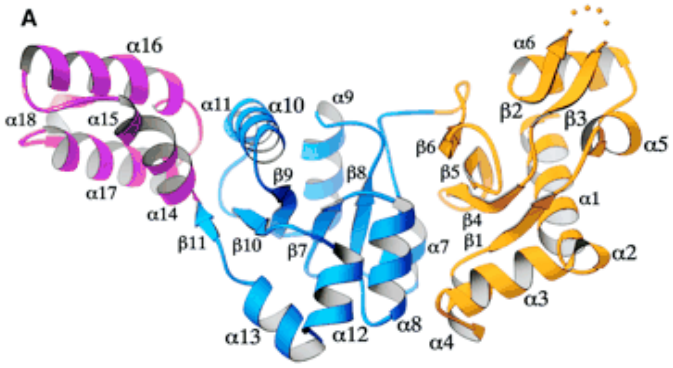
Primers must be made frequently on the lagging strand template



The lagging strand is made as Okazaki fragments

E. coli primase DnaG

Ribbon diagram



Okazaki fragment synthesis in E. coli

RNA primer synthesis by DnaG

DNA synthesis from RNA primer by DNA pol III

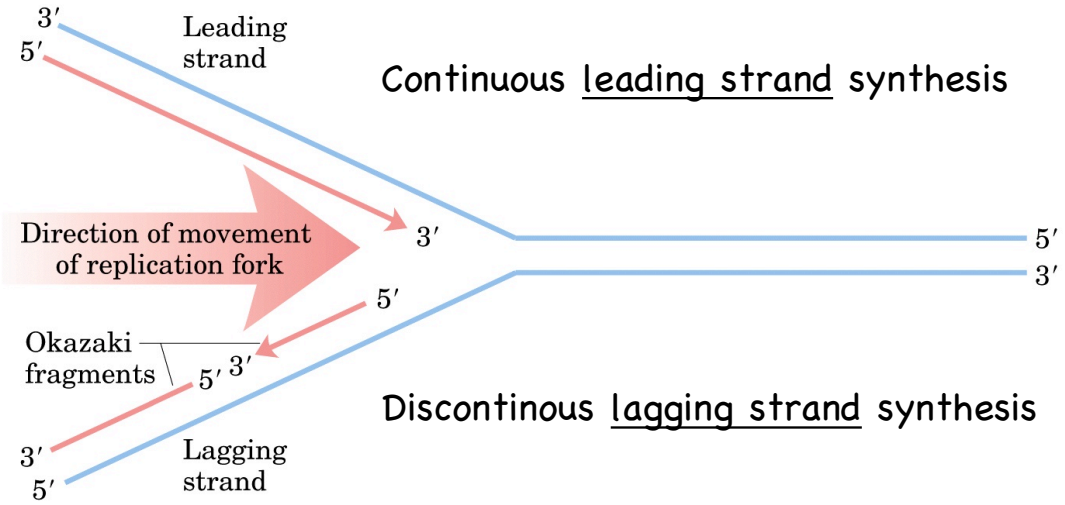
Okazaki fragment synthesis in eukaryotes has an extra step

RNA synthesis by primase (a primase-pol alpha complex)

Extension of RNA with DNA by pol alpha

DNA synthesis by pol delta

Coupled separation of parental strands and new strand synthesis, anti-parallel parental DNA strands, and 5′-3′ polarity of new DNA synthesis:



Replication factors

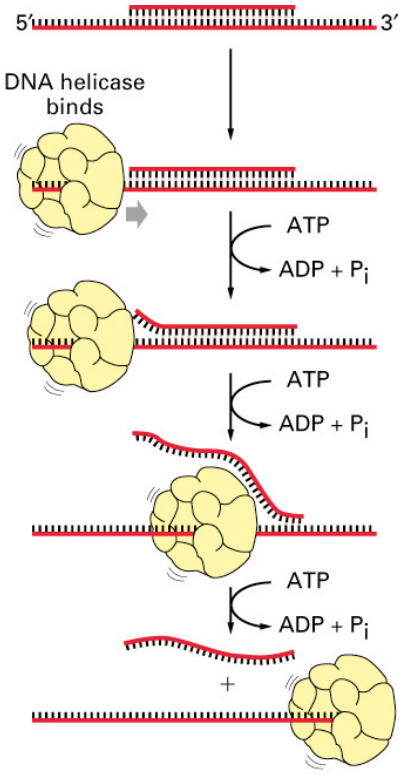
Function	*E. coli*	Eukaryotes
Helicase	DnaB	MCM
Primase	DnaG	pol α ◦primase
Primer removal	pol I 5'-3'exo	FEN1 5'-3' exo RNaseH
Polymerase		
Core	pol III (α, ε, θ subunits)	pol δ, ε
Clamp loader	γ complex	RF-C
Sliding clamp	β	PCNA
ssDNA binding	SSB	RF-A
Ligase	DNA ligase	DNA ligase I

DNA helicases separate DNA strands

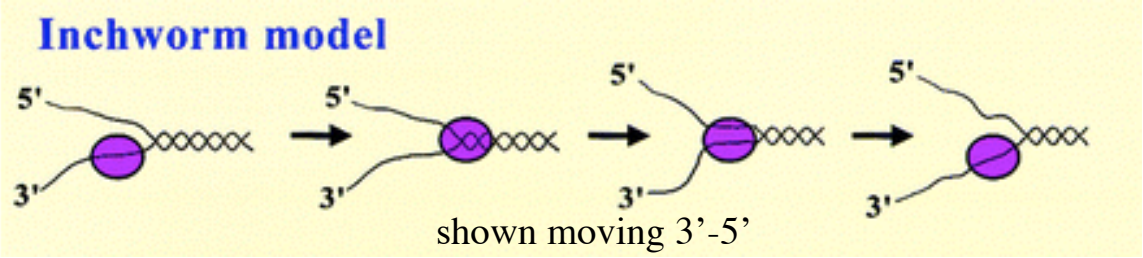
Although dsDNA is the substrate, helicases interact primarily with one strand. Helicases load onto substrates by associating with single-stranded DNA. At right, the helicase can load by sliding onto the single-stranded 5' overhang.

Unwinding occurs by helicase tracking either 5'-3' or 3'-5' on this strand (each helicase has either 5'-3' or 3'-5' polarity). The helicase at right has 5'-3' polarity.

Step-wise movement of the helicase is powered by repeated cycles of ATP hydrolysis. The double helix is unpaired as the helicase powers along the single strand.

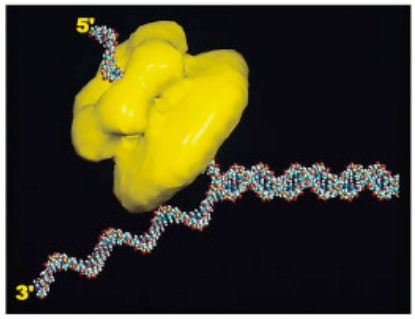


The E. coli replication fork helicase is hexameric DnaB: 5'-3'polarity



shown moving 3'-5'

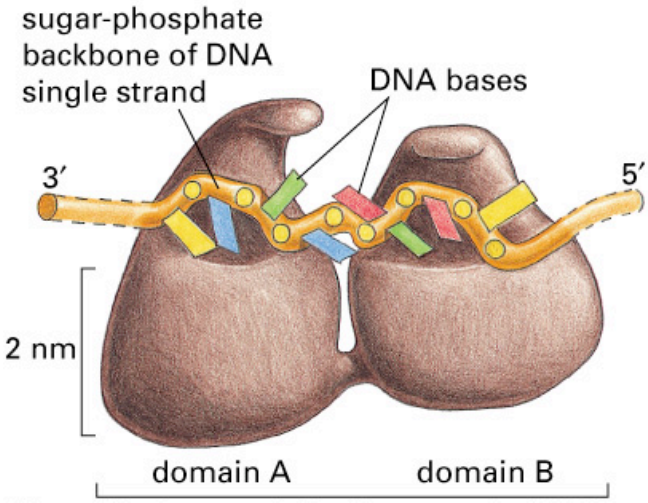
A model for helicase action. Helicase bound to ssDNA translocates along one DNA strand. If dsDNA is encountered, helix destabilization and release of one ssDNA strand takes place as ATP is hydrolyzed.

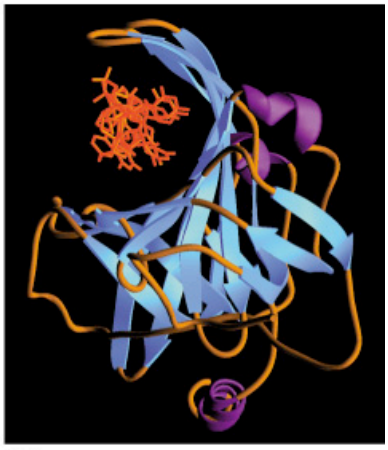


this would move 5'-3'

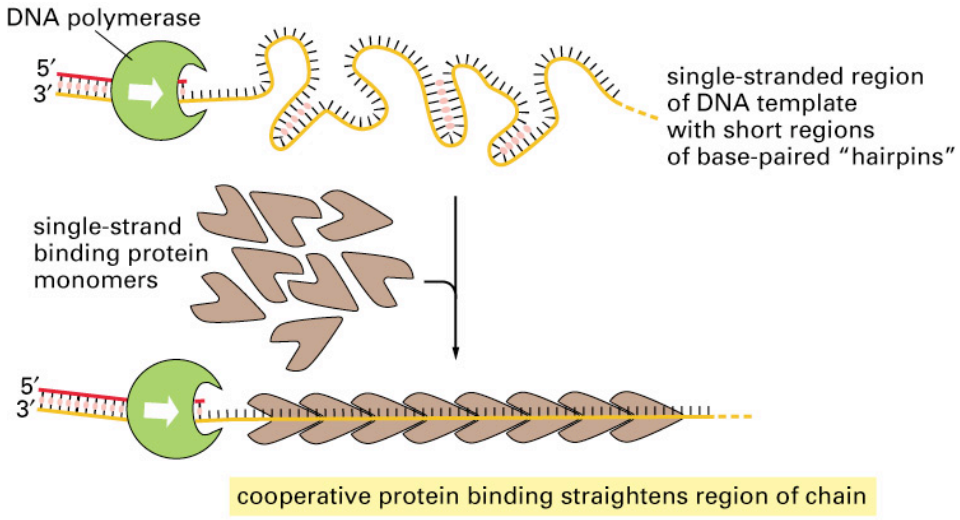
E. coli single-stranded DNA binding protein (SSB)

The eukaryotic equivalent is replication factor A (RF-A)



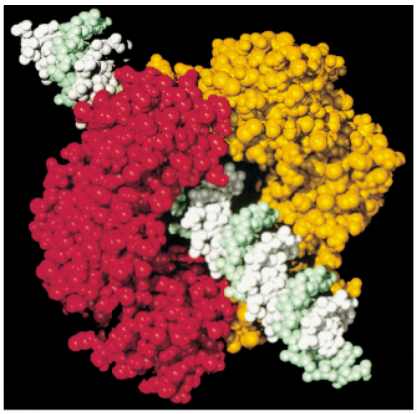


Each SSB prefers to bind DNA next to a previously DNA-bound molecule. This **cooperative binding** keeps a ssDNA template generated by the helicase from reforming dsDNA.



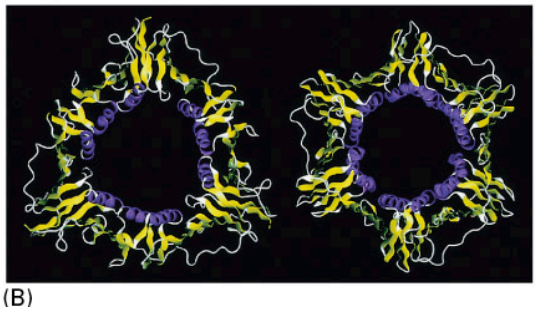
Some DNA polymerases prefer SSB on the template, some are blocked by it.

The processivity factor for DNA pol III in E. coli or DNA pols delta and epsilon in eukaryotes encircles the DNA as a "**sliding clamp**."

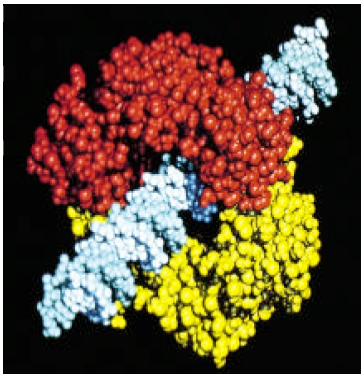

(A)

E. coli beta is a dimer (shown above, red/orange).

Eukaryotic PCNA is a trimer. BOTH form a 6-lobed ring.


(B)

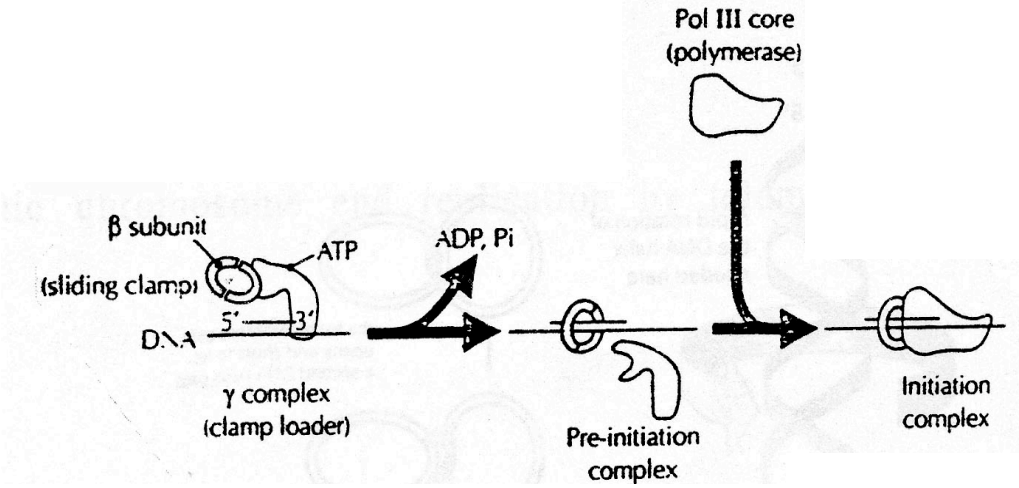
How does this improve processivity?
One surface binds polymerase; if polymerase dissociates from DNA, it cannot diffuse away; it will rebind the same template-primer junction and continue synthesis.



How does the clamp get on DNA?
The closed ring is the most stable conformation.
Therefore, it needs help from an enzyme:
the "clamp loader" complex.

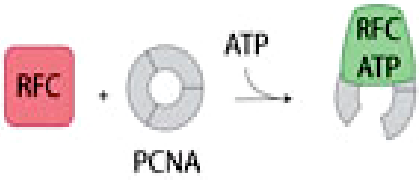
The clamp loader complex puts the sliding clamp where DNA polymerase should bind

γ **complex** in E. coli, **RF-C** in eukaryotes

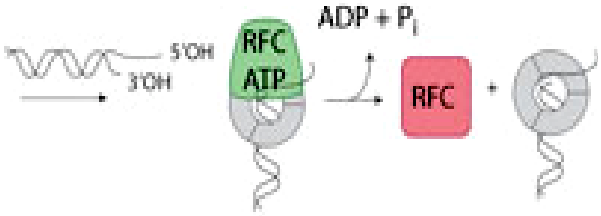


The clamp loader reaction

1. Clamp loader + clamp + ATP opens clamp

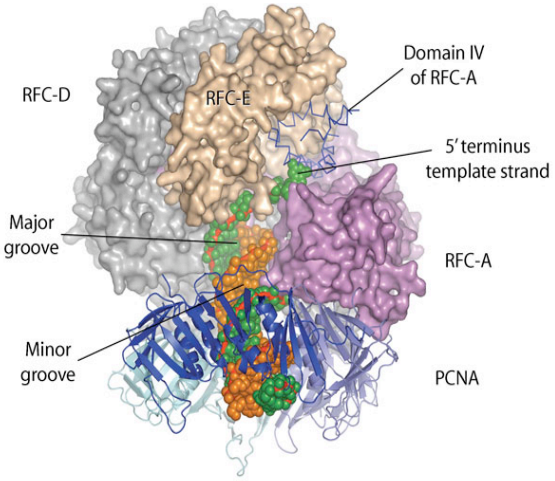


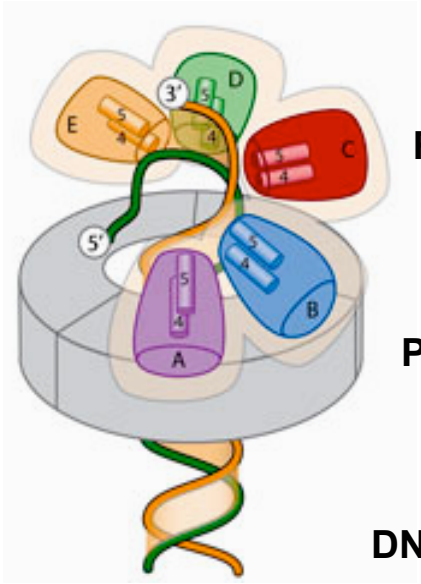
2. Ternary complex + DNA/RNA closes clamp with ATP hydrolysis and release of clamp loader



The clamps have to be unloaded later

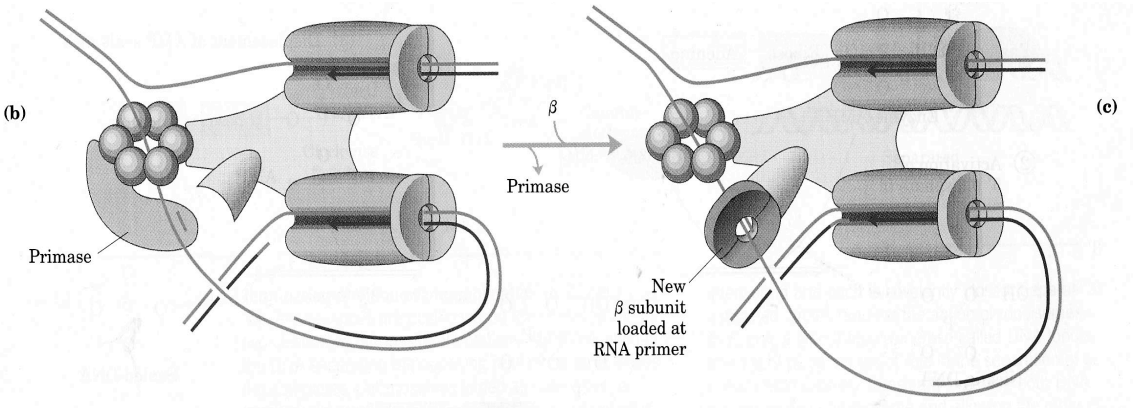
RF-C + PCNA structure

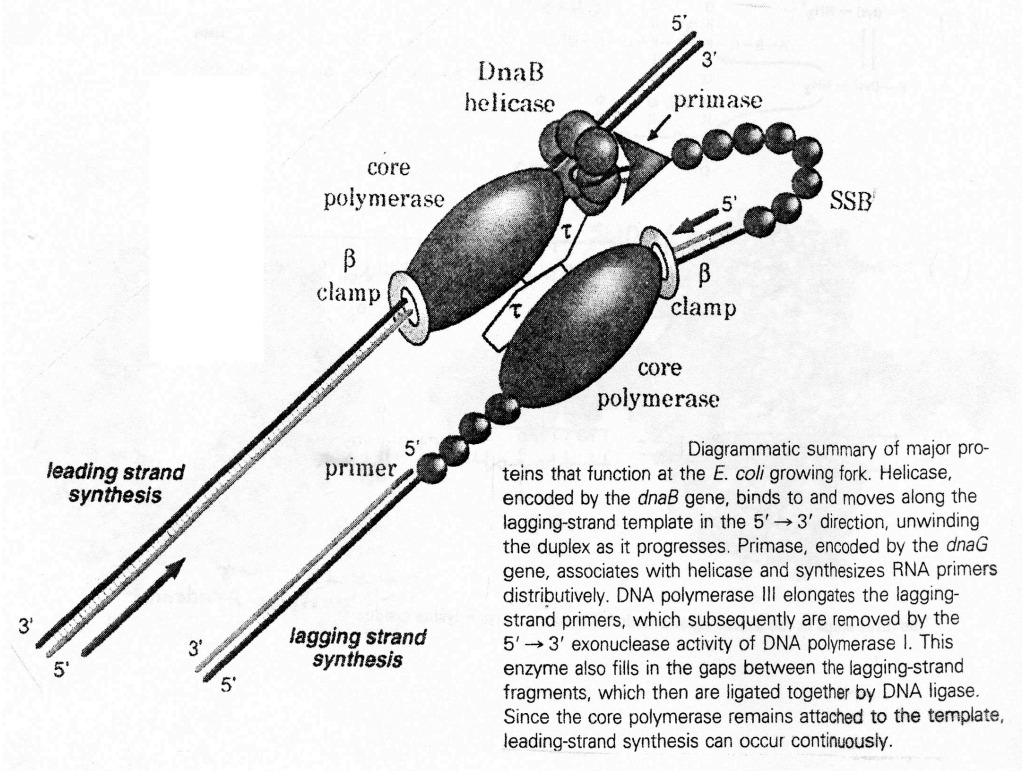




RF-C contains 5 similar subunits that spiral around DNA.
The RF-C helix tracks the DNA or DNA/RNA helix.

There is a coordination of factors at the replication fork: physical interaction of both polymerases, the lagging strand primase and clamp loader, and helicase



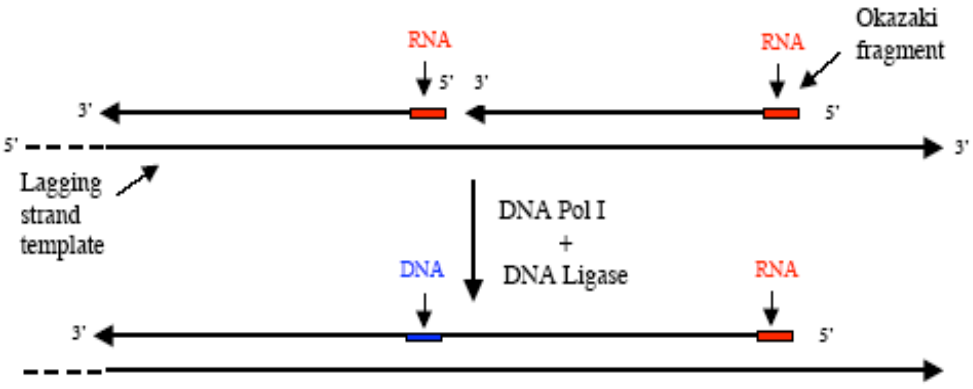


Diagrammatic summary of major proteins that function at the *E. coli* growing fork. Helicase, encoded by the *dnaB* gene, binds to and moves along the lagging-strand template in the 5′ → 3′ direction, unwinding the duplex as it progresses. Primase, encoded by the *dnaG* gene, associates with helicase and synthesizes RNA primers distributively. DNA polymerase III elongates the lagging-strand primers, which subsequently are removed by the 5′ → 3′ exonuclease activity of DNA polymerase I. This enzyme also fills in the gaps between the lagging-strand fragments, which then are ligated together by DNA ligase. Since the core polymerase remains attached to the template, leading-strand synthesis can occur continuously.

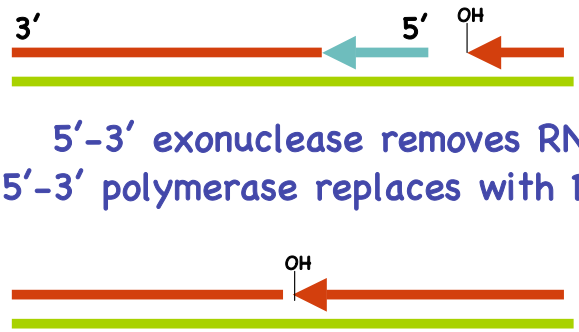
Replication factors

Function	*E. coli*	Eukaryotes
Helicase	DnaB	MCM
Primase	DnaG	pol α ◦primase
Primer removal	pol I 5'-3'exo	FEN1 5'-3' exo RNaseH
Polymerase		
Core	pol III ($\alpha, \varepsilon, \theta$ subunits)	pol δ, ε
Clamp loader	γ complex	RF-C
Sliding clamp	β	PCNA
ssDNA binding	SSB	RF-A
Ligase	DNA ligase	DNA ligase I

Maturation of Okazaki fragments occurs to form a continuous lagging strand

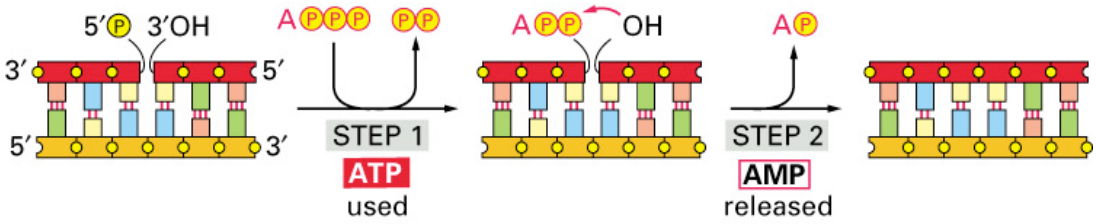


RNA primer removal by E. coli DNA pol I



A different 5′-3′ exonuclease FEN1 is used in eukaryotes.
Perhaps there is also some role for RNase H,
an endonuclease that cleaves the RNA strand
of DNA-RNA hybrids.

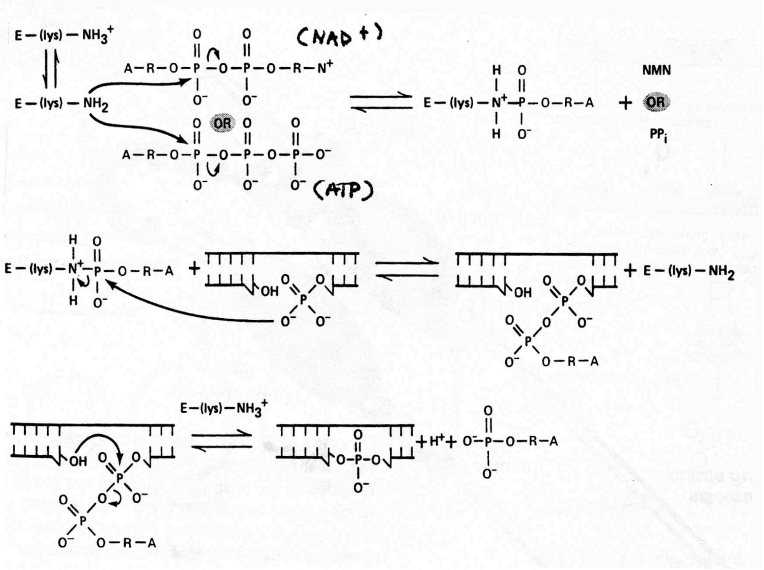
A DNA ligase is required to join DNA strands with discontinuous phosphodiester backbone



In the *E. coli* DNA ligase reaction, the AMP added to the 5′ phosphate is derived from NAD^+. The DNA ligases from bacteriophage and eukaryotic sources use ATP instead (as shown). *Only bacteriophage DNA ligase can join strands at a dsDNA break; all others join DNA strands with no missing nucleotide at at nick in dsDNA.*

The DNA ligase reaction has a covalent enzyme-AMP intermediate

1. Adenylylate the enzyme
2. Transfer AMP to the PO4 at the nick
3. Seal nick, releasing AMP

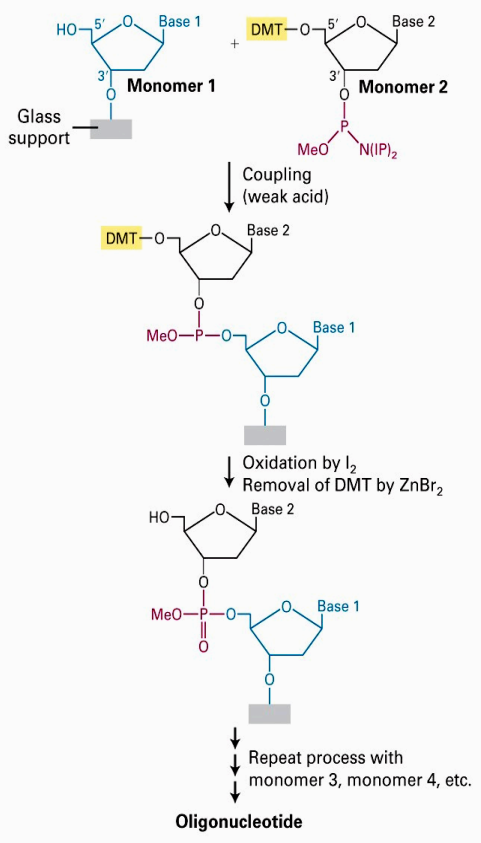


Replication factors

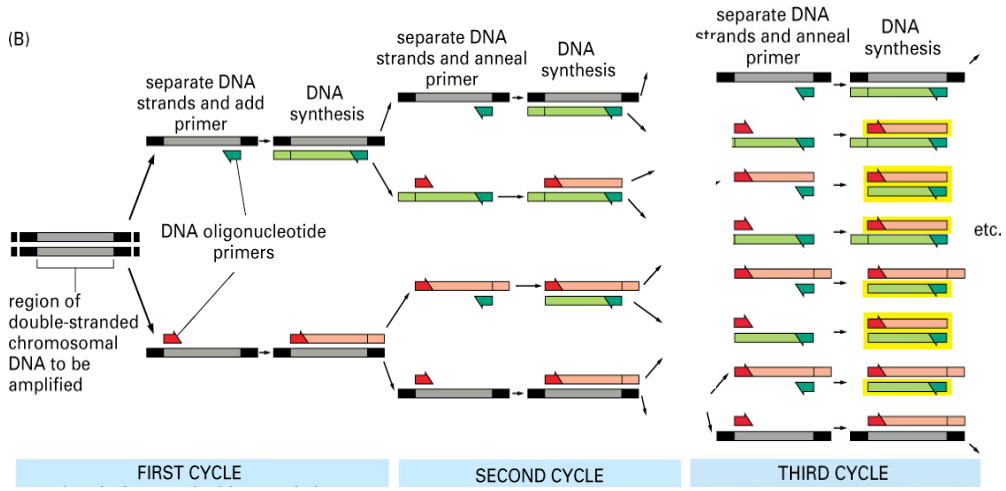
Function	*E. coli*	Eukaryotes
Helicase	DnaB	MCM
Primase	DnaG	pol α ◦primase
Primer removal	pol I 5'-3'exo	FEN1 5'-3' exo RNaseH
Polymerase		
Core	pol III (α, ε, θ subunits)	pol δ, ε
Clamp loader	γ complex	RF-C
Sliding clamp	β	PCNA
ssDNA binding	SSB	RF-A
Ligase	DNA ligase	DNA ligase I

Chemical synthesis of DNA oligonucleotides

Nucleotide blocks are joined from 3′-5′.
Each block is protected at the 5′ side so that only one block can add to a chain.
Sequential rounds of coupling and deprotection build the oligonucleotide.



PCR (Polymerase Chain Reaction) can isolate and amplify any DNA sequence



1 2 4 8

N cycles amplifies the target sequence 2^N-fold