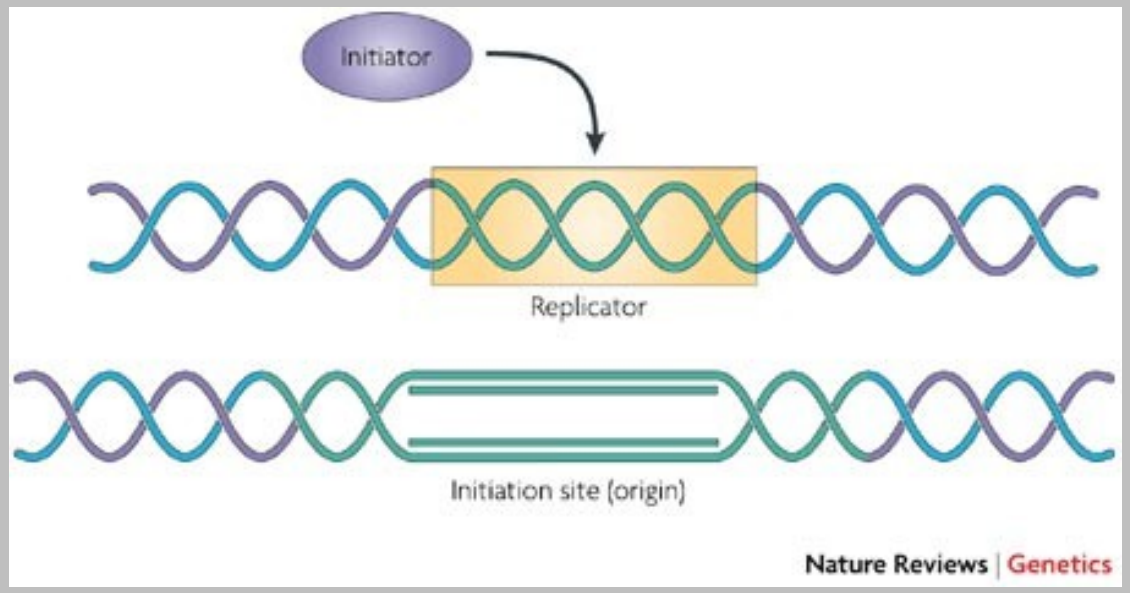
Replikonski model



Nature Reviews Genetics 8, 588-600 (2007)

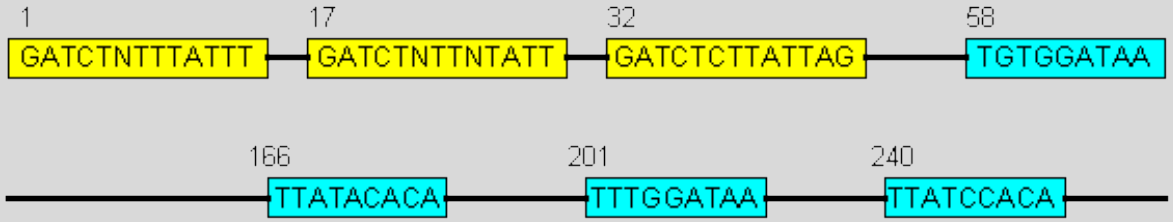
replikator … genetski element, na katerem pride do iniciacije podvojevanja DNA

replikon …. tisti del kromatina, ki se podvoji pod kontrolo določenega replikatorja

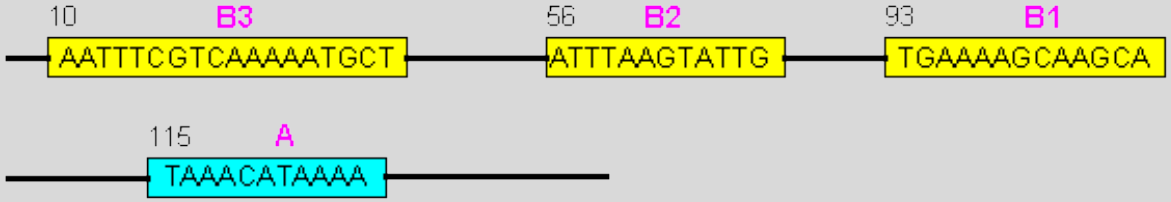
iniciator …. proteini (kompleks), ki se vežejo na replikator in sprožijo podvojevanje

iniciacijsko mesto (ori) …. mesto na kromatinu, kjer se pojavijo replikacijske vilice (lahko sovpadajo z replikatorjem, niso pa vsi replikatorji aktivni v vsakem celičnem ciklu)

(a) Bacterial replication origin



(b) Yeast replication origin (ARS1)



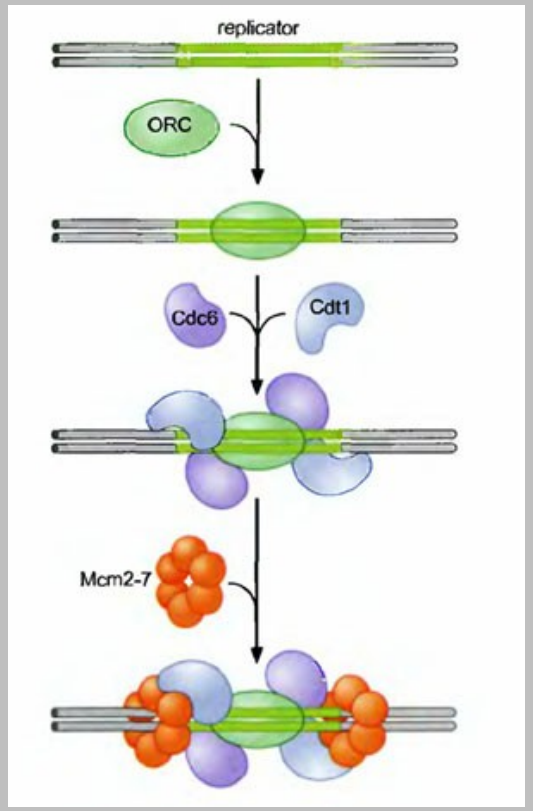
http://www.web-books.com/MoBio/Free/Ch7A.htm

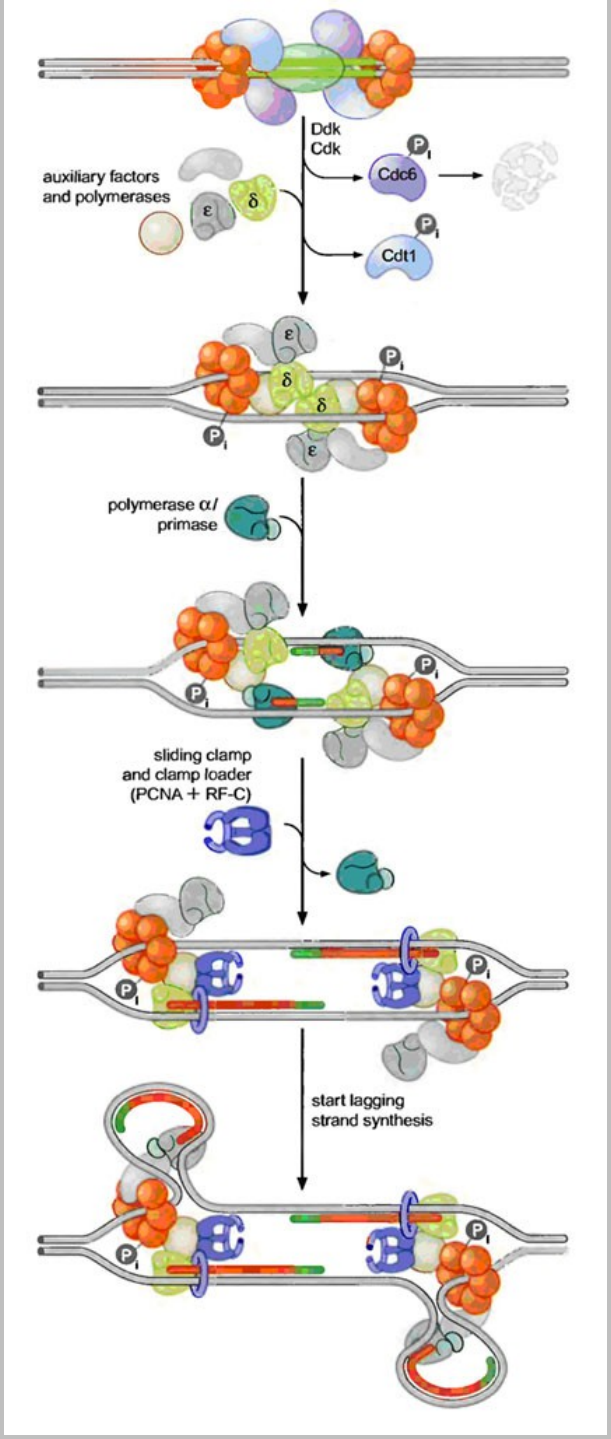
The Current Roster of DNA Polymerases

Greek Name	Human Name	Yeast Name	Proposed Function
α	POLA	*POL1*	Replication
β	POLB	—	BER; ss break repair
γ	POLG	*MIP1*	Mitochondrial replication; Mt BER
δ	POLD1	*POL3*	Replication
ϵ	POLE	*POL2*	Replication
ζ	POLZ	*REV3*	Bypass synthesis
η	POLH	*RAD30*	Bypass synthesis
θ	POLQ	—	Bypass synthesis
ι	POLI	—	Bypass synthesis (?)
κ	POLK	—	Bypass synthesis
λ	POLL	*POL4*	NHEJ
μ	POLM	—	NHEJ (?)
ν	POLN	—	Bypass synthesis
—	REV1	*REV1*	Bypass synthesis

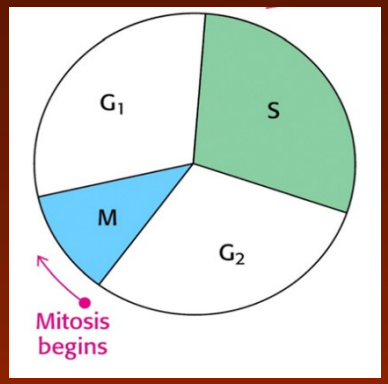
prediniciacija

iniciacija →



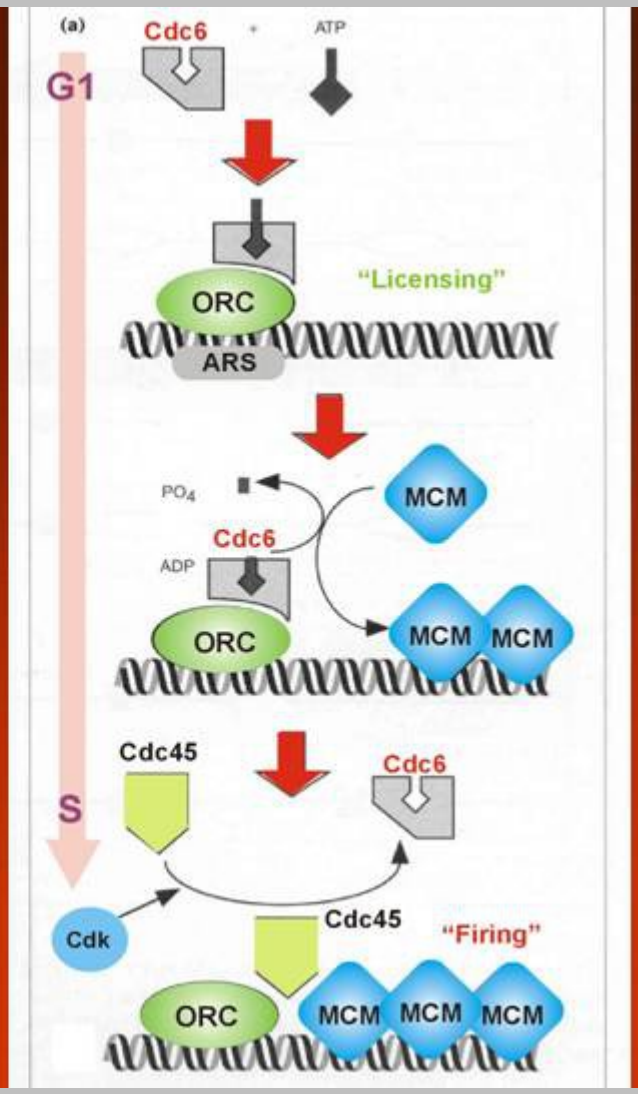


ORC: origin recognition complex
Cdc, Cdt: helicase loading proteins
Mcm2-7: helicase

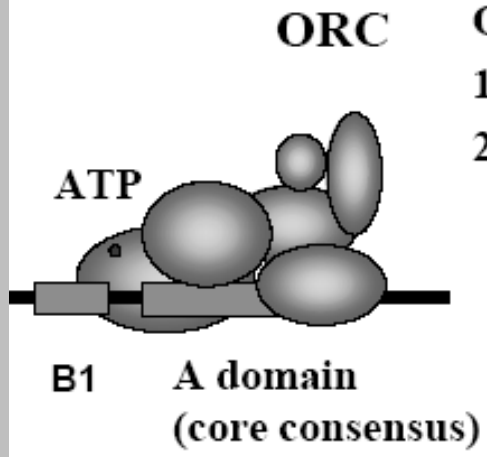


Cdc6= ATPase
ORC= Origin of replication complex
(Orc1 to Orc6)
MCM= Mcm complex
(Mcm1 to Mcm6)

ARS= Origin of replication (autonomously replicating sequence)



http://biochemie.web.med.uni-muenchen.de/Yeast_Biology/10_Cellcycle.htm

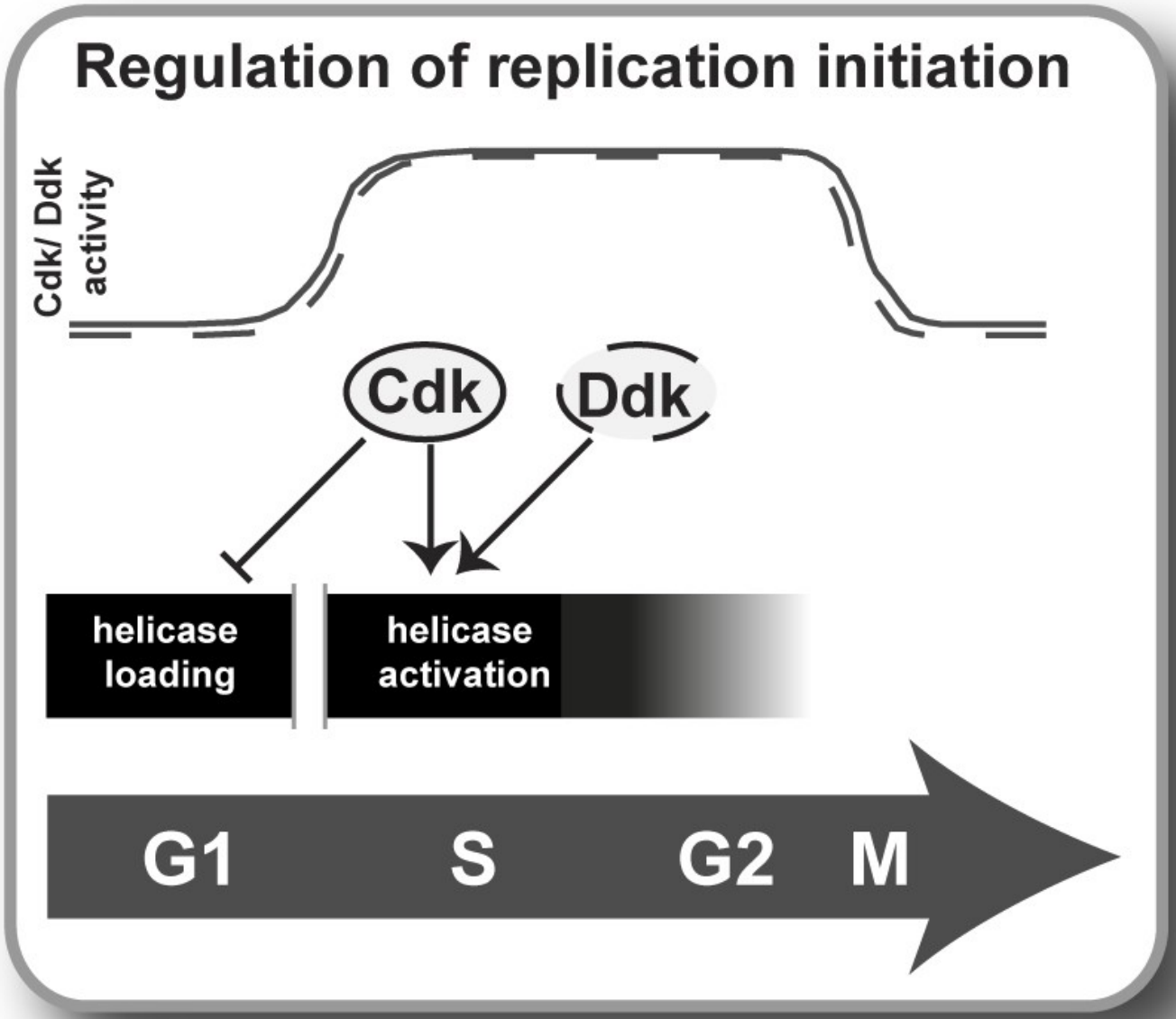


Origin recognition complex:

1. **6 proteins: 400,000 Dalton (400kDa)**
2. **must bind ATP before it can bind to the ARS core consensus.**

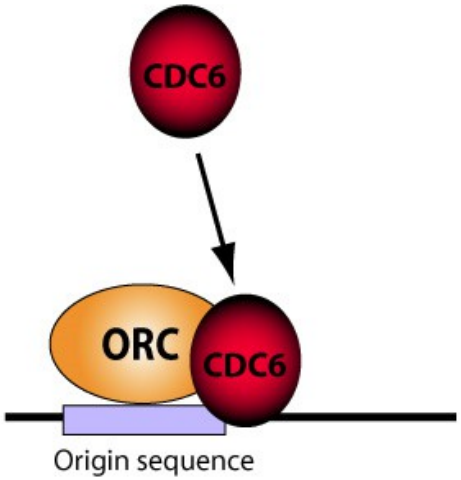
ORC binds to A and B1 domains. Events at A and B1 critical for initiation

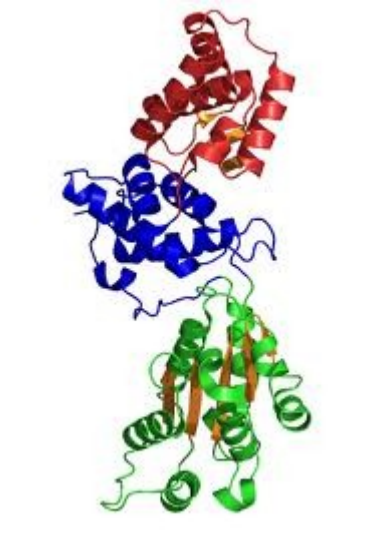
Origin can function effectively with functional A domain and any two B elements



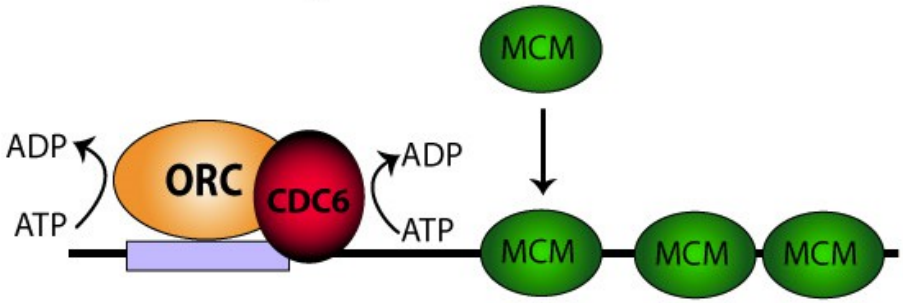
http://www.biochem.mpg.de/en/rg/pfander/Research/index.html

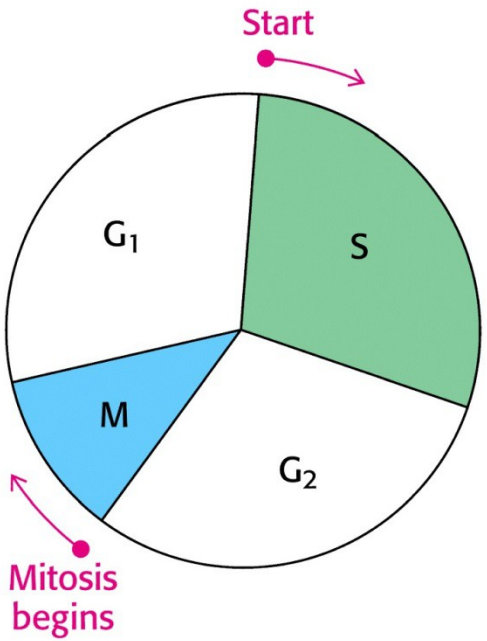
Recruiting of CDC6 to the origin of replication

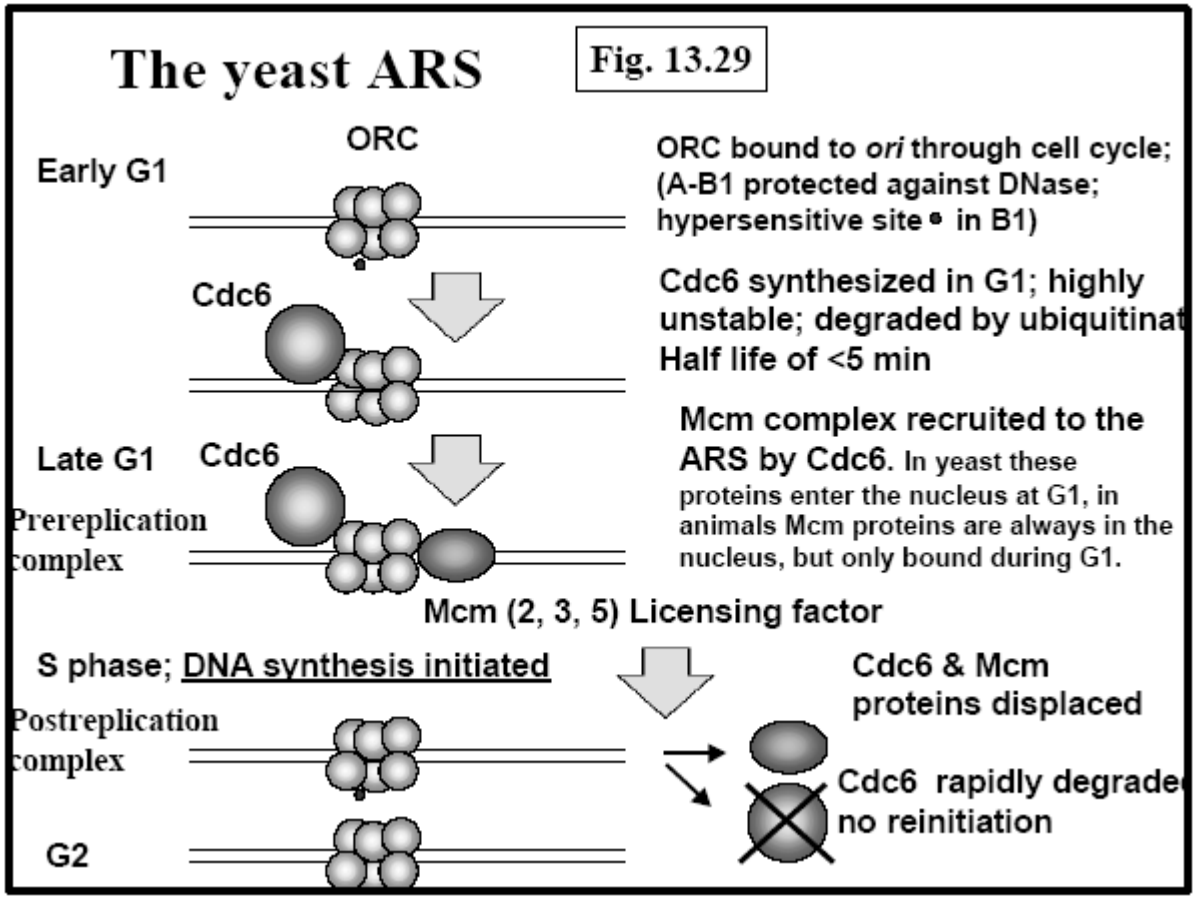




MCM Loading

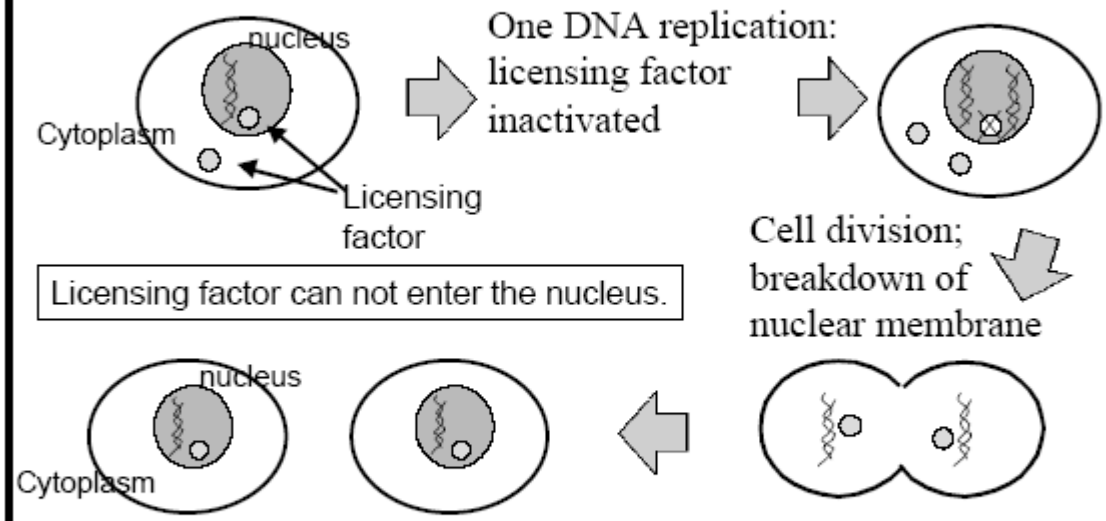






http://nersp.nerdc.ufl.edu/~arabian/mcb3020s/lecture3.pdf

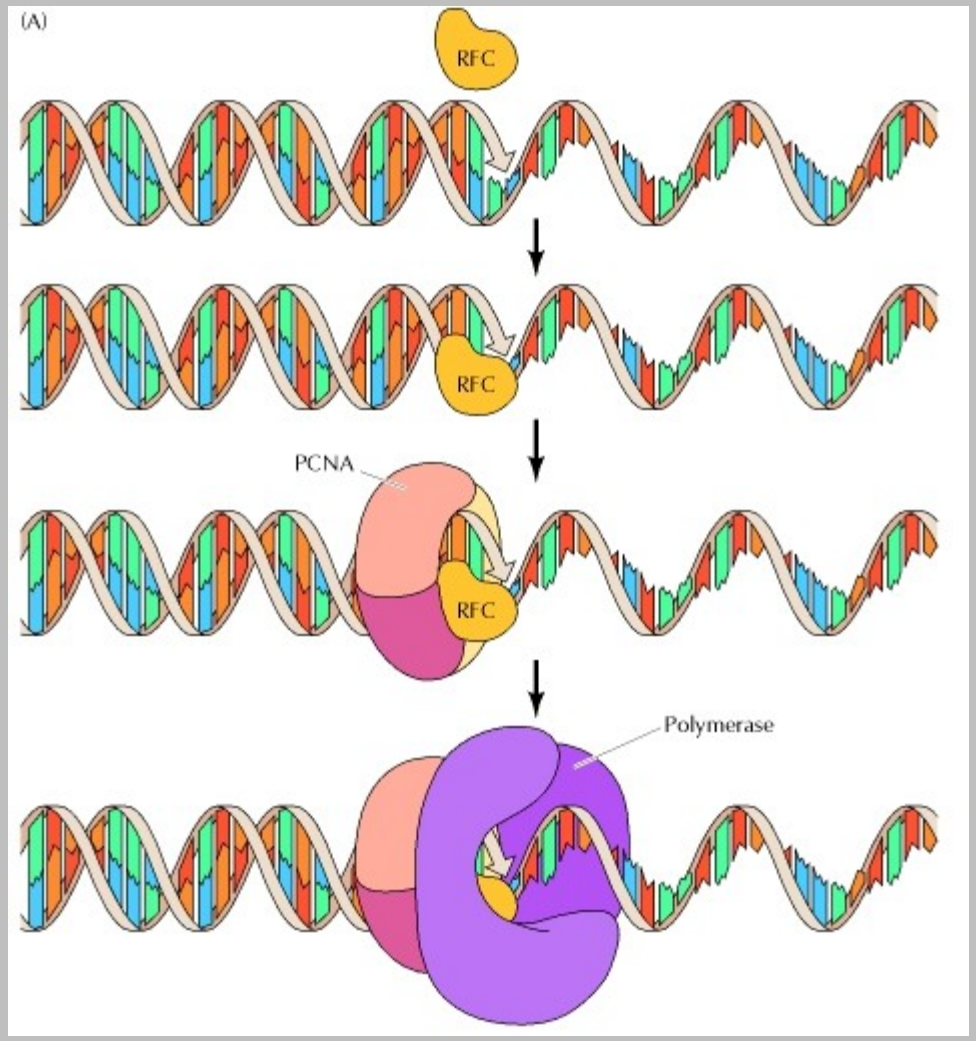
Licensing factor controls eukaryotic replication



Licensing factor can not enter the nucleus.

new licensing factor enters the nucleus

Licensing factor ensures that only single round of DNA replication occurs.



RFC: podajalec drsne vponke
(*clamp-loading protein*)

PCNA: drsna vponka
(*sliding-clamp protein*)

Cooper 2

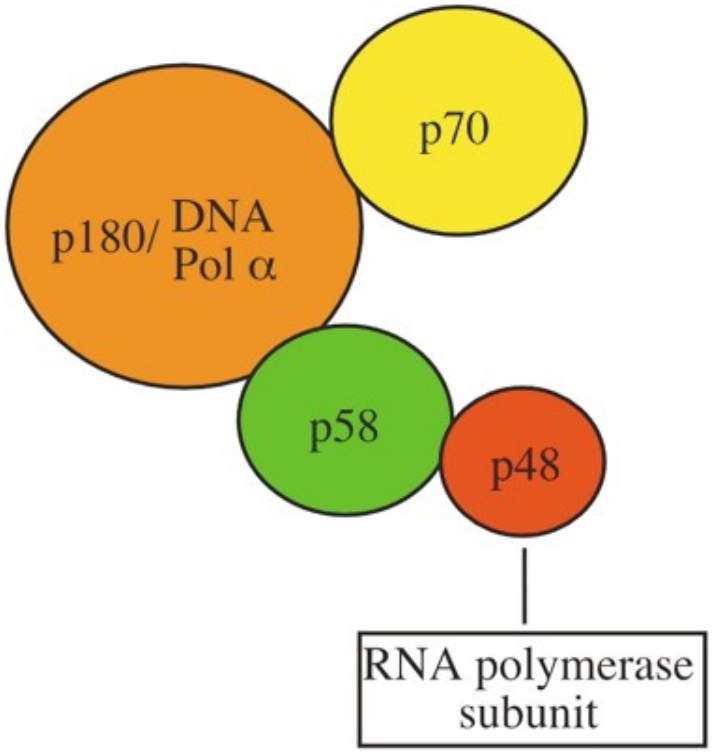
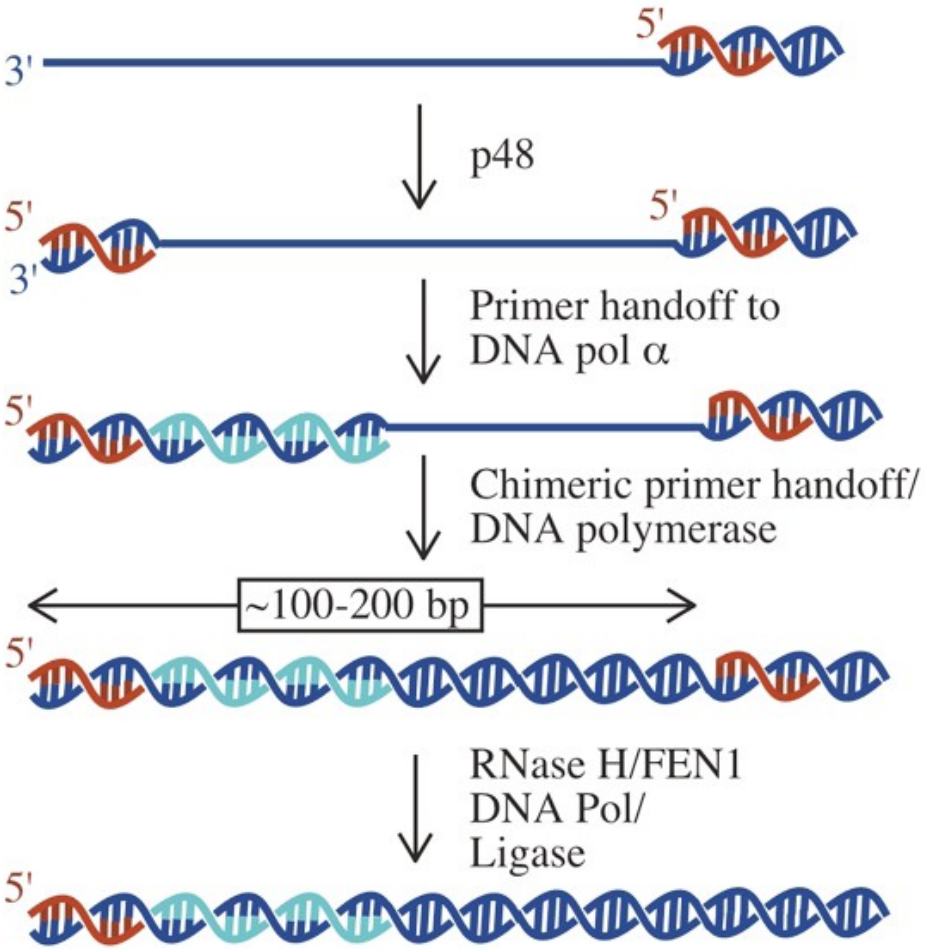
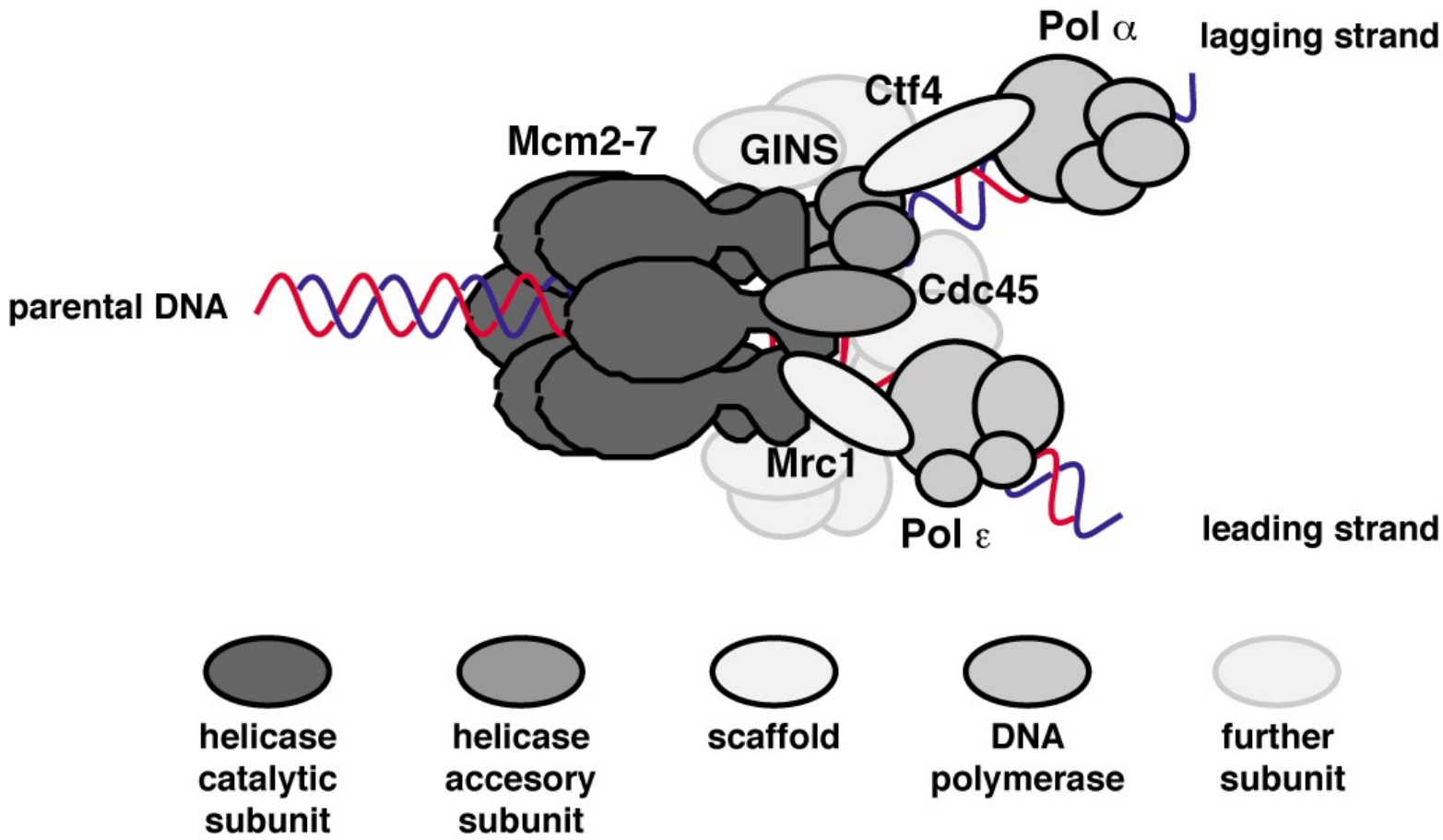


Table 1 Cellular replication proteins

Protein	Subunit (kD)[a]	Replicative function
DNA polymerases		
pol-α:primase	180, 70, 58, 48	DNA polymerase, primase
pol-δ	125, 48	DNA polymerase, 3′ to 5′ exonuclease
pol-ε	258, 55	DNA polymerase, 3′ to 5′ exonuclease
Accessory proteins		
RP-A	70, 32, 14	single-stranded DNA binding
PCNA	36	pol-δ/ε processivity factor
RF-C	145, 40, 38, 37, 36.5	loads PCNA onto template
Nucleases		
ribonuclease H1	89	Okazaki fragment maturation
FEN-1 (MF-1)	44	Okazaki fragment maturation



The eukaryotic replisome



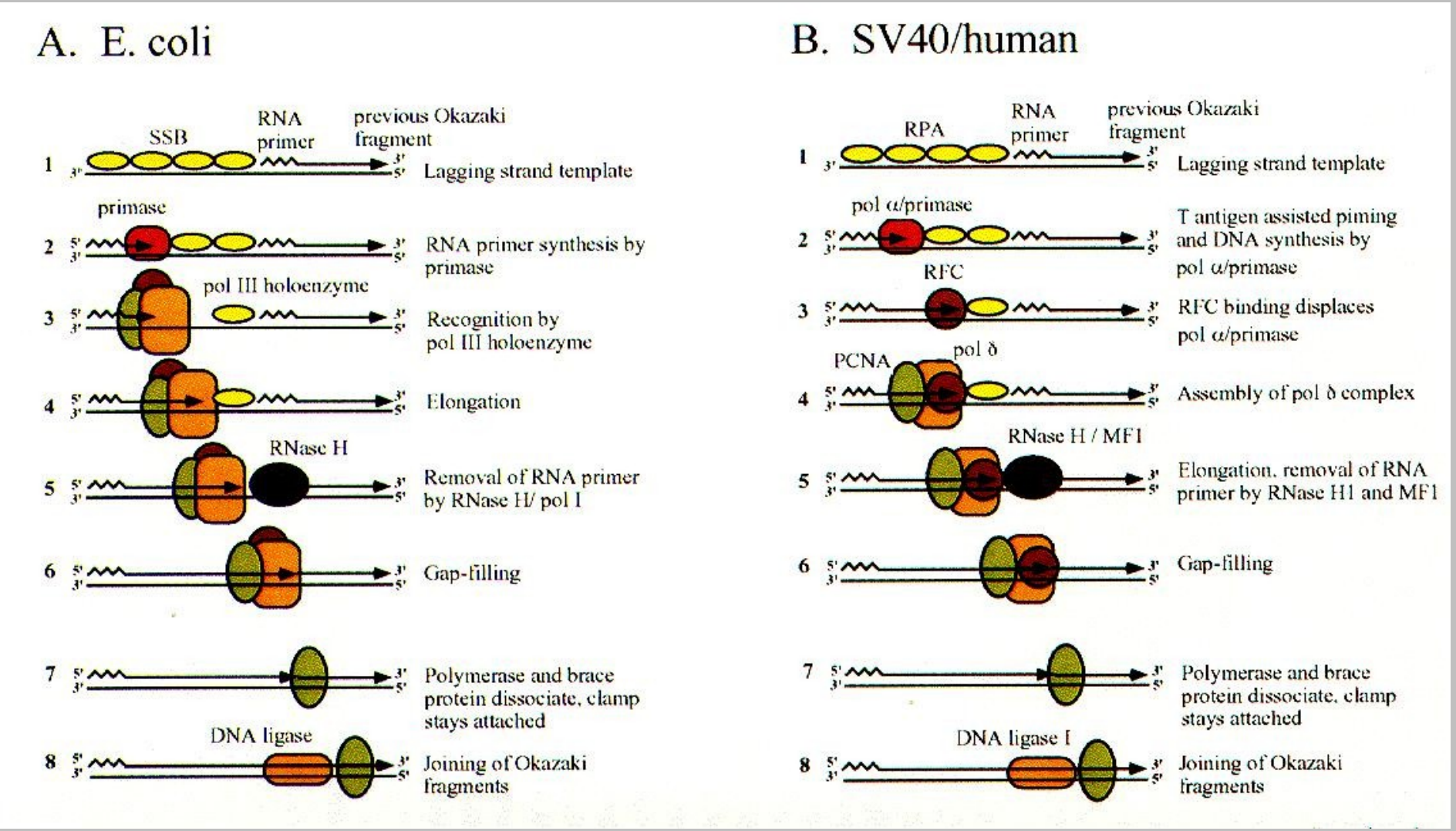
http://www.biochem.mpg.de/en/rg/pfander/Research/index.html

	Replication Process	
	E. coli	Human
DNA content, number of nucleotide pairs per cell	3.9×10^6	ca. 10^9
Rate of replication fork progression, μm/minute	30	3
DNA replication rate, nucleotides/second per replication fork	850	60–90
Number of replication origins per cell	1	10^3–10^4
Hours required for complete genome replication	0.67	8
Hours required for one complete cell division	0.33	24

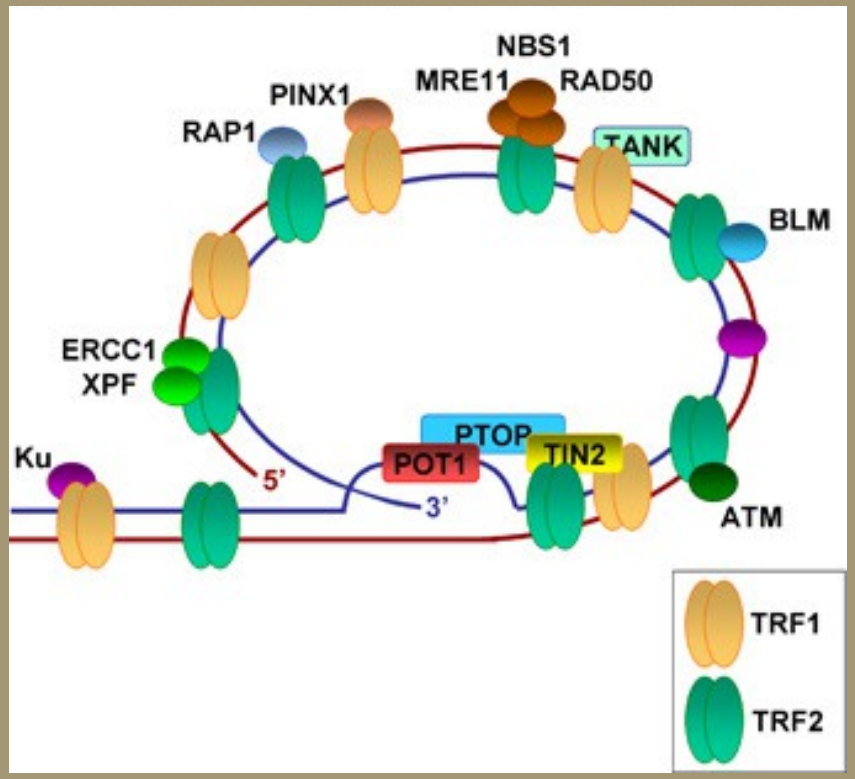
Note: The data are for an *E. coli* cell optimally nourished and cultured at 37°C. The values for human cells represent data from HeLa cells, which were originally derived from a tumor and have been maintained in culture for many years.

	α	β	γ	δ	ε
Cell compartment	Nucleus	Nucleus	Mitochondrion	Nucleus	Nucleus
Associated primase	Yes	No	No	No	No
Biological function	Lagging strand replication	DNA repair	Mitochondrial DNA replication	Leading strand replication	Replication
Number of subunits	4	1	4 (identical)	2	?
M_r of catalytic subunit, kilodaltons	160–185	40	125	125	210–230 or 125–140
K_M for dNTPs, μM	2–5	10[a]	0.5	2–4	?
Processivity (inherent)	Moderate	Low	High	Low	High
Processivity (with PCNA)	Moderate	Low	High	High	High
3′ exonuclease	No[b]	No	Yes	Yes	Yes
Sensitivity to 2′,3′-dideoxy-NTPs	Low	High	High	Low	Moderate
Sensitivity to arabinosyl-CTP	High	Low	Low	High	?
Sensitivity to aphidicolin	High	Low	Low	High	High

[a] K_M values for repair synthesis in permeabilized cells are much lower.
[b] A cryptic, or masked, 3′ exonuclease activity is associated with polymerase α from *Drosophila*.



http://cmgm.stanford.edu/biochem201



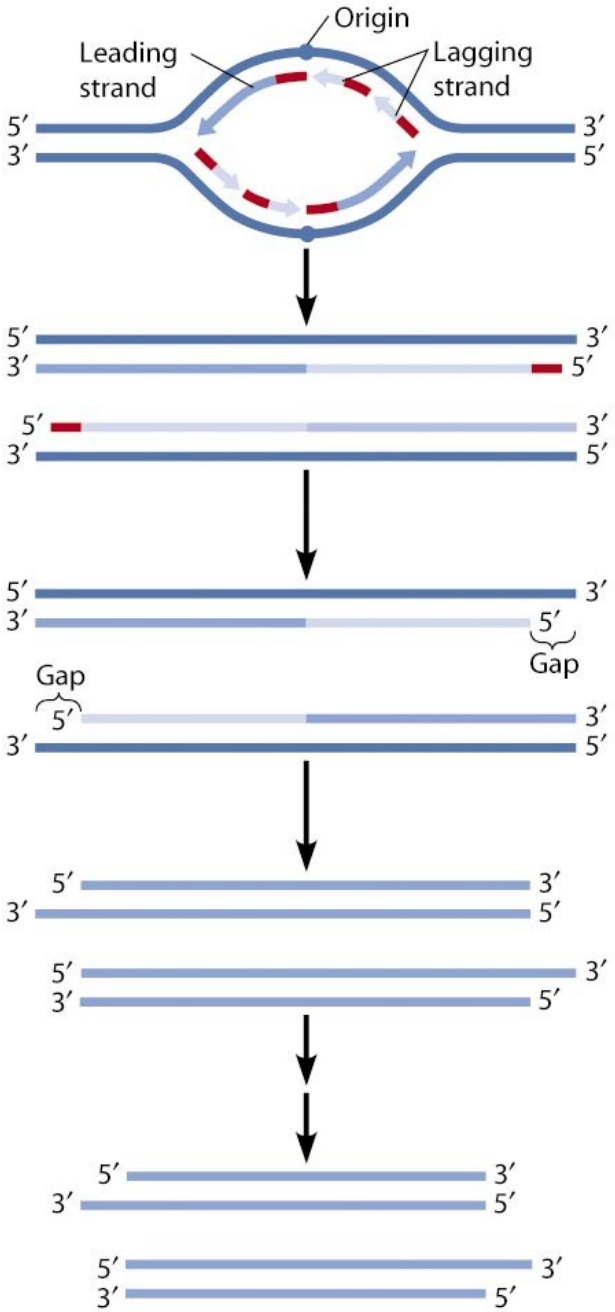
http://ftp.uke.uni-hamburg.de/kliniken/medizinische-klinik-1/index_43074.php

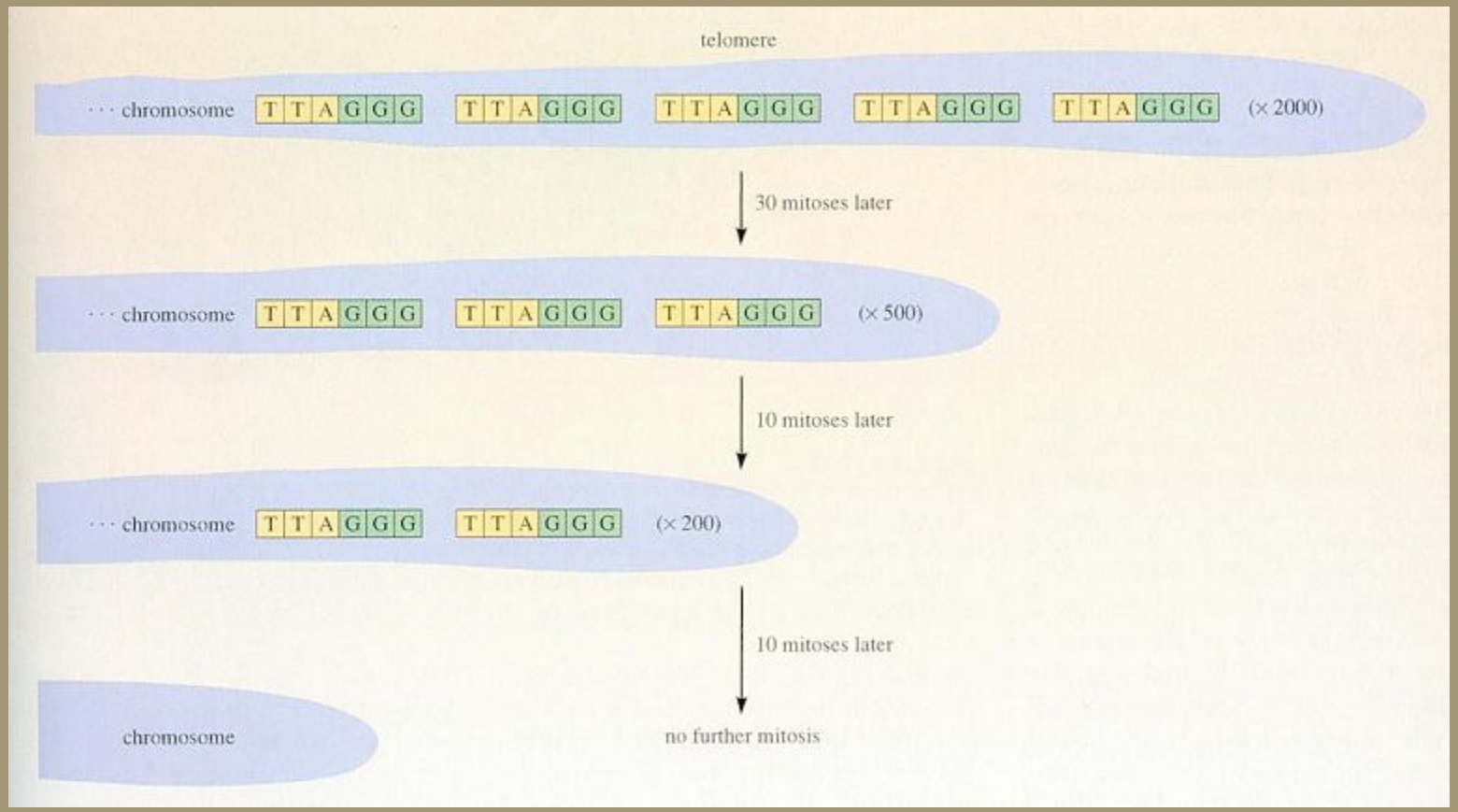
1. DNA replication is initiated at the origin; the replication bubble grows as the two replication forks move in opposite directions.

2. Finally only one primer (red) remains on each daughter DNA molecule.

3. The last primers are removed by a 5′→3′ exonuclease, but no DNA polymerase can fill the resulting gaps because there is no 3′ OH available to which a nucleotide can be added.

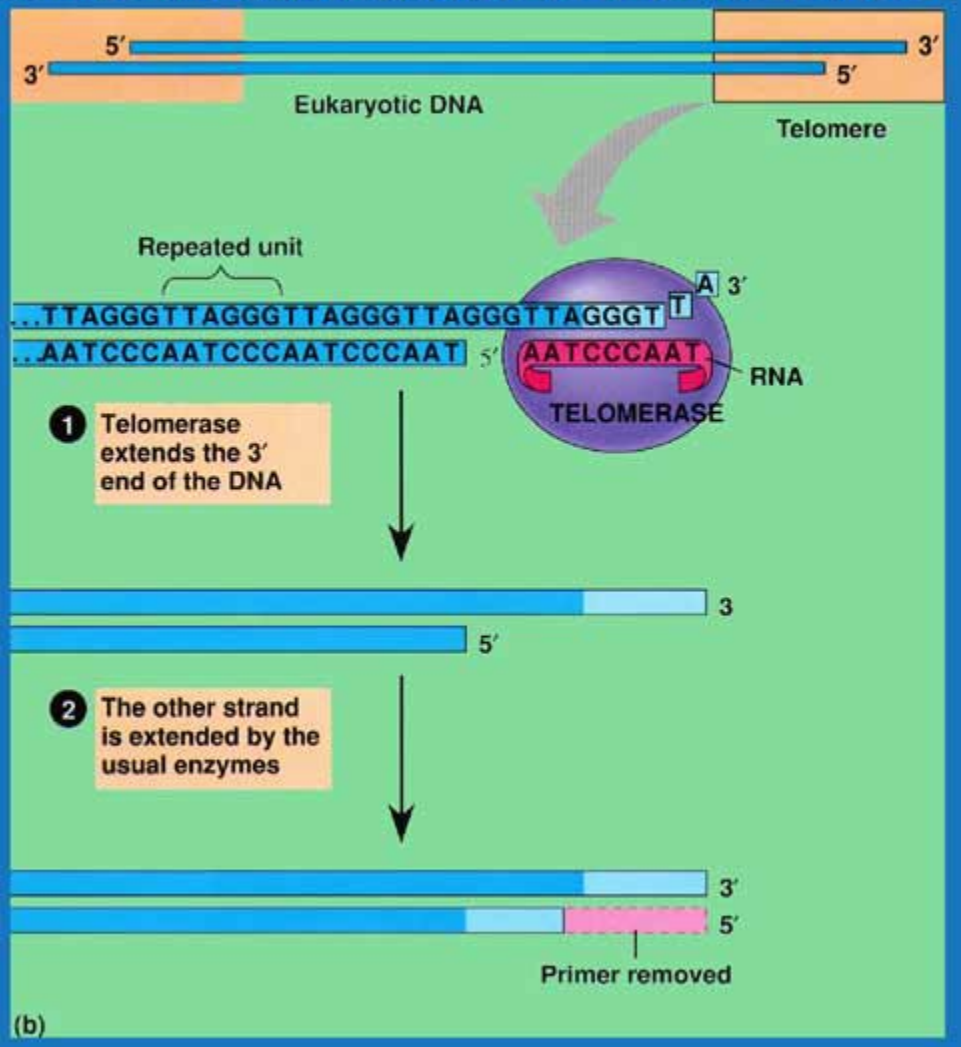
4. Each round of replication generates shorter and shorter DNA molecules.

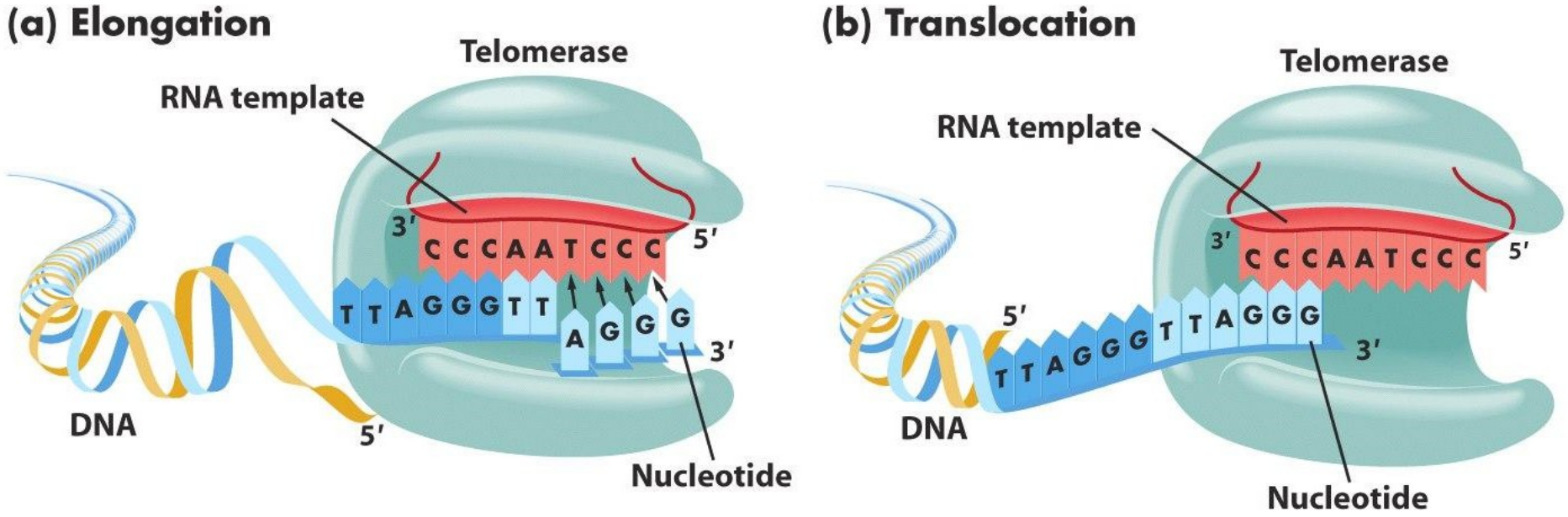




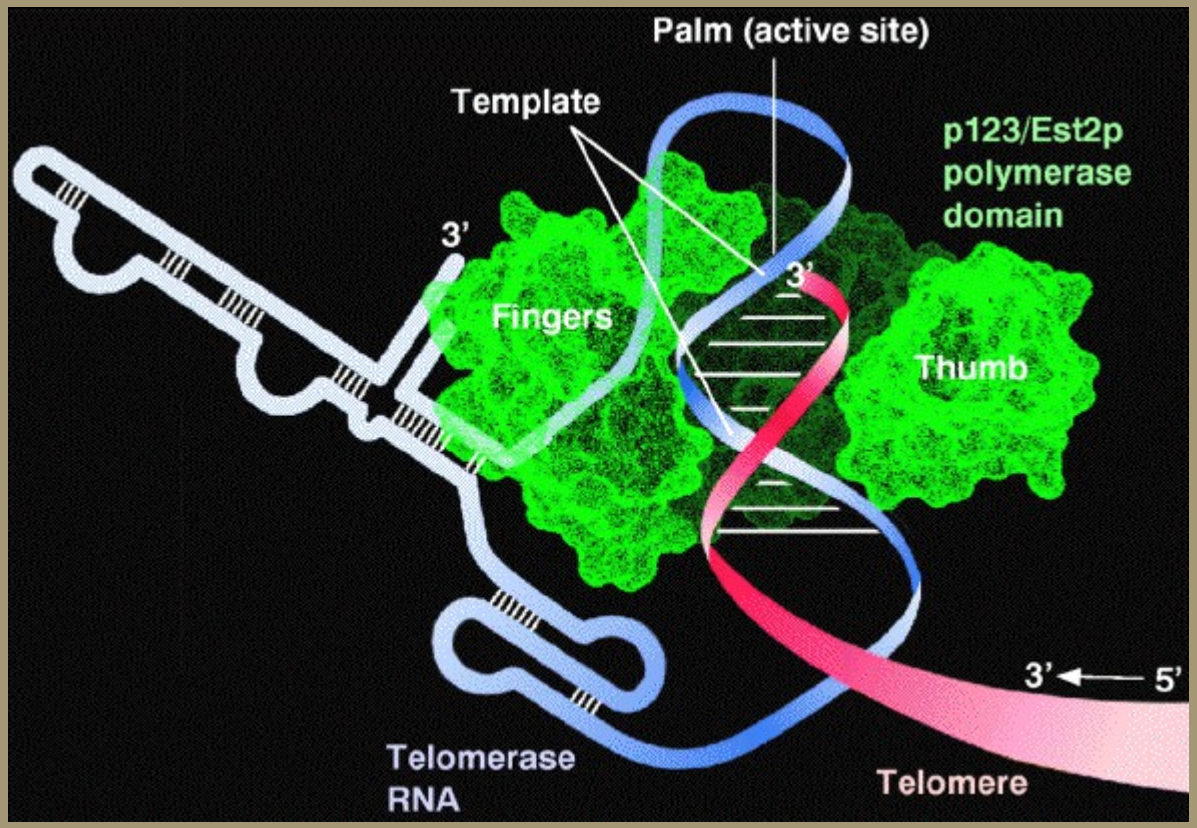
Hayflickov fenomen

TELOMERES AND TELOMERASE





http://barleyworld.org/css430_09/lecture%207-09/figure-07-25.JPG



http://cechlab.colorado.edu/telomodel.html