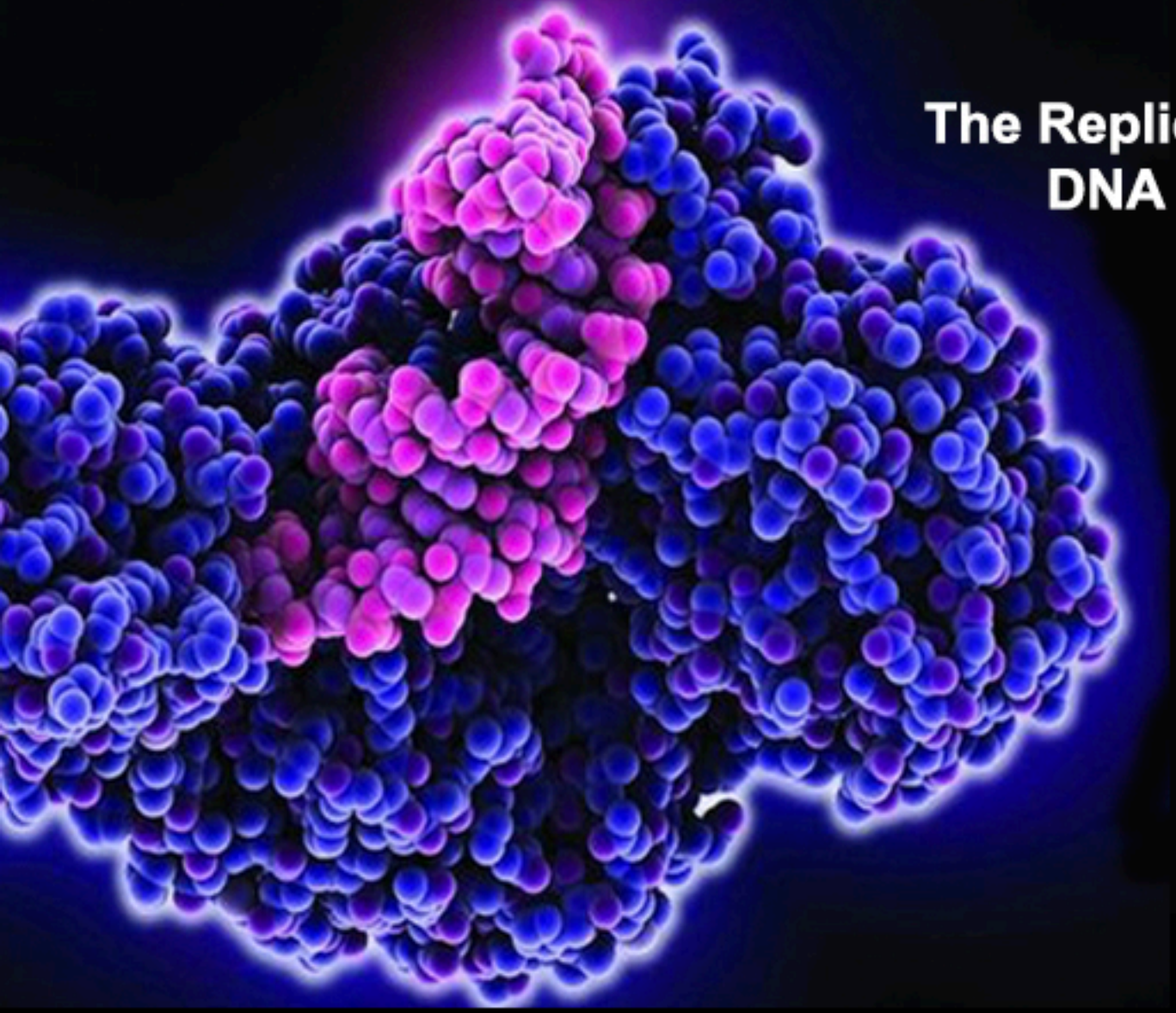
BIOL8620 Eukaryotic Genetics



The Replicon & DNA Replication

Chapter 10
most of 11
& parts of 12

Cell Research (2008) **18:** 148–161. doi: 10.1038/cr.2008.4; published online 1 January 2008

The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases

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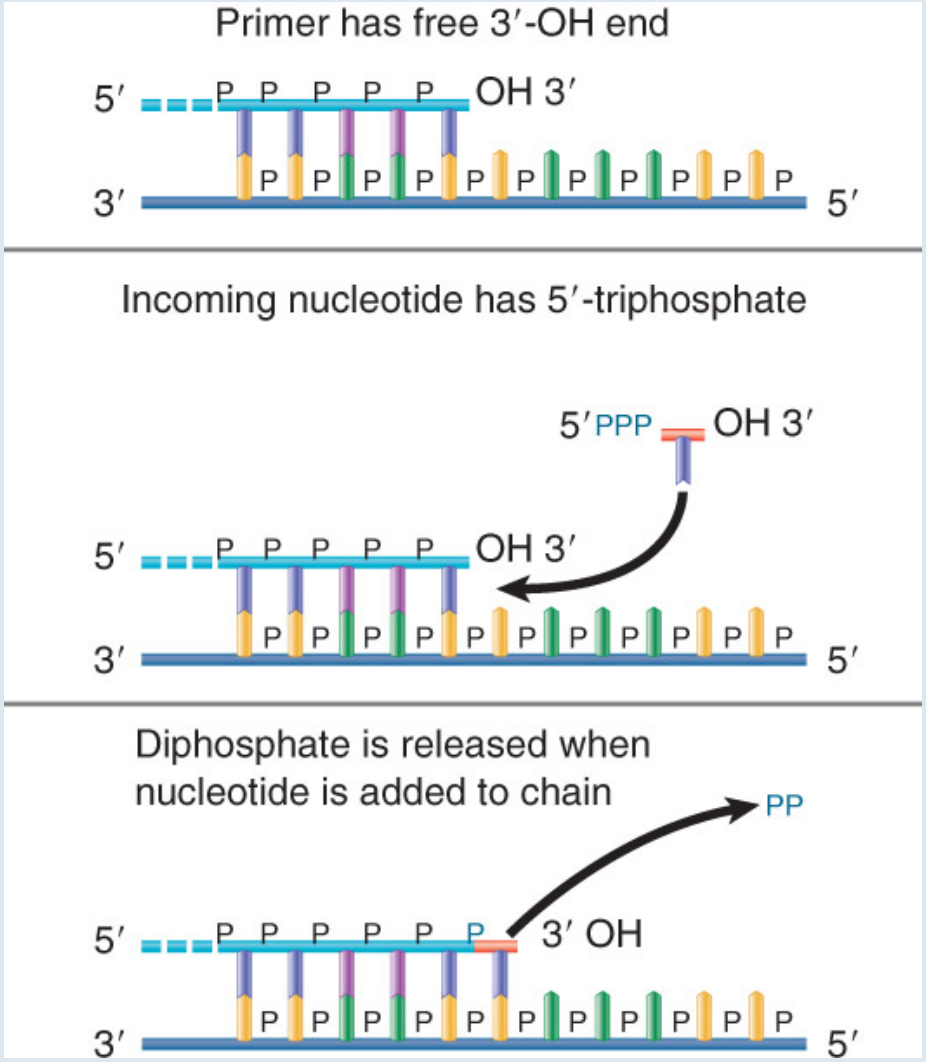
Abstract

Top

In their seminal publication describing the structure of the DNA double helix [1], Watson and Crick wrote what may be one of the greatest understatements in the scientific literature, namely that "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Half a century later, we more fully appreciate what a huge challenge it is to replicate six billion nucleotides with the accuracy needed to stably maintain the human genome over many generations. This challenge is perhaps greater than was realized 50 years ago, because subsequent studies have revealed that the genome can be destabilized not only by environmental stresses that generate a large number and variety of potentially cytotoxic and mutagenic lesions in DNA but also by various sequence motifs of normal DNA that present challenges to replication. Towards a better understanding of the many determinants of genome stability, this chapter reviews the fidelity with which undamaged and damaged DNA is copied, with a focus on the eukaryotic B- and Y-family DNA polymerases, and considers how this fidelity is achieved.

All DNA Polymerases synthesize DNA in one direction 5’ -> 3’



DNA is synthesized by adding nucleotides to the 3’–OH end of the growing chain, so that the new chain grows in the 5’ to 3’ direction

DNA Polymerases Are the Enzymes That Make DNA

- Both pro- and eukaryotic cells have several different **DNA polymerase** (**DNA replicase**) enzymes

- One bacterial DNA polymerase undertakes semiconservative replication of the genome; the others are involved in repair reactions.

Enzyme	Gene	Function
I	*polA*	major repair enzyme
II	*polB*	replication restart
III	*polC*	replicase
IV	*dinB*	translesion replication
V	*umuD′ $_2$C*	translesion replication

Only one DNA polymerase in prokaryotes is the “true” replication enzyme, or “replicase”

DNA Polymerases Are the Enzymes That Synthesize DNA

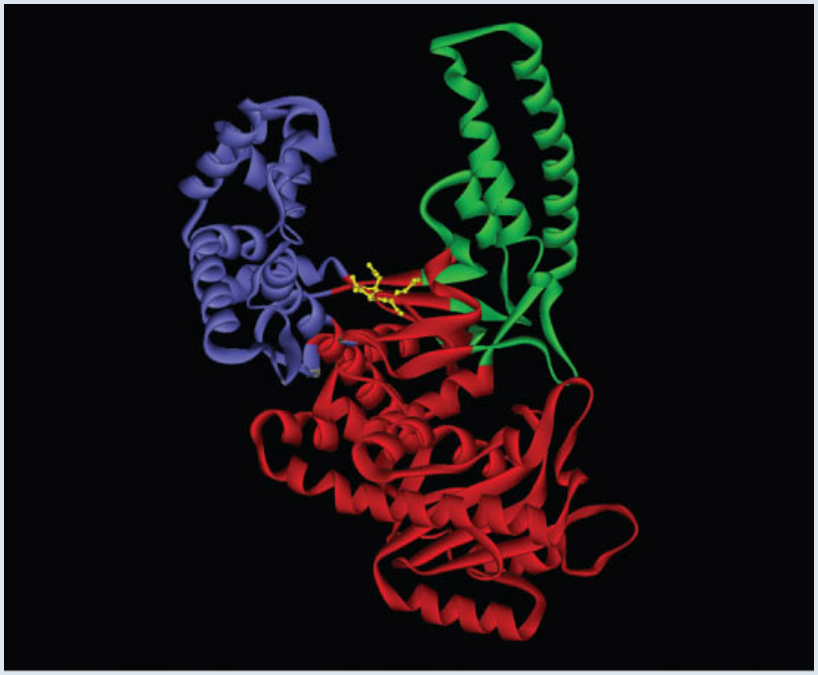
- Both pro- and eukaryotic cells have several different **DNA polymerase** (**DNA replicase**) enzymes

- In eukaryotes replication of the genome and repair are achieved by a wider variety of enzyme subunits… plethora of greek alphabet names

DNA polymerase	Function	Structure
	High fidelity replicases	
α	Nuclear replication	350 kD tetramer
δ	Lagging strand possibly replication initiation	250 kD tetramer
ε	Leading strand	350 kD tetramer
γ	Mitochondrial replication	200 kD dimer
	High-fidelity repair	
β	Base excision repair	39 kD monomer
	Low-fidelity repair	
ζ	Base damage bypass	heteromer
η	Thymine dimer bypass	monomer
ι	Required in meiosis	monomer
κ	Deletion and base substitution	monomer

DNA Polymerases Have a Common Structure

- Many DNA polymerases have a large cleft composed of three domains that resemble a hand.
- DNA lies across the “palm” in a groove created by the “fingers” and “thumb.”



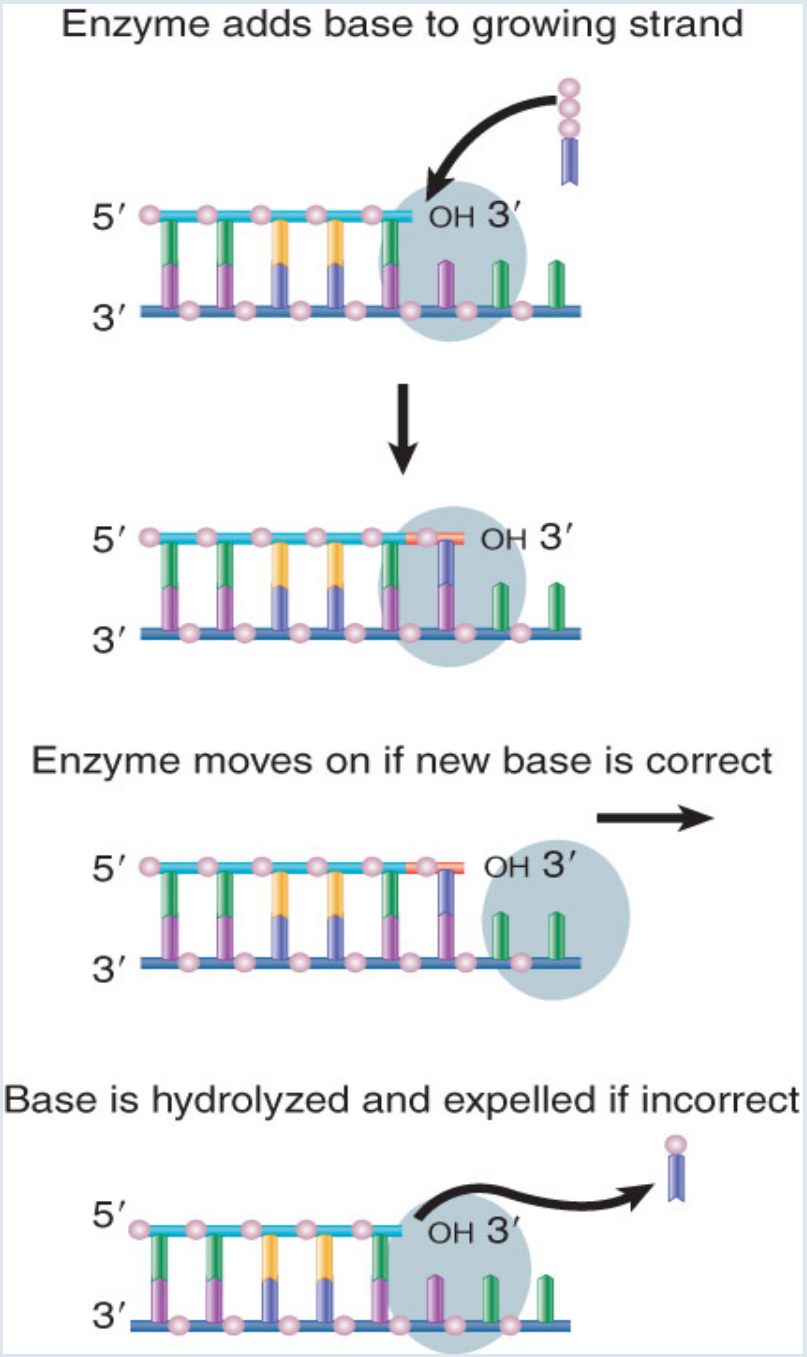
The structure of the Klenow fragment from *E. coli* DNA polymerase I

Structure from Protein Data Bank 1KFD. L. S. Beese, J. M. Friedman, and T. A. Steitz, Biochemistry 32 (1993): 14095-14101.

DNA Polymerases Control the Fidelity of Replication

- High-fidelity DNA polymerases involved in replication have a precisely constrained active site that favors binding of Watson–Crick base pairs.

- **processivity** – The ability of an enzyme to perform multiple catalytic cycles with a single template instead of dissociating after each cycle.



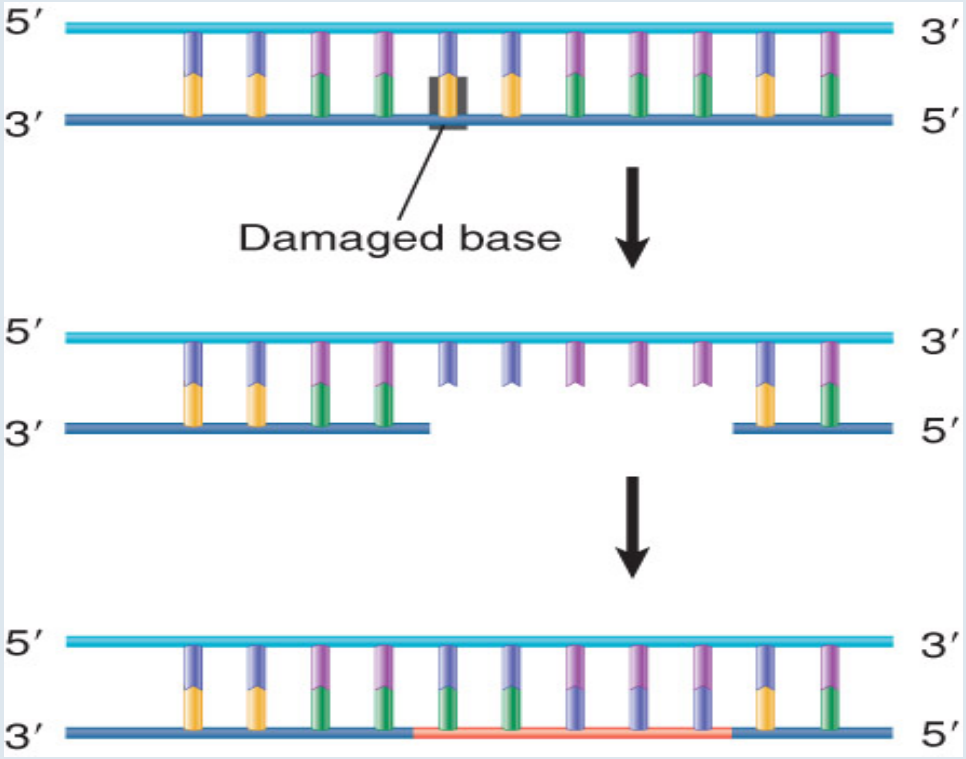
Type of DNA Polymerases Controls the Fidelity of Replication

- DNA polymerases (including the replicase) often have a 3′–5′ exonuclease activity that is used to excise incorrectly paired bases.
- The fidelity of replication is thus improved by **proofreading** ability unto ~100.

Bacterial DNA polymerases are able to “scrutinize” the base pair at the end of the growing chain and excise the nucleotide added in the case of a misfit

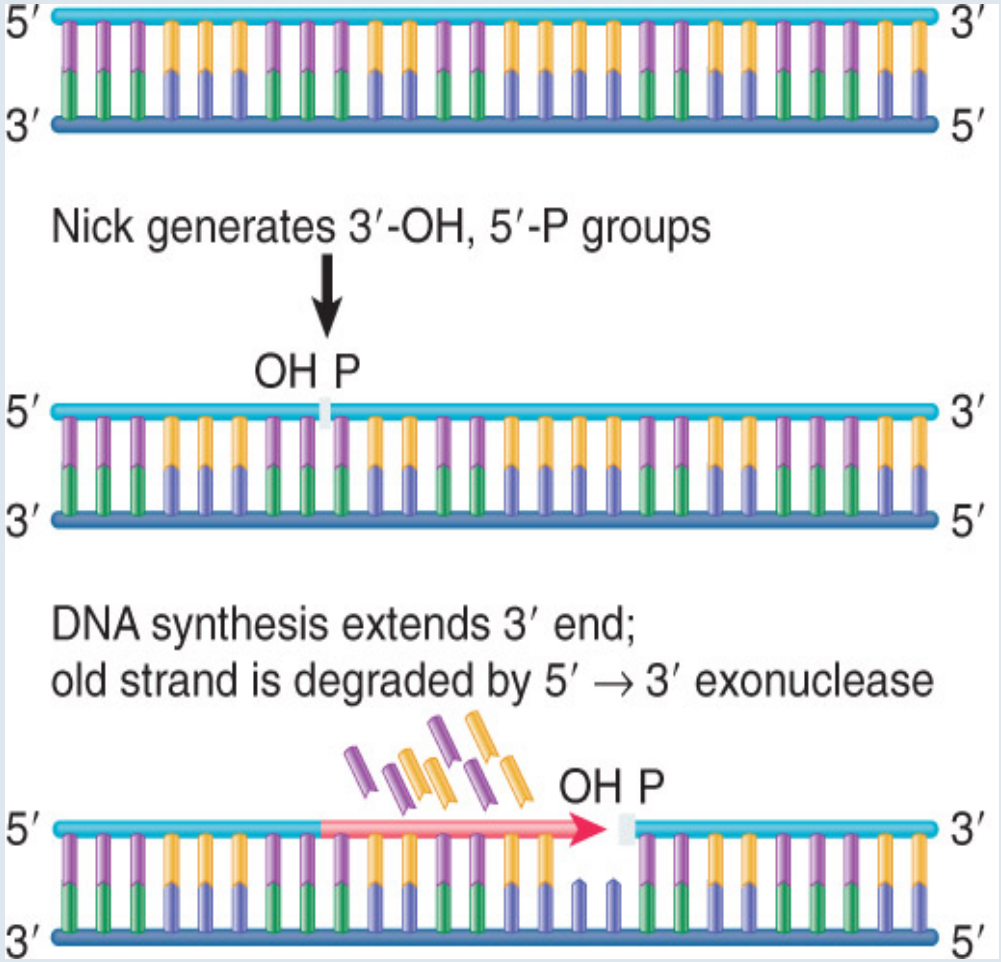
DNA Polymerases Are the Enzymes That Synthesize DNA

- DNA is synthesized in both **semiconservative replication** of entire chromosomes (replisomes)…. and dispersive replication of relatively short patches of DNA -including damaged DNA sequences



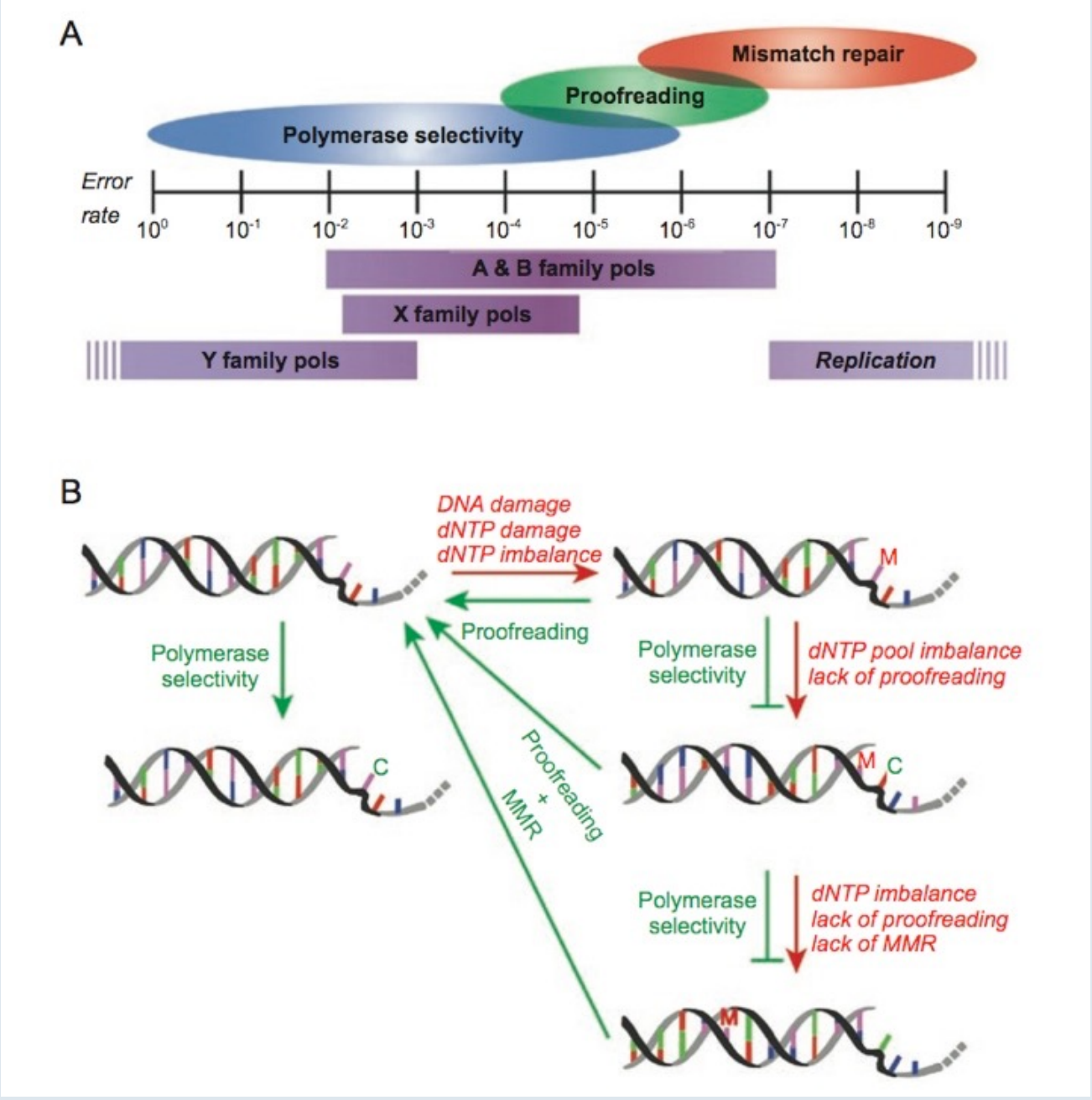
Each parental strand of DNA acts as a template

Different DNA Polymerases Have different Nuclease Activities, as their function dictates.



- **DNA polymerase I** (prokaryotes) has a unique 5′->3′ exonuclease activity that can be combined with DNA synthesis to perform “nick translation” in which the polymerase replaces part of a pre-existing strand of duplex DNA with newly synthesized material.

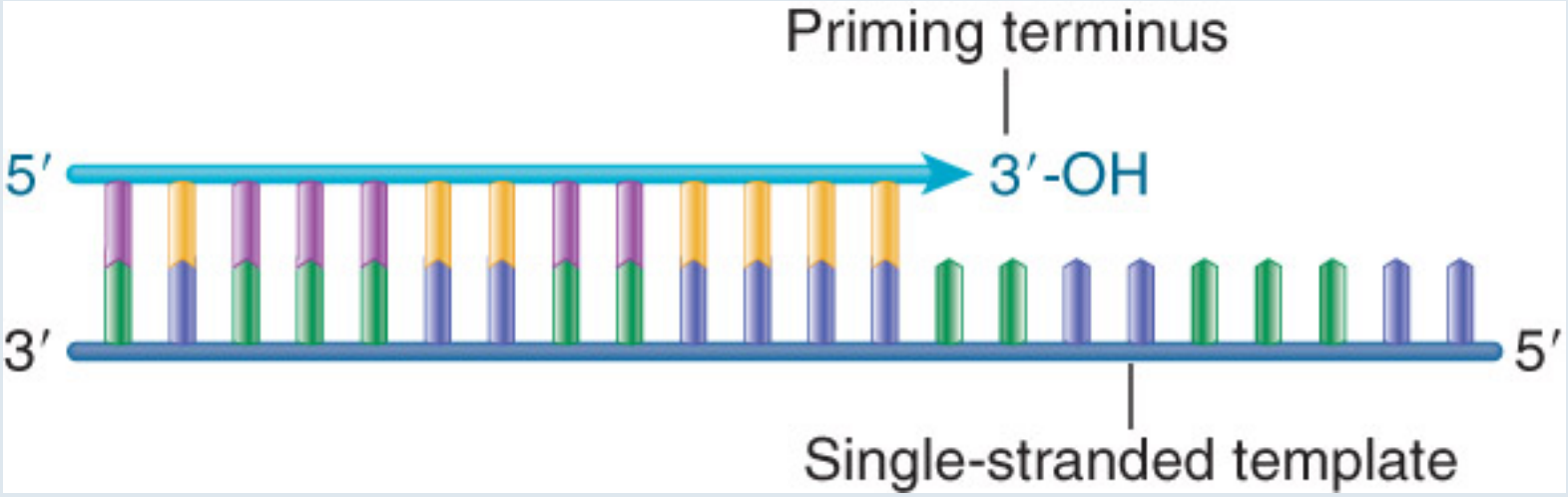
- This exonuclease activity can also be achieved by specific exonuclease


A
Mismatch repair
Proofreading
Polymerase selectivity
Error rate
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
10^-6
10^-7
10^-8
10^-9
A & B family pols
X family pols
Y family pols
Replication
B
DNA damage
dNTP damage
dNTP imbalance
M
Proofreading
Polymerase selectivity
Polymerase selectivity
dNTP pool imbalance
lack of proofreading
C
M C
Proofreading + MMR
Polymerase selectivity
dNTP imbalance
lack of proofreading
lack of MMR
M

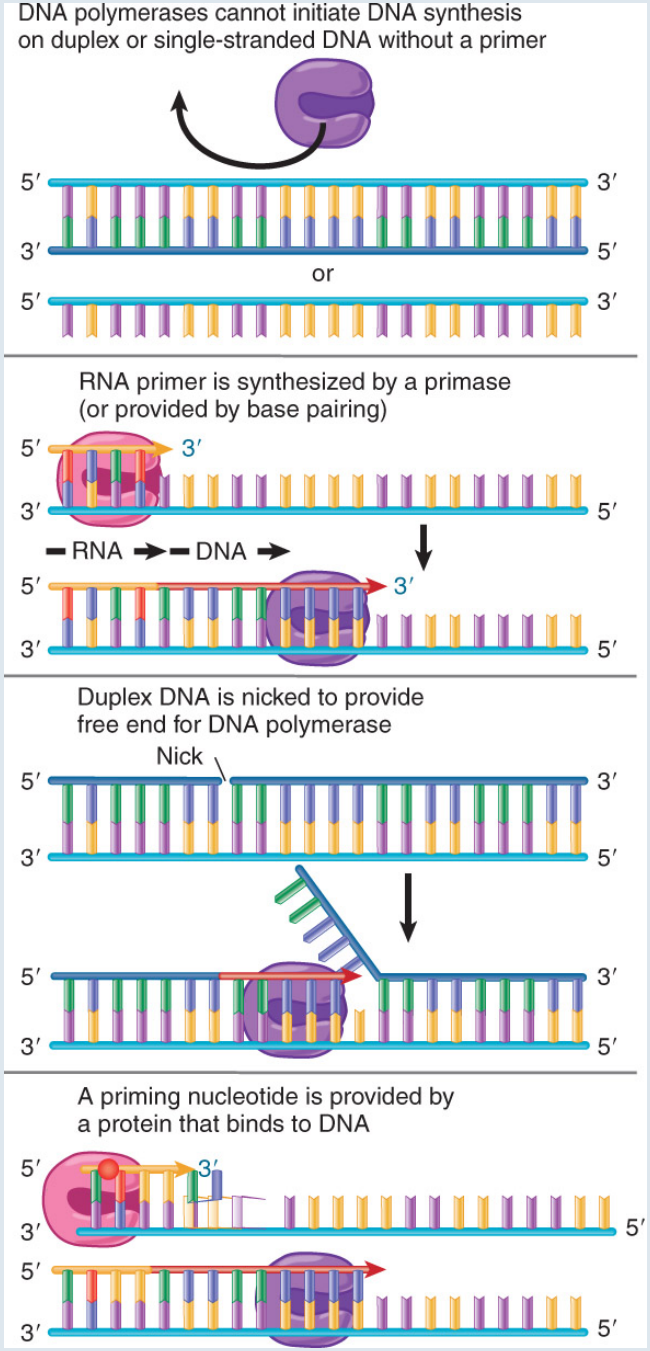
Fidelity of replication comes at a price…

Priming Is Required to Start DNA Synthesis

- All DNA polymerases require a 3′–OH priming end to initiate DNA synthesis.



A DNA polymerase require a 3’–OH end to initiate replication

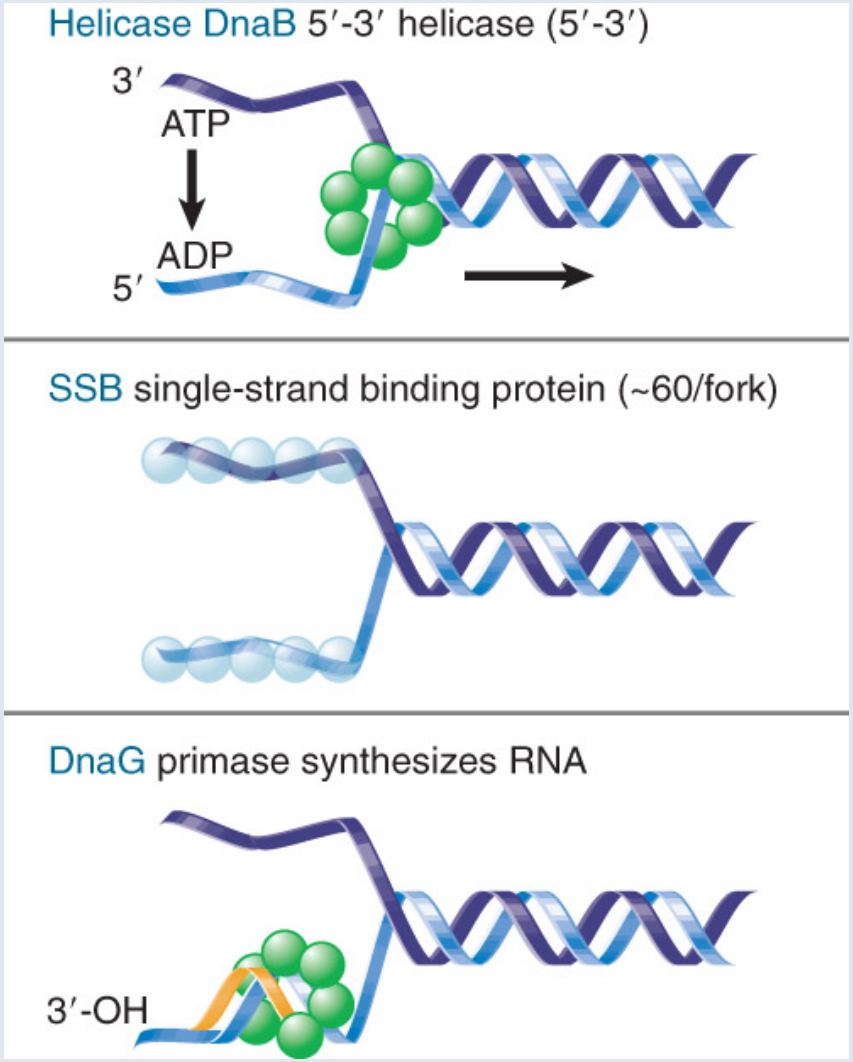


Priming Is Required to Start DNA Synthesis

- The priming end can be provided by an RNA **primer**, a nick in DNA, or a priming protein.

There are several methods for providing the free 3'–OH end that DNA polymerases require to initiate DNA synthesis…. DNA or RNA 3' OH

Priming Is Required to Start chromosomal DNA Synthesis

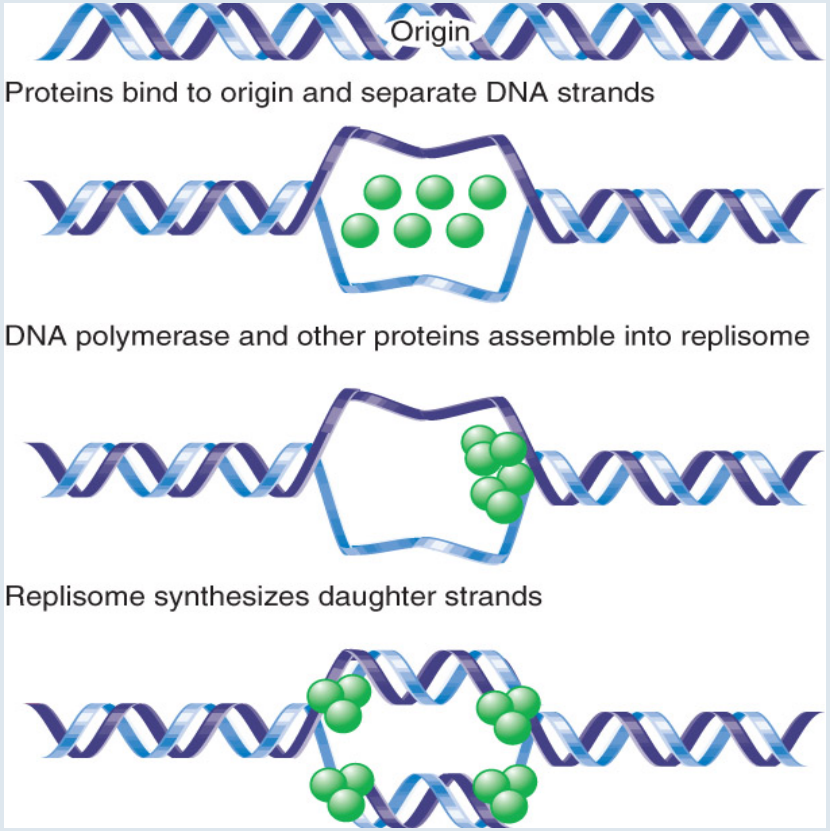


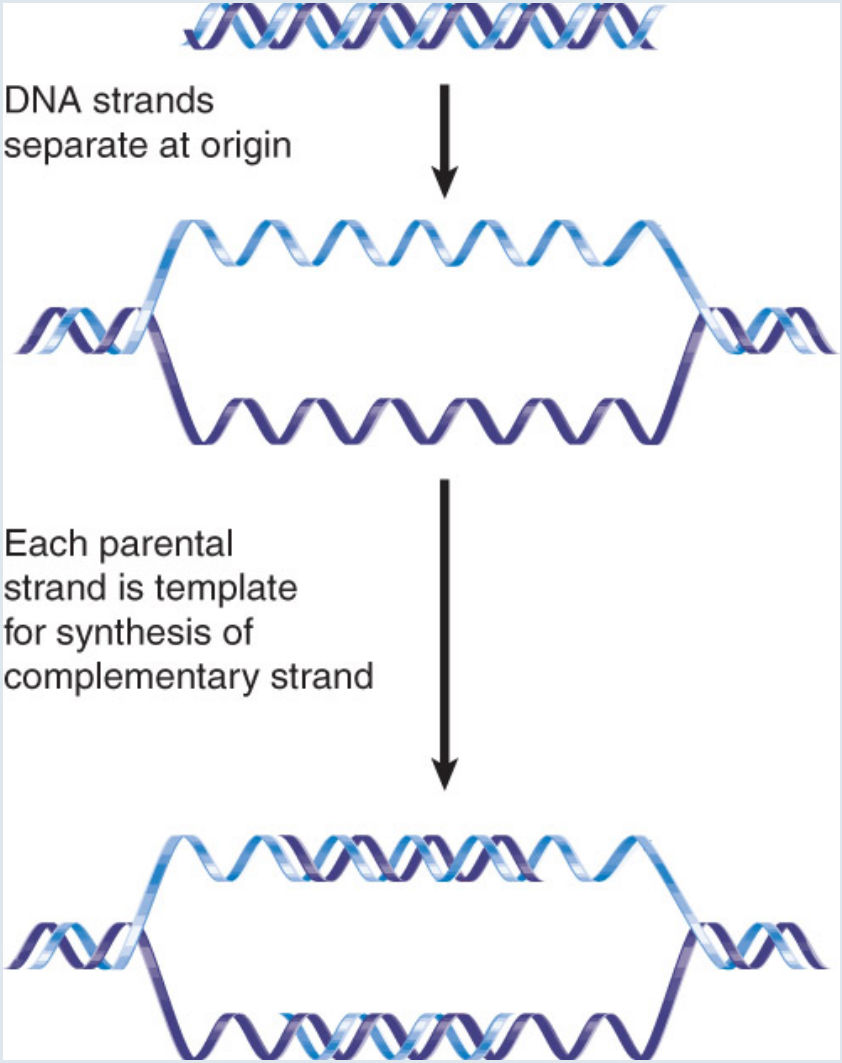
- Priming of replication on double-stranded DNA always requires a **replicase**, **SSB**, and a **primase**.
- **DnaB** is the helicase that unwinds DNA for replication in *E. coli*.

Initiation requires several enzymatic activities, including helicases, single-strand binding proteins, and synthesis of the primer

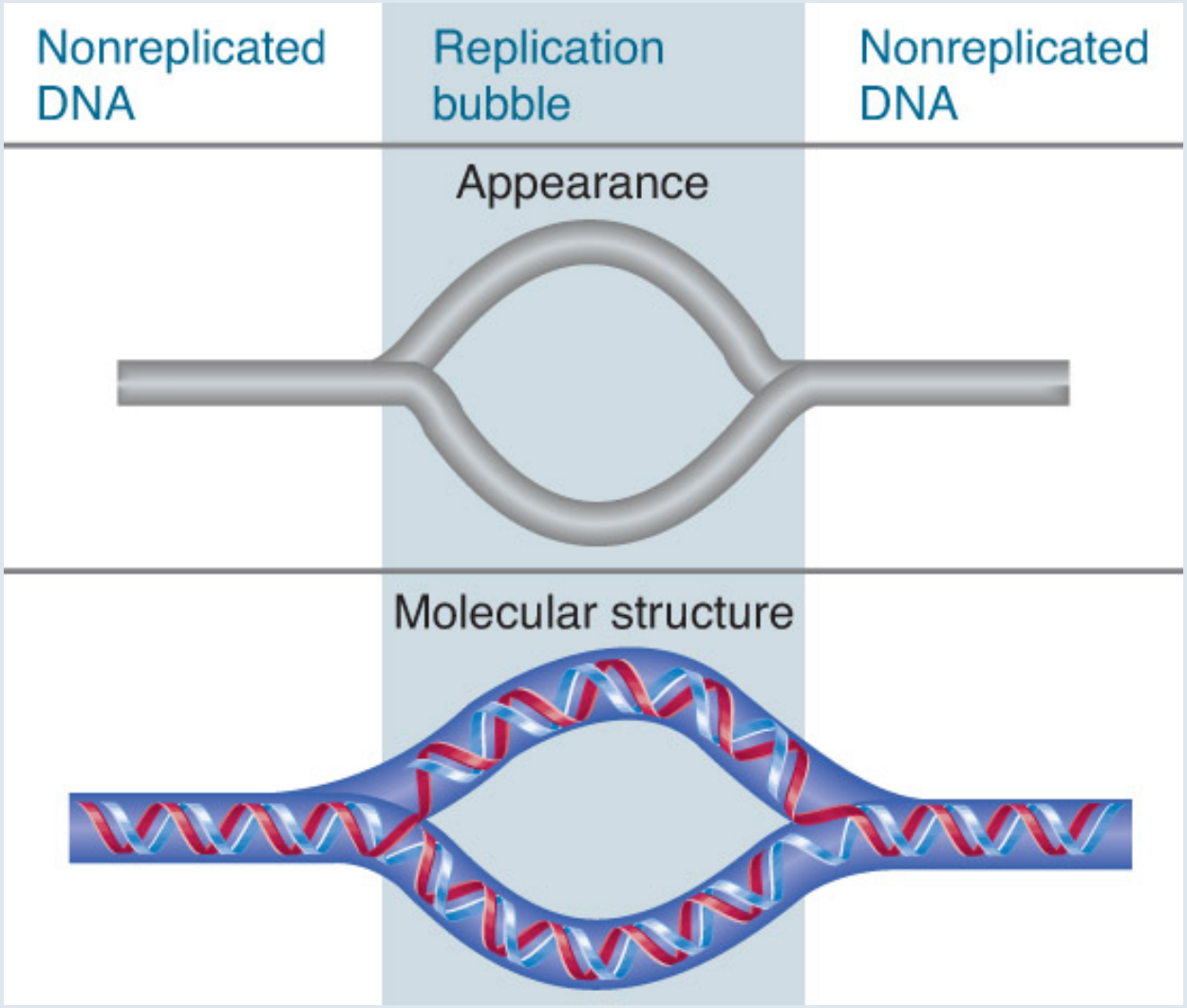
- The **replisome** – Comprises a multi-protein complex that assembles at the bacterial replication fork to undertake synthesis of DNA.

- It contains DNA polymerase and other enzymes.

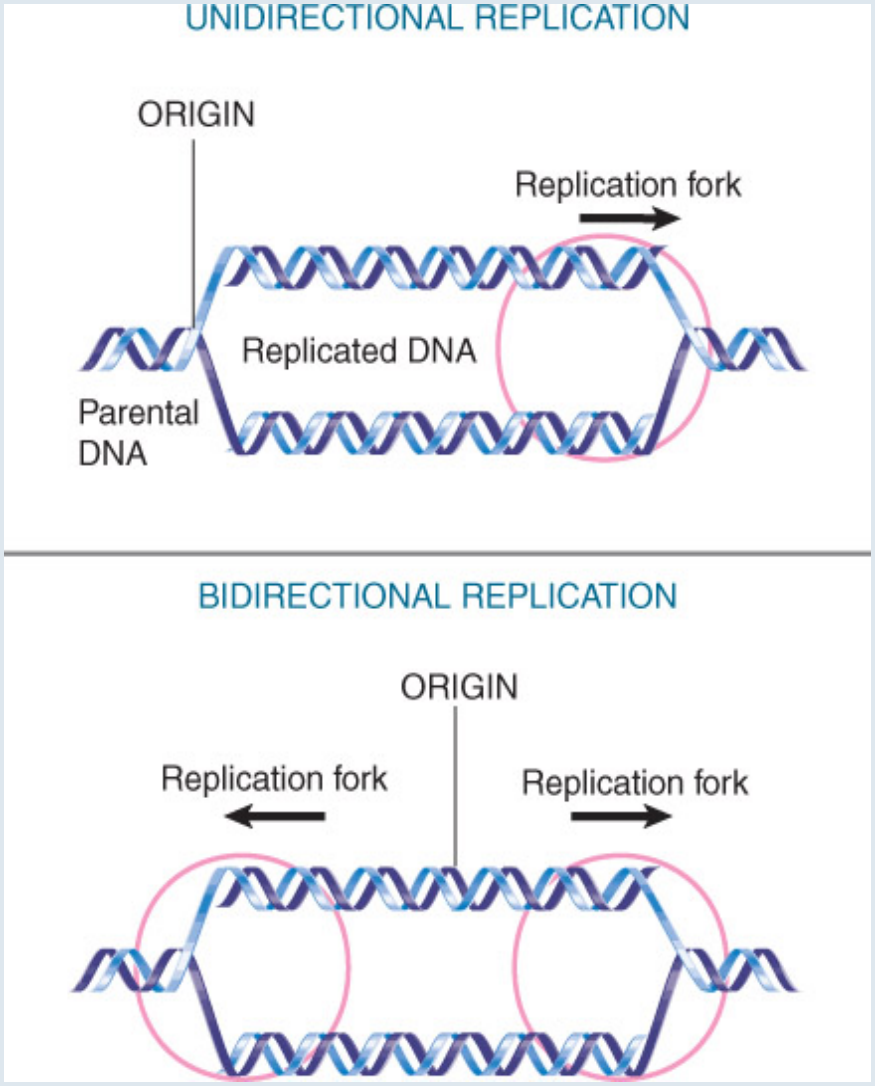




Replication eyes can be uni- or bidirectional.



Replicated DNA is seen as a replication bubble flanked by non-replicated DNA



- Replication is **unidirectional** when a single replication fork is created at an origin.
- Replication is **bidirectional** (with respect to the chromosome) when an origin forms it creates **two replication forks** that move in opposite directions.

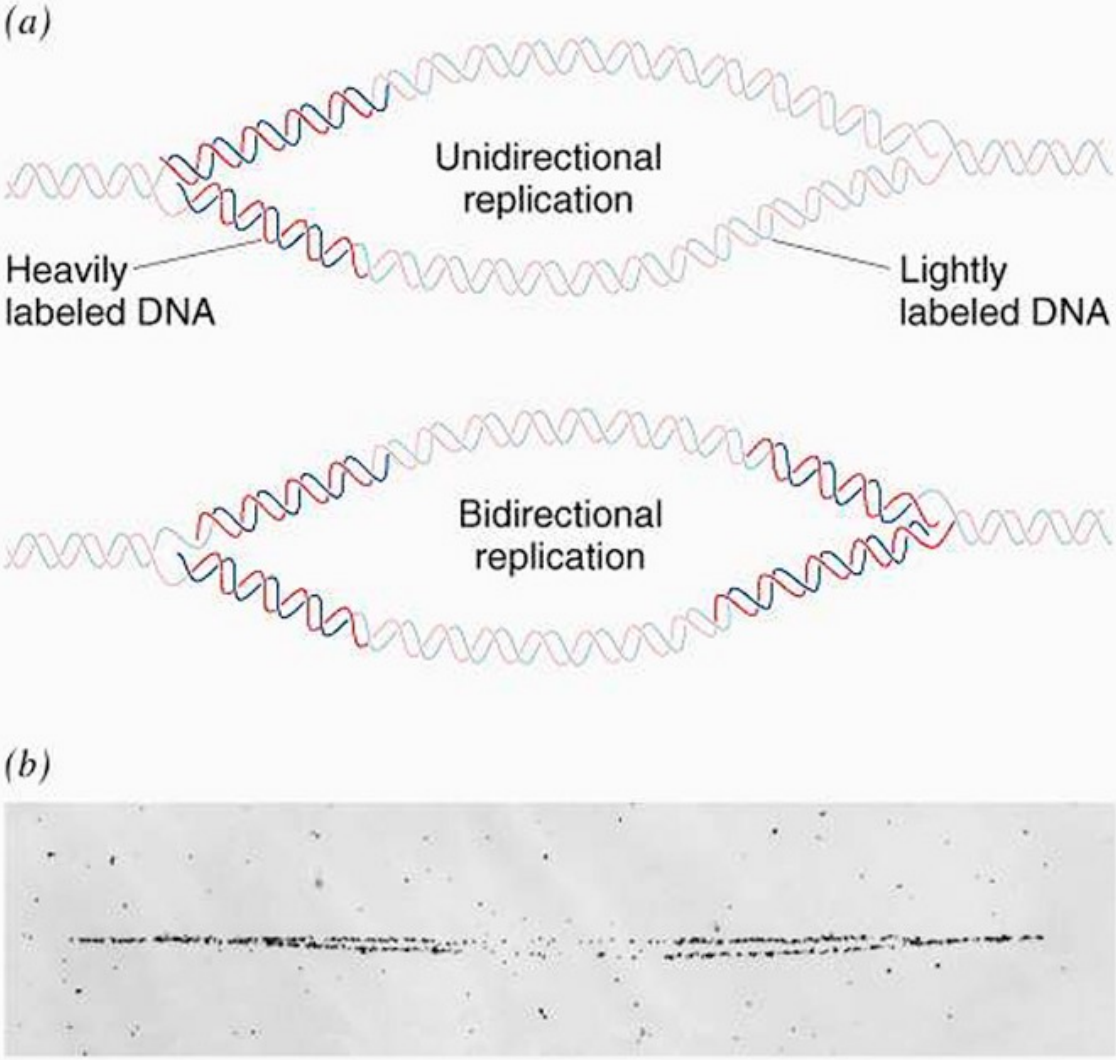
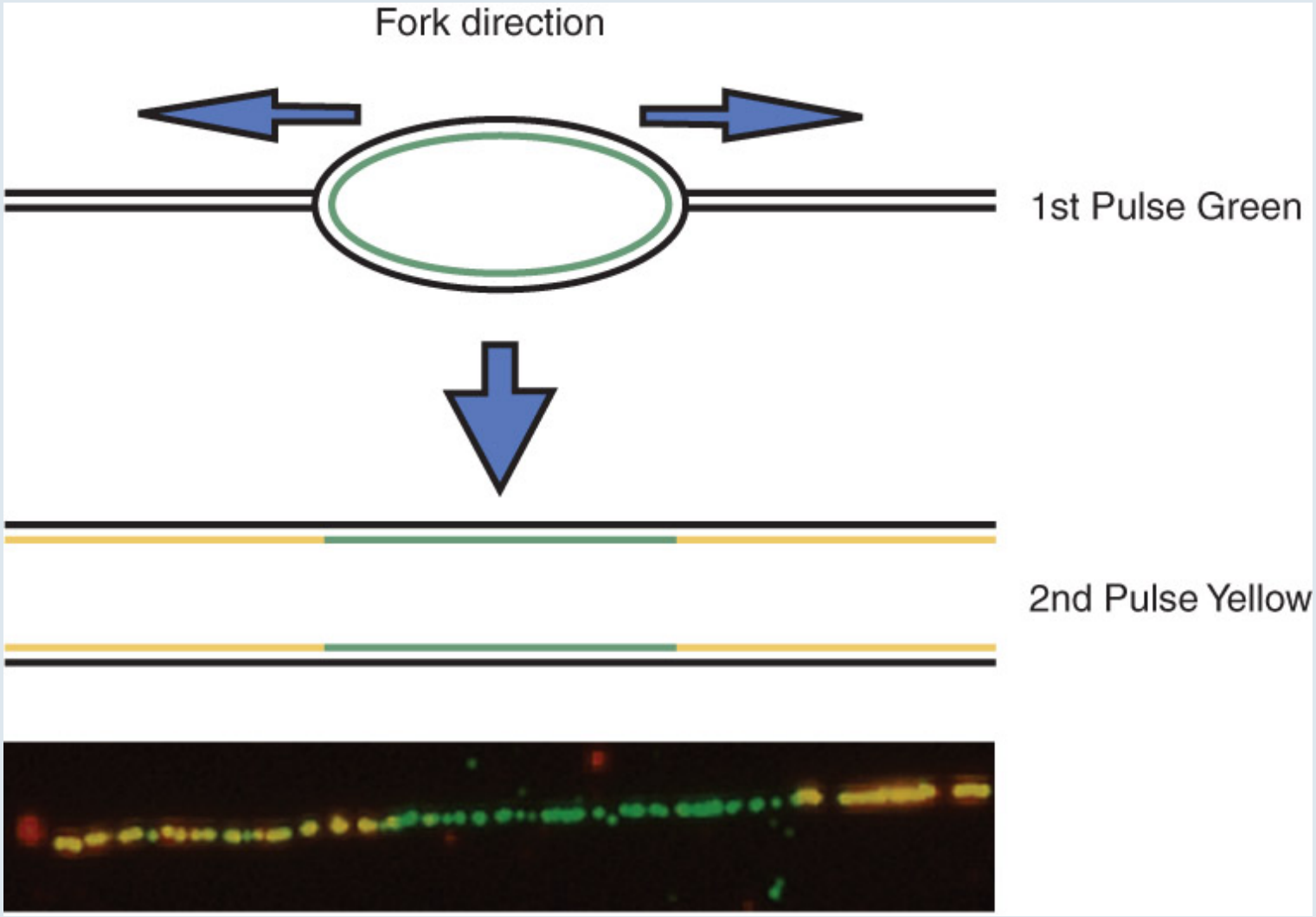
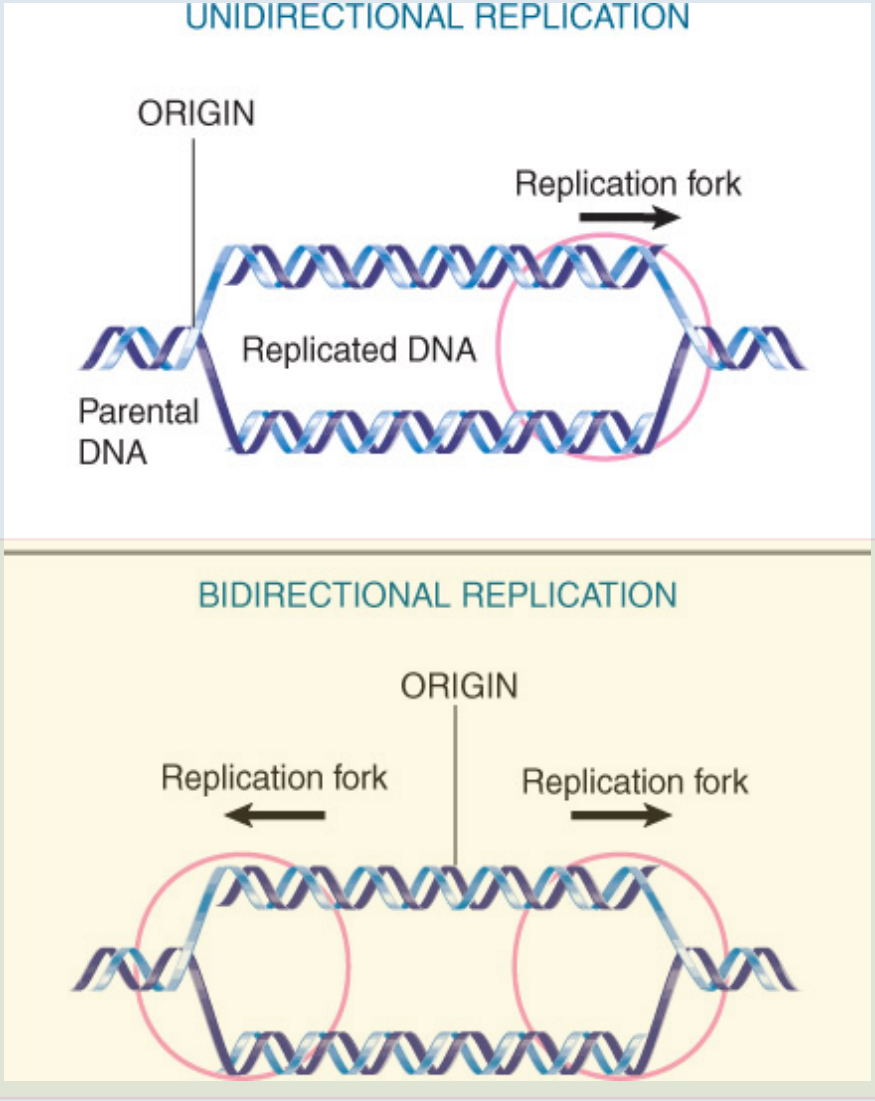


Figure 24-4. The autoradiographic differentiation of unidirectional and bidirectional θ replication of DNA. [Courtesy of David M. Prescott and P.L. Kuempel, University of Colorado.]



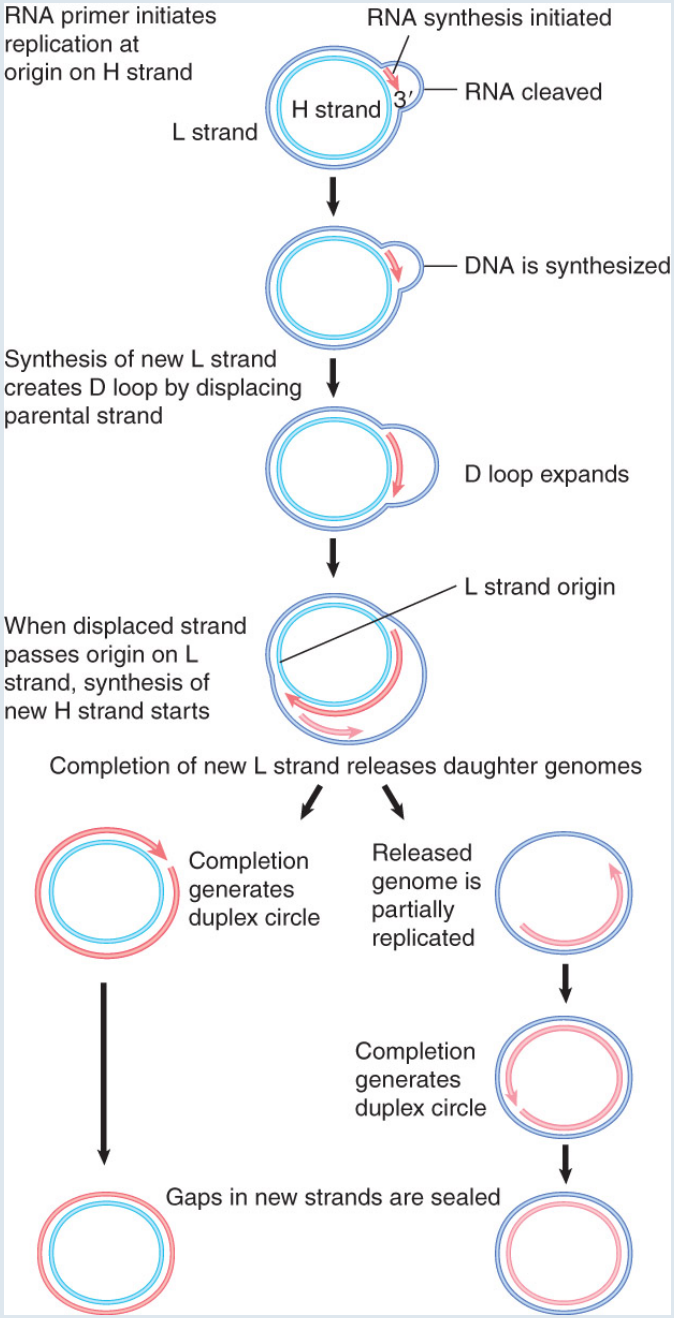
Initial replication is detected by incorporation of green label

Photo reproduced from K. Marheineke, et al., Visualization of bidirectiona
chromosomal DNA replication in a human cell free system, Nucleic Acids
(21), pp. 6931-3941, by permission of Oxford University Press. Photo c
Kathrin Marheineke and Torsten Krude.

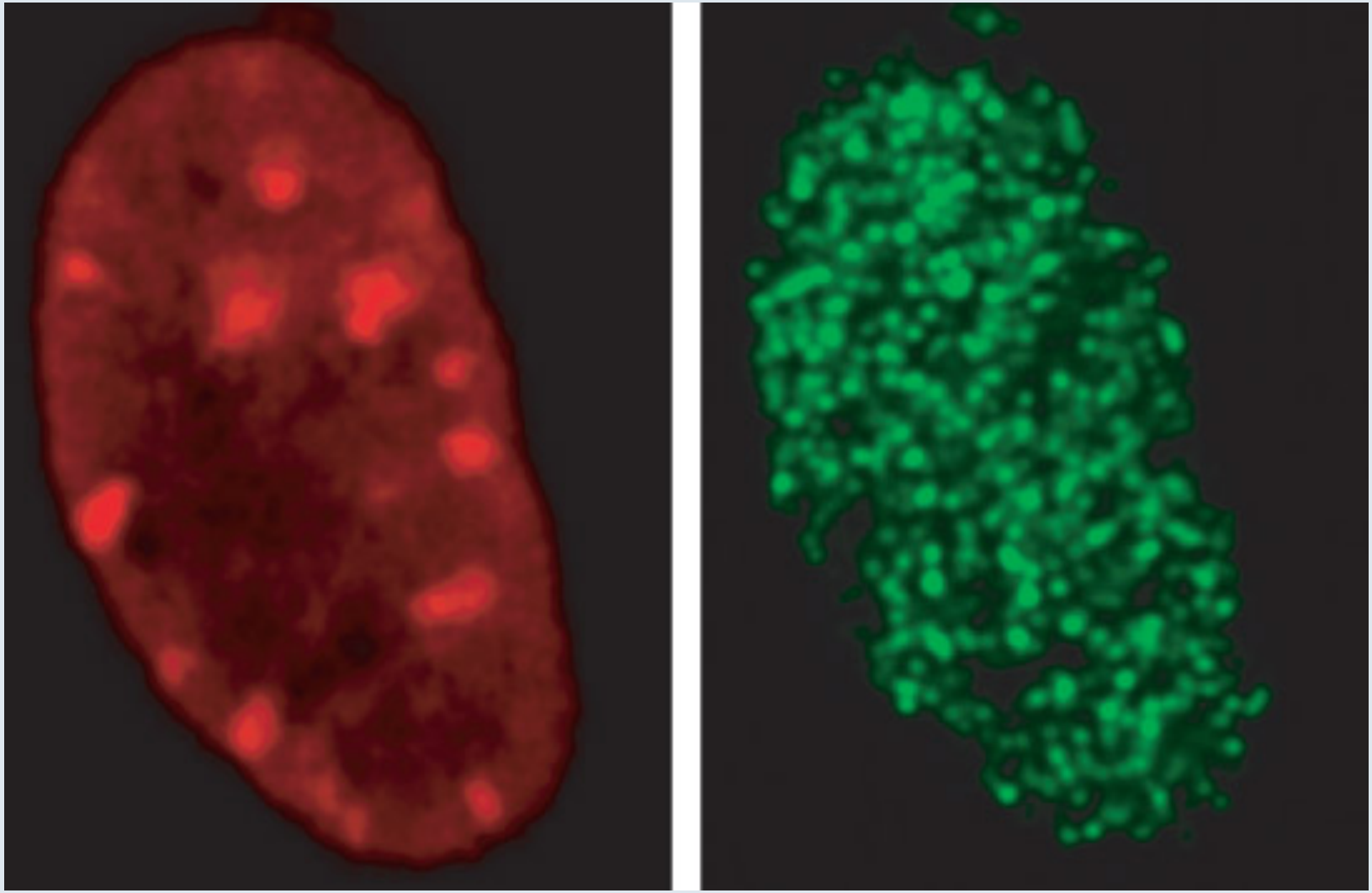


Replicons may be unidirectional or bidirectional, depending on whether one or two replication forks are formed at the origin.

- Replication is **unidirectional** when a single replication fork is created at an origin.
- Replication is **bidirectional** when an origin creates two replication forks that move in opposite directions.



The D loop maintains an opening in mammalian **mitochondrial DNA**, which has separate origins for the replication of each strand



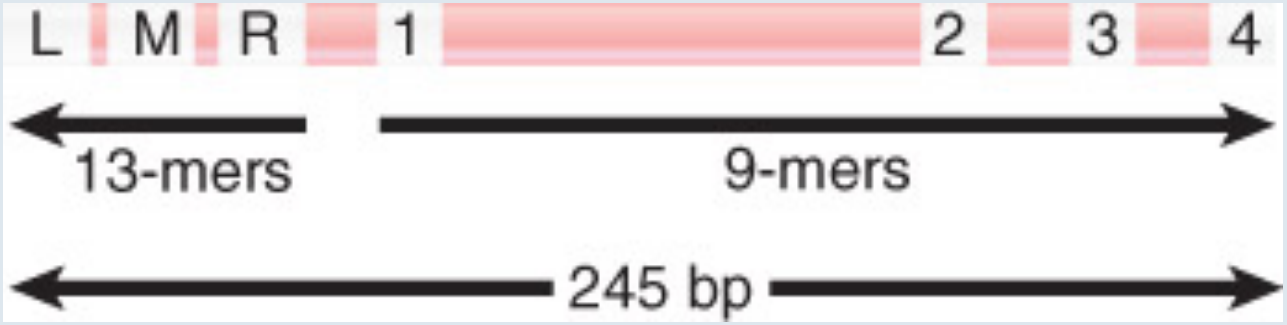
Replication forks are organized into “foci” in the nucleus

Photos courtesy of Anthony D. Mills and Ron La
Research Center, University of Cambridge.

DNA Replication: *youtube.com/watch?v=yqESR7E4b_8*

Initiation: Creating the Replication Forks at the Origin *oriC in prokaryotes*

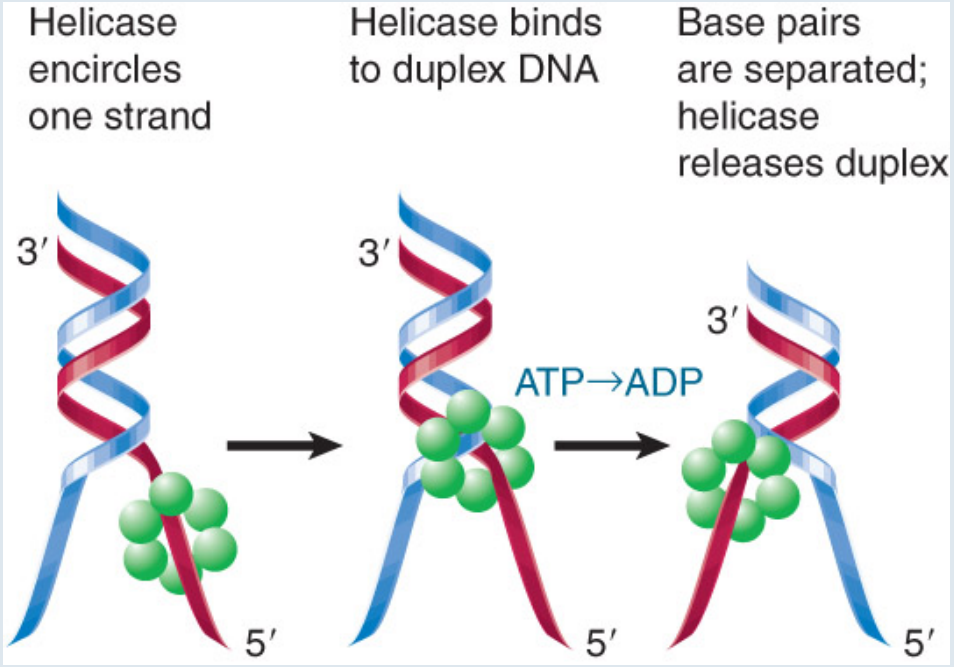
- Initiation takes place at the singular ***oriC*** and requires the sequential assembly of a large protein complex on the membrane.
- **to initiate *oriC*** must be **fully methylated to initiate.**
- **DnaA**- ATP (a **licensing factor**) binds to short repeated sequences and forms an oligomeric complex that melts DNA.



The minimal origin is defined by the distance between the outside members of the 13-mer and 9-mer repeats.

Replication Requires a Helicase and a Single-Strand Binding Protein

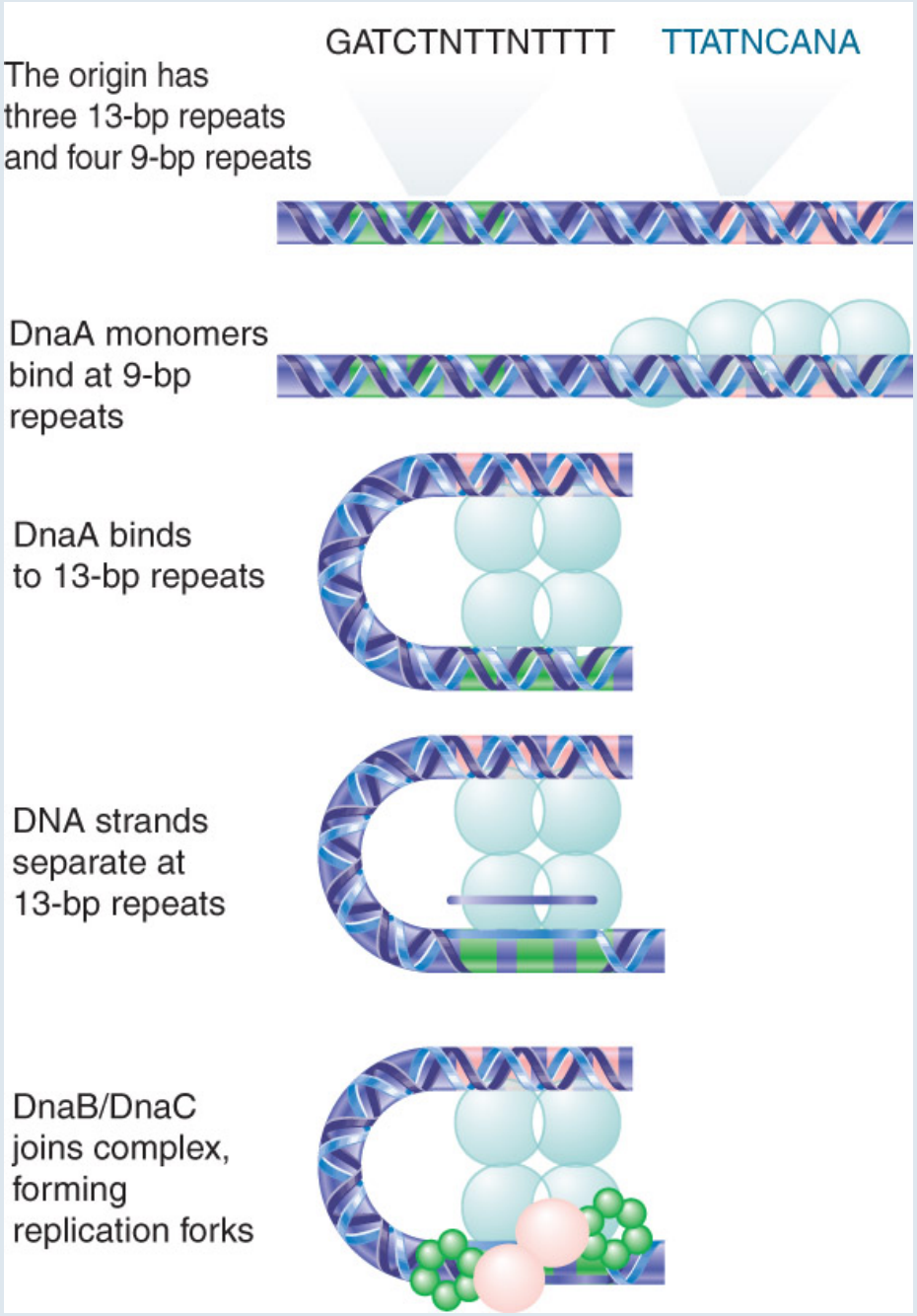
- Replication requires a helicase to separate the strands of DNA using energy provided by hydrolysis of ATP (very expensive).
- A single-stranded binding protein is required to maintain the separated strands.



A hexameric helicase moves along one strand of DNA

Initiation: Creating the Replication Forks at the Origin *oriC*

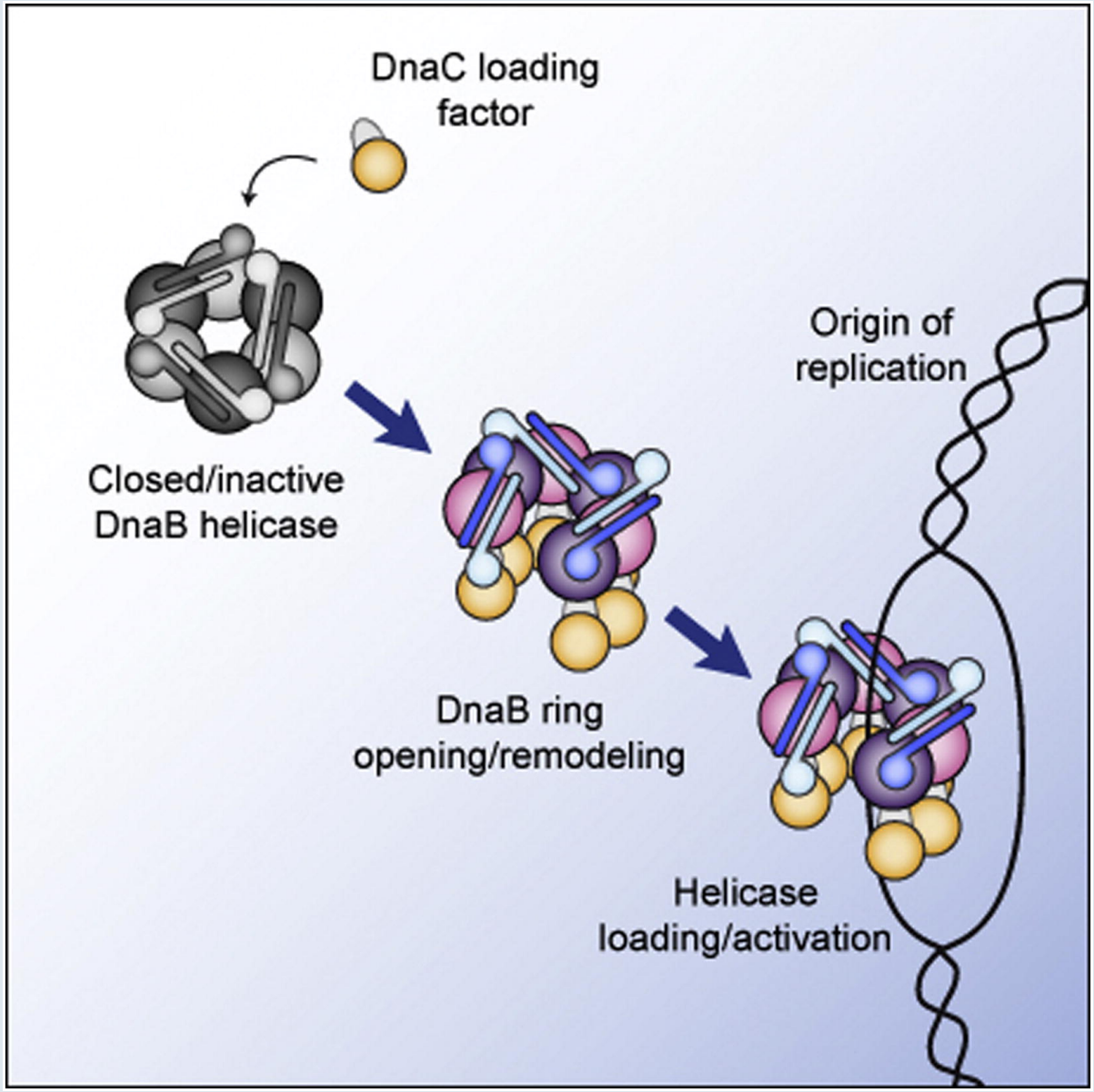
- After **DnaA** has opened up the DNA, the hexameric **DnaB** arrives at the replication fork. **Gyrase** and **SSB** are also required, as well as Hu proteins. The gyrase is a topoisomerase II, which is required to offset the localized stress caused by the action of the 2 helicases
- A short region of A-T -rich DNA is melted.
- **DnaG** is bound to the **helicase** complex and expands away from the ***oriC*** creating the replication forks.
- **DnaG** is an **RNA primase** – A type of RNA polymerase that synthesizes short segments of RNA that will be used as primers for DNA replication.



Initiation: Creating the Replication Forks at the Origin *oriC*

- Six **DnaC** monomers escort each hexamer of **DnaB**, and this complex binds to the origin.

Pre-priming involves formation of a complex by sequential association of proteins, which leads to the separation of DNA strands



Cell 2013 153, 438-448DOI: (10.1016/j.cell.2013.03.006)

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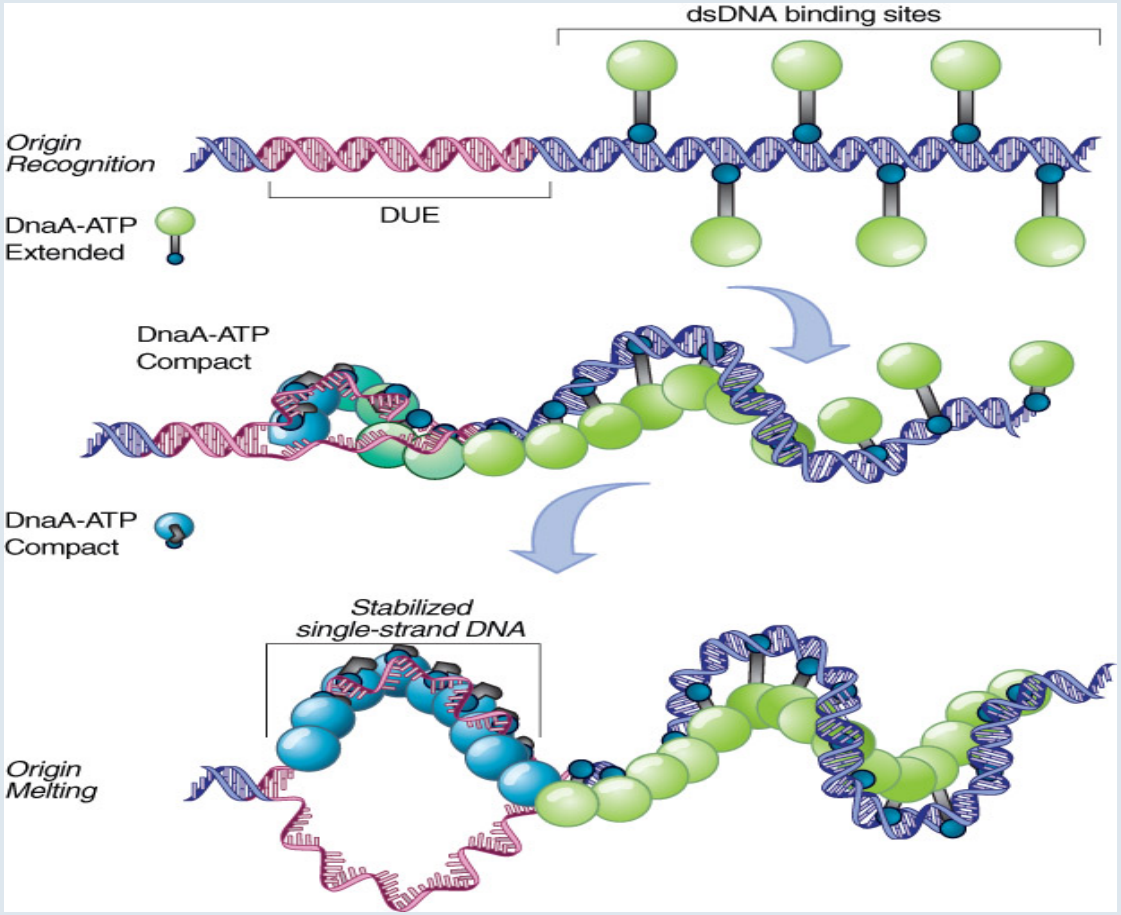
Initiation: Creating the Replication Forks at the Origin *oriC*

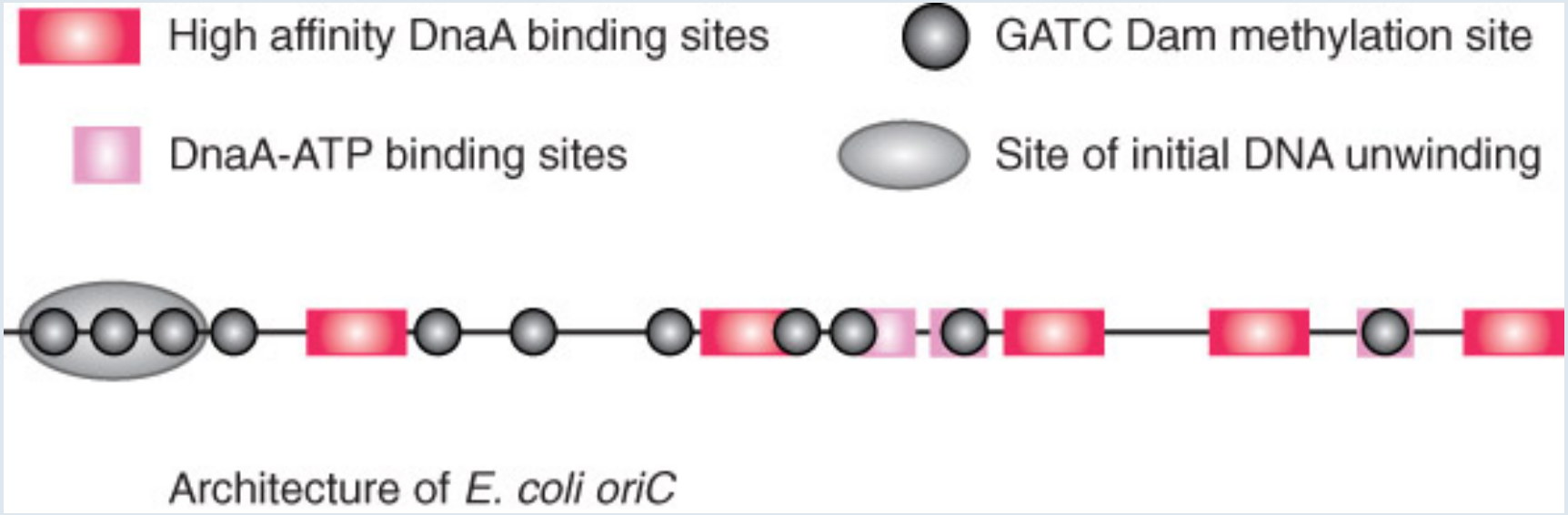


A two-state assembly model of DNA during initiation.

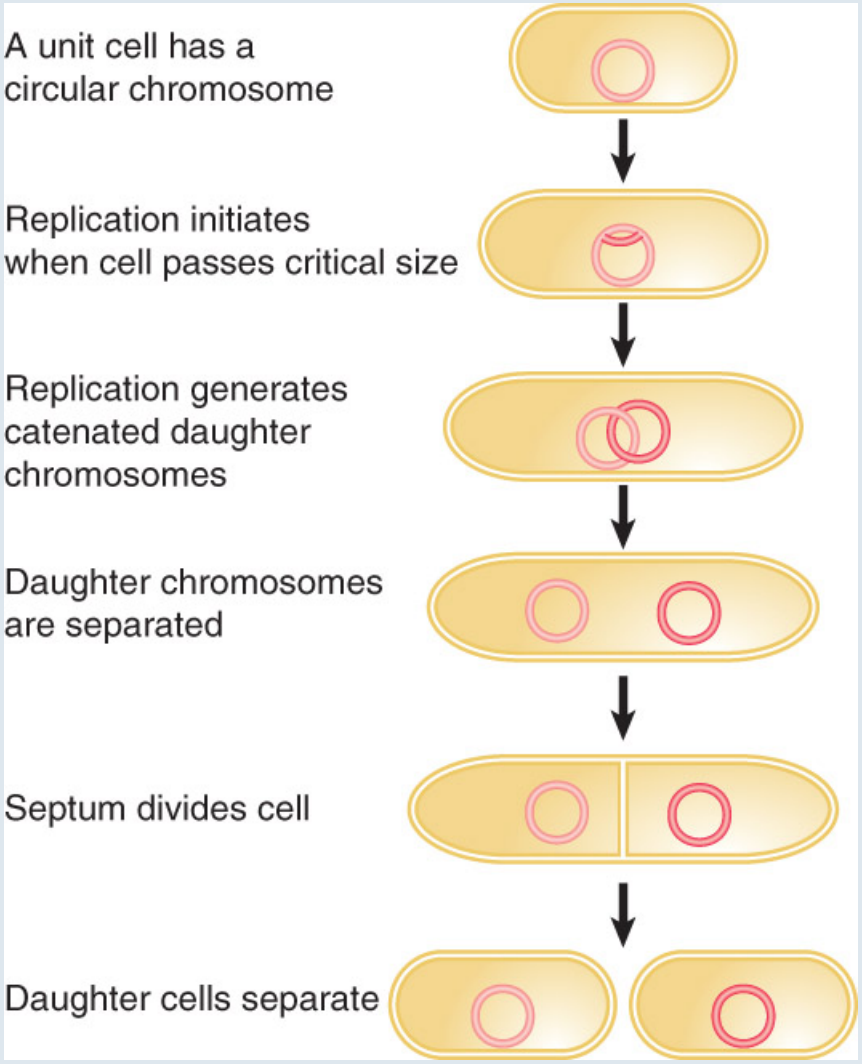
Adapted from Journal of Biological Chemistry, vol 285, Karl E. Duderstadt, et al., Origin Remodeling and Opening in Bacteria..., pp. 28229-28239. © 2010 The American Society for Biochemistry and Molecular Biology

- ***oriC*** contains binding sites for DnaA: *dnaA*-boxes.

- ***oriC*** also contains 11 GATC/CTAG repeats that are **methylated** on **Adenine** residues on both strands.



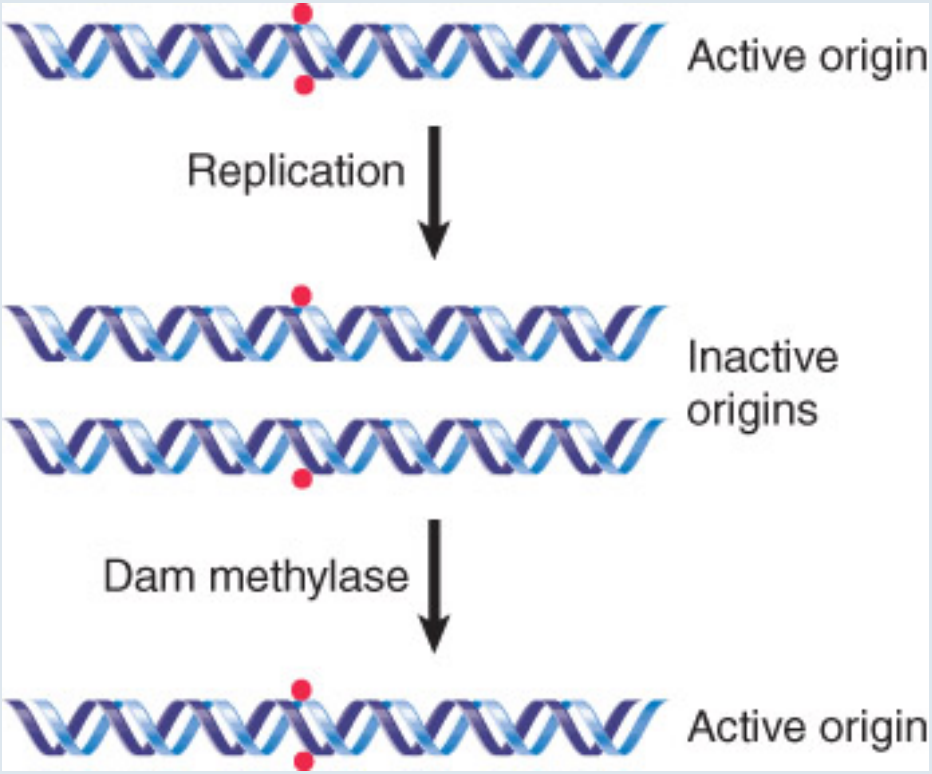
The E. coli origin of replication, oriC, contains multiple binding sites for the DnaA initiator protein.



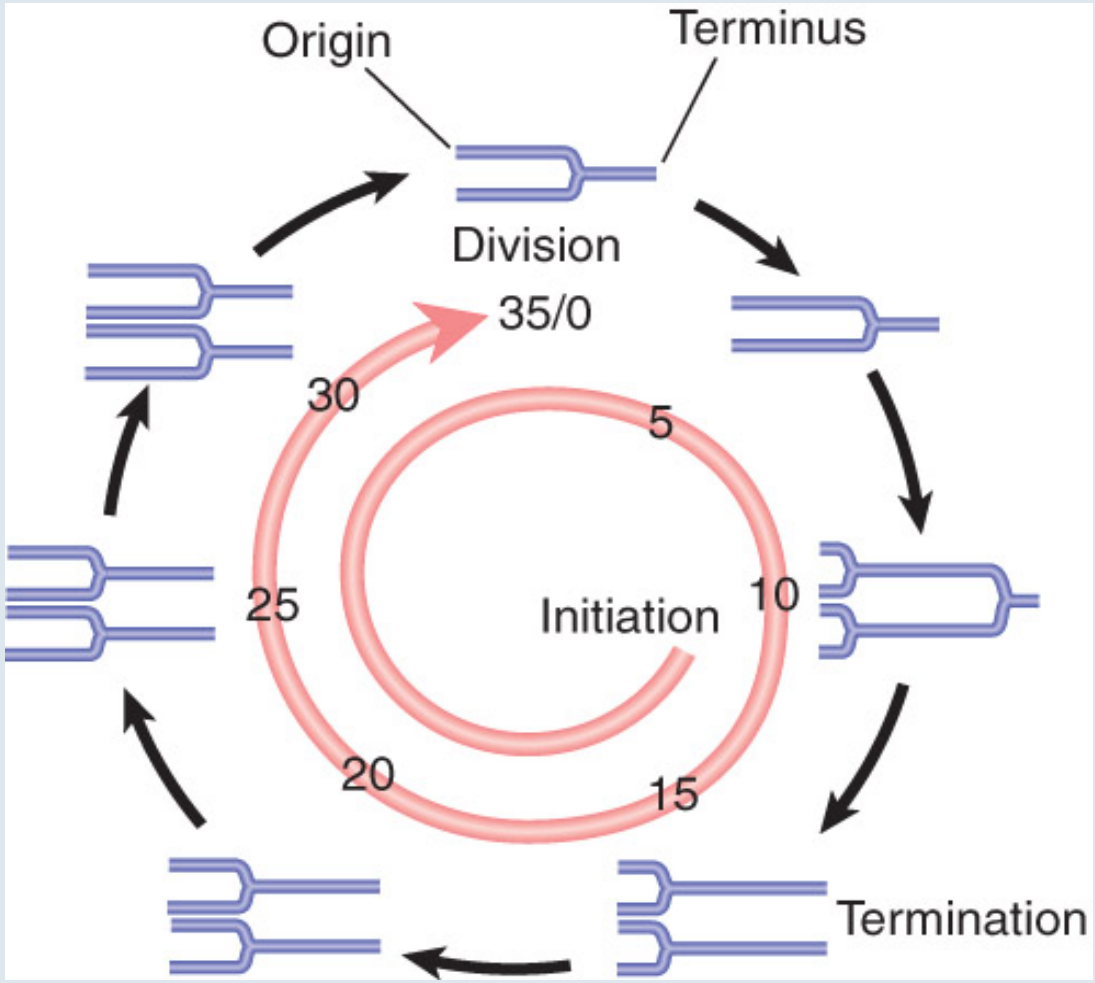
Replication initiates at the bacterial origin when a cell passes a critical threshold of size

Methylation of the Bacterial Origin Regulates Initiation

- Replication generates **hemi-methylated DNA**, which cannot initiate replication.
- There is at least a 13-minute delay before the GATC/CTAG repeats are remethylated.



Only fully methylated origins can initiate replication.



The fixed interval of 60 minutes between initiation of replication and cell division produces multi-forked chromosomes in rapidly growing cells

Multiple Mechanisms Exist to Prevent Premature Re-initiation of Replication

- **SeqA** binds to hemi-methylated DNA and is required for delaying rereplication.
- **SeqA** may interact with **DnaA**.
- As the origins are hemi-methylated they bind to the cell membrane and may be unavailable to methylases.
- The *dat* locus contains DnaA-binding sites that titrate availability of DnaA protein.
- ***Hda*** protein is then recruited to the replication origin to convert **DnaA**-ATP to **DnaA**-ADP.

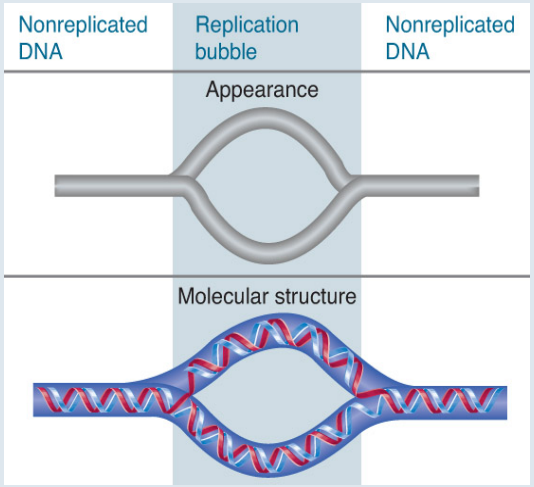
Initiation: A little more complex in Eukaryotes.....

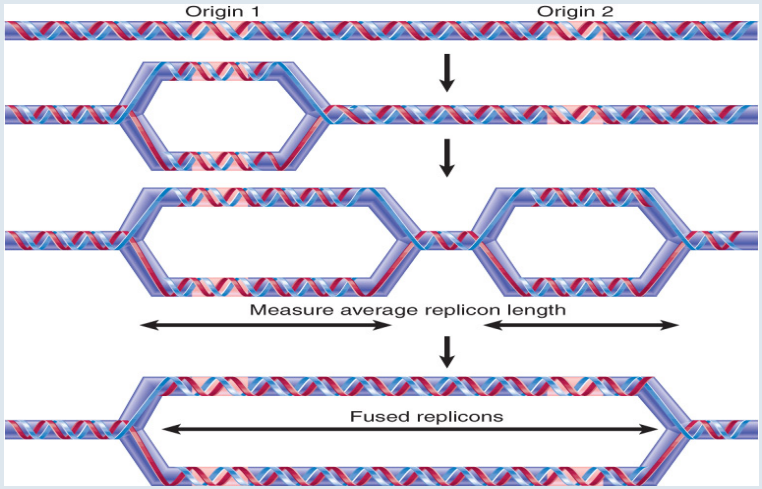
- **Cell Cycle dependency**

- **licensing factor required??**– factors located in the nucleus and necessary for replication that is inactivated or destroyed after one round of replication.
  - New factors must be provided for further rounds of replication to occur.

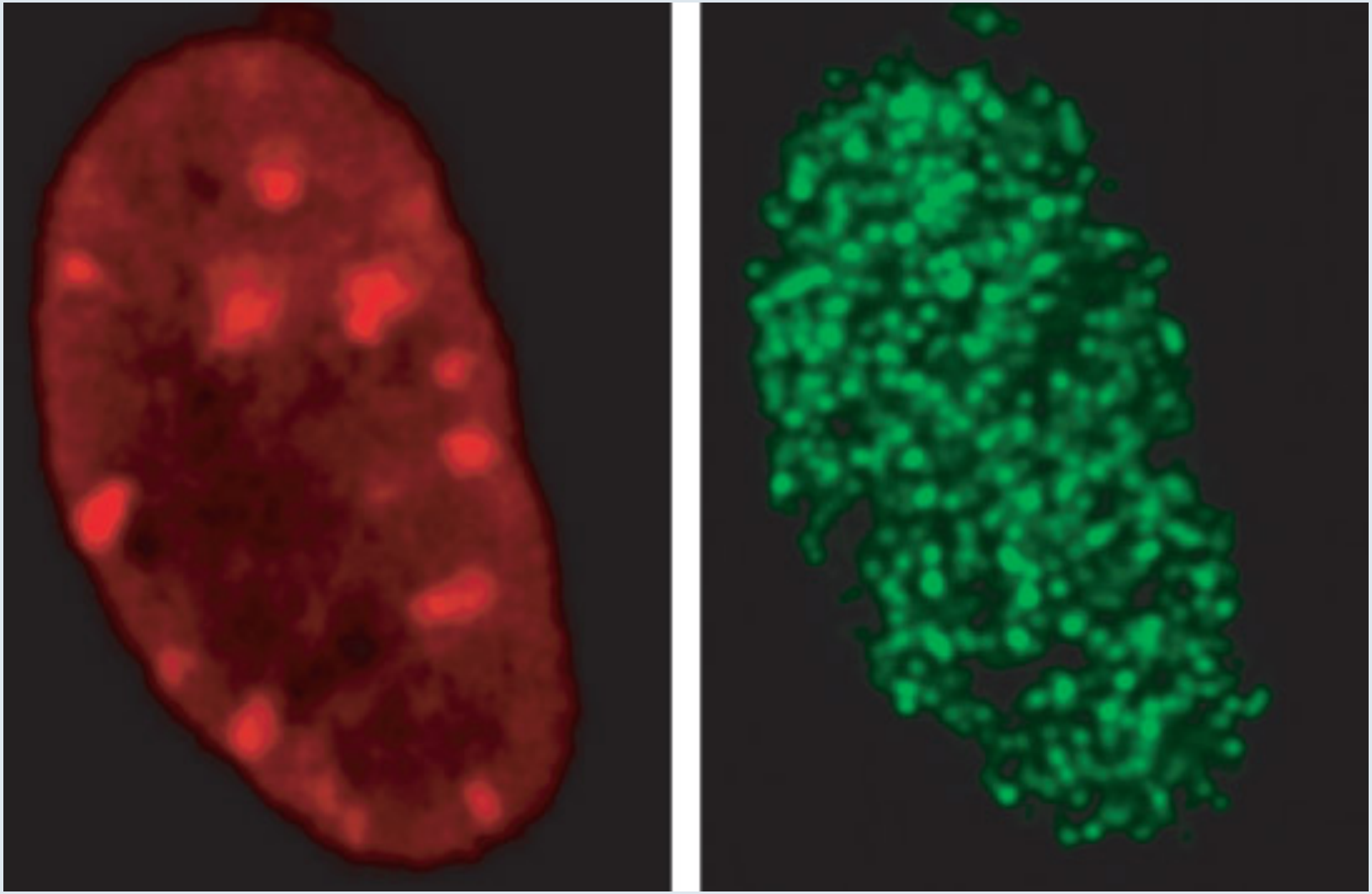
Each Eukaryotic Chromosome Contains Many Replicons

- A chromosome is divided into many replicons.





- The progression into **S phase** is tightly controlled.
- **checkpoint –** A biochemical control mechanism that prevents the cell from progressing from one stage to the next



Replication forks are organized into “foci” in the nucleus

Photos courtesy of Anthony D. Mills and Ron Laskey, Hutch
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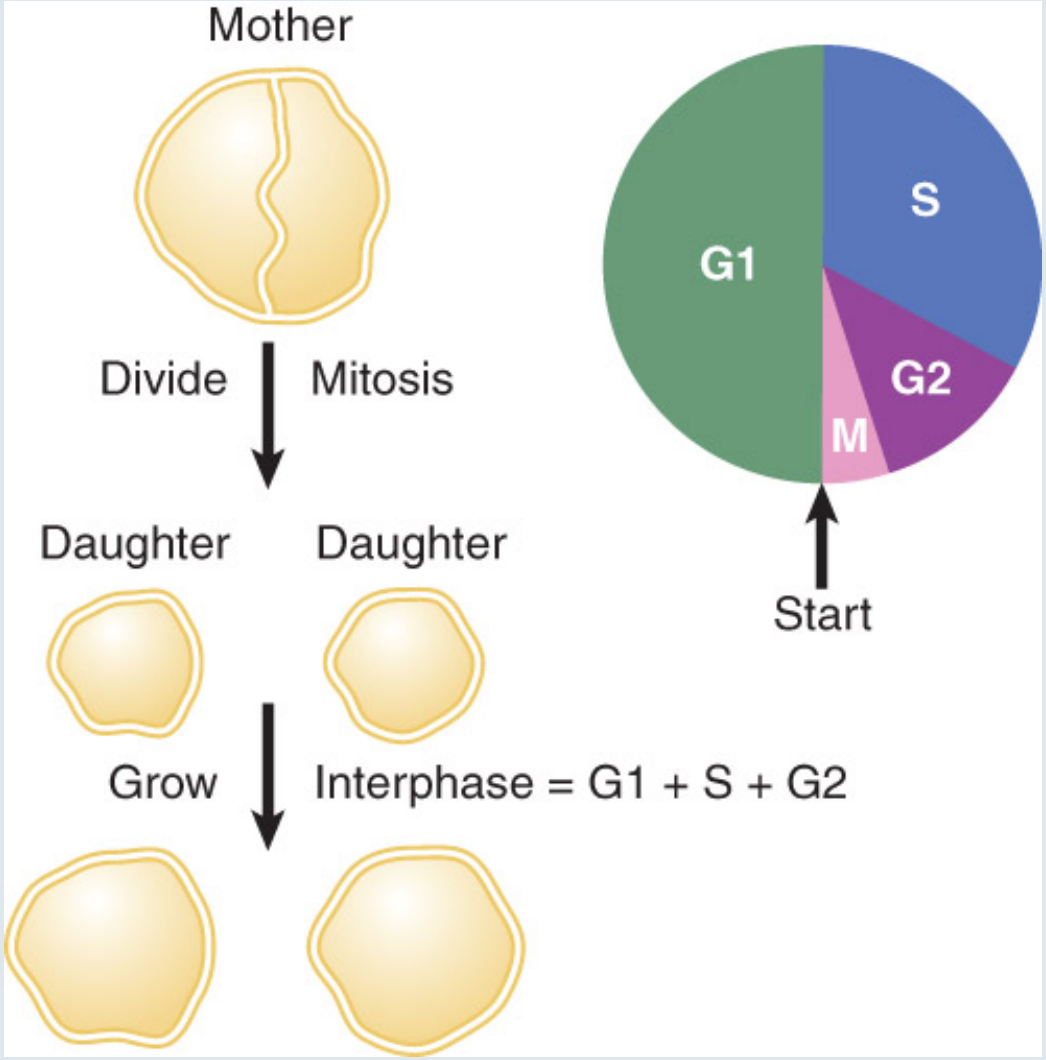
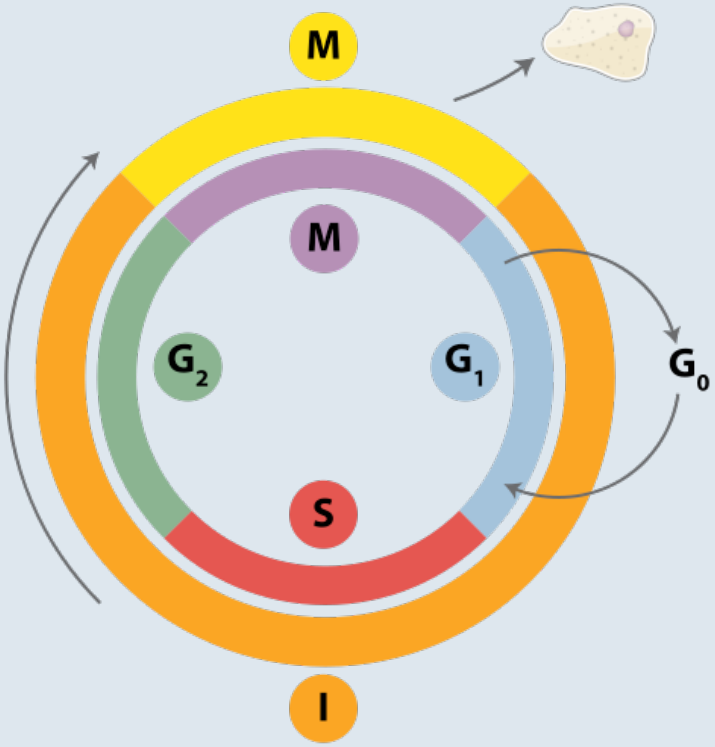
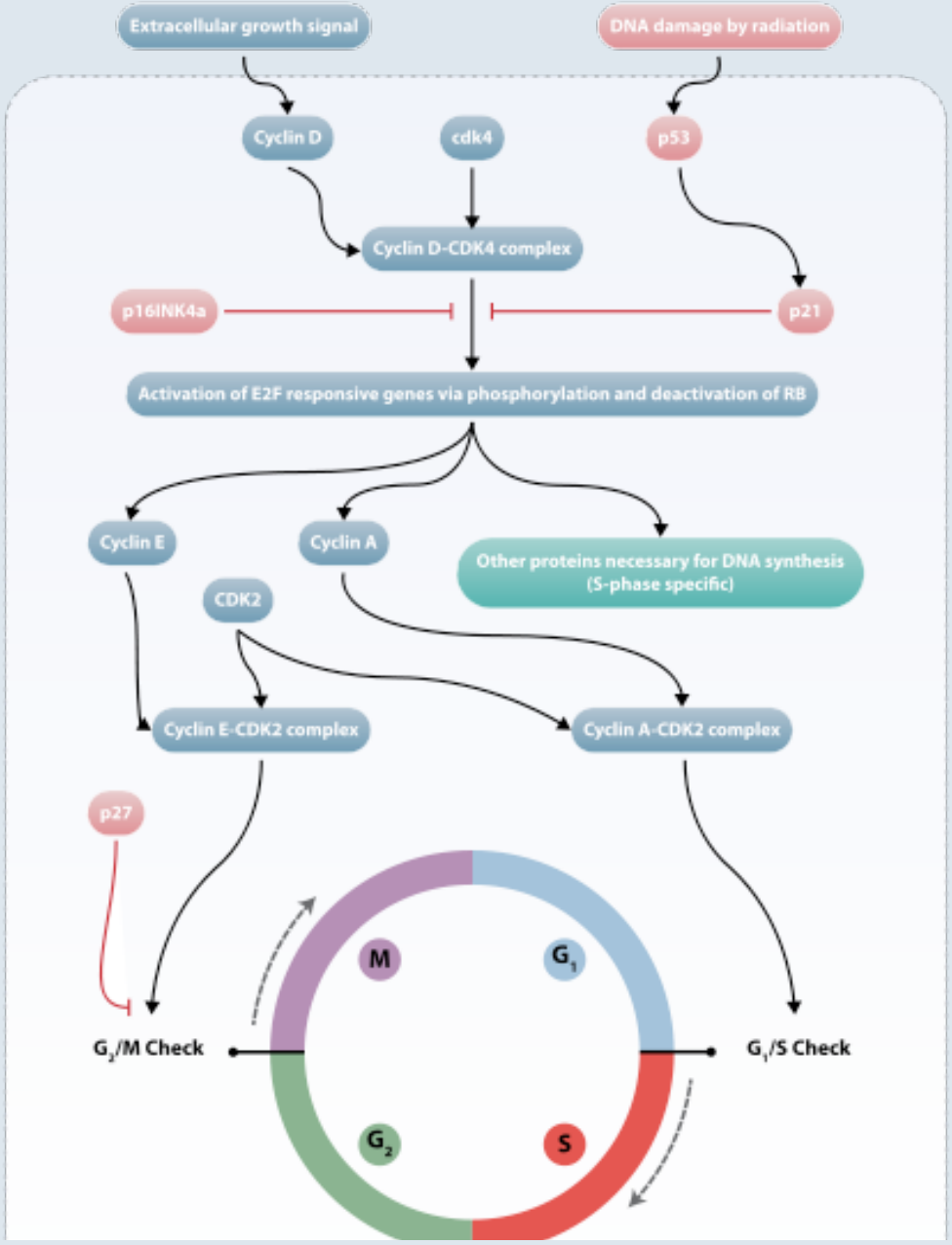


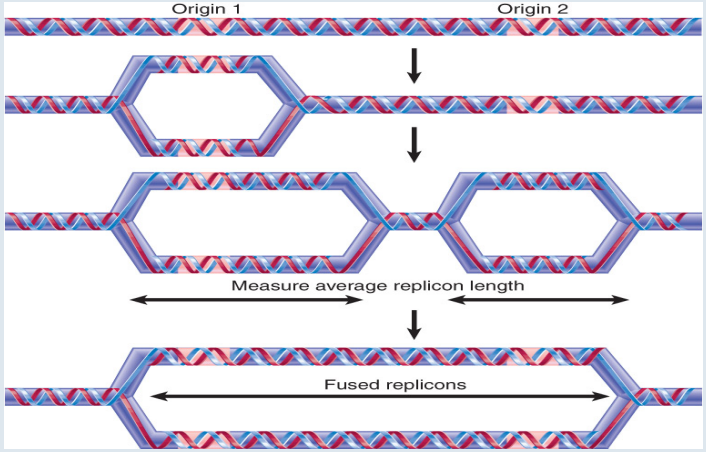
The eukaryote cell cycle


M
M
G2
G1
G0
S
I


Extracellular growth signal
DNA damage by radiation
Cyclin D
cdk4
p53
Cyclin D-CDK4 complex
p16INK4a
p21
Activation of E2F responsive genes via phosphorylation and deactivation of RB
Cyclin E
Cyclin A
Other proteins necessary for DNA synthesis (S-phase specific)
CDK2
Cyclin E-CDK2 complex
Cyclin A-CDK2 complex
p27
M
G1
G2
S
G2/M Check
G1/S Check

Each Eukaryotic Chromosome Contains Multiple Replicons

- A chromosome is divided into many replicons.

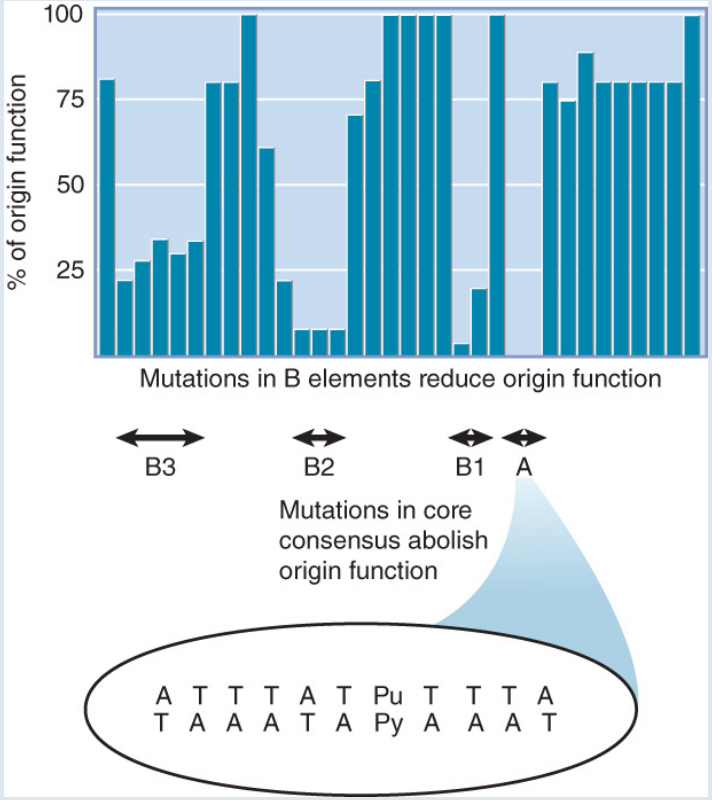


- The progression into **S phase** is tightly controlled.

- **checkpoint –** A biochemical control mechanism that prevents the cell from progressing from one stage to the next unless specific goals and requirements have been met.

Replication Origins Can Be Isolated in Yeast

- Origins in *S. cerevisiae* are short A-T sequences that have an essential 11-bp sequence.
- The **ORC** is a complex of six proteins **ORC**1-6 that binds to an **ARS**.



An ARS extends for ~50 bp and includes a consensus sequence (A) and additional elements (B1–B3).

Replication Origins Can Be Isolated in Yeast

- **A domain** – The conserved 11-bp sequence of A-T base pairs in the yeast ARS element that comprises the replication origin.

- Related ORC complexes are found in multicellular eukaryotes.

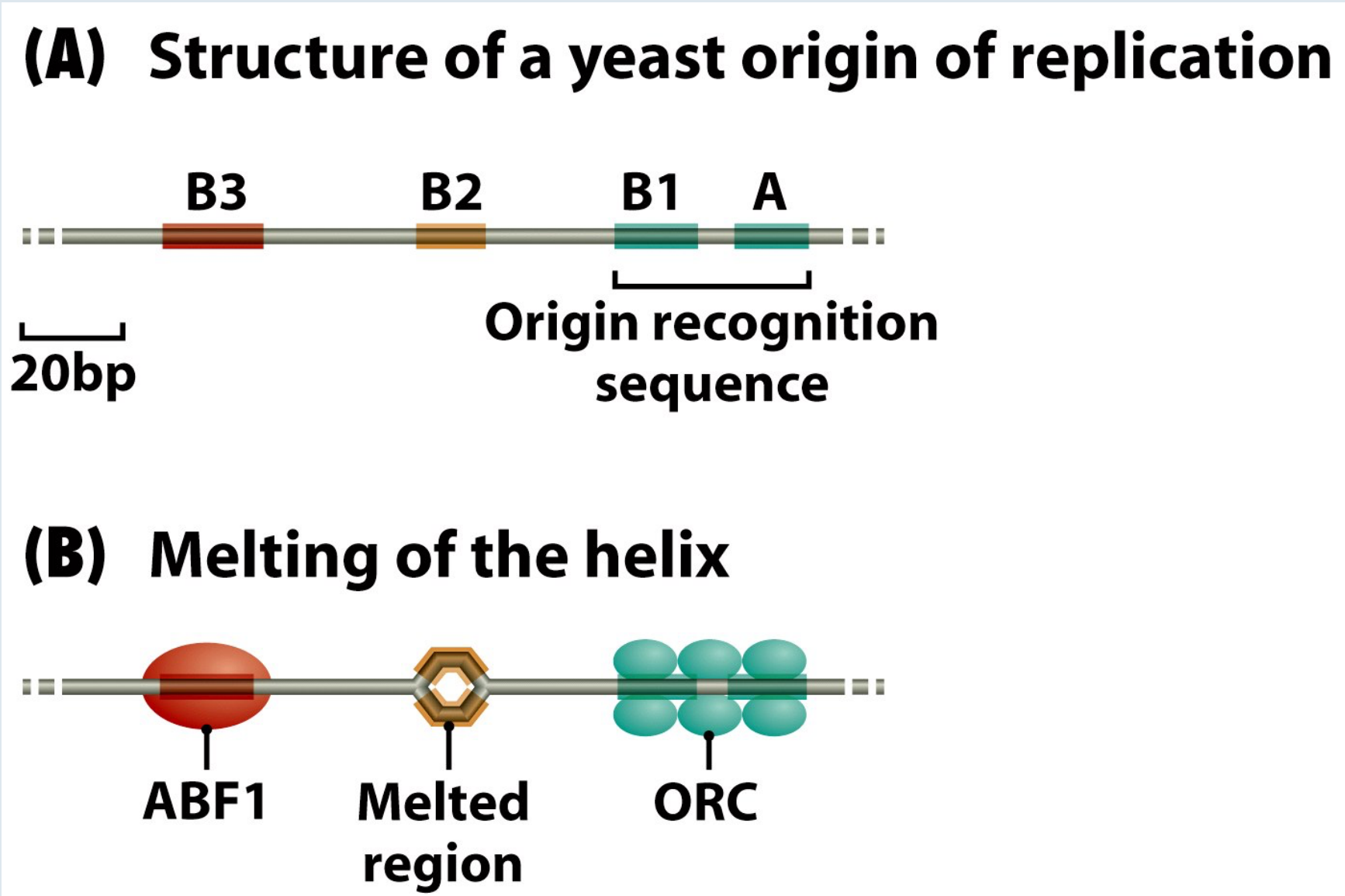
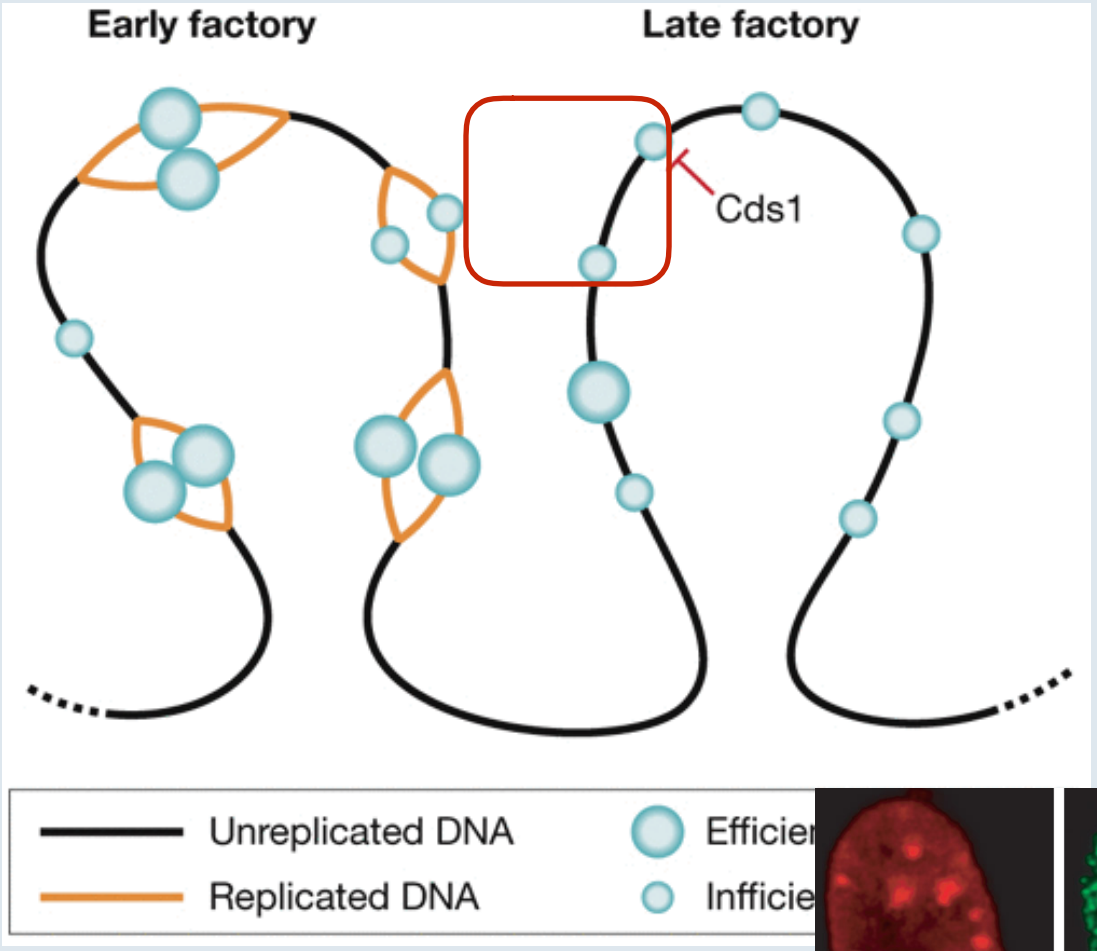


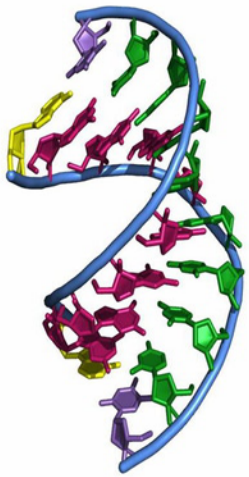
Figure 15-10 Genomes 3 (© Garland Science 2007)

The elusive determinants of replication origins

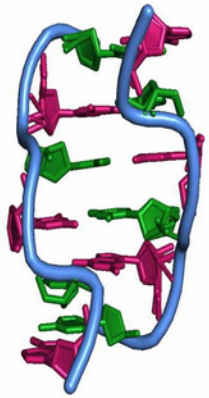


EMBO reports
Volume 8, Issue 4, pages 332-334, 1 APR 2007 DOI: 10.1038/sj.embor.7400954
http://onlinelibrary.wiley.com/doi/10.1038/sj.embor.7400954/full#embr7400954-fig-0002

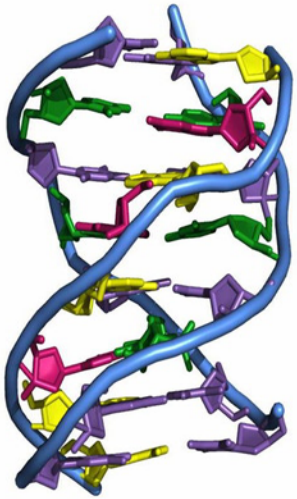
A-DNA



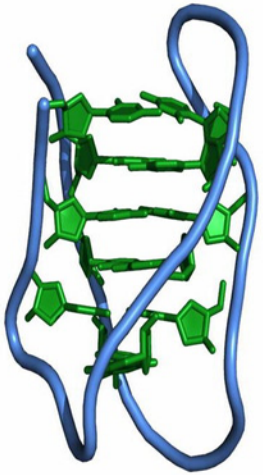
Z-DNA



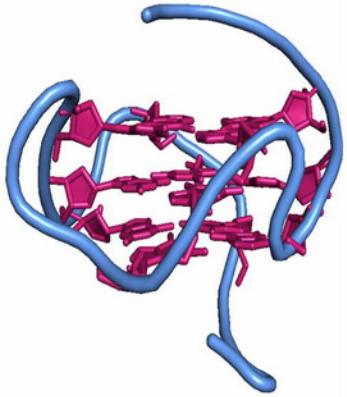
Triplex DNA



i-Motif



G-quadruplex



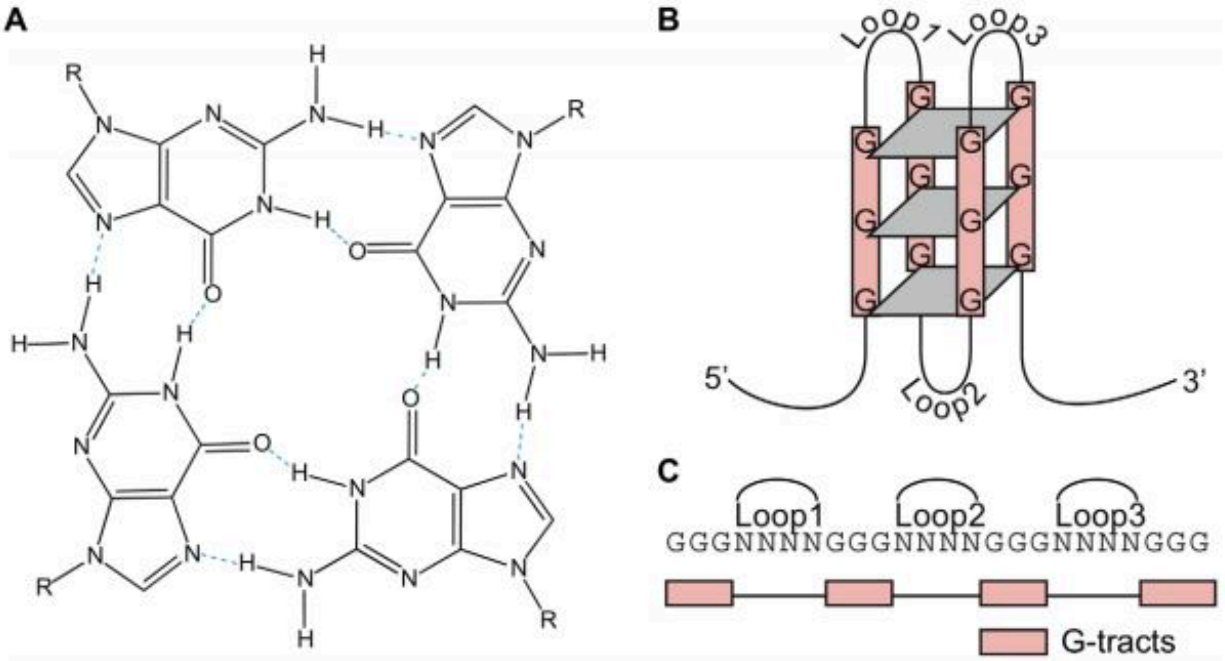
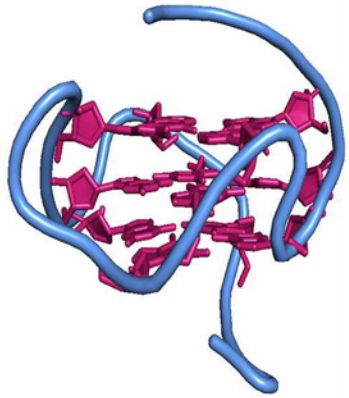


Figure 1. The G4 DNA structure and motif. (**A**) Structure of a G-quartet. The planar ring of four hydrogen-bonded guanines is formed by guanines from different G-tracts, which are separated by intervening loop regions in the intra-molecular G4 DNA structure. (**B**) Schematic of an intra-molecular G4 DNA structure consisting of three G-quartets. Inter-molecular G4 DNA structures can also form from two or four strands. (**C**) The G4 DNA motif sequence used in this study with four G-tracts of three guanines separated by loop regions.
doi:10.1371/journal.pcbi.1000861.g001

G-quadruplex



ARTICLE

https://doi.org/10.1038/s41467-019-11104-0 OPEN

Involvement of G-quadruplex regions in mammalian replication origin activity

Paulina Prorok[1], Marie Artufel[2,8], Antoine Aze[1,8], Philippe Coulombe[1,8], Isabelle Peiffer[1], Laurent Lacroix[3], Aurore Guédin[4], Jean-Louis Mergny[4,5], Julia Damaschke[6], Aloys Schepers[6,7], Benoit Ballester[2] & Marcel Méchali[1]

Genome-wide studies of DNA replication origins revealed that origins preferentially associate with an Origin G-rich Repeated Element (OGRE), potentially forming G-quadruplexes (G4). Here, we functionally address their requirements for DNA replication initiation in a series of independent approaches. Deletion of the OGRE/G4 sequence strongly decreased the corresponding origin activity. Conversely, the insertion of an OGRE/G4 element created a new replication origin. This element also promoted replication of episomal EBV vectors lacking the viral origin, but not if the OGRE/G4 sequence was deleted. A potent G4 ligand, PhenDC3, stabilized G4s but did not alter the global origin activity. However, a set of new, G4-associated origins was created, whereas suppressed origins were largely G4-free. In vitro *Xenopus laevis* replication systems showed that OGRE/G4 sequences are involved in the activation of DNA replication, but not in the pre-replication complex formation. Altogether, these results converge to the functional importance of OGRE/G4 elements in DNA replication initiation.

Architecture of *E. coli oriC*

SCIENTIFIC REPORTS
nature research

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5-hydroxymethylcytosine Marks Mammalian Origins Acting as a Barrier to Replication

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In most mammalian cells, DNA replication occurs once, and only once between cell divisions. Replication initiation is a highly regulated process with redundant mechanisms that prevent errant initiation events. In lower eukaryotes, replication is initiated from a defined consensus sequence, whereas a consensus sequence delineating mammalian origin of replication has not been identified. Here we show that 5-hydroxymethylcytosine (5hmC) is present at mammalian replication origins. Our data support the hypothesis that 5hmC has a role in cell cycle regulation. We show that 5hmC level is inversely proportional to proliferation; indeed, 5hmC negatively influences cell division by increasing the time a cell resides in G1. Our data suggest that 5hmC recruits replication-licensing factors, then is removed prior to or during origin firing. Later we propose that TET2, the enzyme catalyzing 5mC to 5hmC conversion, acts as barrier to rereplication. In a broader context, our results significantly advance the understating of 5hmC involvement in cell proliferation and disease states.

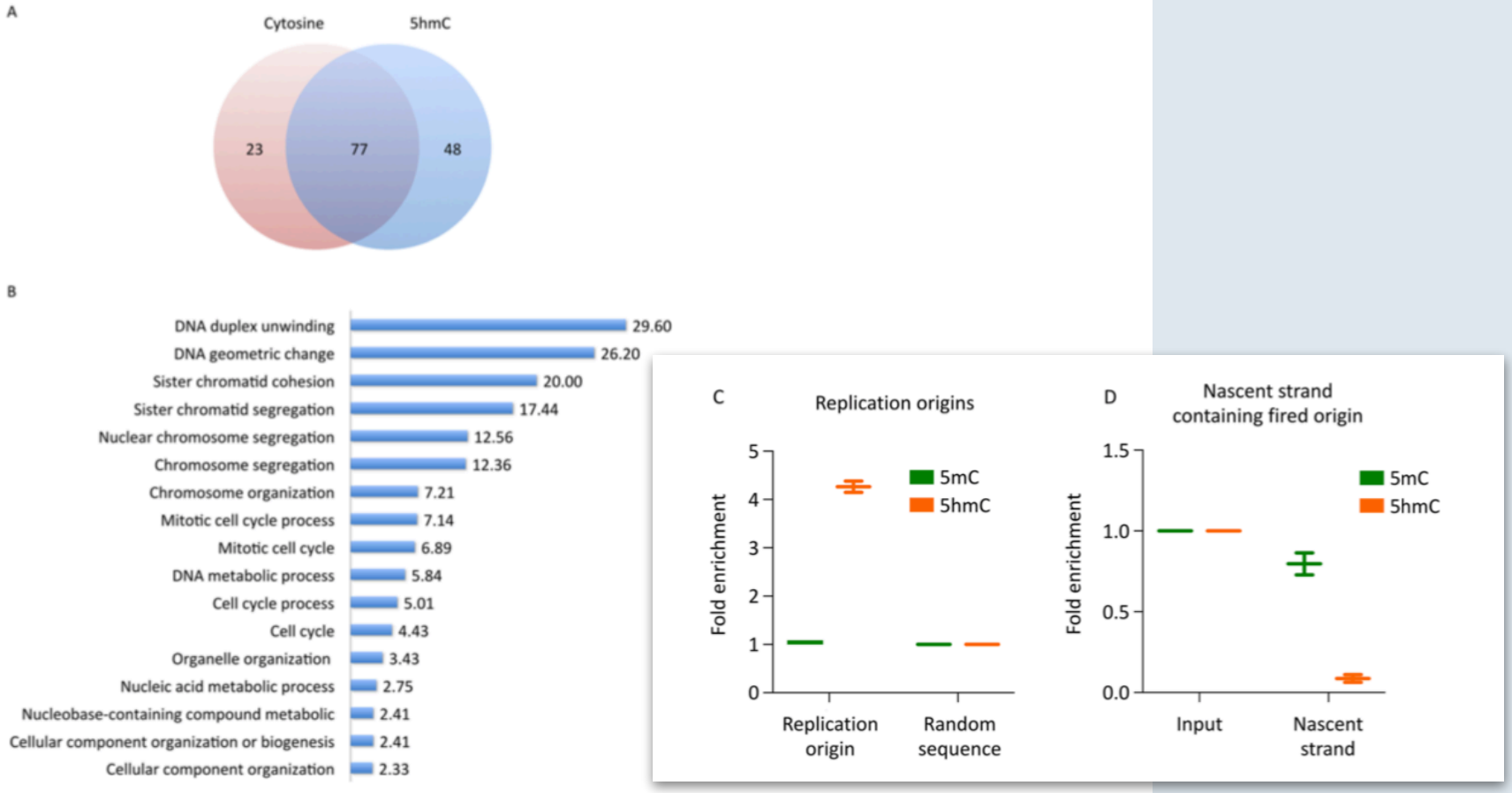


Figure 1. Proteins that exclusively bind 5hmC-modified DNA are involved in cell cycle and chromosome maintenance. (**A**) HeLa nuclear extracts were incubated with unmodified and 5hmC- modified DNA under conditions favoring DNA binding. Proteins that bound to each substrate were resolved using SDS-PAGE and identified by mass spectrometry. A significant fraction of proteins interacted with both unmodified DNA and 5hmC-modified DNA. (**B**) Proteins that interacted exclusively with 5hmC-modified DNA were subjected to a Panther Gene Ontology over-representation test. Results of the over-representation test are displayed as fold above expected for a random protein population.

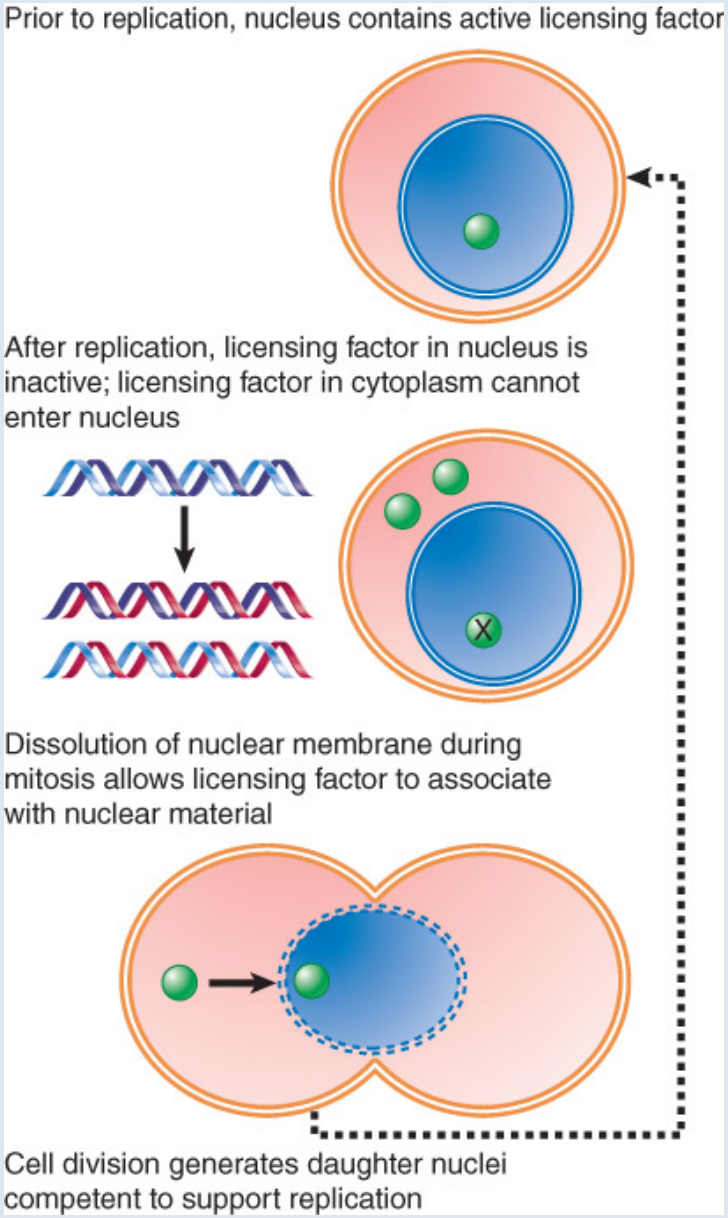
Initiation: A little more complex in Eukaryotes.....

- **Cell Cycle dependency**

- **licensing factors required??**– factors located in the nucleus that are necessary for replication and which are inactivated or destroyed after one round of replication.

  – Consequently, new factors must be provided for further rounds of replication to occur.

Licensing Factor Controls Eukaryotic Re-replication



Licensing factor in the nucleus is inactivated after replication.

- Initiation of another replication cycle becomes possible **only** after **licensing factor(s)** reenters the nucleus following mitosis.

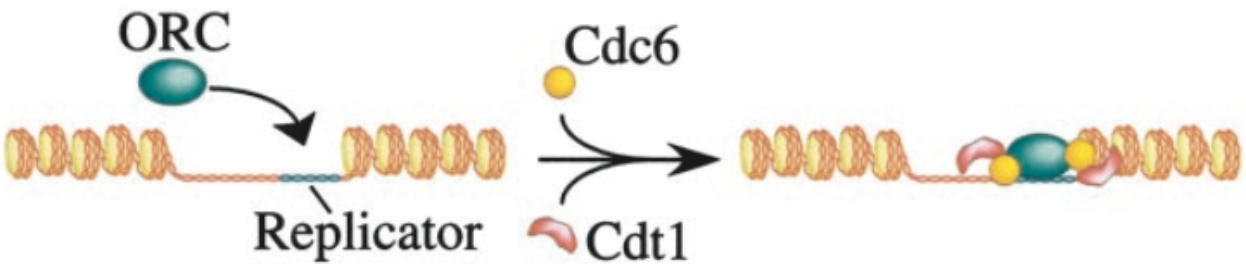


Figure 1 A model for pre-replicative complex formation accommodating much but not all of the current information concerning pre-RC formation in eukaryotes.

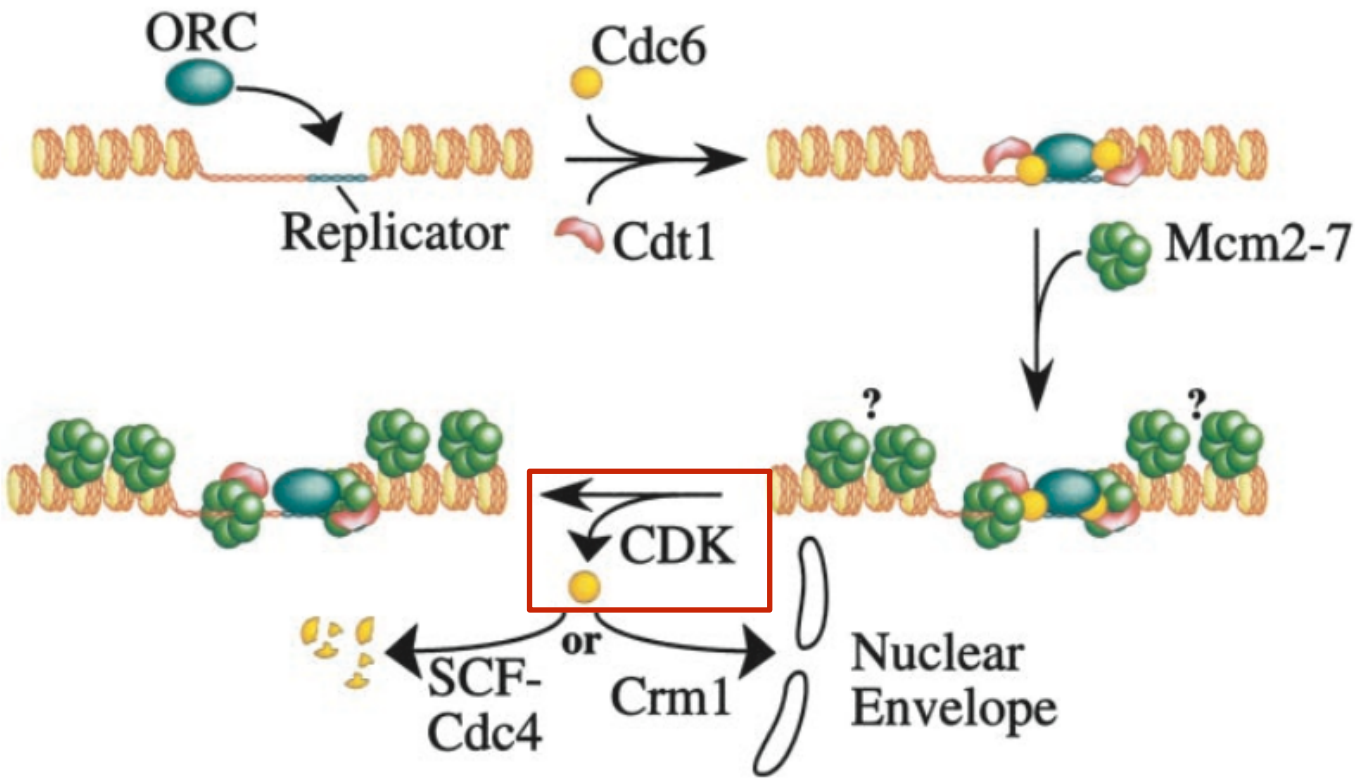
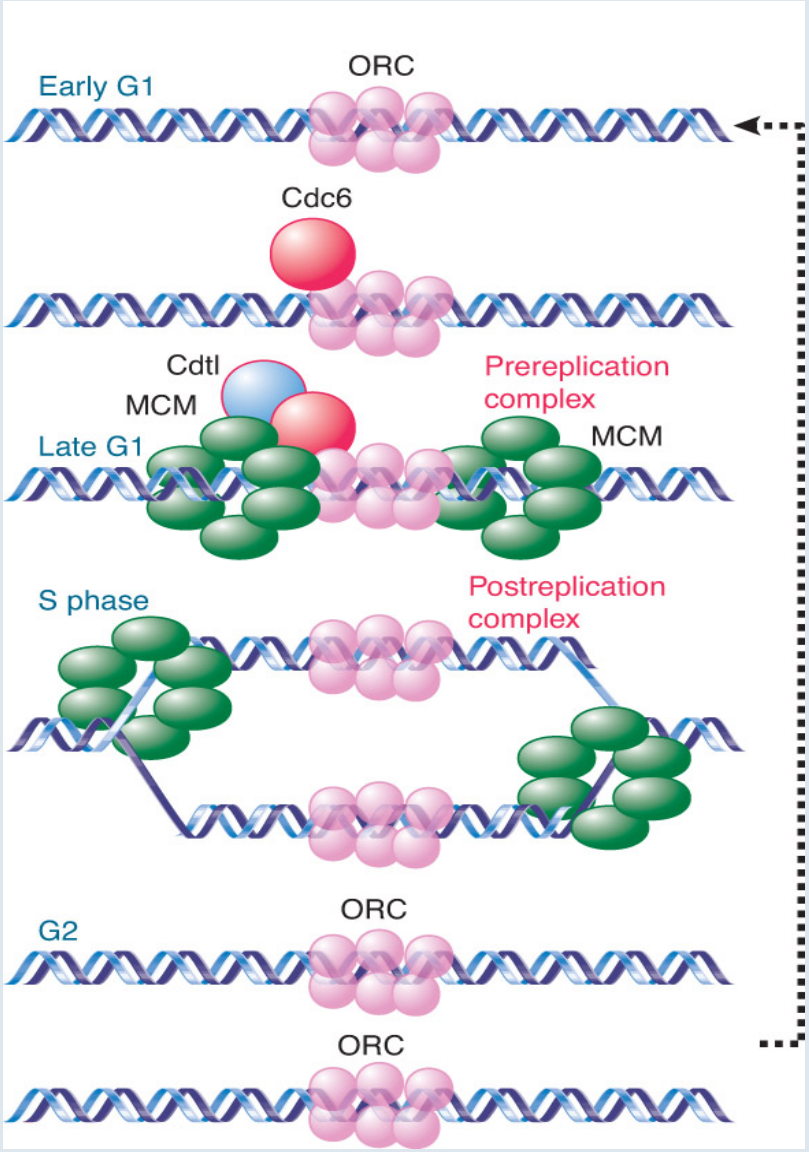
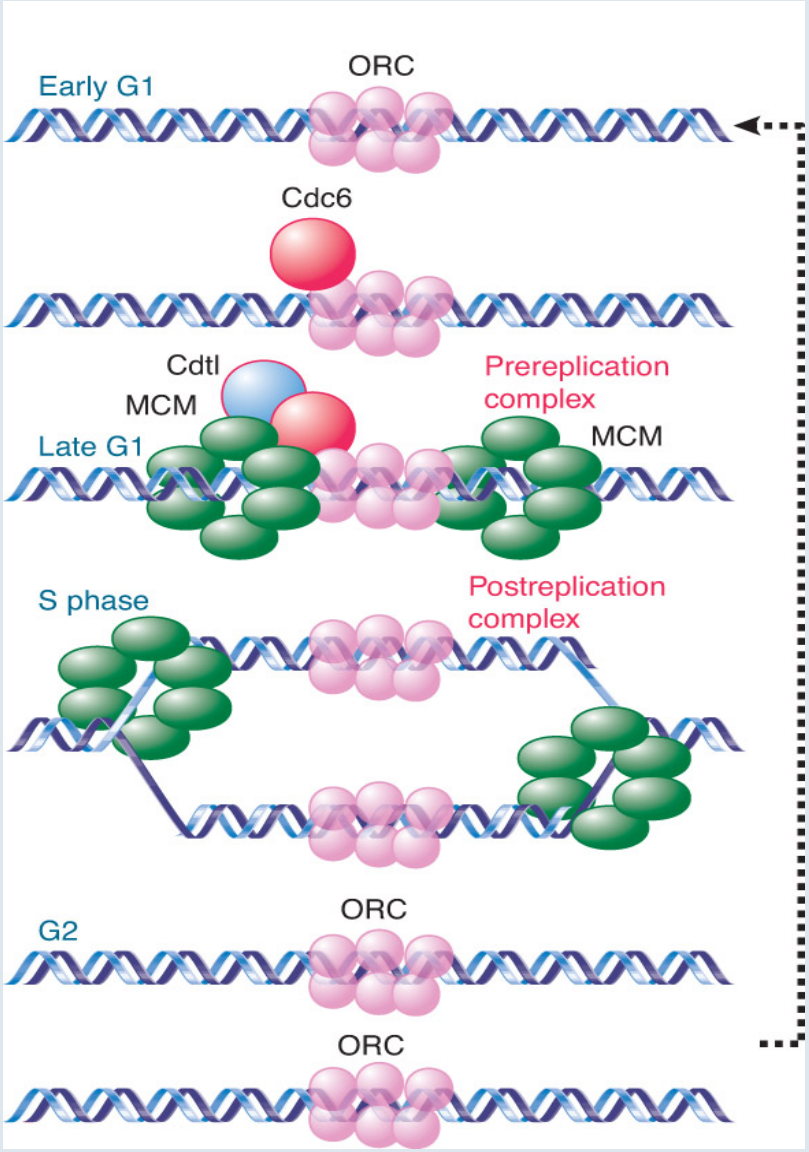


Figure 1 A model for pre-replicative complex formation accommodating much but not all of the current information concerning pre-RC formation in eukaryotes. stoichiometry of the different components is unknown. The apparent overabund of Mcm2–7p relative to other components is illustrated as additional Mcm complexes associated with adjacent chromatin; however, the location relati nucleosomes and the distribution adjacent to the origin have not been determ The fate of Cdc6p after pre-RC formation is distinct in different organisms; possibilities are illustrated. See text for further details.

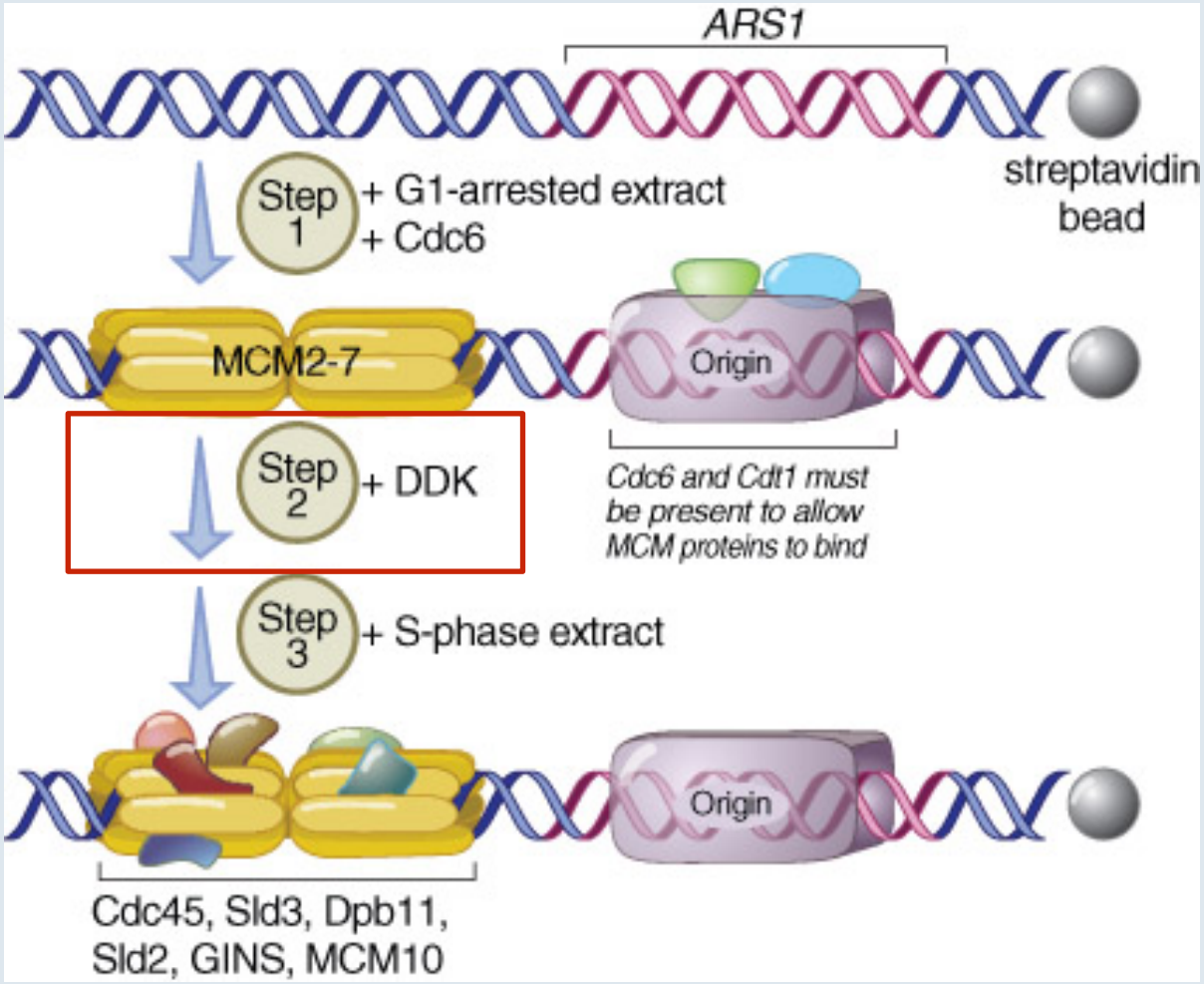


Proteins at the origin control susceptibility to initiation



Proteins at the origin control susceptibility to initiation

Licensing Factor(s) Binds to ORC



Proteins at the origin replication complex (ORC) control susceptibility to initiation.

Adapted from R. C. Heller, et al., Cell 146 (2011): 80-91

Nucleic Acids Research, 2005, Vol. 33, No. 2 ***765–775***
doi:10.1093/nar/gki226

Licensing for DNA replication requires a strict sequential assembly of Cdc6 and Cdt1 onto chromatin in *Xenopus* egg extracts

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ABSTRACT

Replication origins are licensed for a single initiation event by the loading of Mcm2-7 proteins during late mitosis and G1. Sequential associations of origin recognition complex, Cdc6 and Mcm2-7 are essential for completion of the licensing. Although Cdt1 also binds to the chromatin when the licensing reaction takes place, whether the binding is a requirement for Cdt1 to function is unclear. To analyze the relevance of the chromatin association of Cdt1, we carried out chromatin transfer experiments using either immunodepleted *Xenopus* egg extracts or purified proteins. Licensing assay and immunoblotting analyses indicated that Cdt1 could only license DNA replication and load Mcm2-7 onto DNA when it binds to chromatin that has already associated with Cdc6. These results provide evidence supporting that Cdc6 and Cdt1 must bind to chromatin in a strict order for DNA licensing to occur.

(5,6), and finally Mcm2-7 is loaded, which is, in turn, dependent on the binding of Cdc6 (7,8).

Mcm protein complex has been thought of as a replicative helicase and it was previously reported that Mcm4, 6 and 7 form a complex possessing helicase activity (9,10). At present, it is believed that the loading of Mcm2-7 is the entity of the licensing reaction for DNA replication. ORC, Cdc6 and Cdt1 are essential for the loading of Mcm2-7 onto chromatin. Cdc6 was originally identified as a protein essential for cell proliferation in yeast (11). The binding of Cdc6 to the chromatin at the early stage of the cell cycle has been shown to be essential for DNA replication in *Xenopus* egg extract and human cells (6,12). Cdc6 is a member of the AAA+ ATPase family and exhibits significant sequence similarity to the eukaryotic clamp loader, RF-C, and therefore, is thought to act as a clamp loader for the Mcm complex (13,14).

Cdt1 was originally found in *S.pombe* as a protein whose expression was induced by Cdc10, a CDK-dependent transcription factor (15). Later on, Cdt1 was found to possess an activity referred as replication licensing factor-B (RLF-B) that had previously been identified in *Xenopus* egg extract (16–18). Cdt1 also binds to chromatin as a component of pre-RC. The binding of Cdt1 is dependent on the association of ORC but not Cdc6 with the chromatin (5,19). More recently,

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Published online 2010 Jan 28. doi: 10.1016/j.cell.2009.12.049

PMCID: PMC2857569
NIHMSID: NIHMS168500

Treslin Collaborates with TopBP1 in Triggering the Initiation of DNA Replication

Akiko Kumagai,[1] Anna Shevchenko,[2] Andrej Shevchenko,[2] and William G. Dunphy[1,*]

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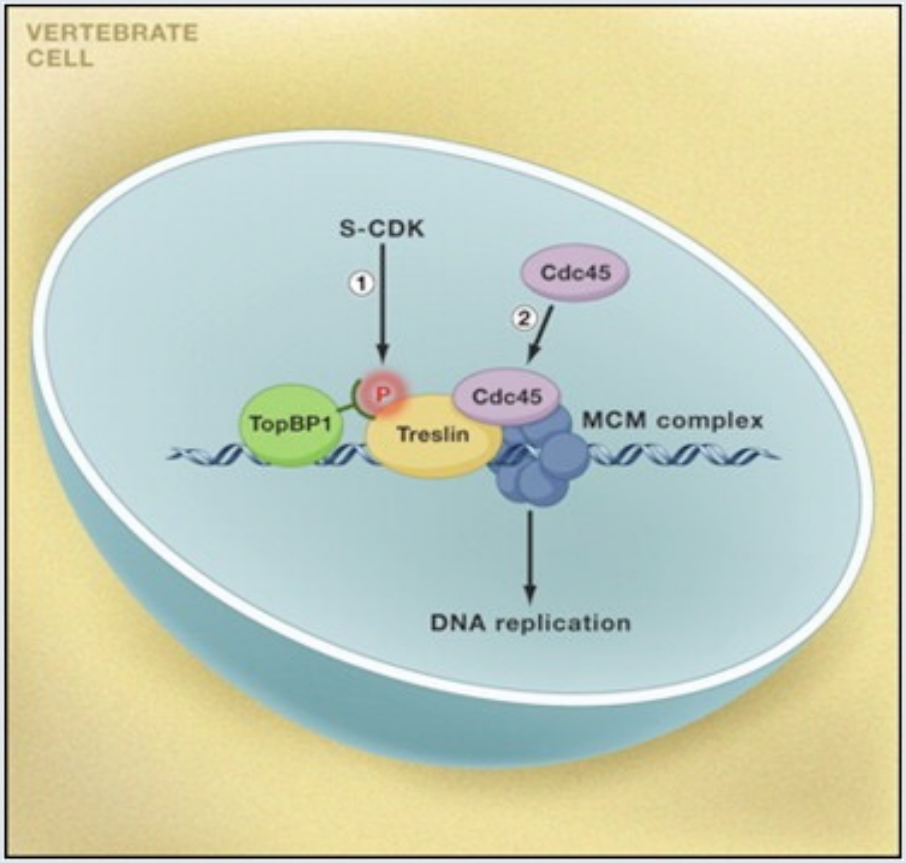
SUMMARY

Go to:

TopBP1 has important roles in both DNA replication and checkpoint regulation in vertebrates. We have identified a protein called Treslin that associates with TopBP1 in *Xenopus* egg extracts. Depletion of Treslin from egg extracts strongly inhibits chromosomal DNA replication. Binding of Treslin to chromatin in egg extracts occurs independently of TopBP1. However, loading of the initiator protein Cdc45 onto chromatin cannot take place in the absence of Treslin. Prior to the initiation of DNA replication, Treslin associates with TopBP1 in a Cdk2-dependent manner. Ablation of Treslin from human cells also strongly inhibits DNA replication. Taken together, these results indicate that Treslin and TopBP1 collaborate in the Cdk2-mediated loading of Cdc45 onto replication origins. Thus, Treslin regulates a pivotal step in the initiation of DNA replication in vertebrates.



Treslin is a relatively newly identified factor for initiation of DNA replication in vertebrates

-**Treslin** interacts with **TopBP1**, a protein required for initiation of DNA replication

-Association of **Treslin** and **TopBP1** requires cyclin-dependent kinase activity

-**Treslin** and **TopBP1** collaborate in the Cdk2-mediated loading of the initiator protein **Cdc45** onto DNA

TABLE 1. Synonyms for conserved replication proteins[a]

Protein	Synonym in:				
	S. cerevisiae	*S. pombe*	*Drosophila*	Human	*Xenopus*
Mcm2	Mcm2	Mcm2, Cdc19, Nda1	DmMcm2	BM28, Mcm2	Mcm2
Mcm3	Mcm3	Mcm3	DmMcm3	Mcm3	Mcm3
Mcm4	Mcm4, Cdc54	Mcm4, Cdc21	DmMcm4 Dpa (disk proliferation abnormal)	Mcm4	Mcm4
Mcm5	Mcm5, Cdc46	Mcm5, Nda4	DmMcm5, DmCdc46	Mcm5	Mcm5
Mcm6	Mcm6,	Mcm6, Mis5	DmMcm6	Mcm6	Mcm6
Mcm7	Mcm7, Cdc47	Mcm7	DmMcm7	Mcm7	Mcm7
Mcm8	No homologue	No homologue	?	Mcm8	?
Dbf4	Dbf4, Dna52	Dfp1, Him1	Chiffon	Dbf4, ASK	Dbf4
Alternative Dbf4 subunit	No homologue	No homologue	No homologue	Drf1	Drf1
Cdc6	Cdc6	Cdc18	Cdc6	Cdc6	Cdc6
Cdt1	Tah11, Sid2	Cdt1	Dup (doubleparked)	Cdt1	Cdt1
Geminin	No homologue	No homologue	Geminin	Geminin	Geminin
ORC1–ORC6	Orc1–Orc6	Orp1–Orp6, Orc1–Orc6	DmORC1–DmORC6	ORC1–ORC6	XOrc1–XOrc6
Cdc45	Cdc45	Sna41, Cdc45	Cdc45	Cdc45	Cdc45
GINS					GINS
PSF2	Psf2, Cdc102	Psf2, Bsh3	?	Psf2, Bsh3	Psf2
PSF3	Psf3	?	?	?	Psf3
SLD5	Sld5, Cdc105	?	?	?	Sld5
PSF1	Psf1, Cdc101	?	?	?	Psf1
Drc1	Drc1, Sld2	Drc1	?	?	?
Sld3	Sld3	Sld3	?	?	?
Dpb11	Dpb11	Rad4, Cut5	Mus101	TopBP1, Cut5	Xmus101
Mcm10	Mcm10, Dna43	Cdc23	Mcm10	Mcm10	Mcm10

[a] For references, see the text.

Mini Chromosome Maintenance

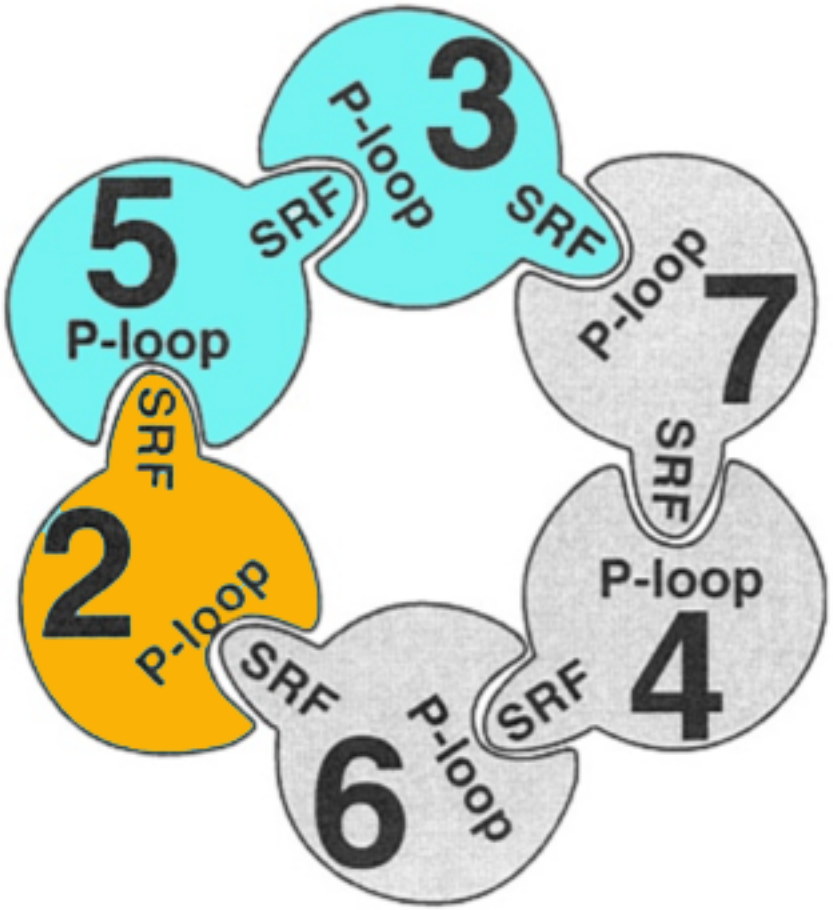
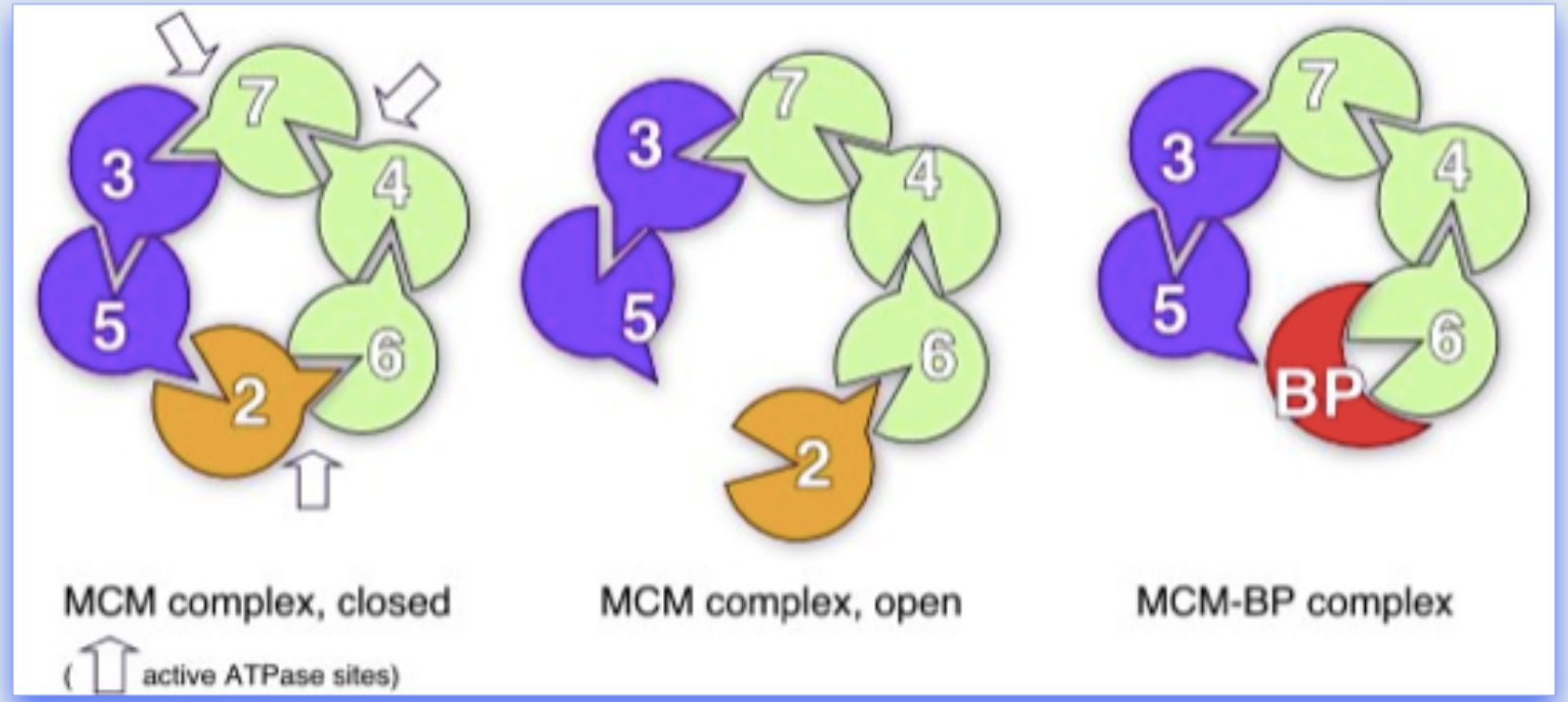


FIG. 3. Pairwise interactions in vitro suggest this organization of the MCM heterohexamer. The P-loop (Walker A motif) and SRF motif (arginine finger) are proposed to act together as ATPase domains. In isolation, this complex does not have helicase activity in vitro (see the text). Reprinted from reference 55 with permission from the publisher.



Forsburg Lab Research

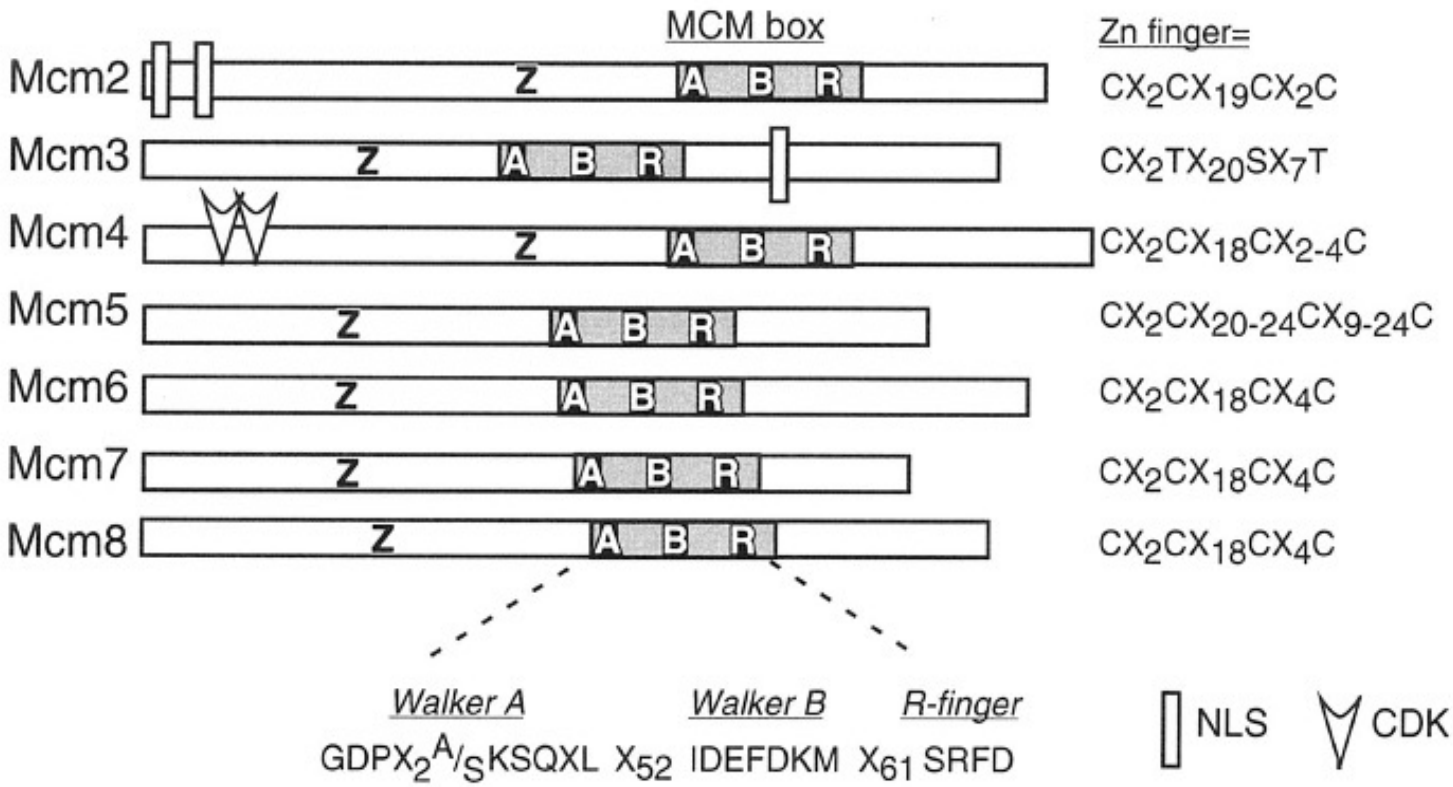
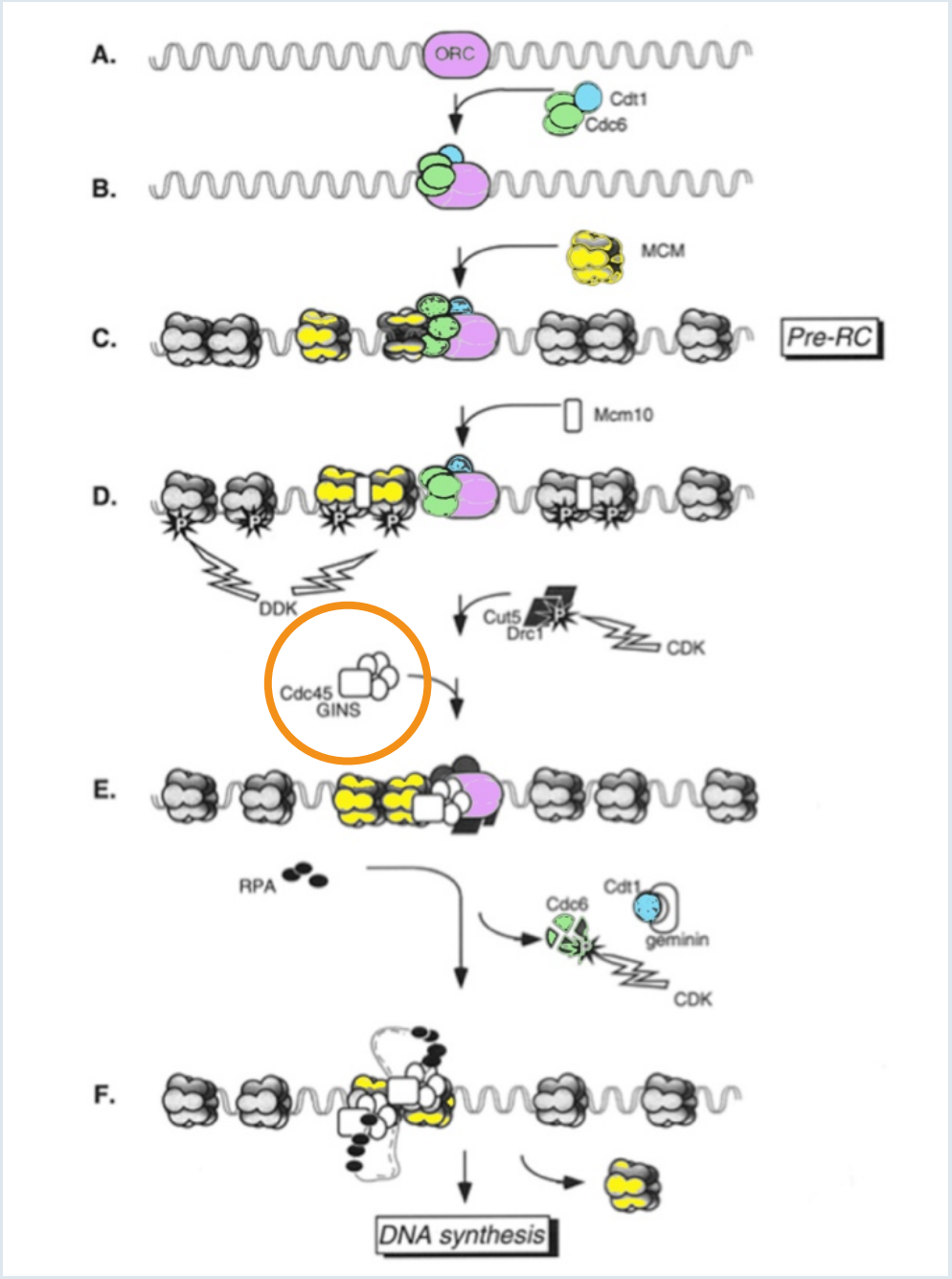


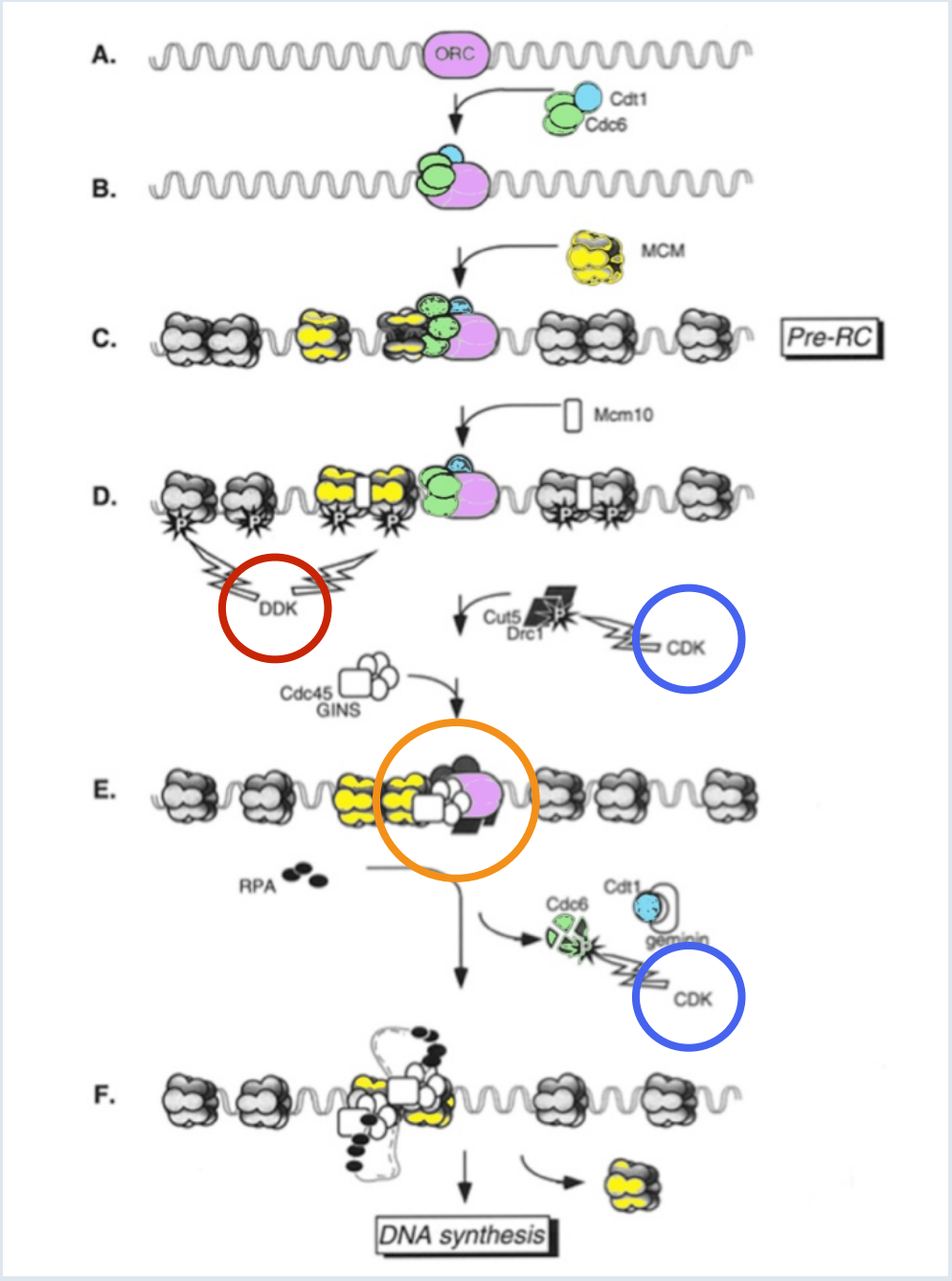
FIG. 2. Structural features of the Mcm protein family, derived from the alignment used in Fig. 1. Abbreviations: Z, zinc finger; A, Walker A ATPase motif; B, Walker B ATPase motif; R, arginine finger motif.



Cdc45p has emerged as a pivotal factor in the transition to replication- involved in loading of the polymerase on to the chromatin

Origin firing is effectively controlled by two kinases: **CDK** and **DDK**

In budding yeast, at least, inactivation of **CDK** has the dramatic effect of causing massive rounds of re-replication; a similar, although less dramatic.



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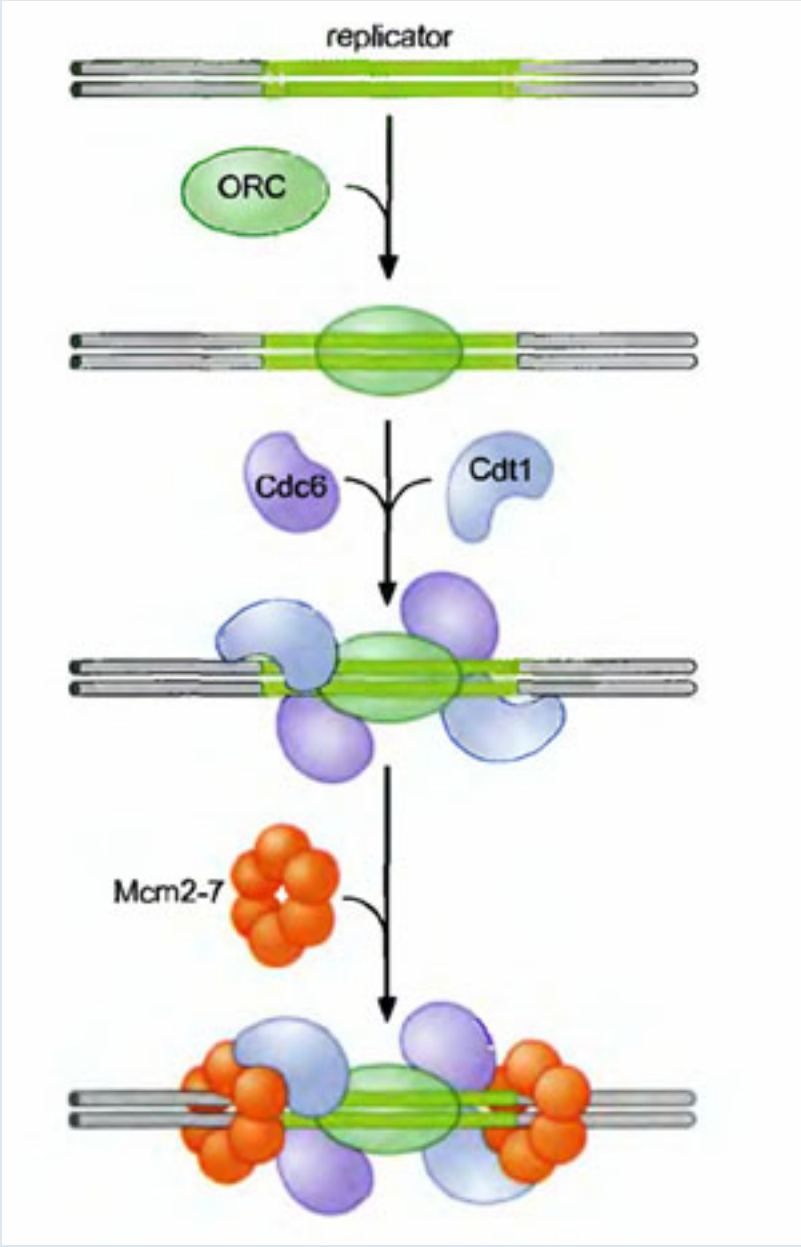
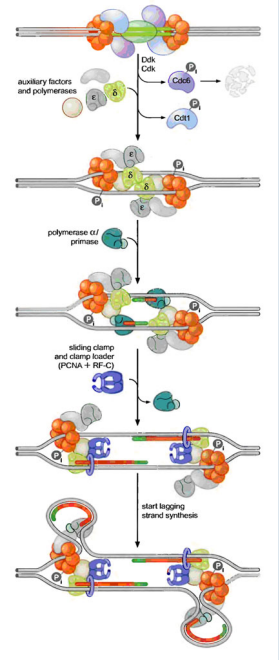
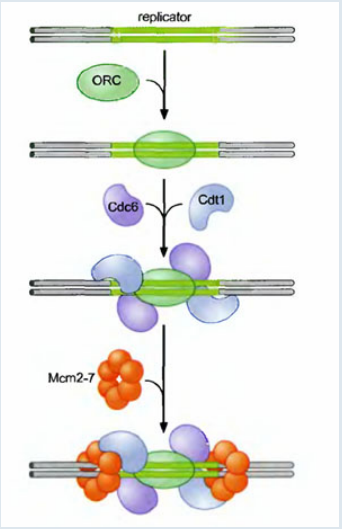
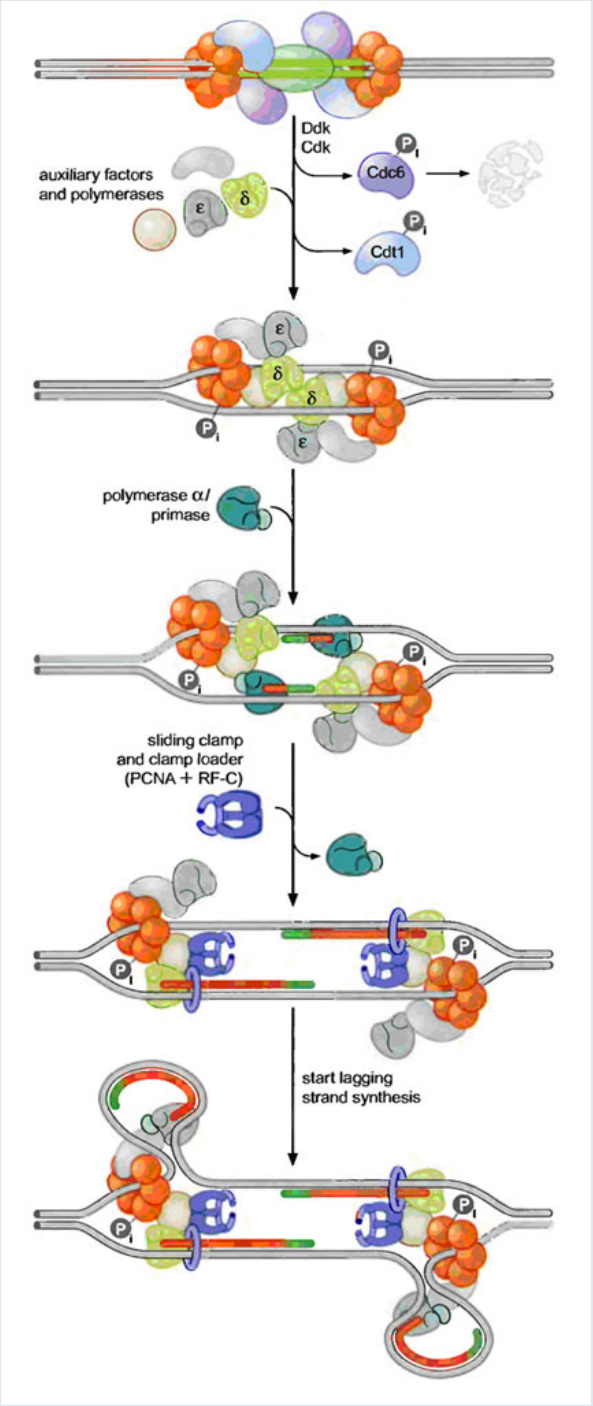

replicator
ORC
Cdc6
Cdt1
Mcm2-7


Ddk
Cdk
auxiliary factors
and polymerases
Cdc6
Cdt1
polymerase α/
primase
sliding clamp
and clamp loader
(PCNA + RF-C)
start lagging
strand synthesis


replicator
ORC
Cdc6
Cdt1
Mcm2-7


Ddk
Cdk
auxiliary factors
and polymerases
ε
δ
Cdc6
Cdt1
P
polymerase α/
primase
sliding clamp
and clamp loader
(PCNA + RF-C)
start lagging
strand synthesis

Variety of Studies -indicate different effects of different replication mutations

- **conditional lethal mutations** – mutations that are lethal under one set of conditions, but are not lethal under a second set of conditions, such as temperature
- ***dna* mutants** - temperature-sensitive replication mutants in *E. coli* that identify a set of loci called the *dna* genes.
- ***in vitro* complementation** – a functional assay used to identify components of a process.
  - the reaction is reconstructed using extracts from different mutant cells.
  - fractions from wild-type cells are then tested for restoration of activity.

- **slow-stop mutants** – temperature-sensitive replication mutants that produce a slowly derived affect on replication events and were subsequently found to be defective in initiation of replication.

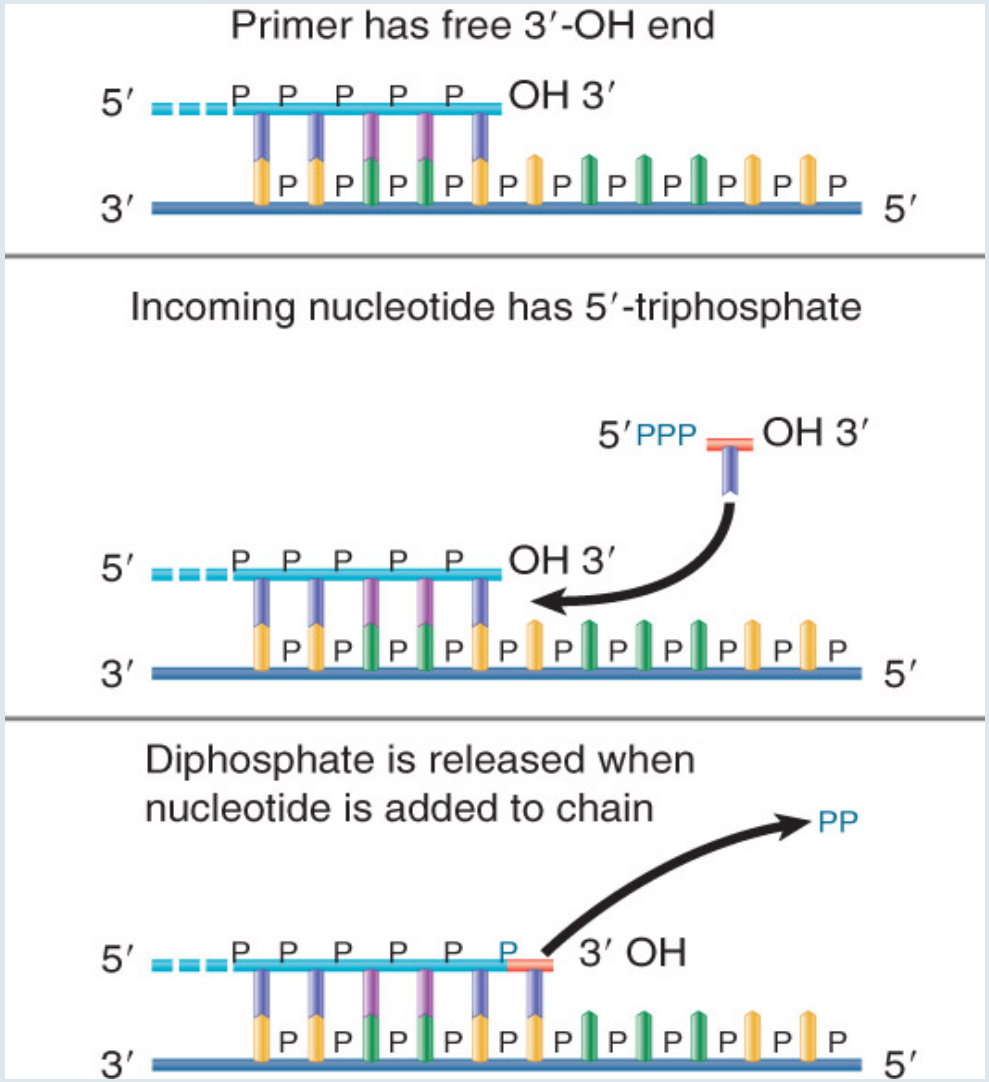
- **quick-stop mutants** – temperature-sensitive replication mutants that produce a rapidly derived affect on replication events when temperature shifted to the non-permissive temperature, and which were found to be defective in replication elongation during synthesis of DNA.

The Two New DNA Strands Have Different Modes of Synthesis

- The DNA polymerase advances continuously when it synthesizes the **leading strand** (5′–3′), but synthesizes the **lagging strand** by making short fragments (**Okasaki fragments**) that are subsequently joined together.

- **semidiscontinuous replication** – The mode of replication in which one new strand is synthesized continuously while the other is synthesized discontinuously.

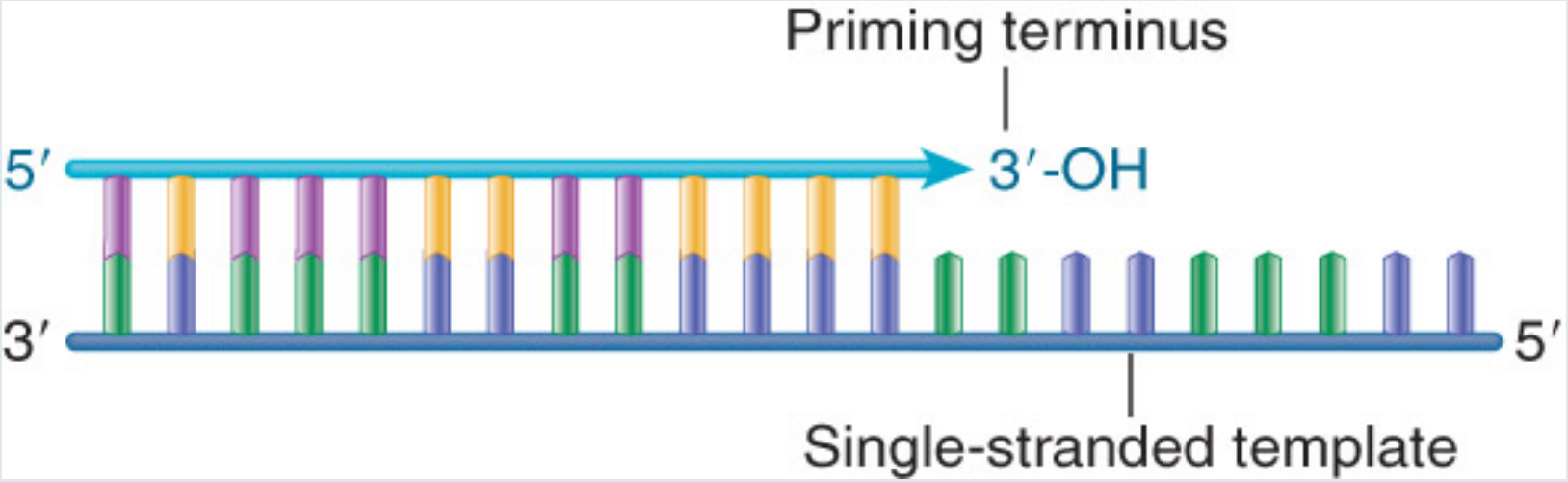
DNA Synthesis:



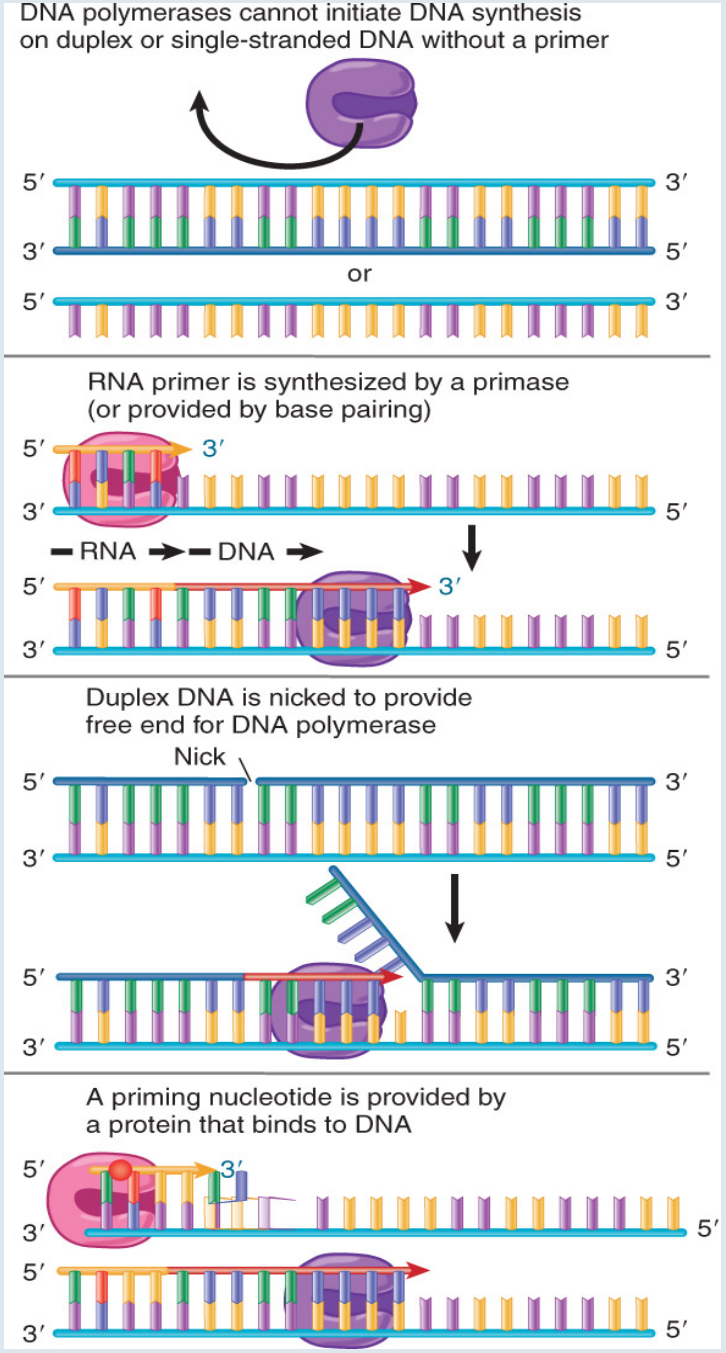
DNA is synthesized by adding nucleotides to the 3'–OH end of the growing chain, so that the new chain grows in the 5' to 3' direction

Priming Is Required to Start DNA Synthesis

- All DNA polymerases require a 3′–OH priming end to initiate DNA synthesis.



A DNA polymerase requires a 3’–OH end to initiate replication

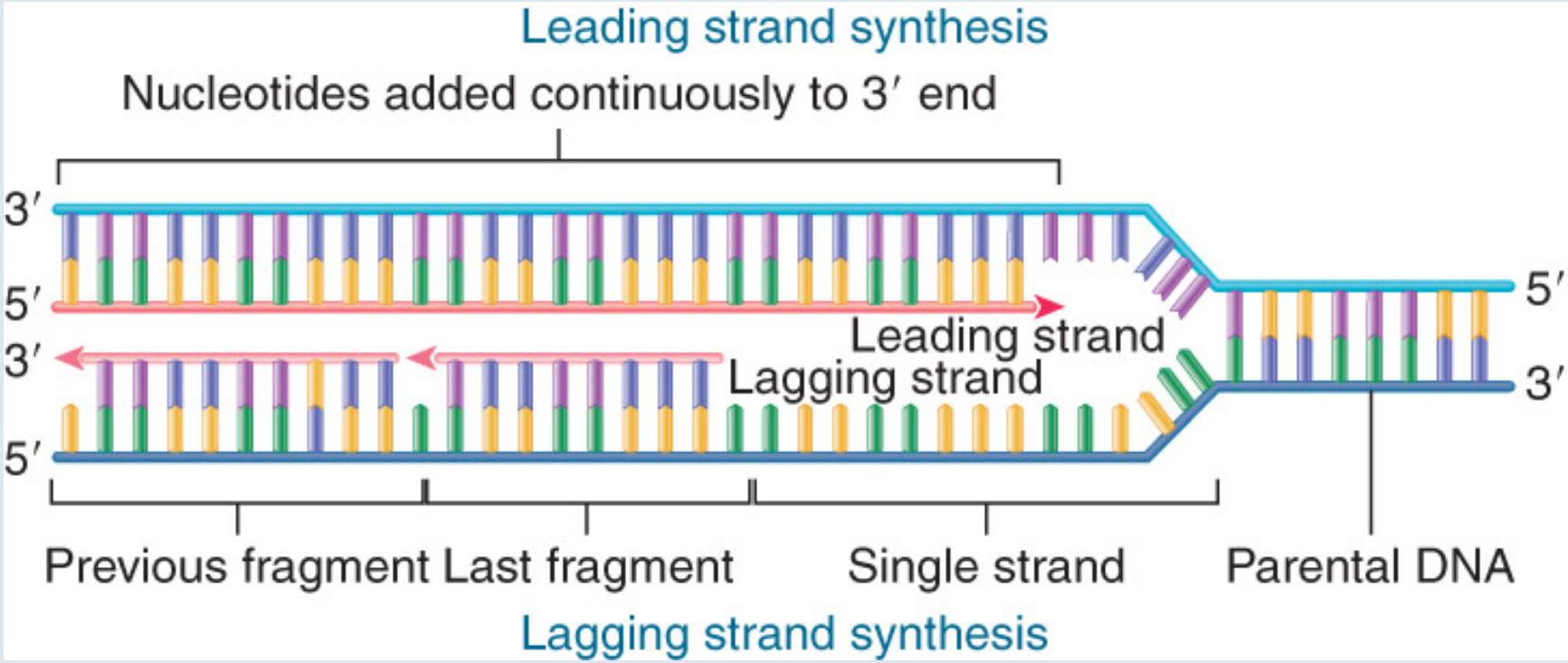


- As such, all DNA Synthesis requires a “primer” of one sort or another.

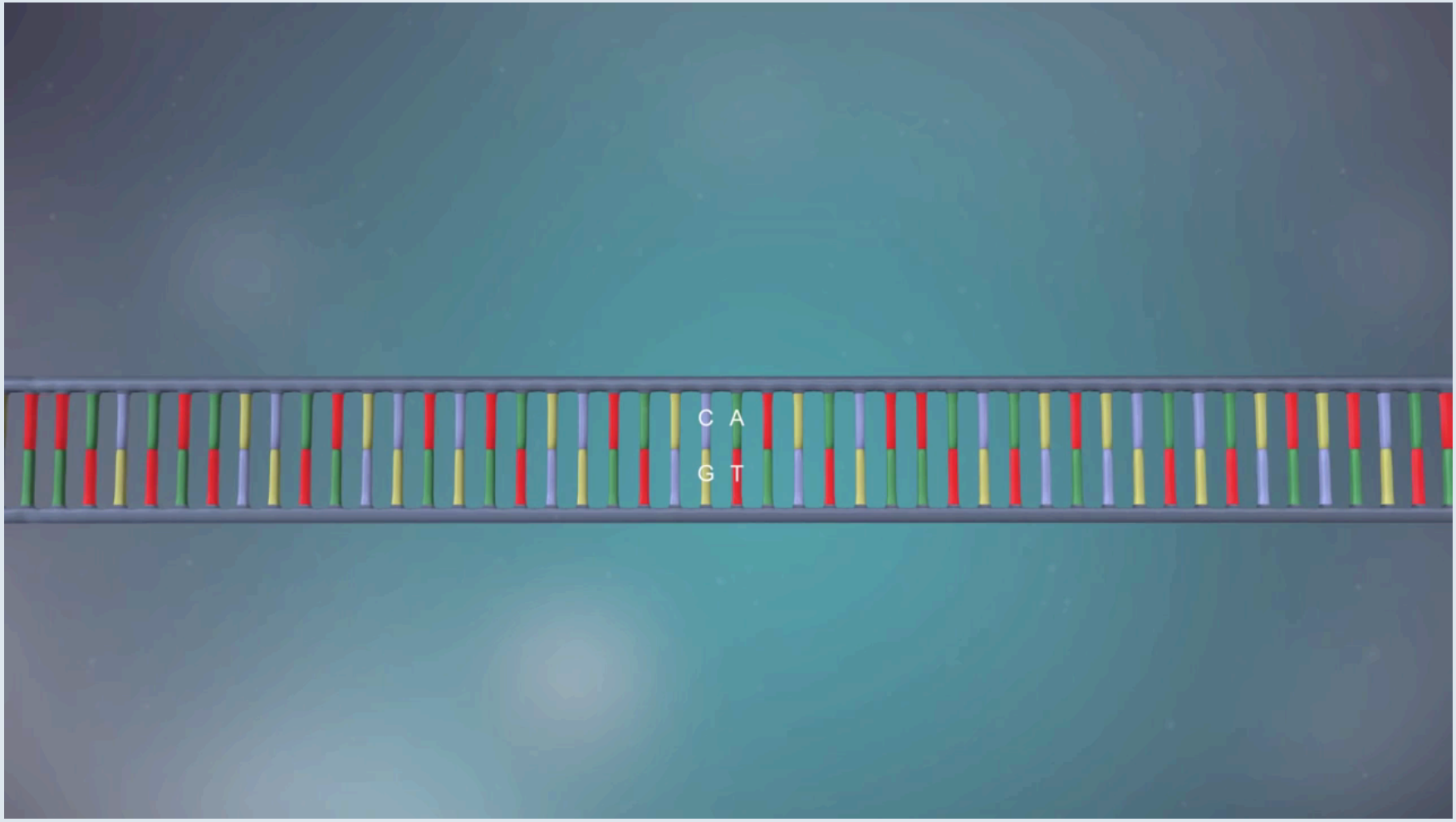
- The priming 3’OH can be provided by an **RNA primer**, a **nick in the DNA**, or a **priming protein**.

There are several methods for providing the free 3’–OH end that DNA polymerases require to initiate DNA synthesis

The Two New DNA Strands Have Different Modes of Synthesis



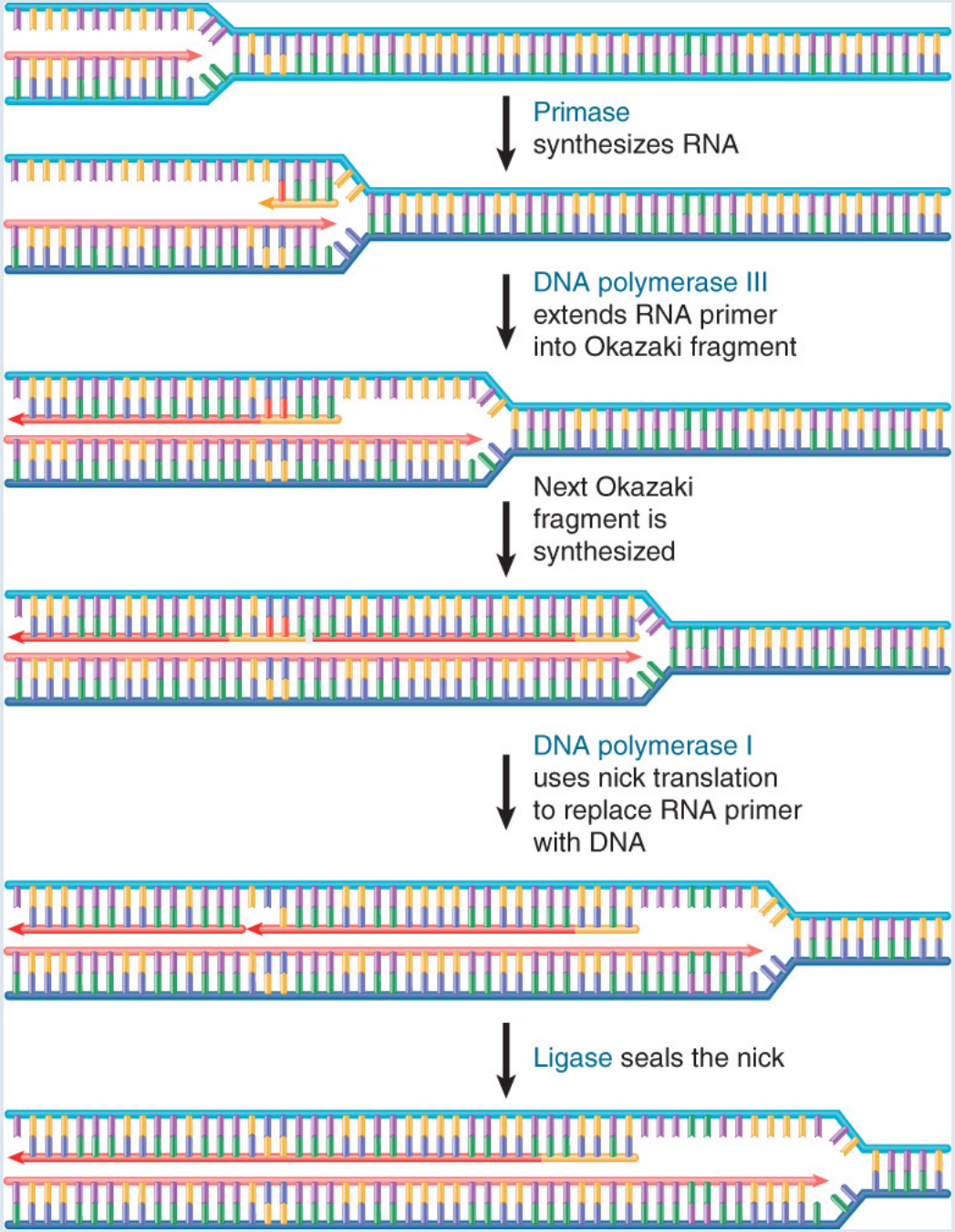
The leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously



Fundamentals of chromosomal DNA replication

https://www.youtube.com/watch?v=TNKWgcFPHqw

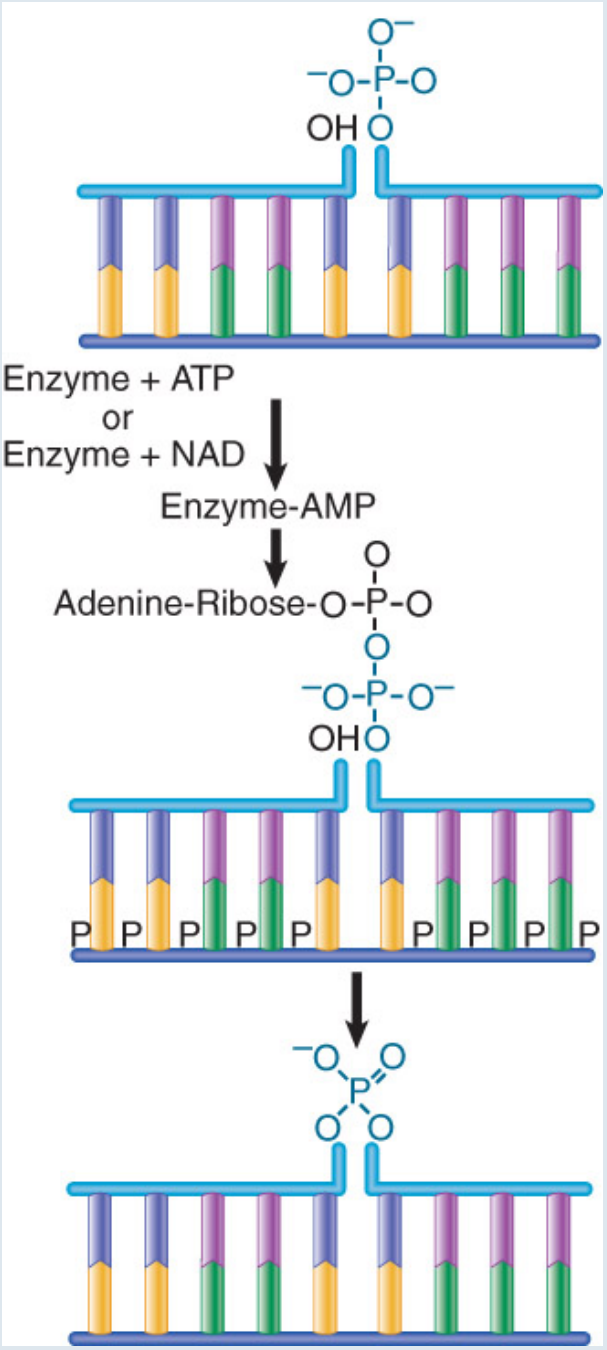
Okazaki Fragments Are Linked by Ligase



- Each Okazaki fragment starts with a primer and stops before the next fragment.
- DNA polymerase I removes the primer and replaces it with DNA.

Synthesis of Okazaki fragments require priming, extension, removal of RNA primer, gap filling, and nick ligation

Okazaki Fragments Are Linked by Ligase

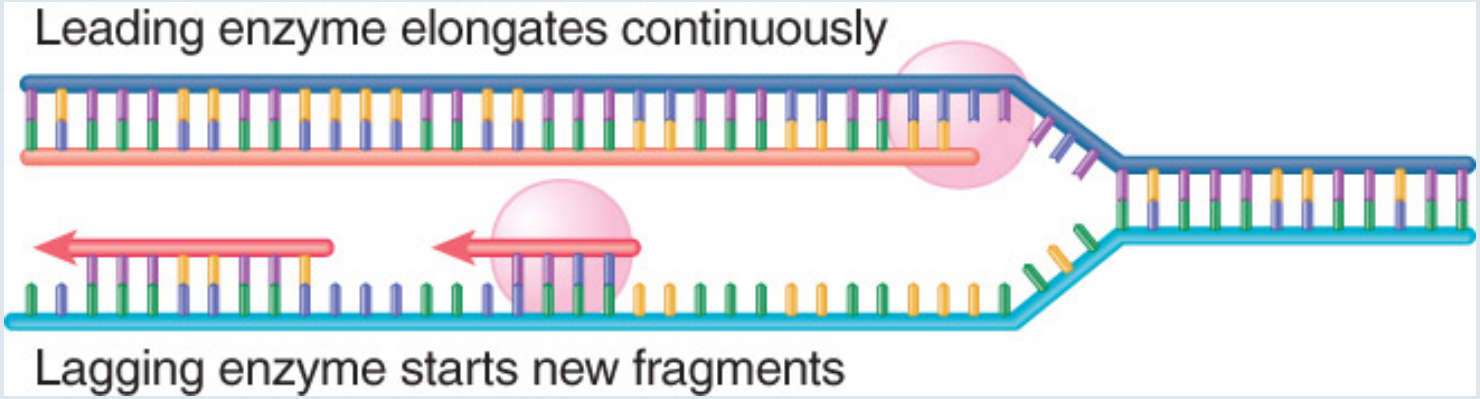


- **DNA ligase** makes the bond that connects the 3′ end of one Okazaki fragment to the 5′ beginning of the next fragment.

DNA ligase seals nicks between adjacent nucleotides by employing an enzyme-AMP intermediate

Coordinating Synthesis of the Lagging and Leading Strands

- Different enzyme units are required to synthesize the leading and lagging strands.
- In *E. coli*, both these units contain the same catalytic subunit (DnaE).
- In other organisms, different catalytic subunits may be required for each strand.

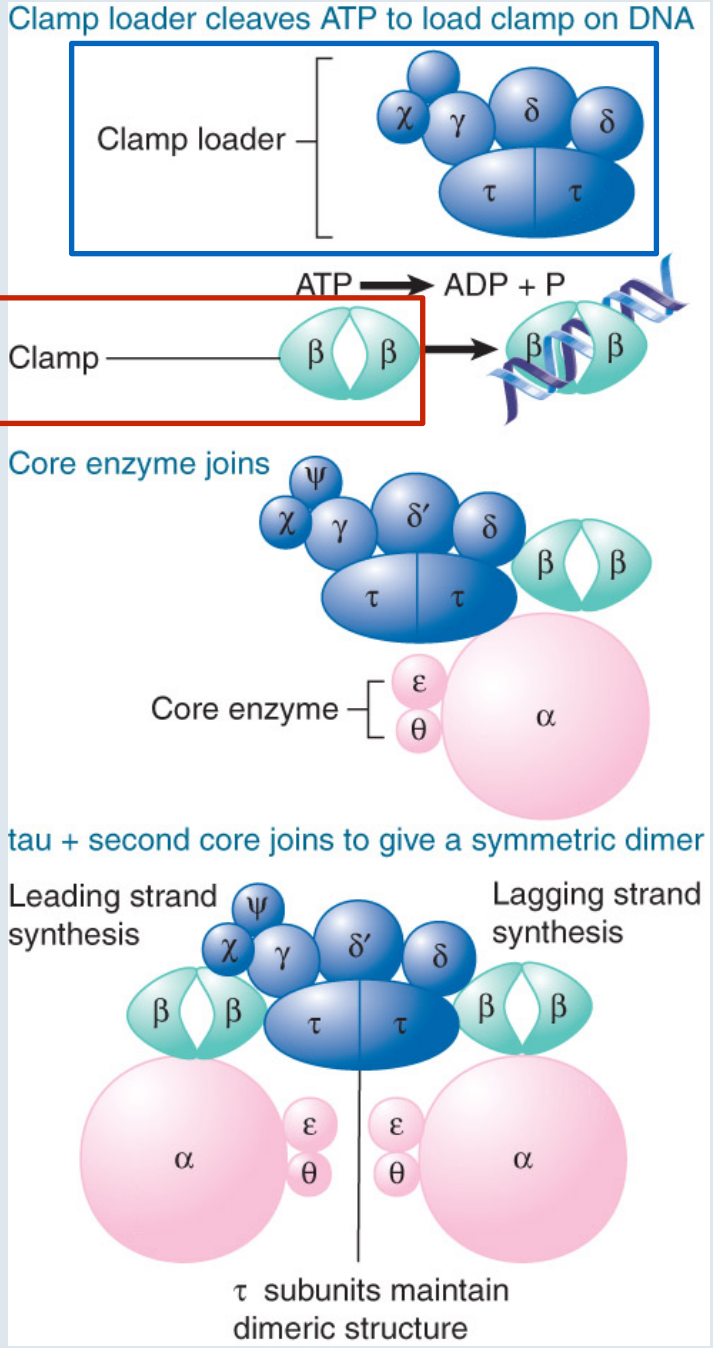


Leading and lagging catalytic units behave differently

In Prokaryotes **DNA polIII** holoenzyme consists of a number of subcomplexes of protein subunits

- The *E. coli* replicase DNA polymerase III is a 900-kD complex with a dimeric structure.

- Each monomeric unit has a catalytic core, a dimerization subunit, and a processivity component.



DNA Polymerase III Holoenzyme Consists of Subcomplexes

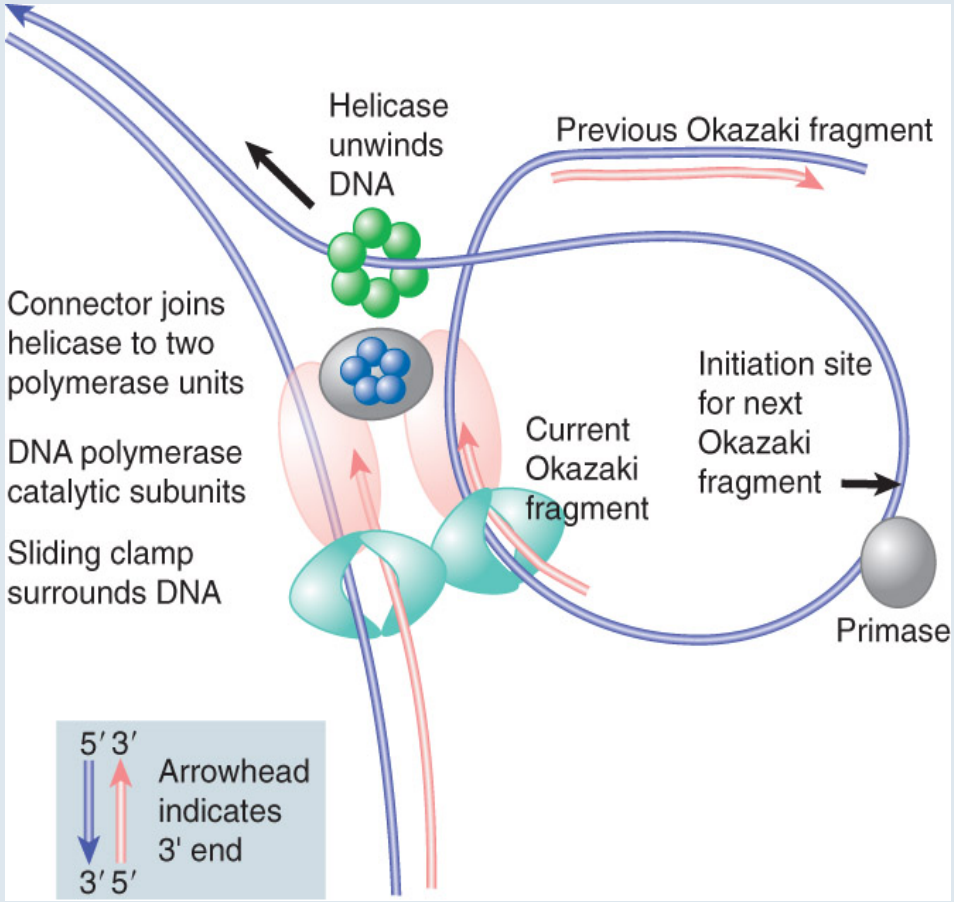
- A **clamp loader** places the processivity subunits on DNA, where they form a circular **clamp** around the nucleic acid.
- One catalytic core is associated with each template strand.

DNA polymerase III holoenzyme assembles in stages, generating an enzyme complex that synthesizes the DNA of both new strands

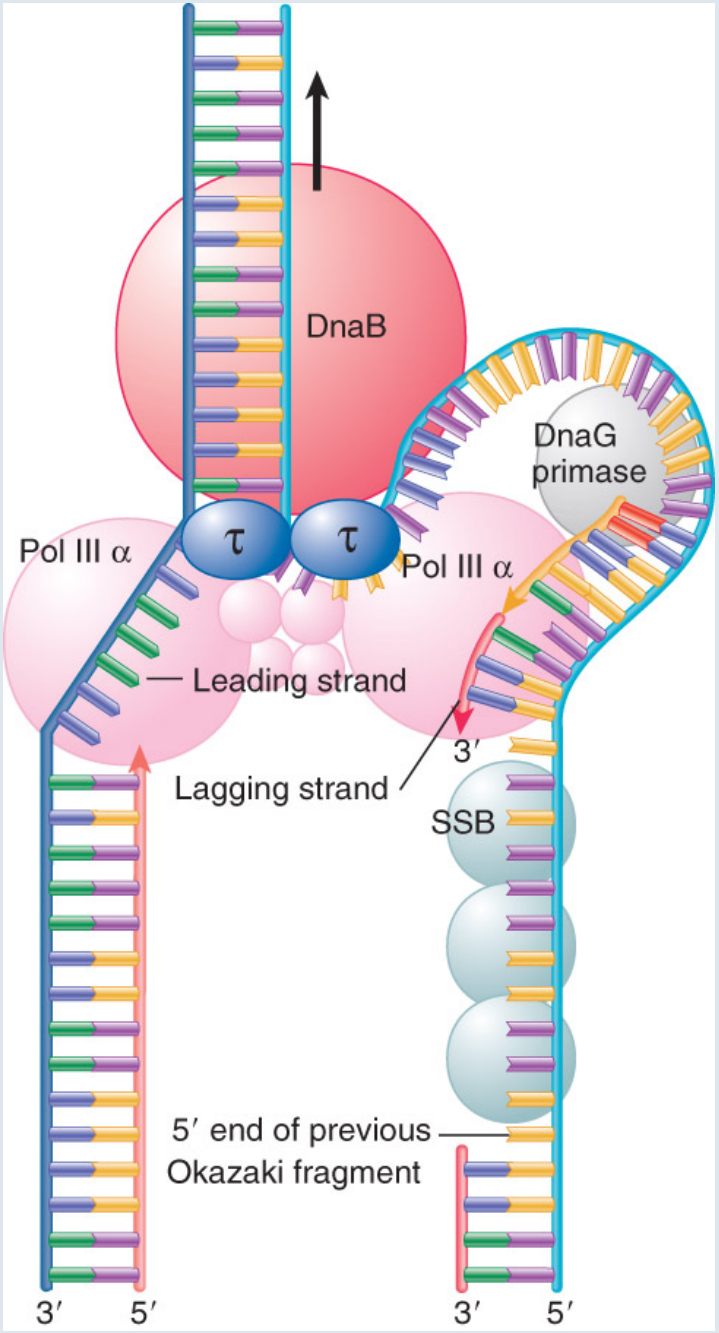
The Clamp Controls Association of Core Enzyme with DNA

- The core on the leading strand is processive because its **sliding clamp** keeps it on the DNA.

- The clamp associated with the core on the lagging strand dissociates at the end of each newly synthesized Okazaki fragment and reassembles for the next fragment.



The dimeric polymerase model

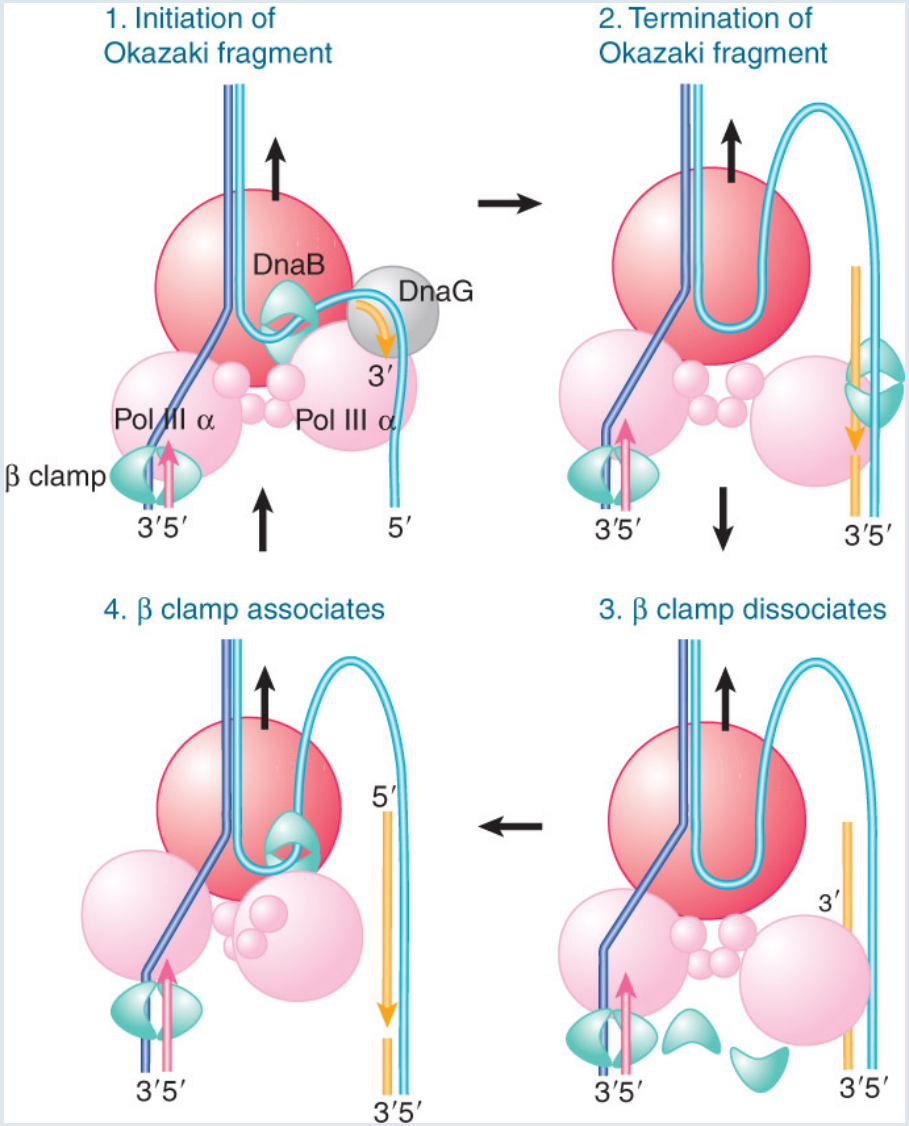


The Clamp Controls Association of Core Enzyme with DNA

- The helicase **DnaB** is responsible for interacting with the primase **DnaG** to initiate each round of Okazaki fragment synthesis.

Each catalytic core of Pol III synthesizes a daughter strand. **DnaB** is responsible for forward movement at the replication fork

The Clamp Controls Association of Core Enzyme with DNA



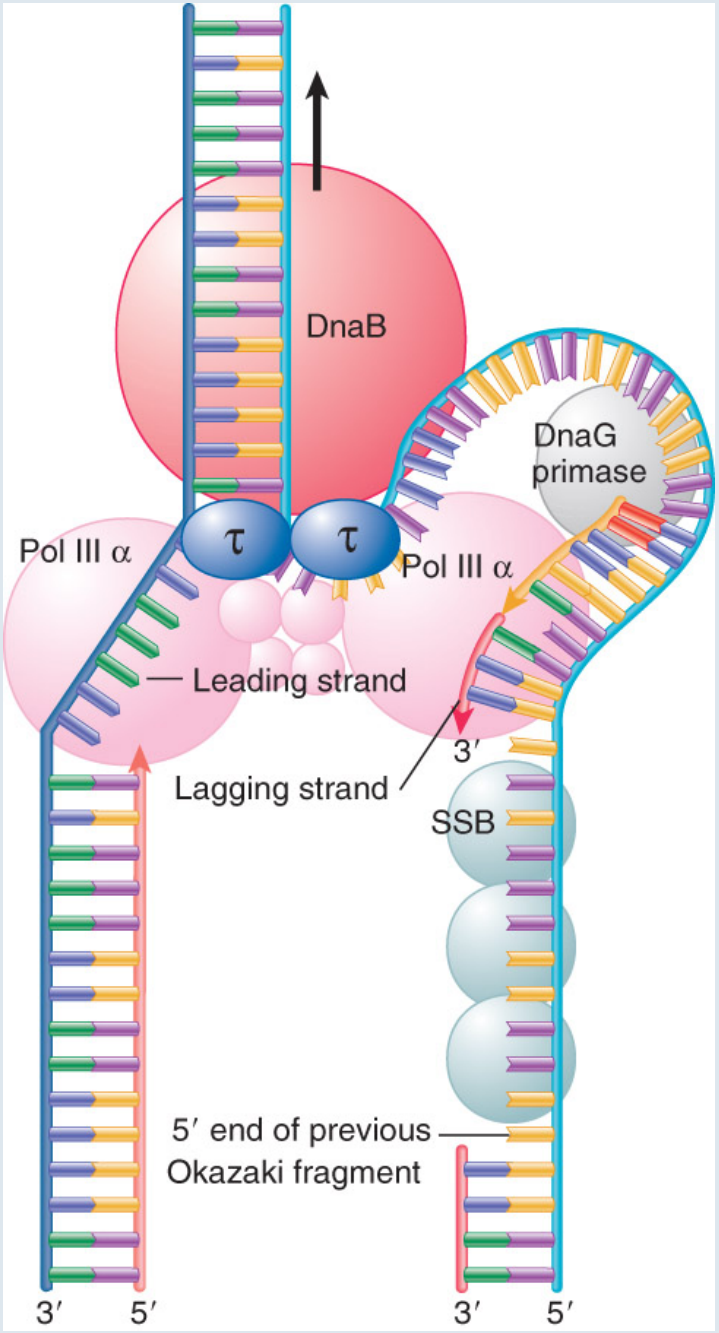
Core polymerase and the **Beta clamp** dissociate at completion of Okazaki fragment synthesis and reassociate at the beginning

In eukaryotes….different DNA Polymerase subunits undertake Initiation and Elongation

- A replication fork has one complex of DNA polymerase **α/primase,** one complex of **DNA polymerase δ**, and one complex of **DNA polymerase ε**.

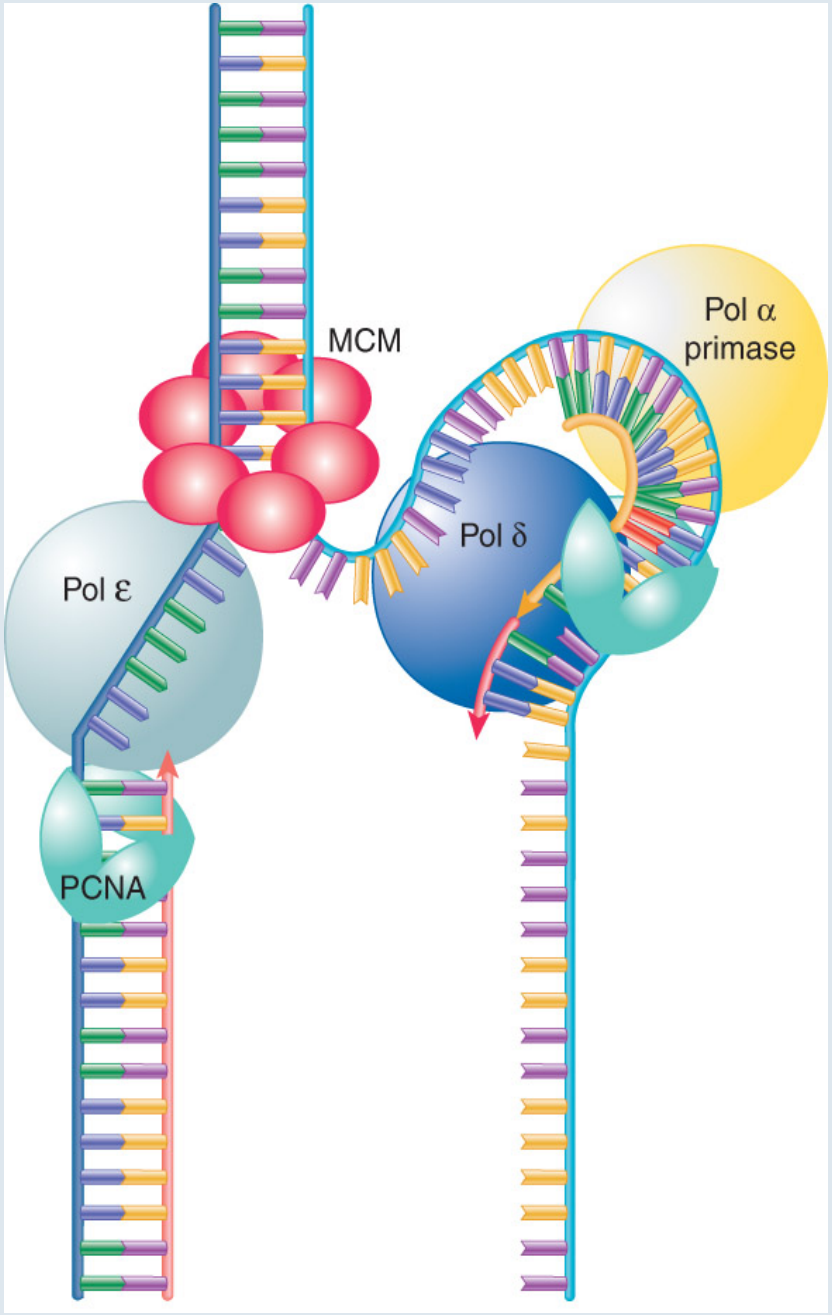
- The DNA polymerase **α/primase** complex initiates the synthesis of both DNA strands.

- DNA polymerase ε elongates the leading strand and the second DNA polymerase δ elongates the lagging strand.



Each catalytic core of the Replicase synthesizes a daughter strand.

The **DnaB** subunit is responsible for forward movement at the replication fork



Each catalytic core of the Replicase synthesizes a daughter strand, with the responsibilities of the different core subunits **ε** and **δ,** defined by leading and lagging strand function, respectively.

The **MCM complex** is responsible for forward movement at the replication fork

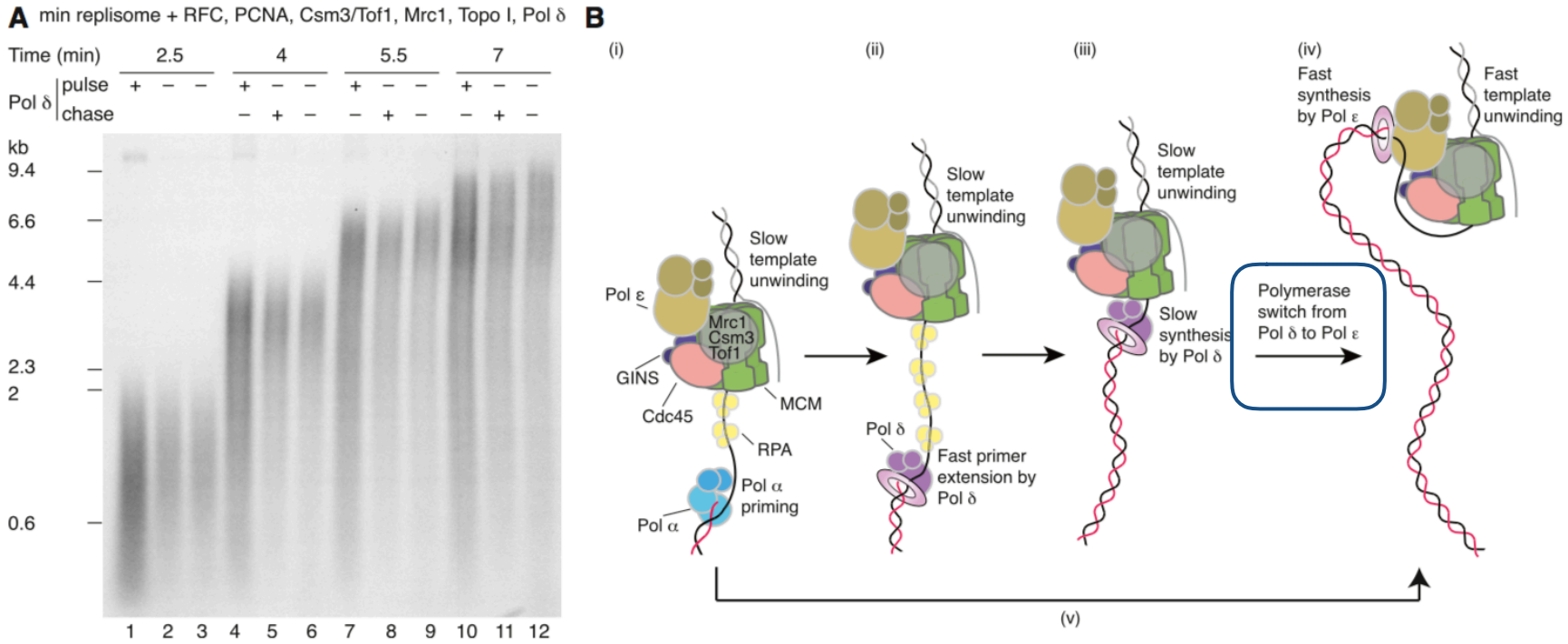
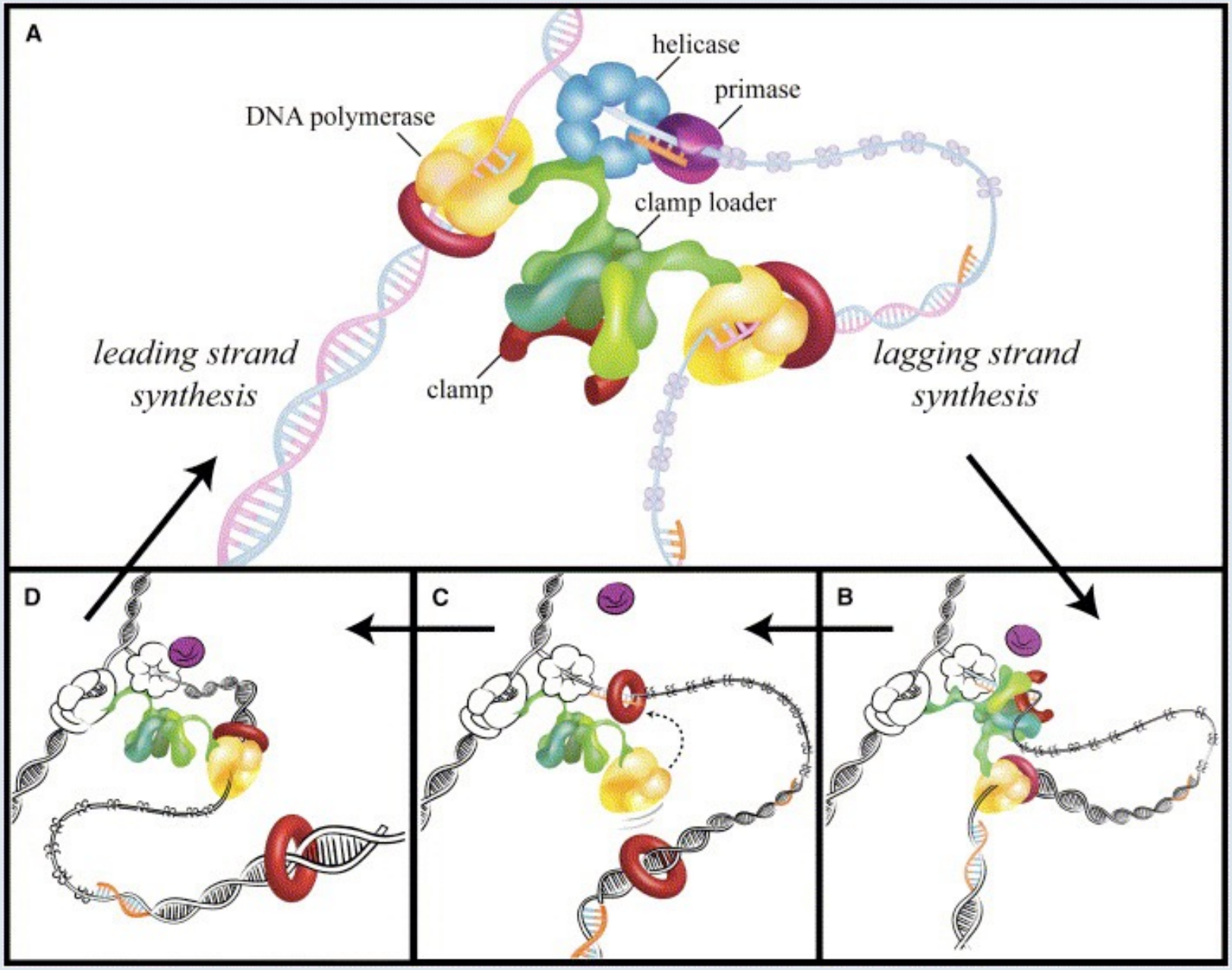


Figure 7. Pol δ Promotes the Establishment of Leading-Strand Synthesis

(A) Pulse-chase reactions conducted as in Figure 2C with 10 nM Pol δ included where indicated. When Pol δ was included in the chase, it was added immediately after the 2-min 30-s time point was removed.

(B) Model for eukaryotic leading-strand synthesis. (i) Following helicase activation the replisome advances slowly, unwinding the template to generate a priming site on the leading strand for Pol α. (ii) Following priming, RFC assembles PCNA around the primer terminus. Pol δ rapidly binds to the primer and commences elongation. The elongation rate of Pol δ is considerably faster than the advancing replisome, so Pol δ quickly catches up with the replication fork. (iii) Once Pol δ has made contact with the replisome, the rate of synthesis is limited by the template-unwinding rate of the replisome. (iv) A polymerase switch transfers the 3′ end of the leading strand together with PCNA from Pol δ to Pol ε. Pol ε-dependent leading-strand synthesis stimulates the template-unwinding rate of the replisome, and DNA synthesis rates of ~2 kb min^{-1} are established. (v) In the absence of Pol δ, Pol ε can take over leading-strand synthesis directly from Pol α, although this process is less efficient than the pathway involving Pol δ.


A
helicase
primase
DNA polymerase
clamp loader
clamp
leading strand synthesis
lagging strand synthesis
D
C
B

Function	*E. coli*	Eukaryote	Phage T4
Helicase	DnaB	MCM complex	41
Loading helicase/primase	DnaC	cdc6	59
Single strand maintenance	SSB	RPA	32
Priming	DnaG	Polα/primase	61
Sliding clamp	β	PCNA	45
Clamp loading (ATPase)	$\gamma\delta$ complex	RFC	44/62
Catalysis	*Pol III core*	Polδ + Pol ϵ	43
Holoenzyme dimerization	τ	?	43
RNA removal	*Pol I*	FEN1	43
Ligation	*Ligase*	Ligase 1	T4 ligase

Similar functions are required at all replication forks

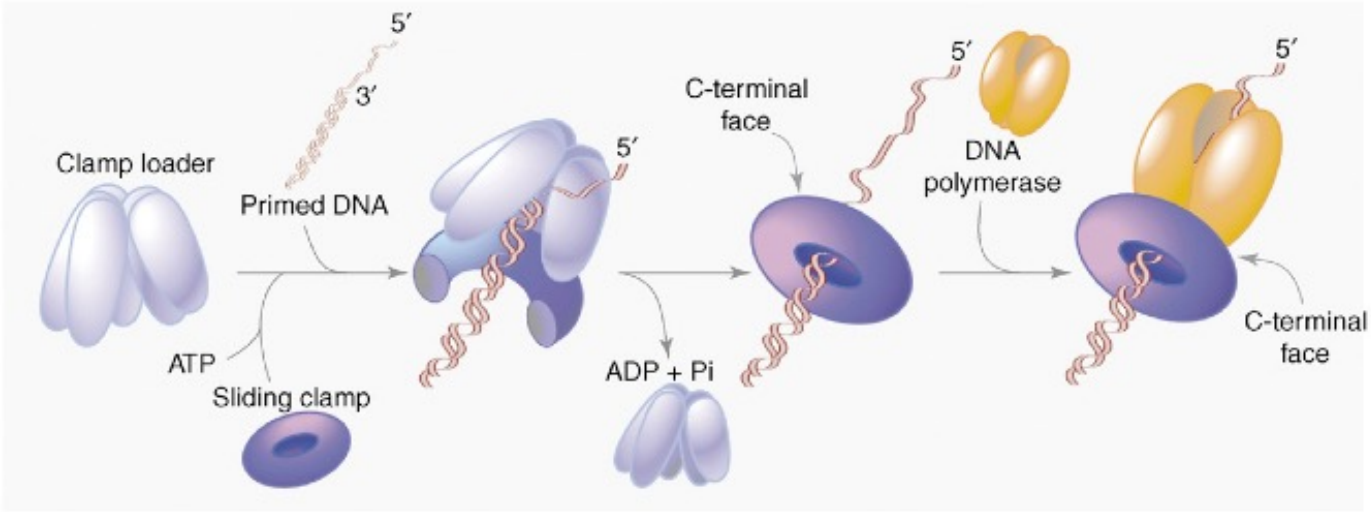


Figure 4. The clamp-loading process. The multisubunit AAA+ clamp loader uses the energy of ATP hydrolysis to assemble sliding clamps at primed sites. In the presence of ATP, the clamp loader binds to and opens a sliding clamp. The clamp loader selectively binds to primer-template junctions, which stimulates ATP hydrolysis and results in dissociation of the clamp loader from the clamp and closure of the sliding clamp around DNA. DNA polymerase is recruited to the primer-template junction by the sliding clamp assembled onto the RNA primer.

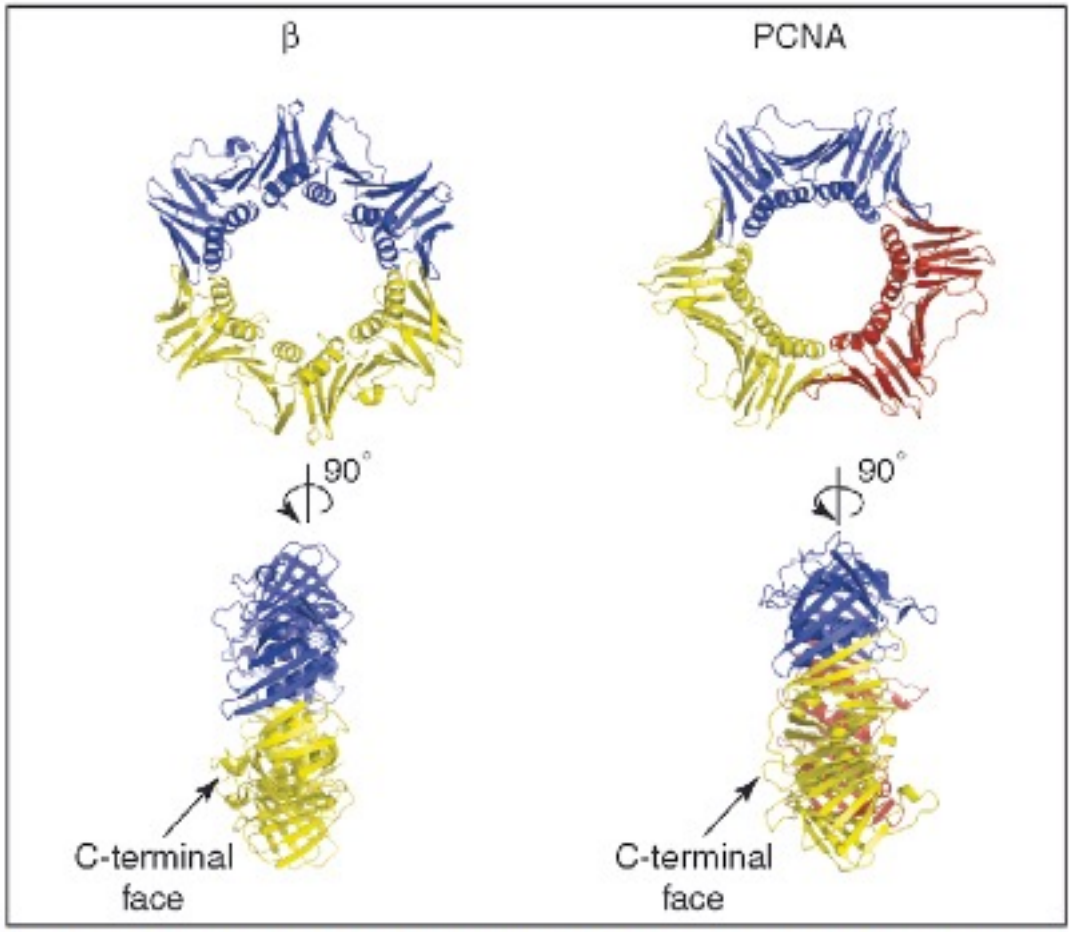


Figure 3. Structures of *Escherichia coli* and eukaryotic sliding clamps β and PCNA. Sliding clamps form ring-shaped structures that encircle duplex DNA and confer processivity to replicative DNA polymerases. β is composed of a homodimer with each protomer containing three subdomains (left). PCNA is composed of a homotrimer with each protomer containing two subdomains (right). The C-terminal face of β and PCNA is the molecular surface that is involved in intermolecular interactions. Protomers are indicated in blue, yellow and red. Protein Data Bank codes: β, 2POL; PCNA, 1PLQ. Figure reproduced, with permission, from *Nature Reviews Molecular Cell Biology* (Ref. [5]; http://www.nature.com/reviews). © (2006) Macmillan Magazines Ltd.

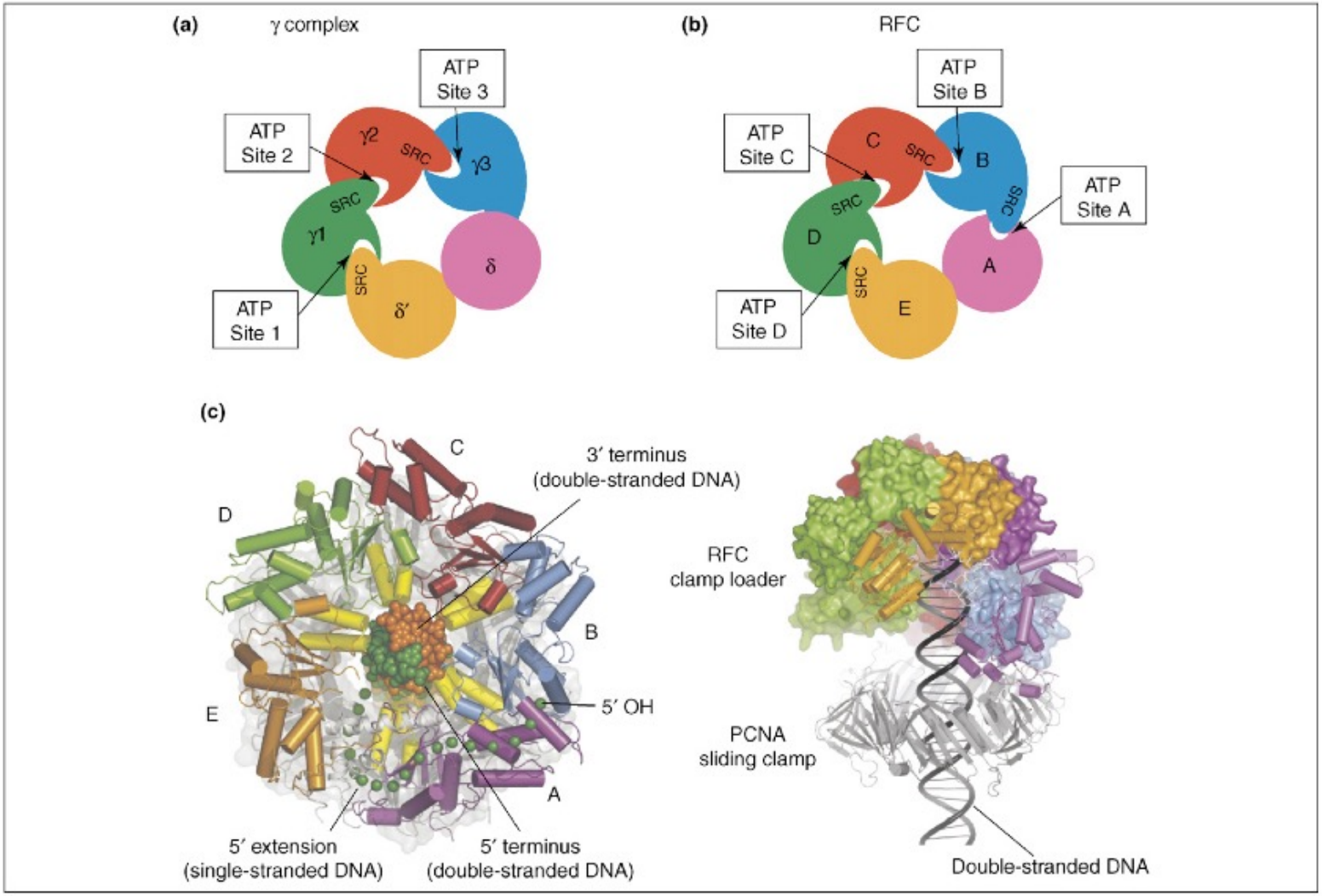
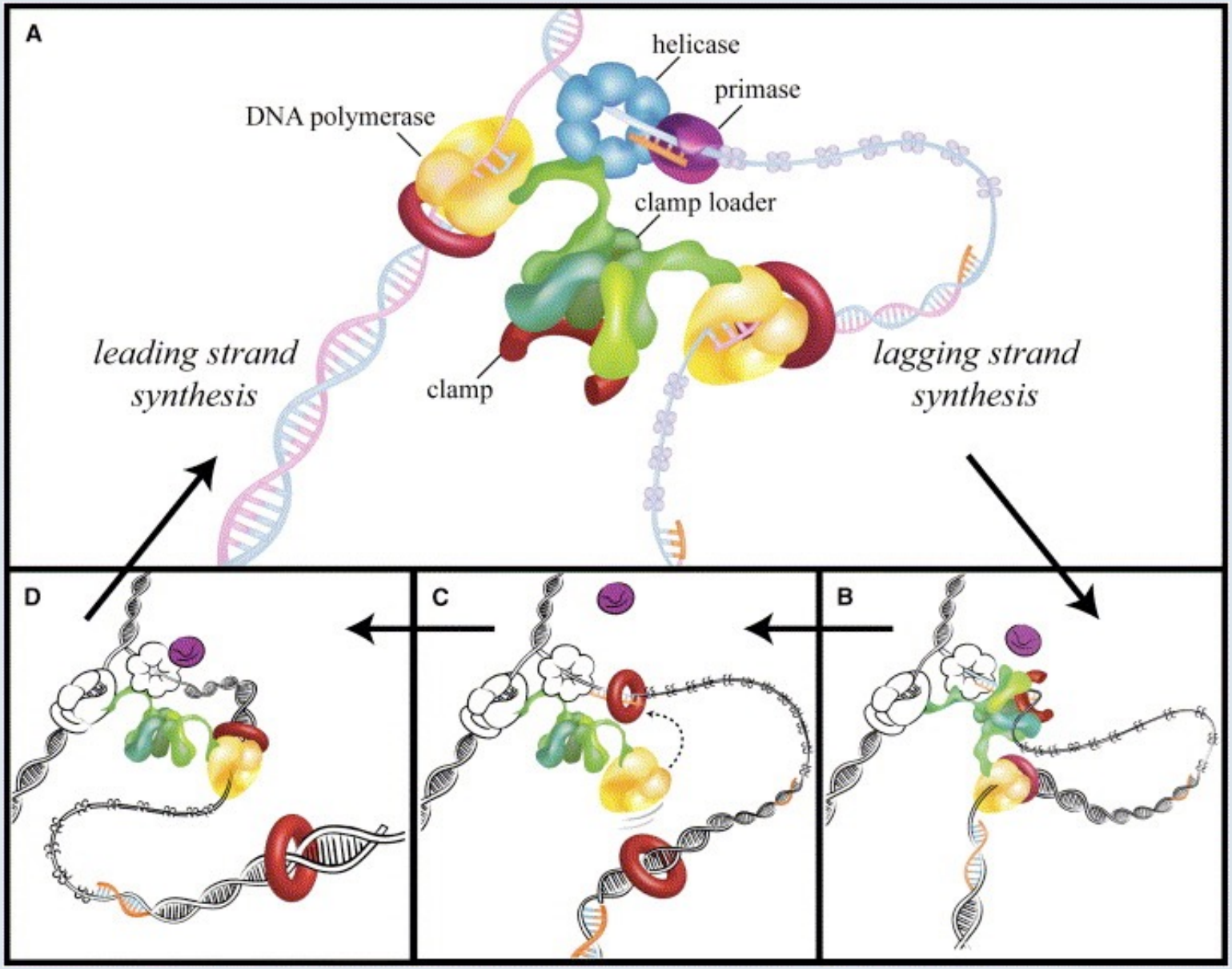


Figure 5. Organization of *Escherichia coli* and eukaryotic clamp loaders. The γ complex and RFC clamp loaders have similar spiral-shaped subunit architectures. **(a)** The γ complex is composed of five subunits (δ,δ',γ_3) and includes three ATP sites. **(b)** RFC is composed of five subunits (A,B,C,D,E) and includes four ATP sites. The ATP sites of γ complex and RFC are located at subunit interfaces and contain an arginine finger embedded in a conserved SRC motif. Parts (a) and (b) adapted, with permission, from reference [42]. **(c)** Model of the RFC–PCNA complex bound to primed DNA. Top (left) and side (right) views of the co-crystal structure of the RFC–PCNA complex are shown modeled in complex with primed DNA. The top and side portions of RFC are removed for clarity in the left and right panels, respectively. RFC–PCNA Protein Data Bank code: 1SXJ. Part (c) adapted, with permission, from *Nature* (Ref. [30]; http://www.nature.com). © (2004) Macmillan Magazines Ltd.

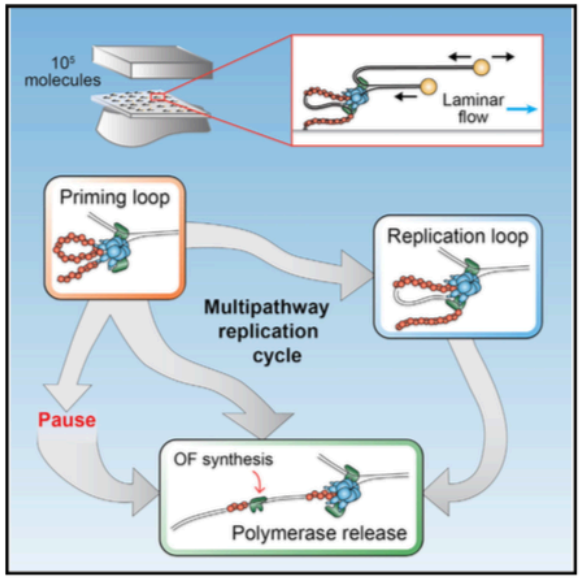

A
helicase
primase
DNA polymerase
clamp loader
leading strand synthesis
clamp
lagging strand synthesis
D
C
B

Article

Molecular Cell

Simultaneous Real-Time Imaging of Leading and Lagging Strand Synthesis Reveals the Coordination Dynamics of Single Replisomes

Graphical Abstract



Highlights

- Simultaneous imaging of leading- and lagging-strand synthesis by single replisomes
- Most loop formation events on the lagging strand occur during primer synthesis
- Polymerases are released from the replisome to complete Okazaki-fragment synthesis
- Multiple pathways and exchange events underlie replisome coordination

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In Brief

Duderstadt et al. report a multidimensional single-molecule approach to simultaneously observe DNA looping and leading-strand synthesis during replication. Working with the bacteriophage T7 replisome, they show that most DNA loops form only during priming to support several parallel pathways that ensure robust coordination of daughter-strand synthesis.

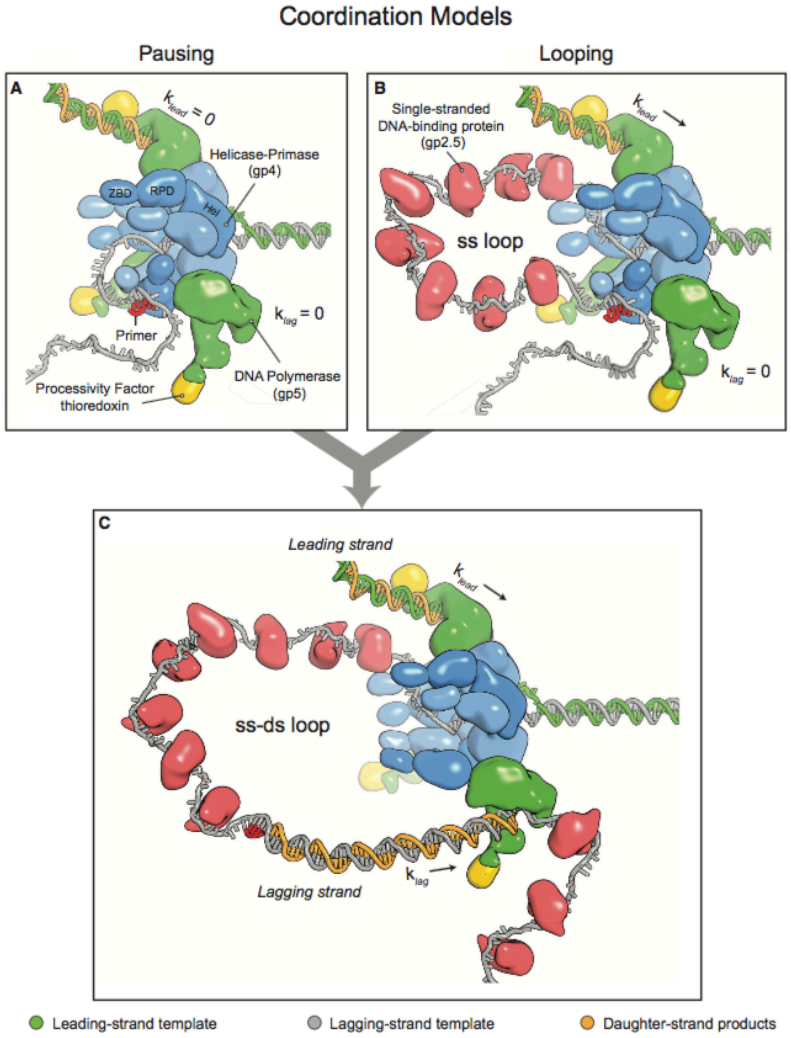
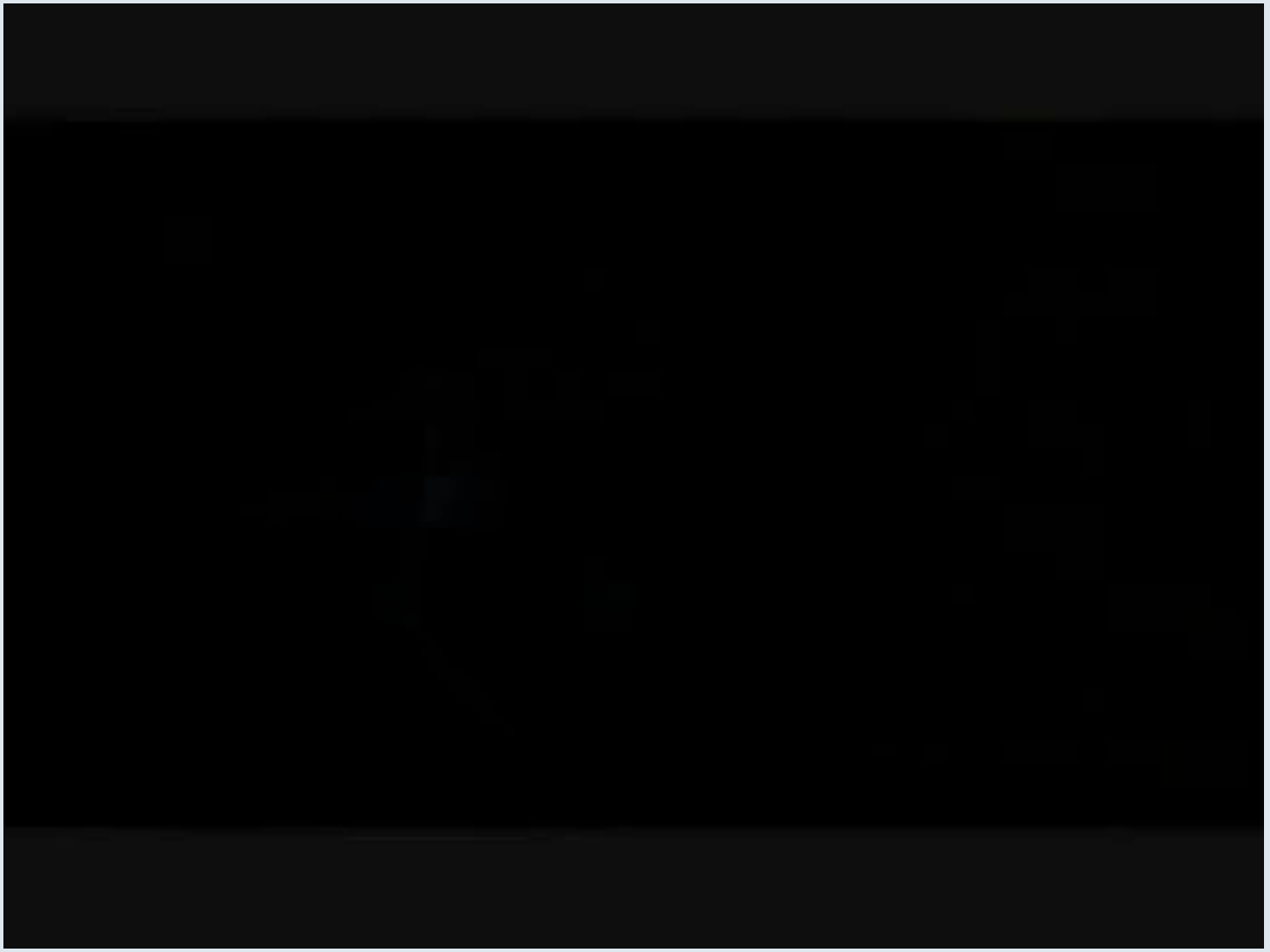


Figure 1. Coordination Models

(A) Priming pauses the replisome to ensure that leading-strand synthesis does not outpace lagging-strand synthesis.

(B) Helicase unwinding and leading-strand synthesis continue during priming by the formation of a lagging-strand ss loop emerging from the helicase. This configuration keeps the primer in close physical proximity to the fork facilitating hand-off to a lagging-strand polymerase.

(C) Coupled leading- and lagging-strand synthesis leads to the formation of a ss-ds loop that grows as the sum of the ssDNA output of helicase activity and the synthesis rate of the lagging-strand polymerase. A detailed description of how the models were constructed can be found in the Supplemental Information.



DNA Replication: *youtube.com/watch?v=yqESR7E4b_8*


newly forming strand. DNA replication will begin at a specific area of the molecule called the origin of replication.

Molecular Cell 23, 155–160, July 21, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.molcel.2006.05.034

DNA Replication: Keep Moving and Don't Mind the Gap

Short Review

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As the replication fork progresses, synthesis of the discontinuous lagging strand requires frequent priming and cycling of the lagging strand polymerase to the new primers. It appears that this mechanism also permits bypass of template lesions on both strands, leaving the damage behind in a single-strand gap and precluding fork stalling or collapse.

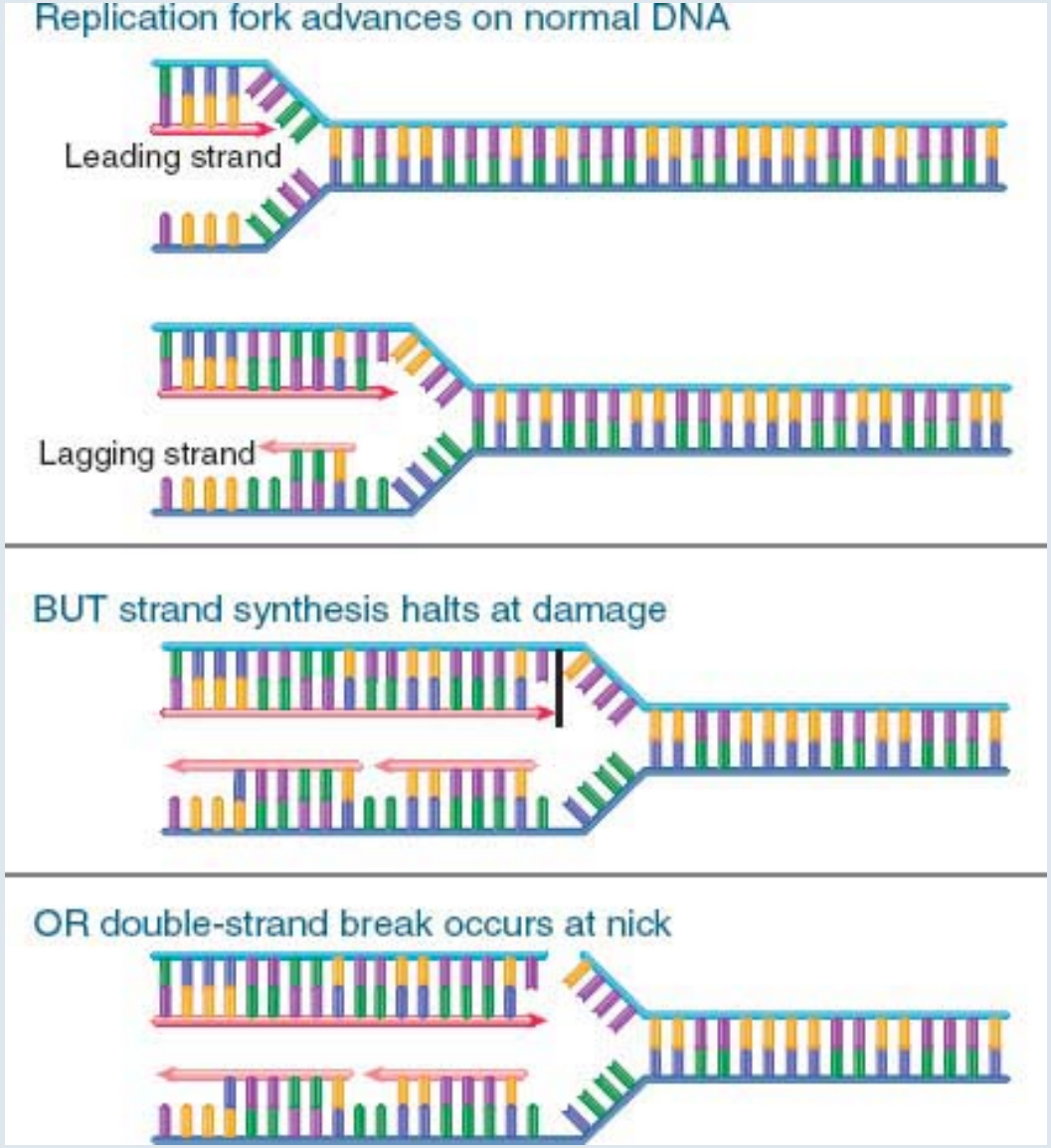
Introduction

The standard view of DNA replication is that it is semi-discontinuous, with the leading strand synthesized as a single uninterrupted chain and the lagging strand as a series of short (<2 kb) Okazaki fragments. Despite the prevalence of this view and the support it has gained from in vitro studies, it is not in agreement with cytological evidence in *E. coli*, which routinely shows discontinuities in both strands during DNA replication (reviewed in Wang [2005]). This does not necessarily imply that there is a regular cycle of reinitiation on the leading strand, but it suggests that priming can and does recur on the leading strand as the replication fork moves away from the origin, most likely in response to damage on the tural bridge between the replicative helicase and the leading and lagging strand polymerases at the prow of the replication fork.

Cellular DNA polymerases cannot initiate synthesis in the absence of a nucleic acid primer, so the first step in DNA synthesis is the formation of a short RNA primer (~10 nt) by specialized RNA polymerases known as primases (Kornberg and Baker, 1992). In principle, leading strand synthesis requires only a single priming event, whereas frequent repriming is the hallmark of discontinuous lagging strand synthesis. The distribution of primers, ~1–2 kb apart on the lagging strand, is governed by dynamic interactions between DnaB and the DnaG primase (Tougu and Marians, 1996) and possibly, according to a recent report, by interactions between separate primase molecules bound to a single DnaB on the lagging strand (Corn et al., 2005).

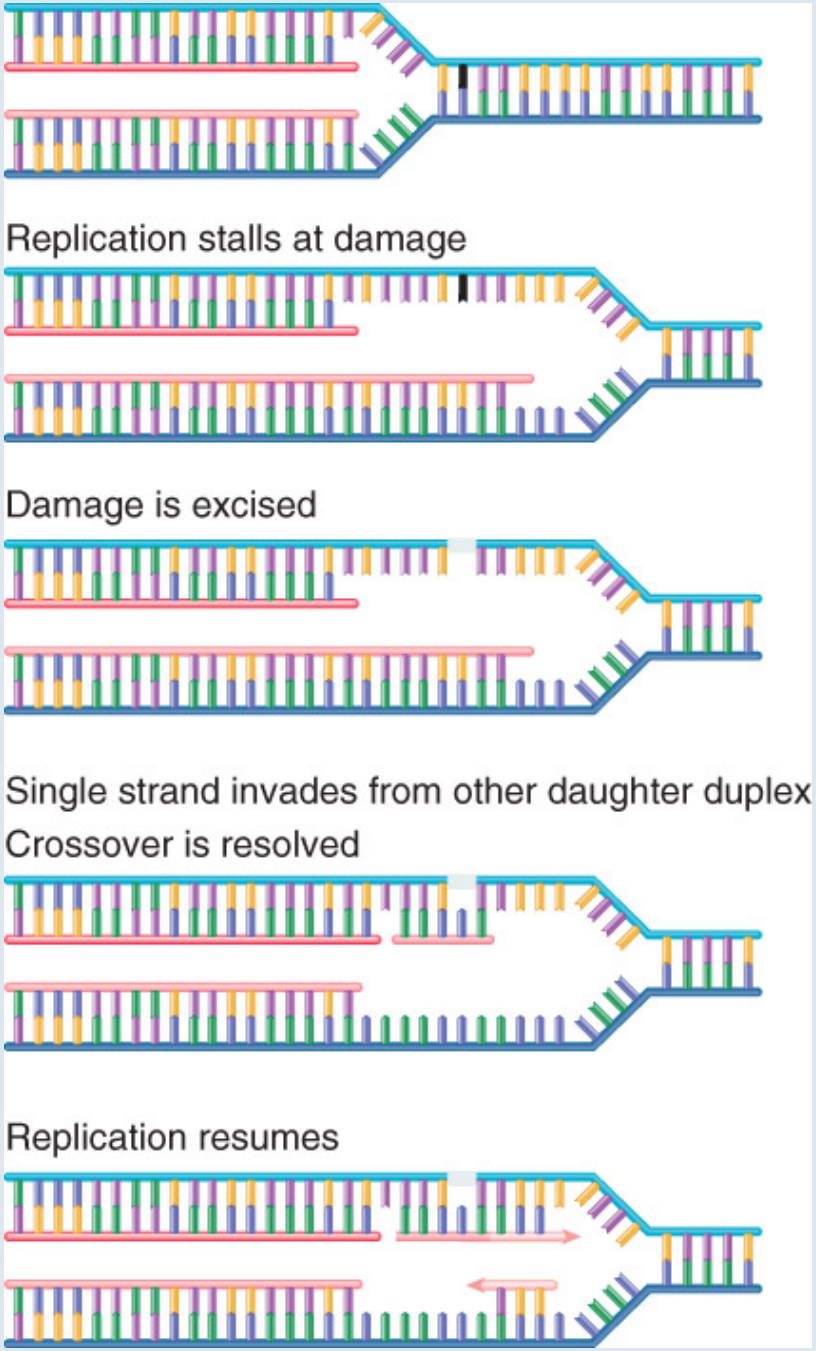
Primase remains bound to the 3′ terminus of the RNA primer through contact with SSB, the single-strand DNA (ssDNA) binding protein, which binds and protects ssDNA ahead of the primer (Figure 1A). The Pol III core polymerase then replaces primase at the primer terminus in a three-part switch activated by the χ subunit of the γ complex clamp loader (Yuzhakov et al., 1999). χ displaces primase by competitive binding to SSB, whereas the clamp-loading subunits of γ complex (the AAA+ proteins γ, τ_2, δ, and δ') form a helical structure that completely encases the newly cleared primer-template junction (for a detailed review of clamp loaders and how their structures confer specificity for the primed

Lesion Bypass Requires Polymerase Replacement



- A replication fork stalls when it arrives at damaged DNA.
- The replication complex must be replaced by a specialized DNA polymerase for **lesion bypass**.

The replication fork stalls and may collapse when it reaches a damaged base or a nick in DNA. Arrowheads indicate 3′ ends.

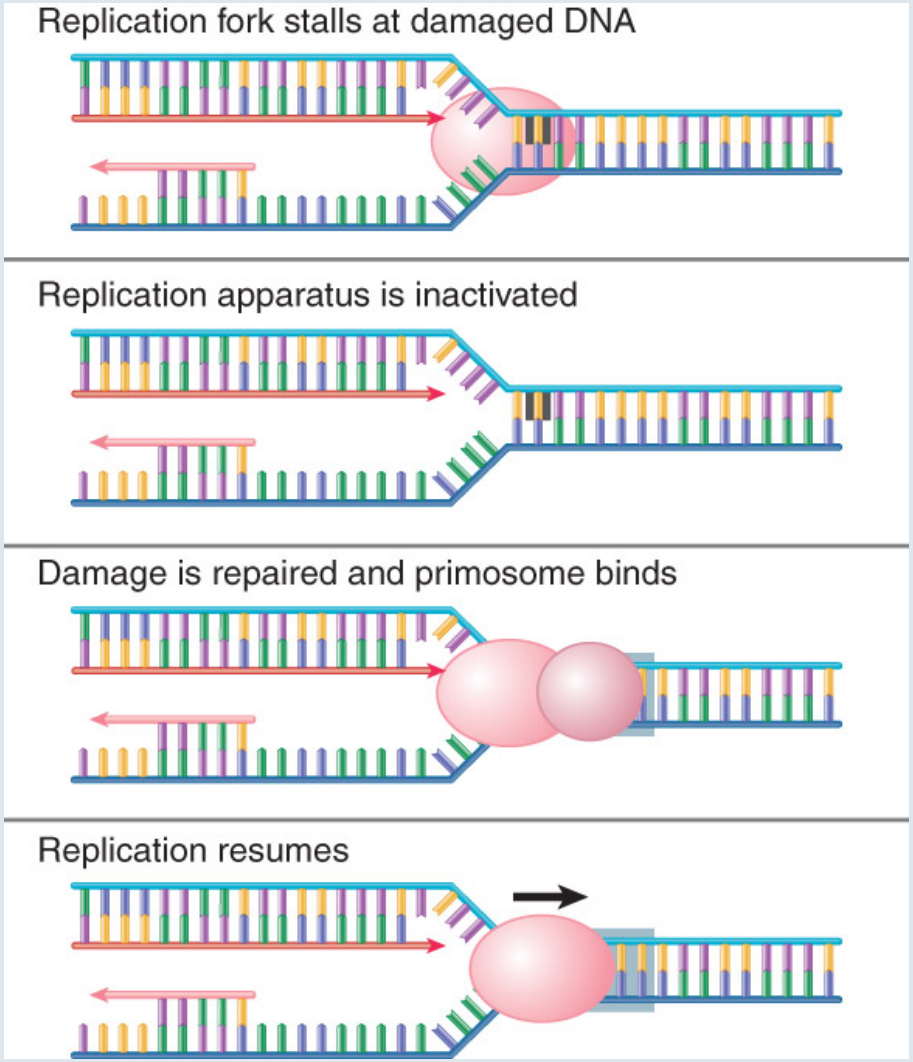


Lesion Bypass Requires Polymerase Replacement

- A replication fork stalls when it arrives at damaged DNA.
- The replication complex must be replaced by a specialized DNA polymerase for **lesion bypass**.

When replication halts at damaged DNA, the damaged sequence is excised and the complementary (newly synthesized) strand of the other daughter duplex crosses over to repair the gap. Replication can now resume, and the gaps are filled in

Lesion Bypass Requires Polymerase Replacement



- After the damage has been repaired, the **primosome** is required to reinitiate replication by reinserting the replication complex.

The primosome is required to restart a stalled replication fork after the DNA has been repaired

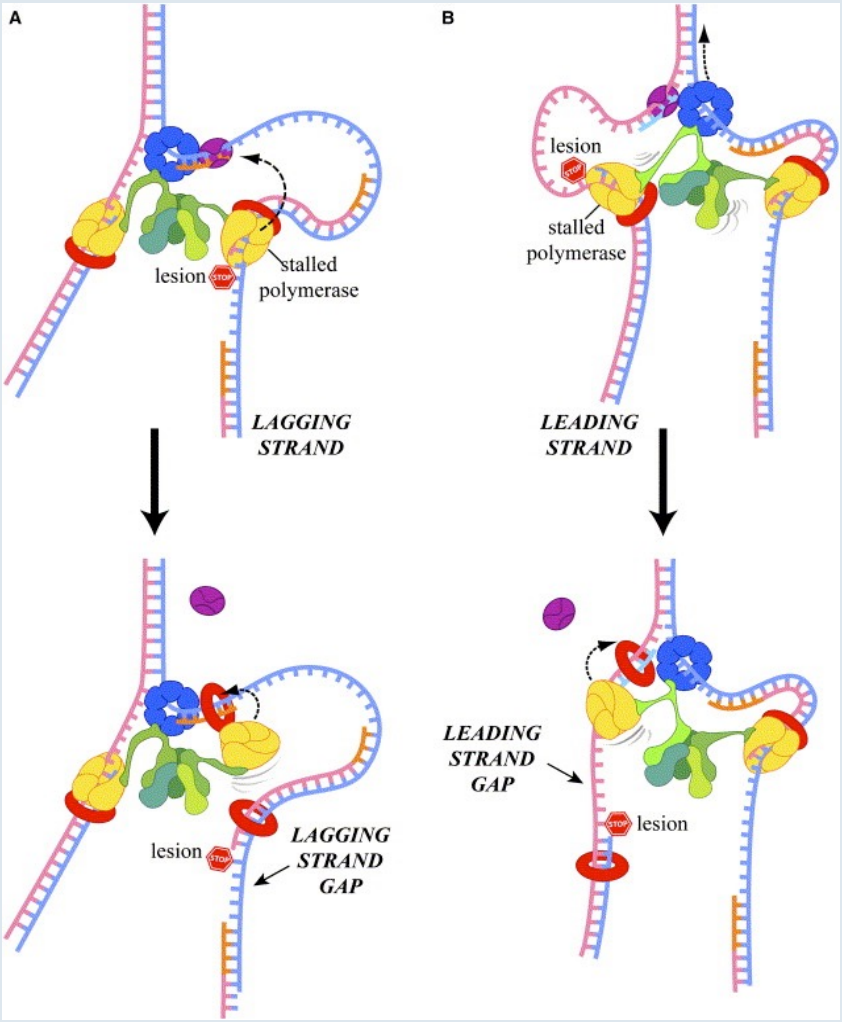


Figure 2. Replisomes Bypass Template Damage

(A) Upon encountering a lesion on the lagging strand template (stop sign), leading strand synthesis continues and the stalled lagging strand polymerase recycles (dotted arrow) to a new primer/template junction, leaving a single-strand gap with a template lesion (bottom).

(B) The leading strand polymerase stalls upon encountering a lesion (top). The helicase recruits primase to reinitiate leading strand synthesis ahead of the lesion, leaving a single-strand gap (bottom). If stalling causes the replication fork to collapse, additional factors (e.g., PriA or PriC) are required to reload the helicase at the collapsed fork. For (A) and (B), gaps that are left behind in either strand can be repaired with high fidelity by recombination processes using the new sister chromatid as a template. Artwork by Dr. Nina Yao.

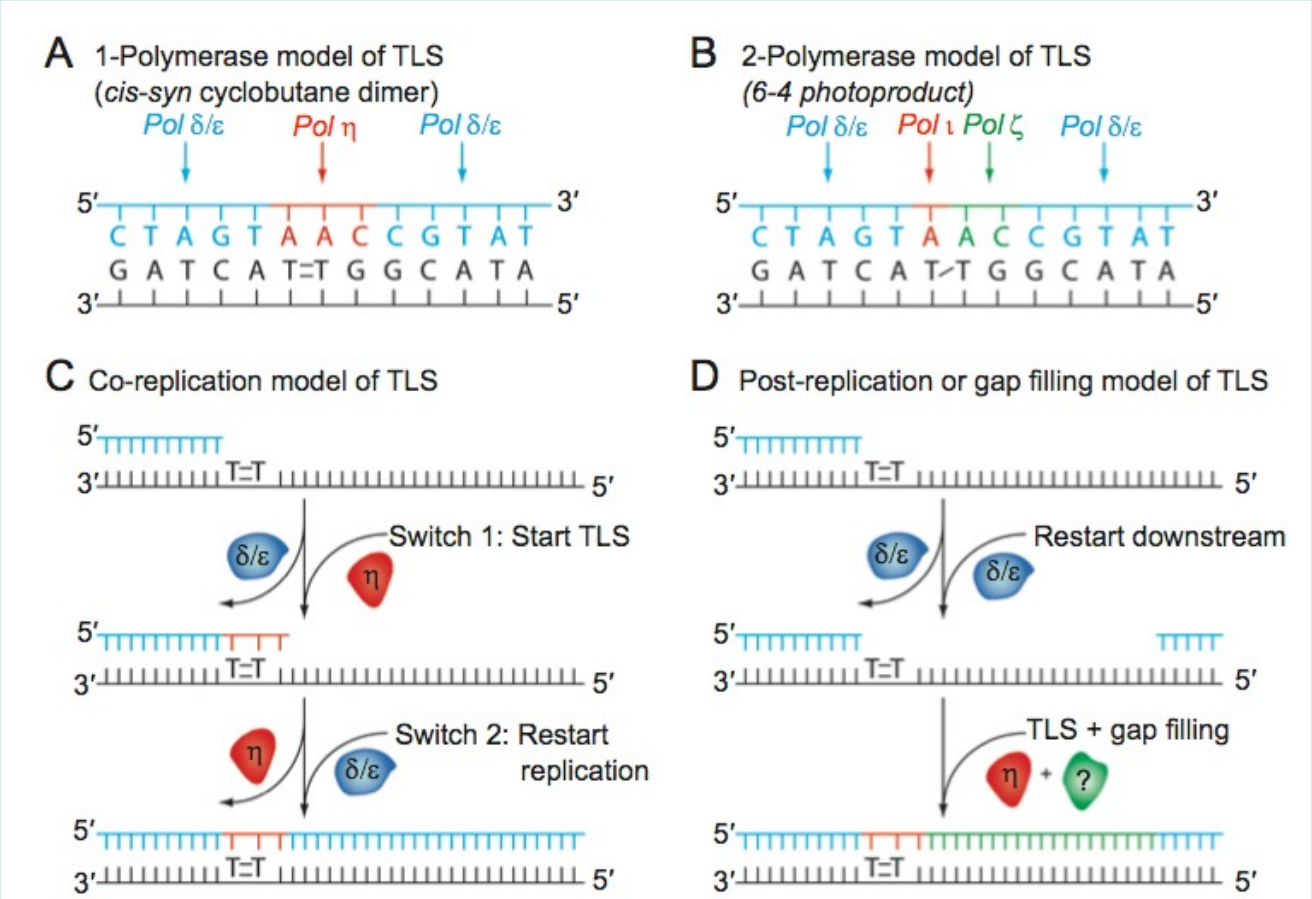


Figure 3 Models of translesion synthesis. **(A)** The 1-polymerase model of TLS, shown here for a thymine-thymine dimer, states that a single polymerase is responsible for the complete bypass of a lesion, including insertion opposite all lesion bases and extension from the primer terminus opposite a damaged template base. **(B)** The 2-polymerase model of TLS, shown here for a thymine-thymine 6-4 photoproduct, states that different polymerases are responsible for the insertion steps at the various lesion positions. In the example given, note that while pol ζ is responsible for extension from the template-3′ T primer terminus, it also carries out an insertion at the 5′ T position of the lesion. For a single base lesion, the insertion step would be opposite undamaged DNA. A more comprehensive listing of 2-polymerase/lesion combinations is given elsewhere [11]. Note that for both examples given, the actual TLS reaction is flanked relatively closely both upstream (1-2 bases) and downstream (1-5 bases) of the lesion by replicative polymerase synthesis. **(C)** Model for TLS that occurs at a replication fork during the process of ongoing synthesis. **(D)** Model for TLS that takes place as a "gap-filling" reaction, away from the main replication machinery. Note that both of these models are consistent with either the 1- or 2-polymerase model of TLS given in panels **A** and **B**. In both cases, post-translational modification of PCNA and possible other proteins is critical for the polymerase switch. Note that panels A and B are models of the actual TLS process while panels **C** and **D** depict models for the timing of TLS. As such (and as noted in the text), there is overlap between the panels.