# Objectives

- For each topic below – consider the evolutionary implications!
- Connect DNA replication to structure of genetic material
- Describe the role of individual proteins in DNA replication
- Know what the telomere is and why its special
- Describe the role of telomerase in cellular immortality
- Articulate ways that telomere biology can be used for diagnostics, therapies, and biotechnology
- Propose evolutionary rationales for biologically important molecular phenomena

# Importance of DNA Replication

• DNA must be replicated every time a cell divides

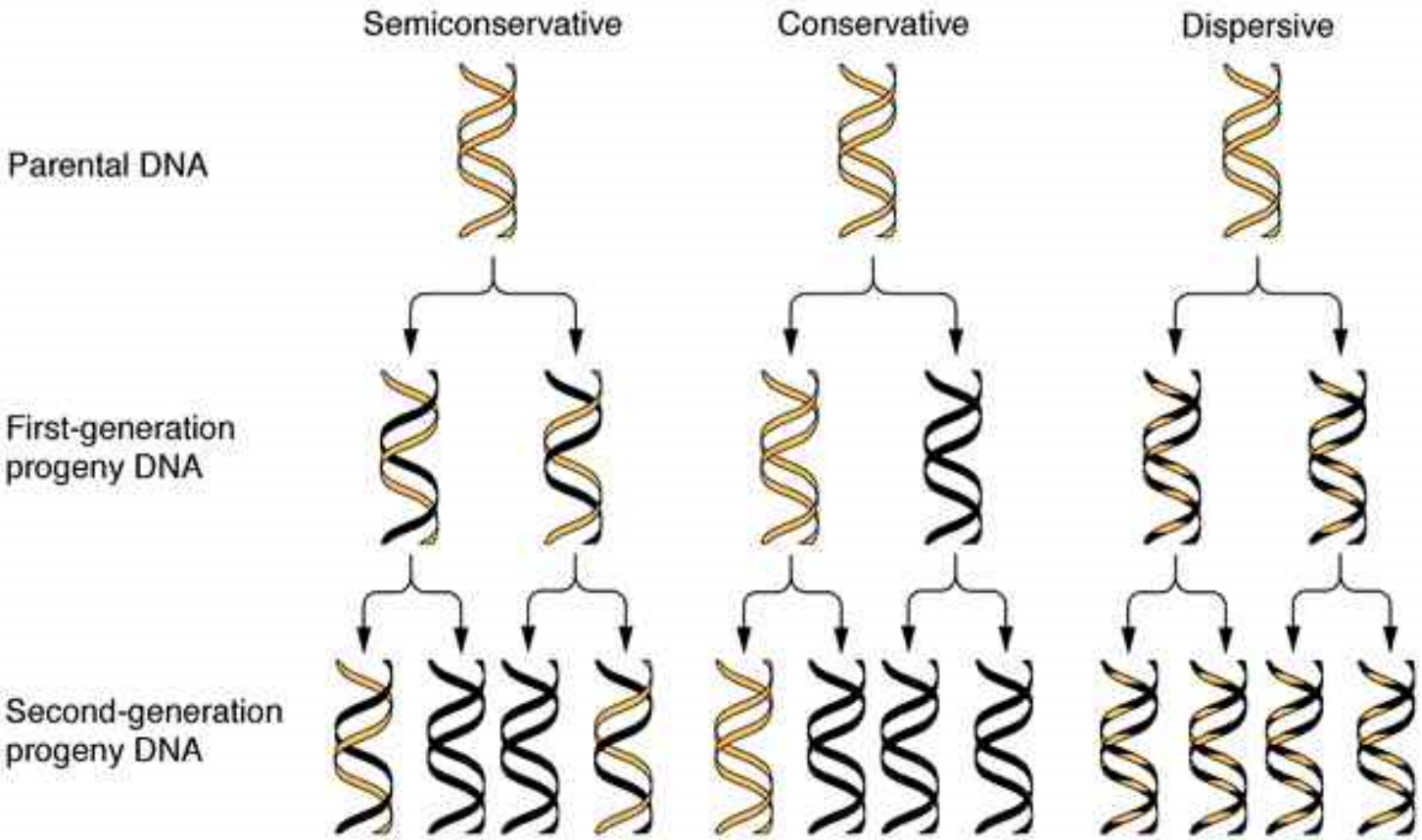
– Errors in replication can cause mutation, thus replication must be accurate; fidelity

– Eukaroytic genomes are large, therefore replication must be fast and efficient

• Replication takes advantage of the “instructive” aspect of base-pairing in DNA synthesis

– incorporation of nucleotide only occurs if proper Watson-Crick base pairs can be made

# Replication is Semi-Conservative

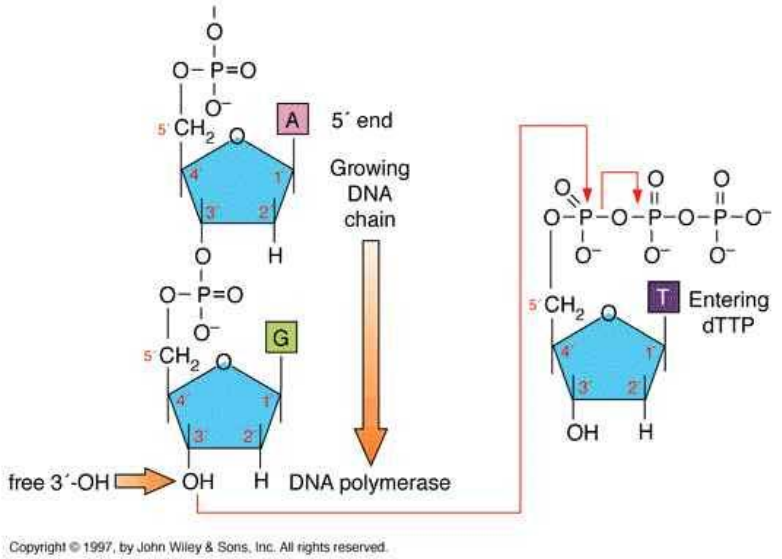


# Much is Conserved in DNA Replication

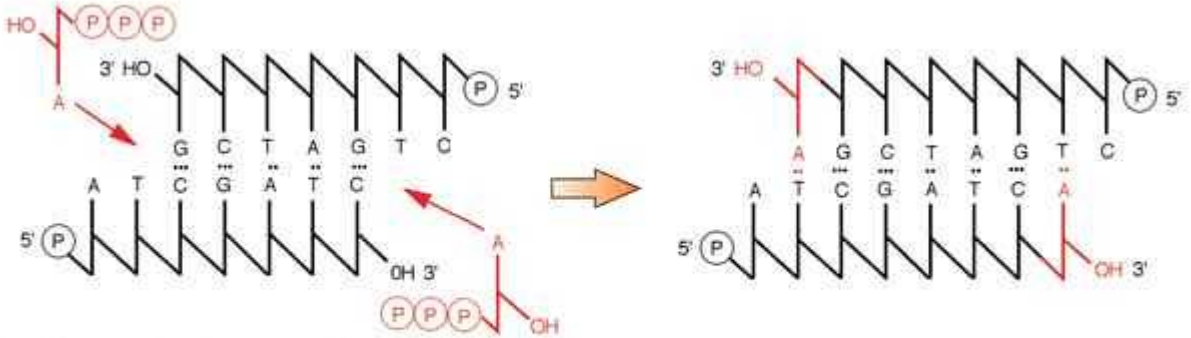
Direction

Template

Primer





# DNA Replication Steps

**Initiation**:

Assembly of a replication fork (bubble)
Fork is generated by primosome

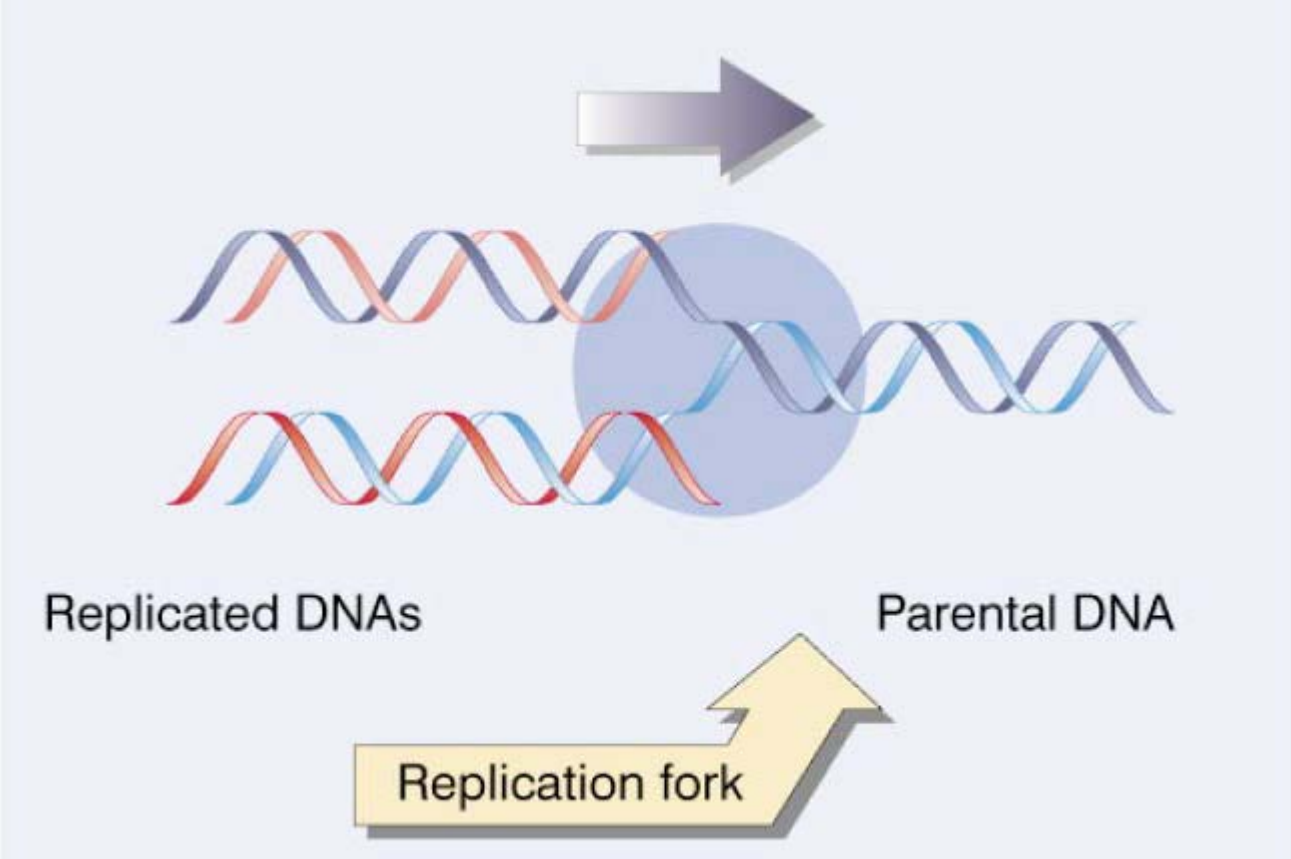
**Elongation**:

Addition of bases
Catalyzed by the replisome.
Parental strands unwind and daughter strands are synthesized

**Termination**:

Duplicated chromosomes are separated

# The Replication Fork



# Initiation: Work of the Primosome

At origins of replication the primosome goes to work

**Helicase**

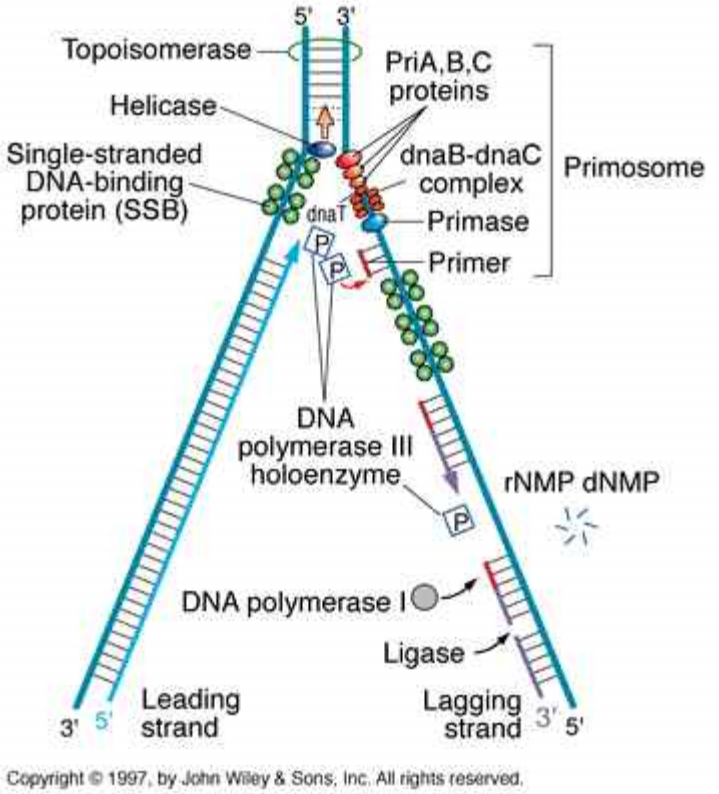
Separates the DNA Strands

**Single-strand DNA binding proteins**

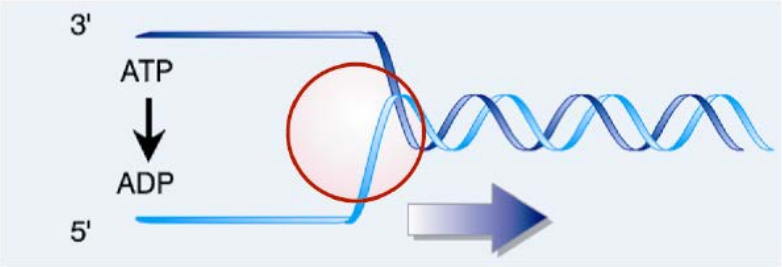
Bind strands in this region as single-stranded

**Primase**

Makes the primers

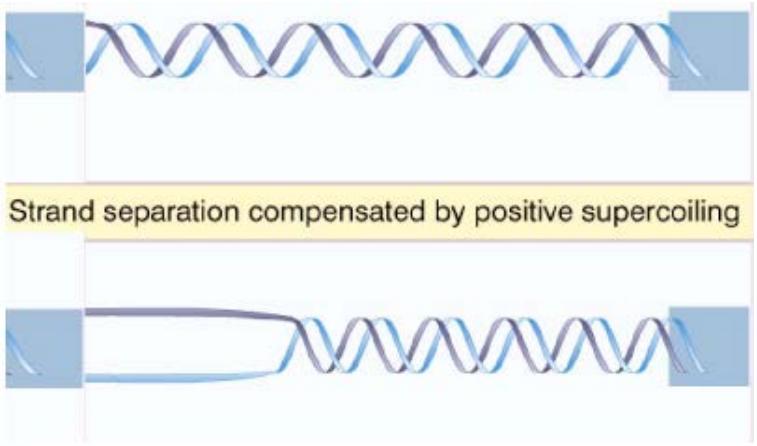


# Helicase



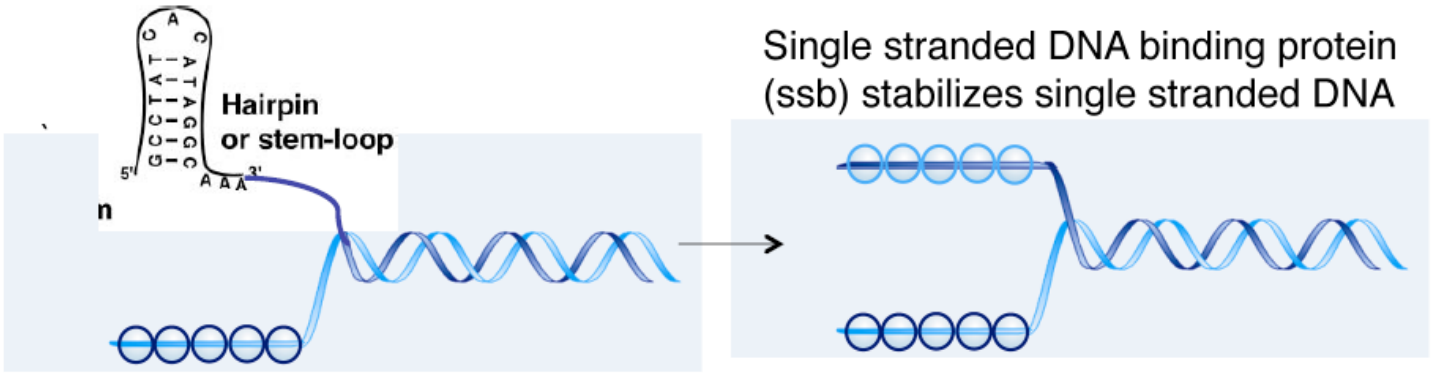

Unwinds DNA to present template

Unwinding at the fork supercoils DNA in advance of the fort

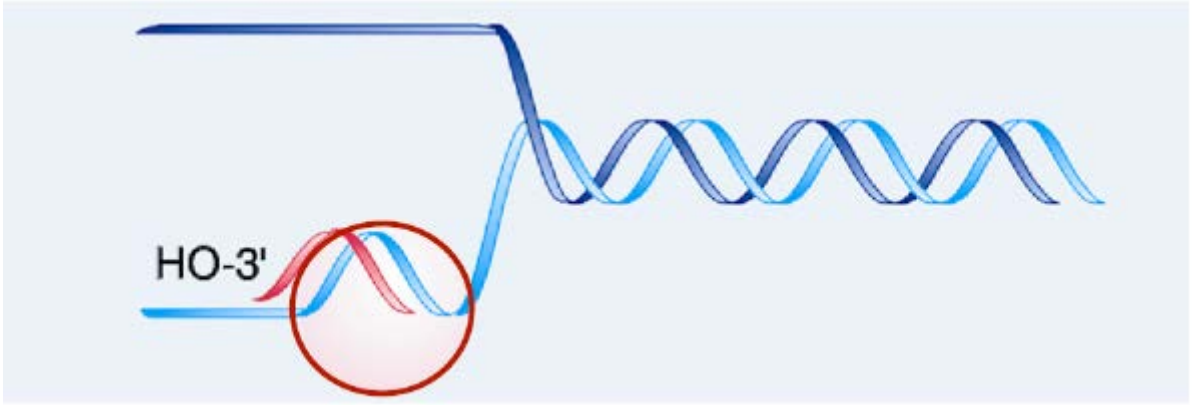



Topoisomerase relaxes supercoil (ATP dependent)

# Initiation: Isolate Template; Make Primer

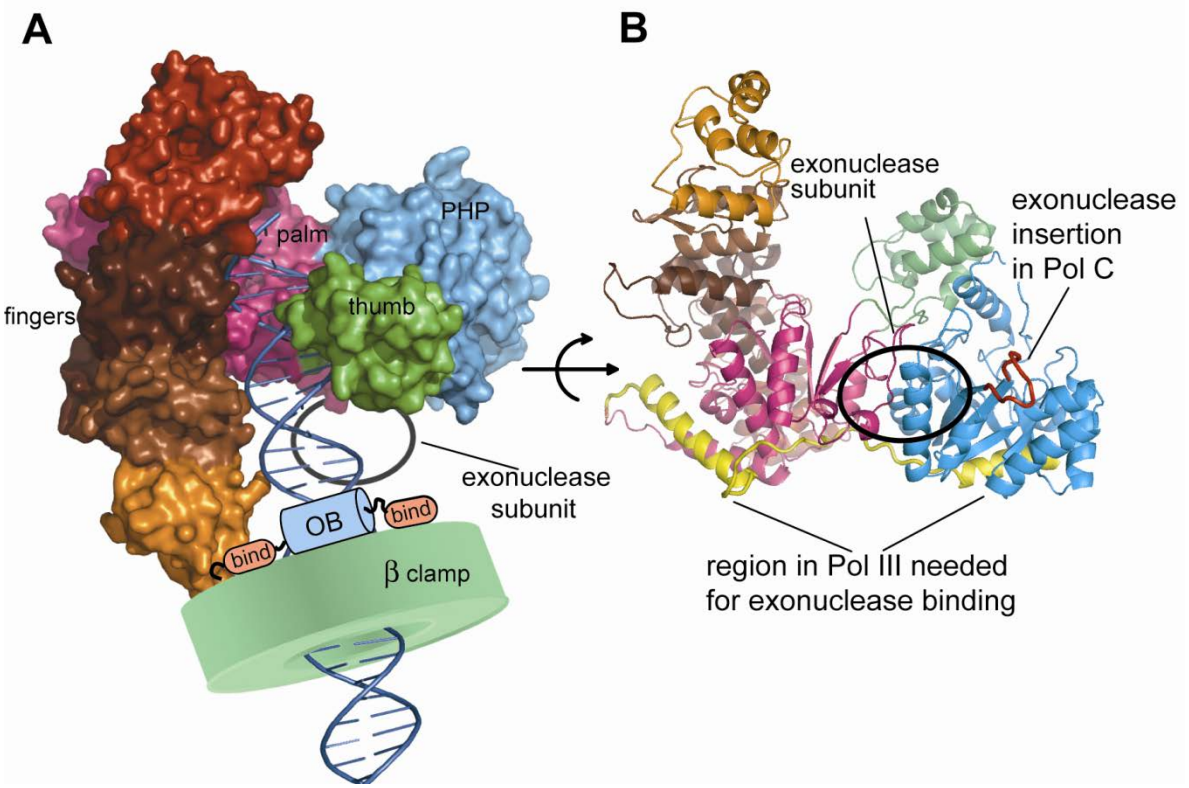



ssDNA-Binding proteins keep template single stranded

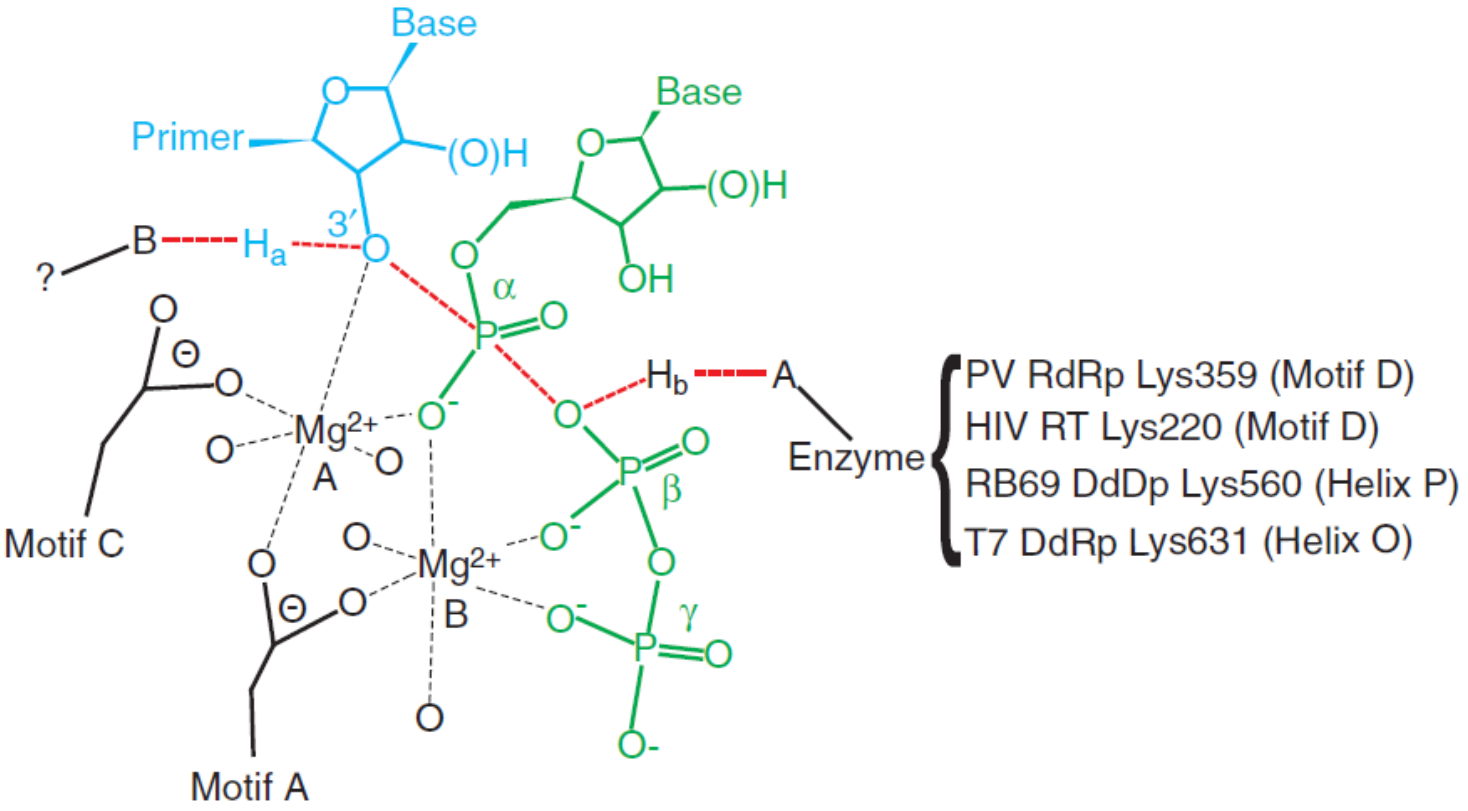



Primase makes an RNA primer

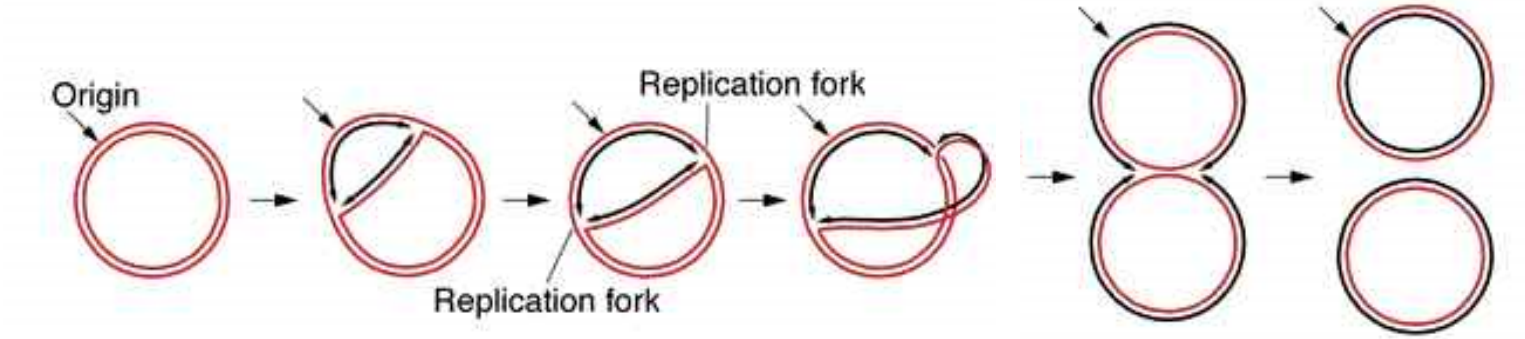
# Elongation: The Work of DNA Polymerase



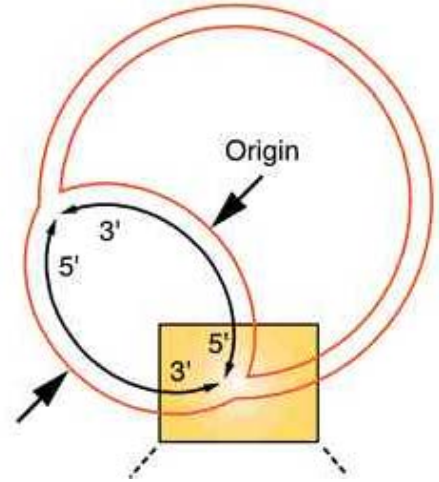
# Chemical Mechanism of Nucleotide Addition

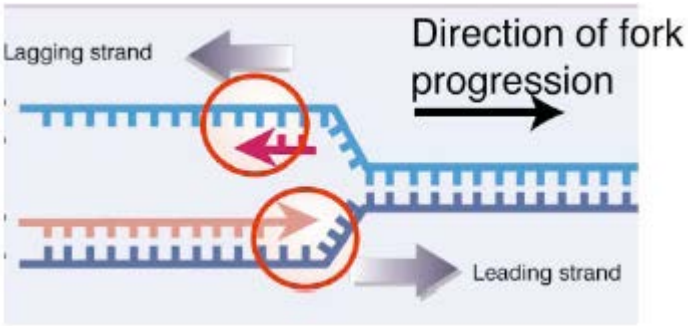


# Elongation: Rolling Circle

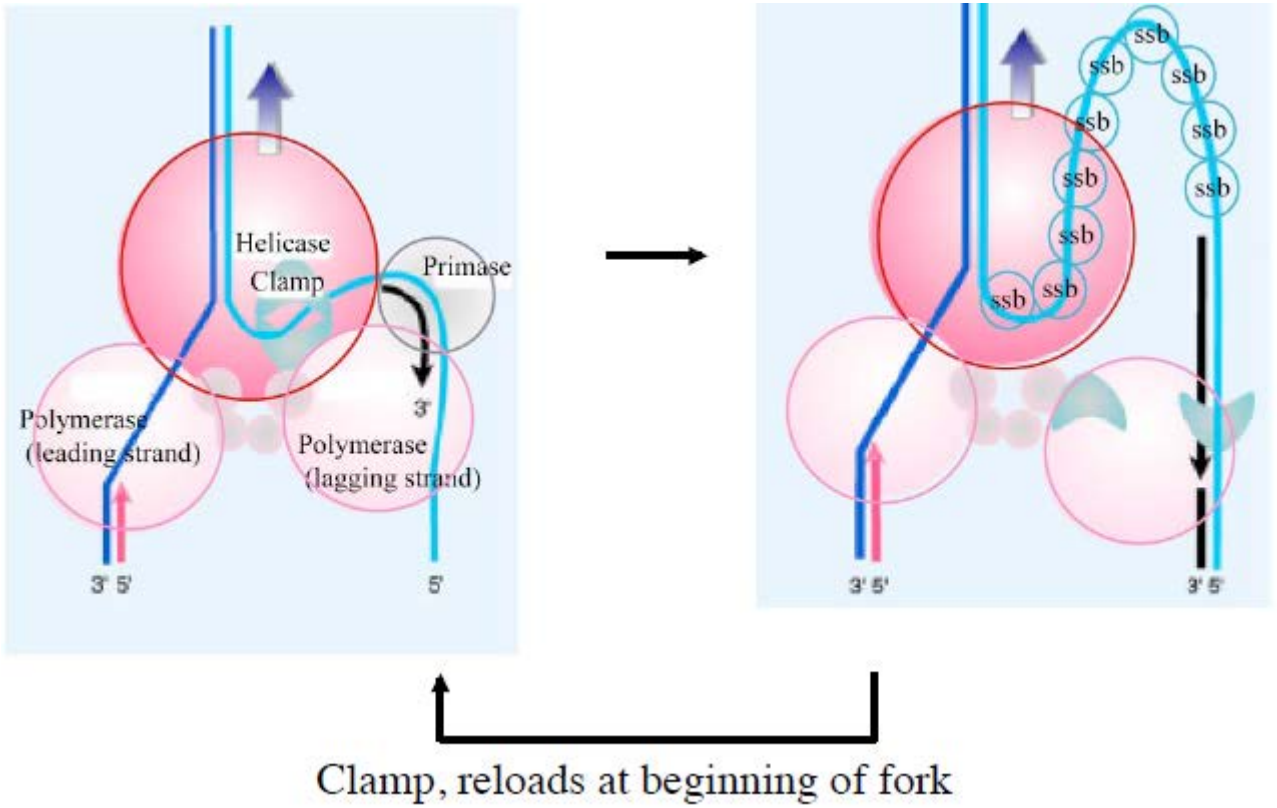



Two polymerase moving in opposite directions



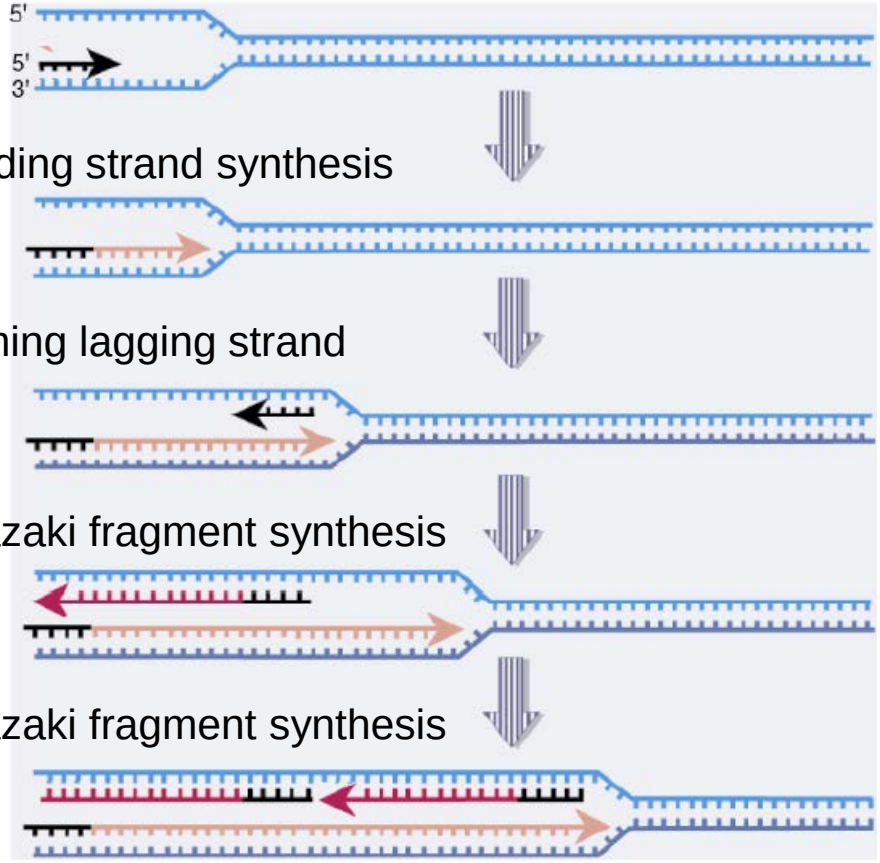
# Leading and Lagging Synthesis are Coupled




http://www.youtube.com/watch?v=5VefaI0LrgE&feature=related

# Semi-Discontinuous Synthesis and Primer Removal

Priming leading strand

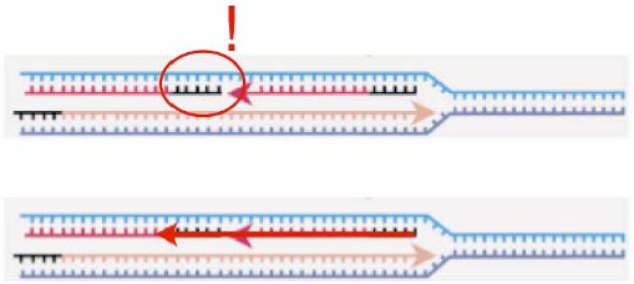



Leading strand synthesis

Priming lagging strand

Okazaki fragment synthesis

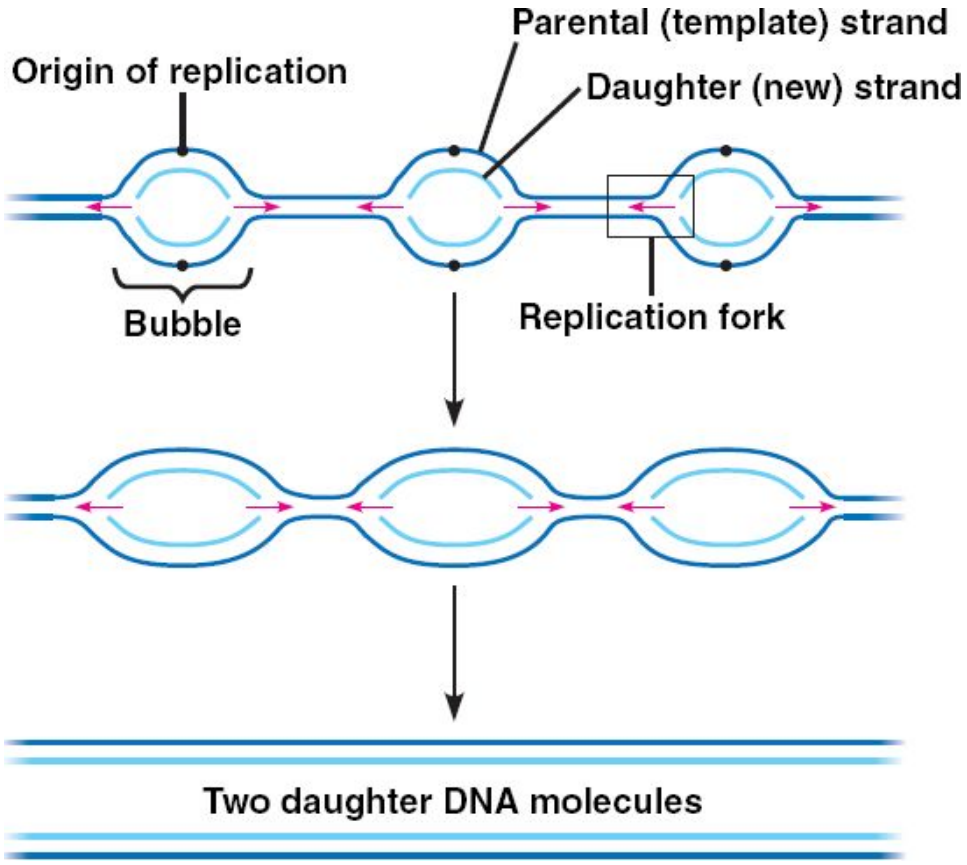
Okazaki fragment synthesis

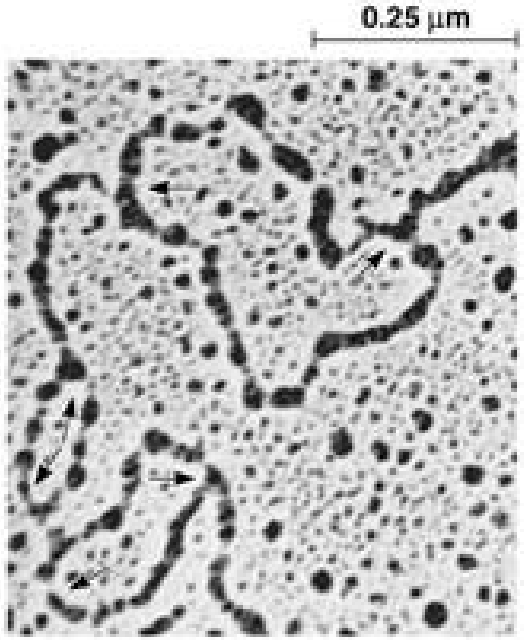



Primers removed

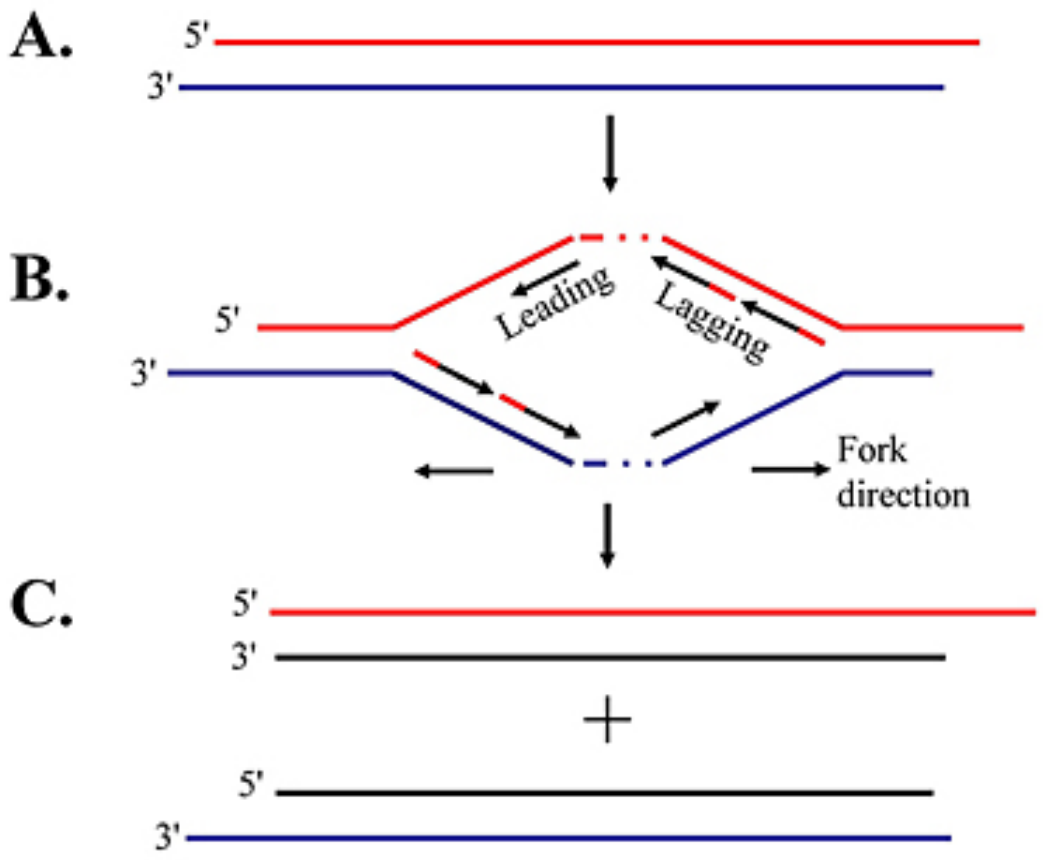
Resulting DNA fragments joined

# How Can Polymerase Copy The Whole Chromosome in a Few Hours?

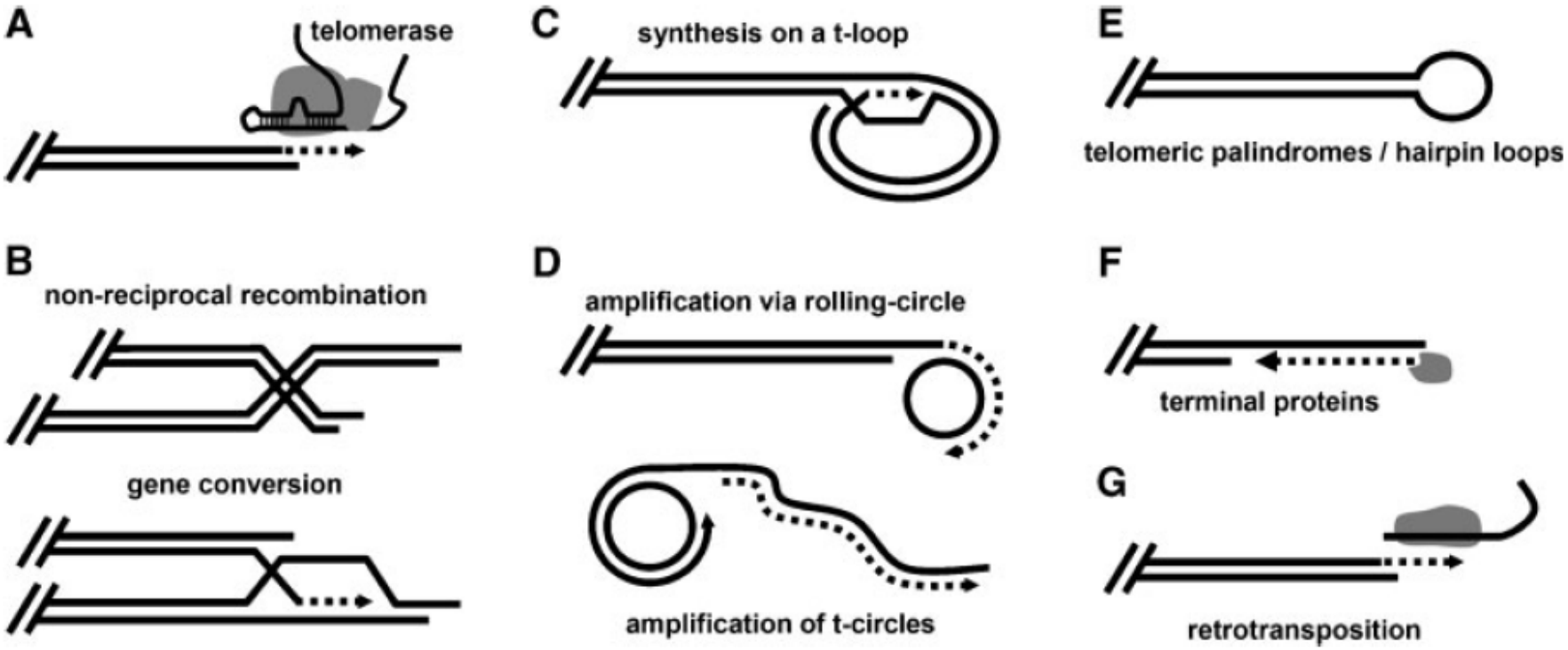


# Normal DNA Replication Fails to Replicate the Telomere



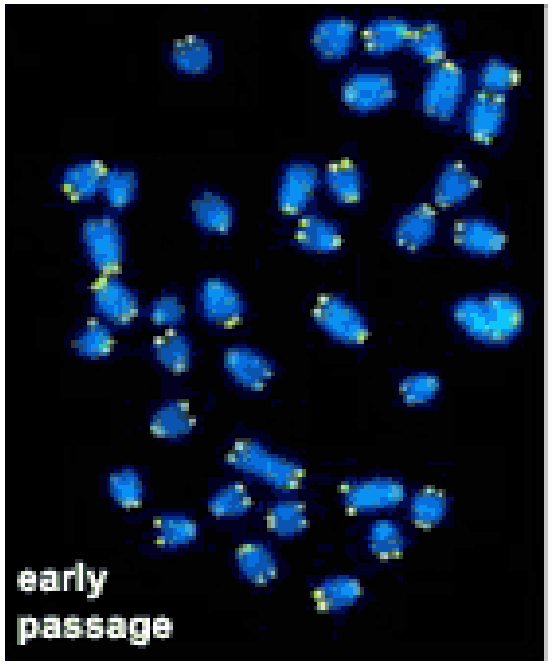
# How to Overcome the End-Replication Problem




Nosek, J.; Kosa, P.; Tomaska, L.BioEssays (2006) 28:182–190

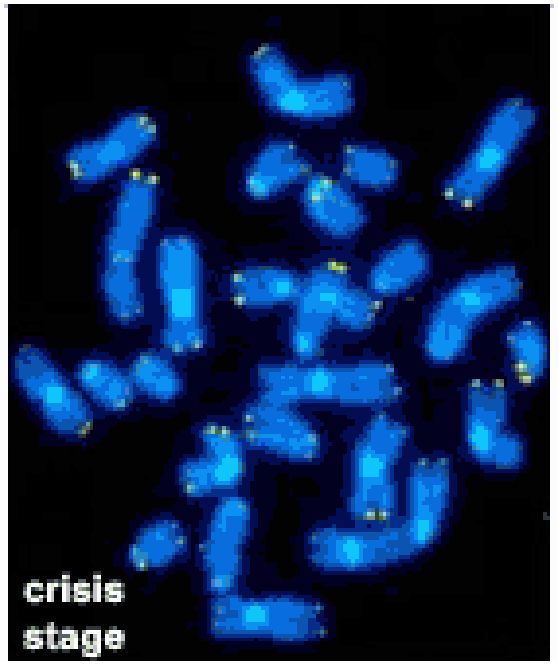
# The Telomere is the Chromosome Aglet

FISH analysis of healthy chromosomes



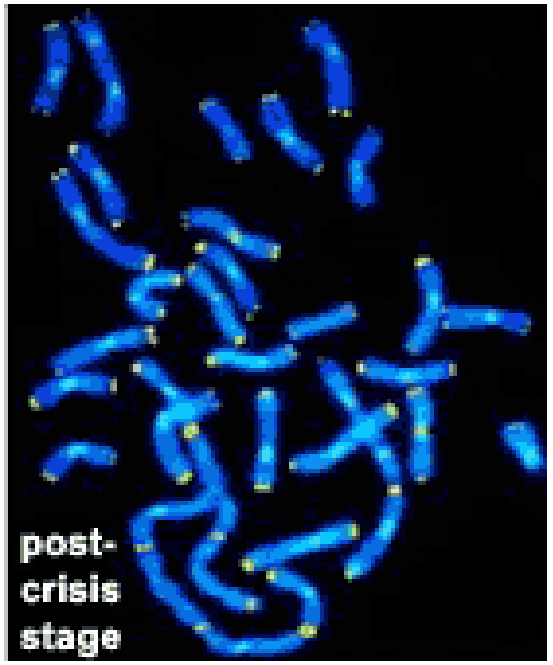

Telomeres can be visualized

FISH analysis of chromosomes with short telomeres



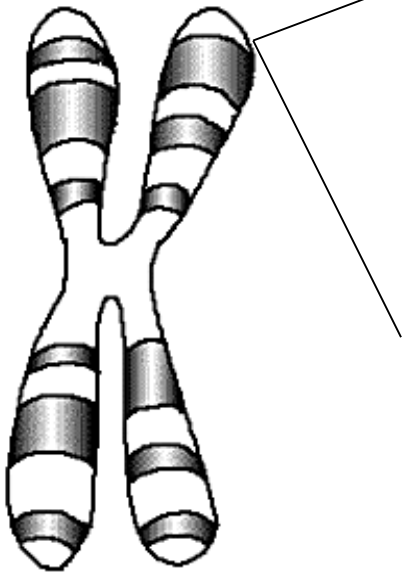

Telomeres signal is decreased

FISH analysis of damaged chromosomes




Chromosomes are fused and damaged

# The Chromosome End



3’ Overhang

...TTAGGGTTAGGGTTA**GGGTTAGGGTTAGGGTTAGGG**-3’

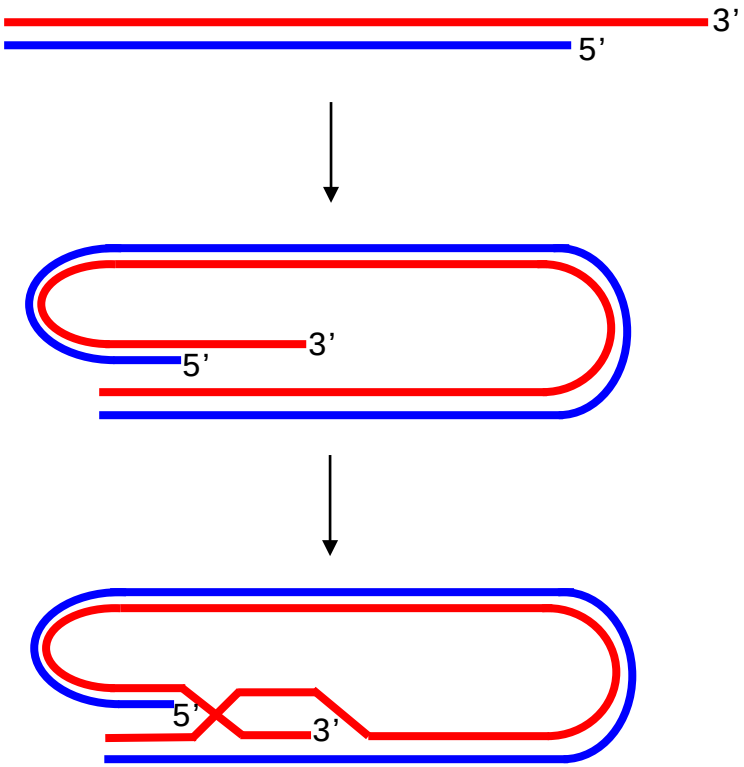
...AATCCCAATCCCAATC-5’

Telomeric DNA, TTAGGG in humans

Double stranded region is 5,000 to 20,000 bases
Single strand region is 150-300 bases

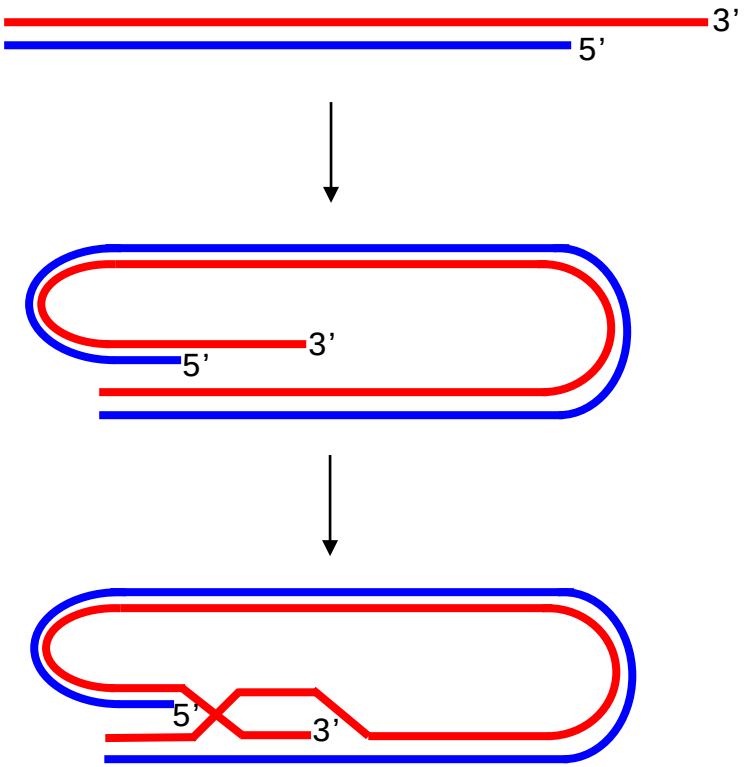
Human Chromosome

# The Chromosome Ends in a Loop

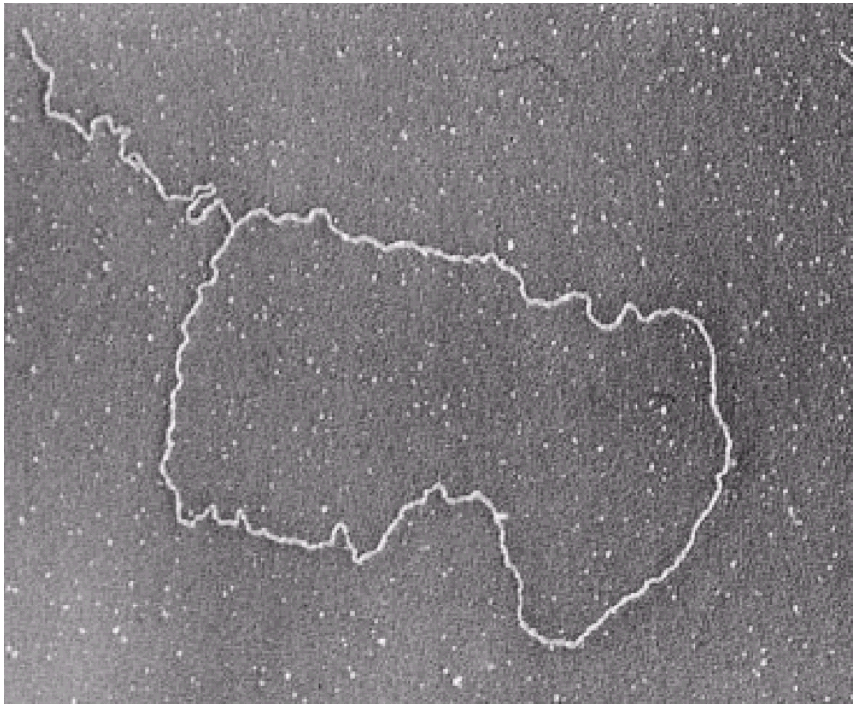



Griffith, J.D. et al. *Cell* **1999**, *97*, 503-14.

# The Chromosome Ends in a Loop

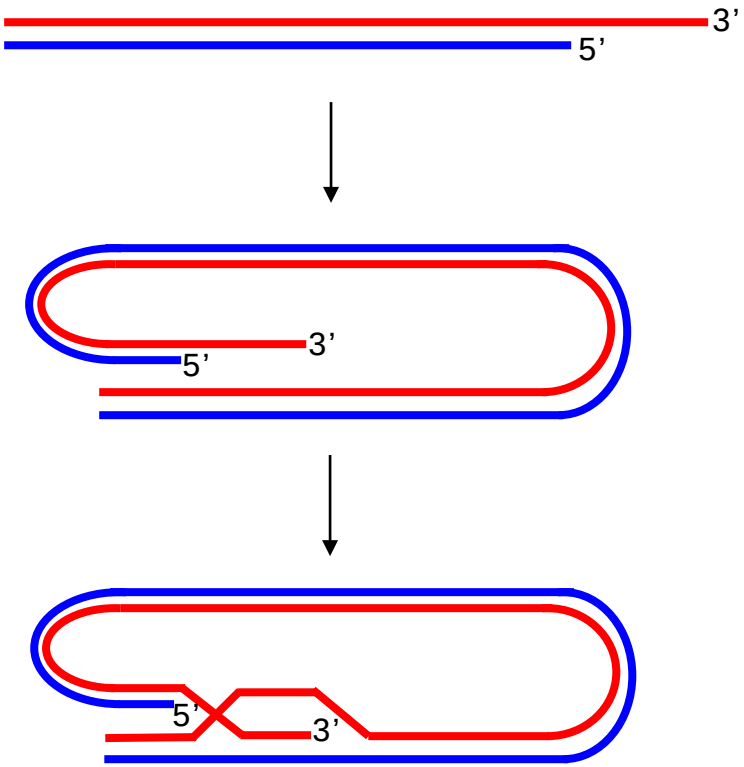


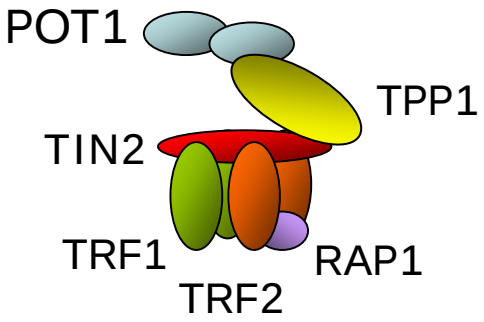

Electron micrograph of a telomere



Griffith, J.D. et al. Cell 1999

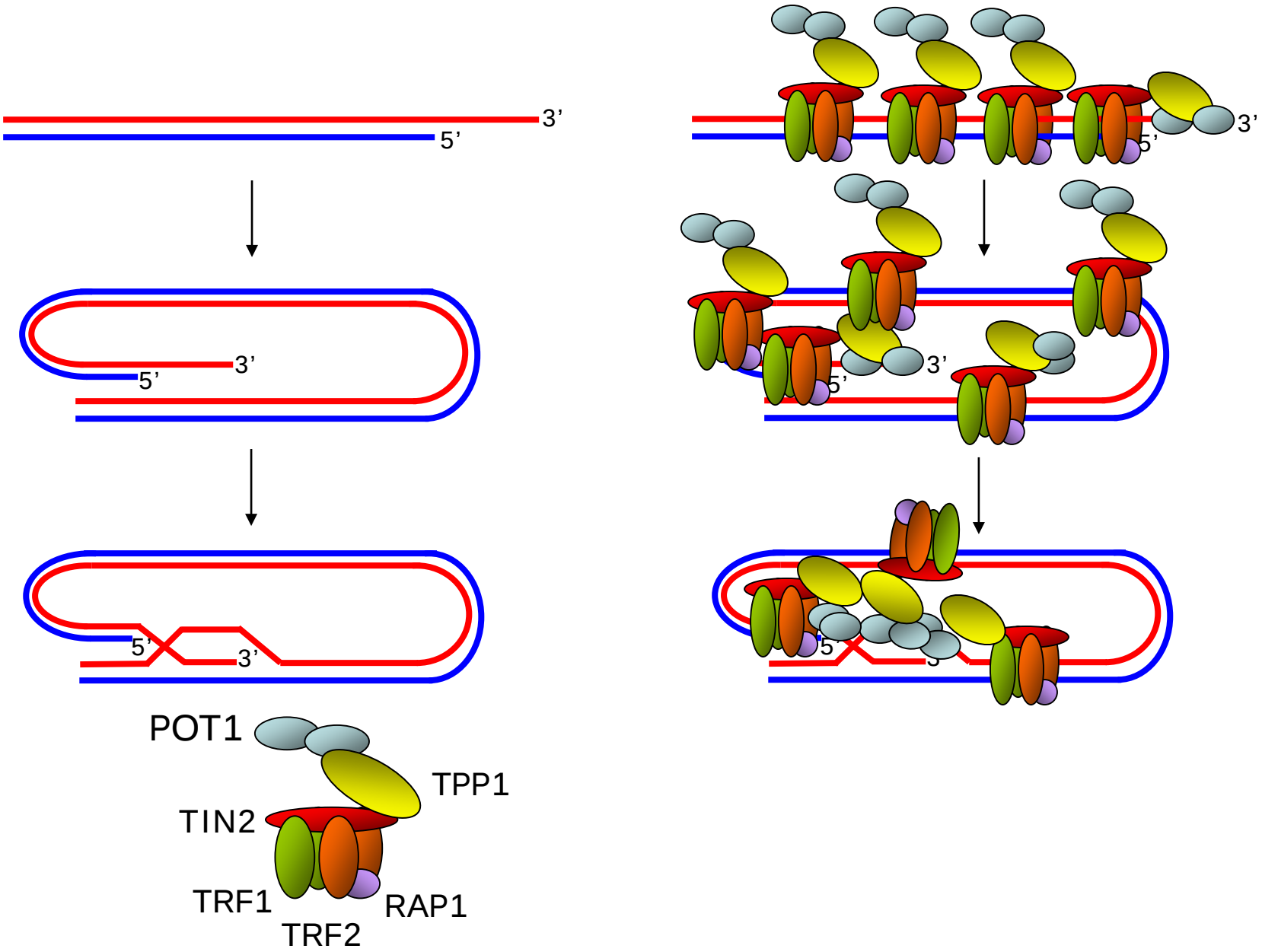
# The Telomere is a Protein-DNA Complex



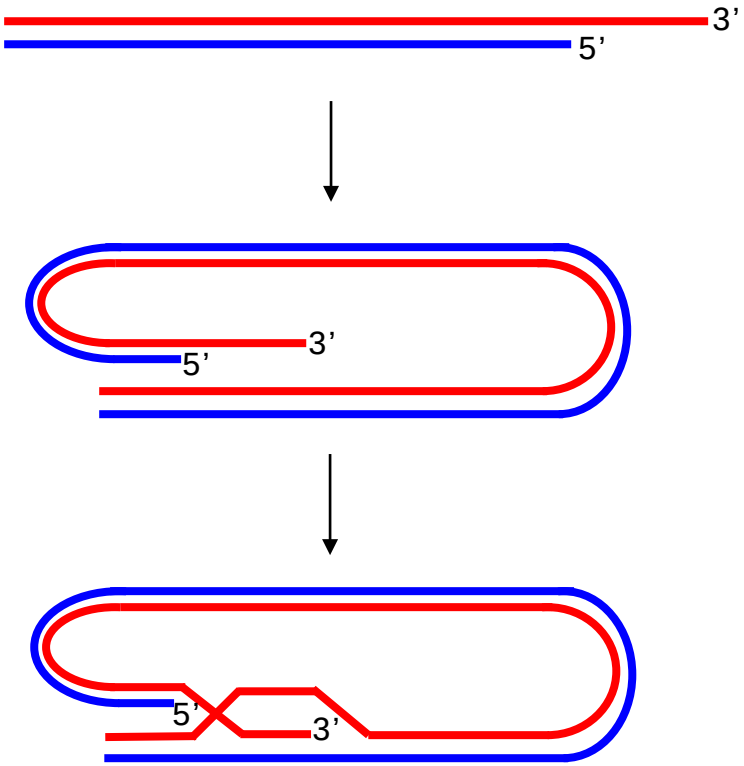



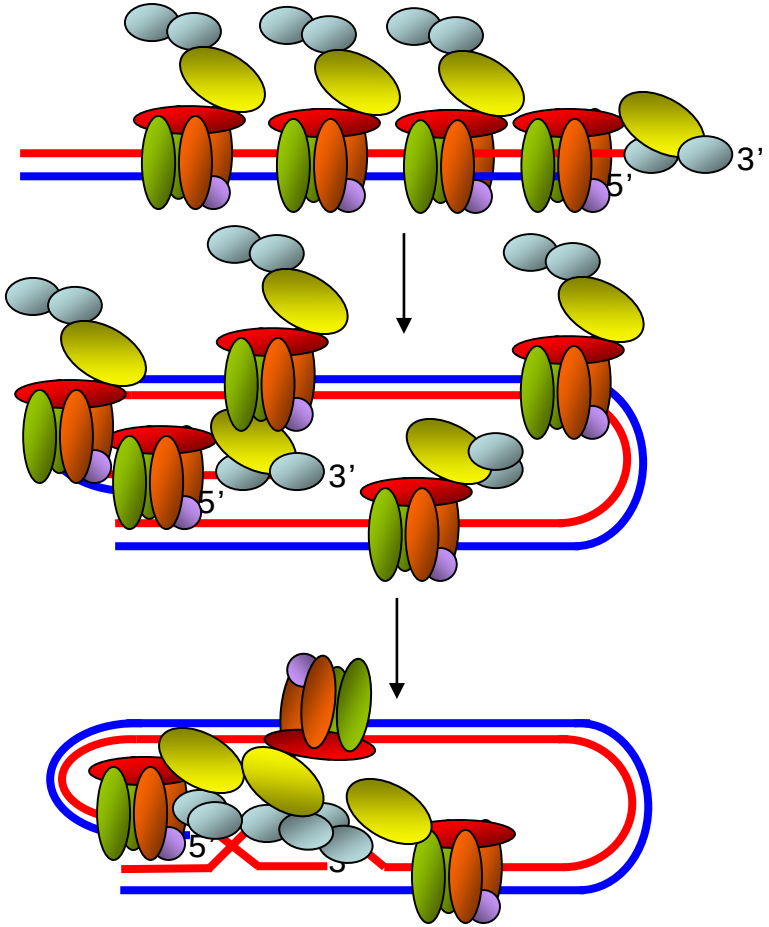

de Lange Genes Dev. 2005

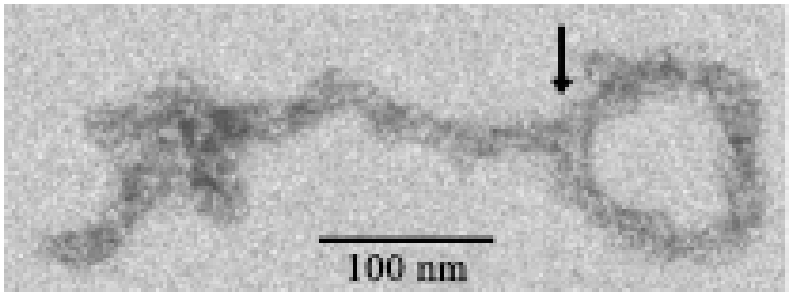
# The Telomere is a Protein-DNA Complex



# The Telomere is a Protein-DNA Complex

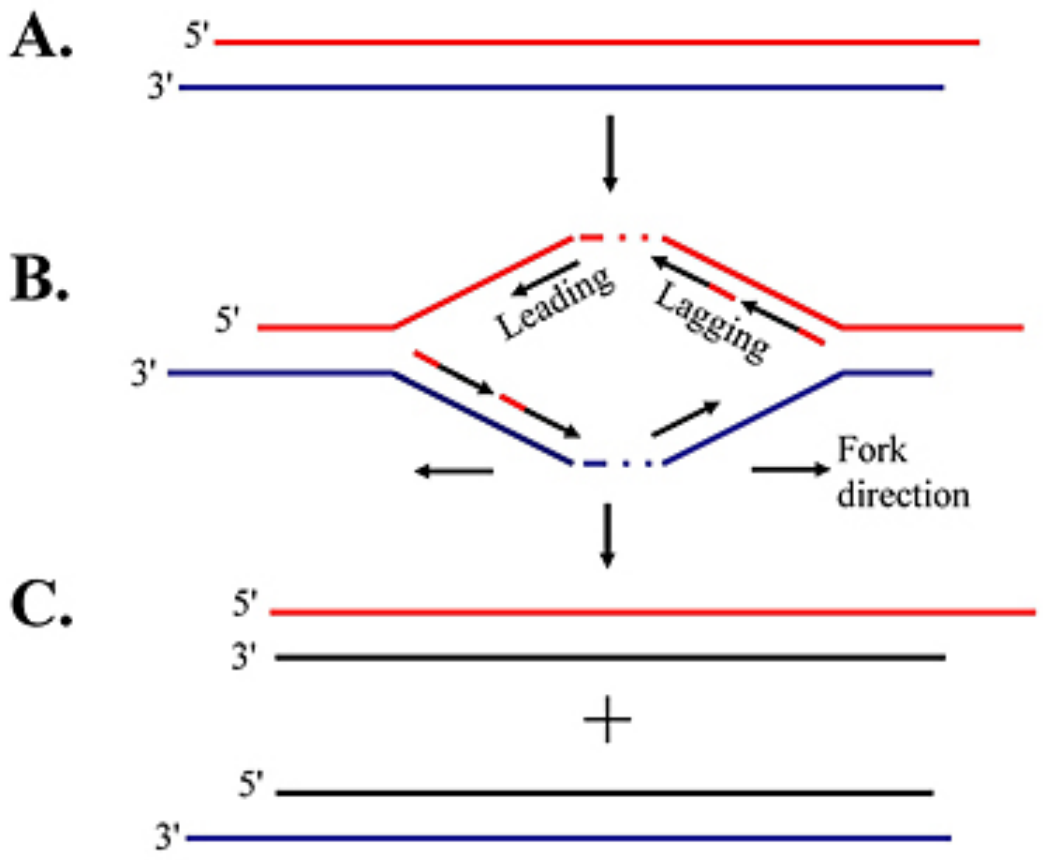


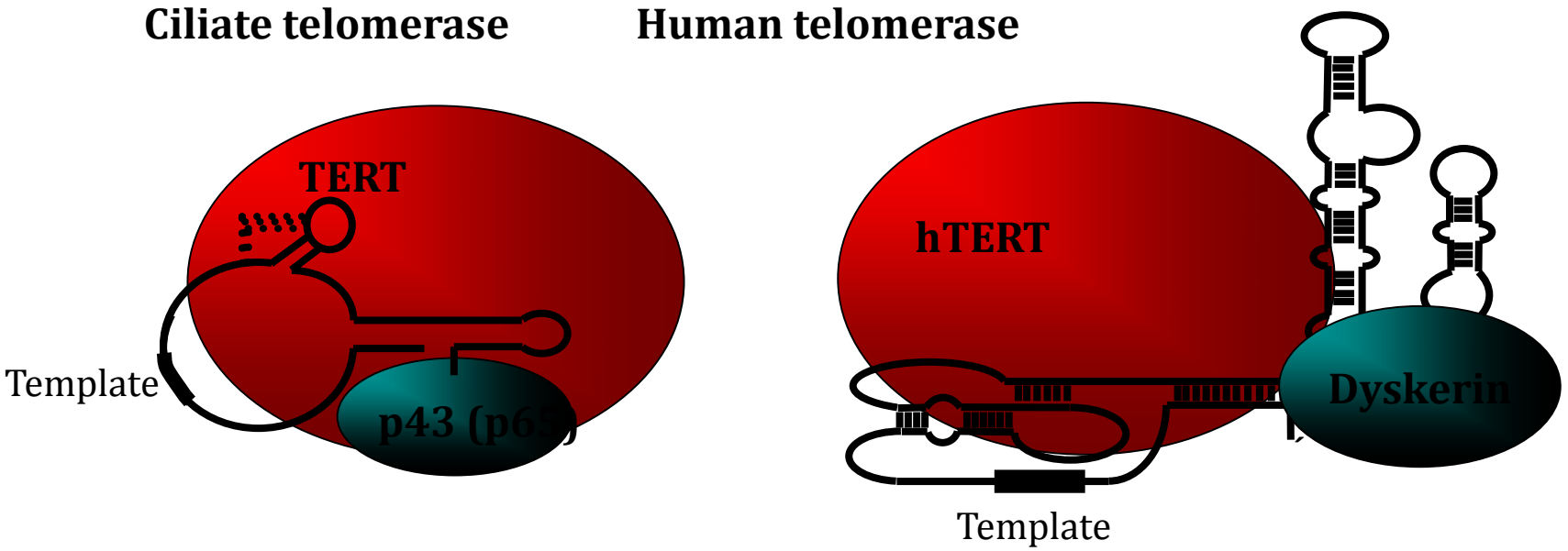




Nikitina and Woodcock J. Cell. Biol. 2004

# Normal DNA Replication Fails to Replicate the Telomere



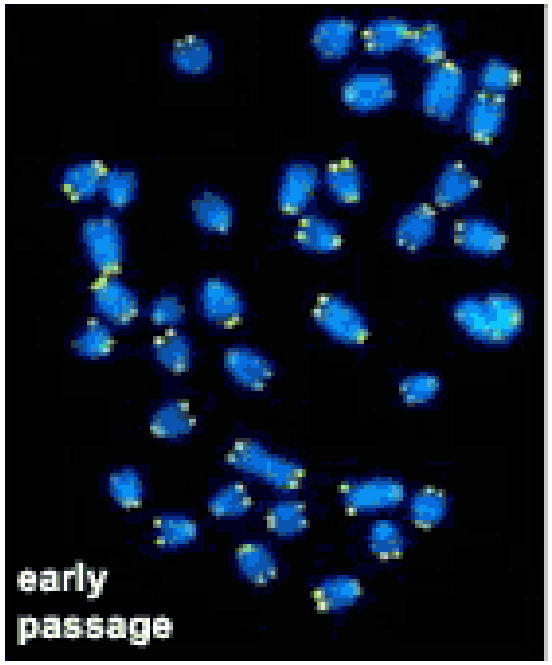
# Telomerase is a Ribonucleoprotein Complex




Common features of all telomerase complexes:
RNA supplies the template
TERT is the catalytic subunit
RNA-binding protein is present

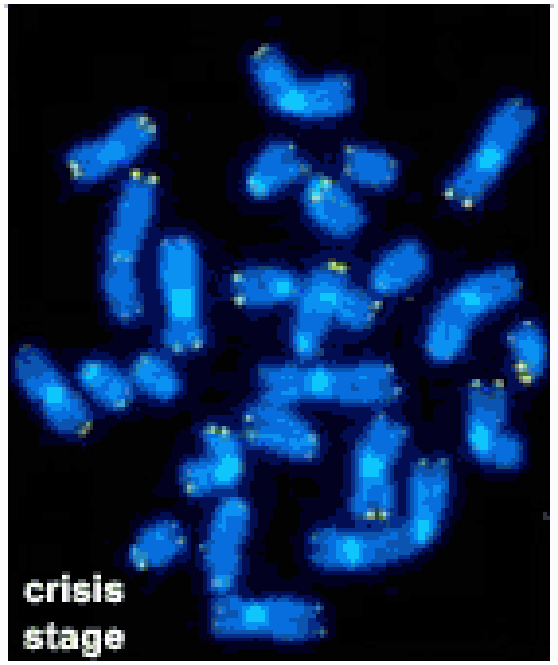
# The Telomere is the Chromosome Aglet

FISH analysis of healthy chromosomes



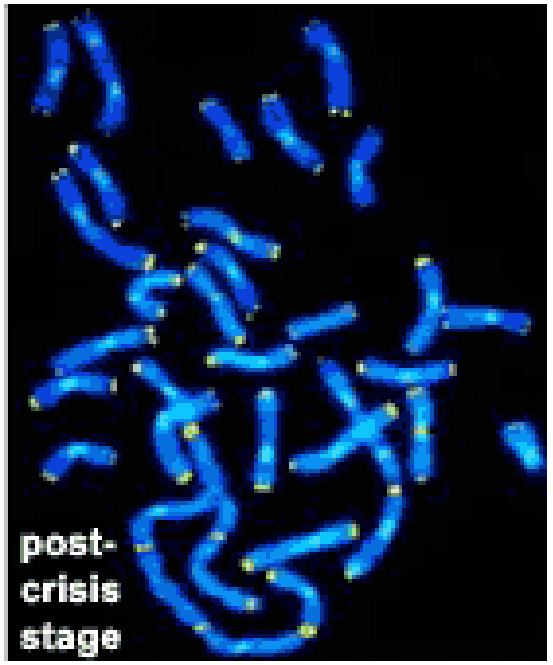

Telomeres can be visualized

FISH analysis of chromosomes with short telomeres



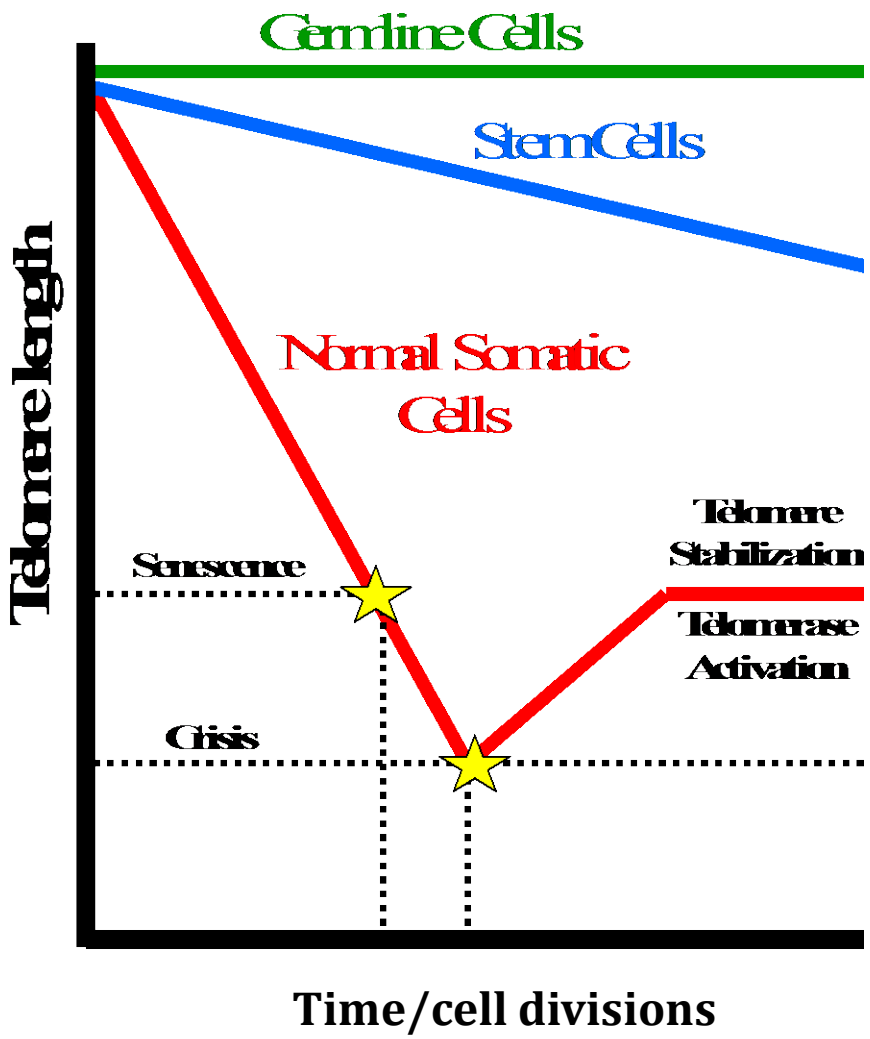

Telomeres signal is decreased

FISH analysis of damaged chromosomes

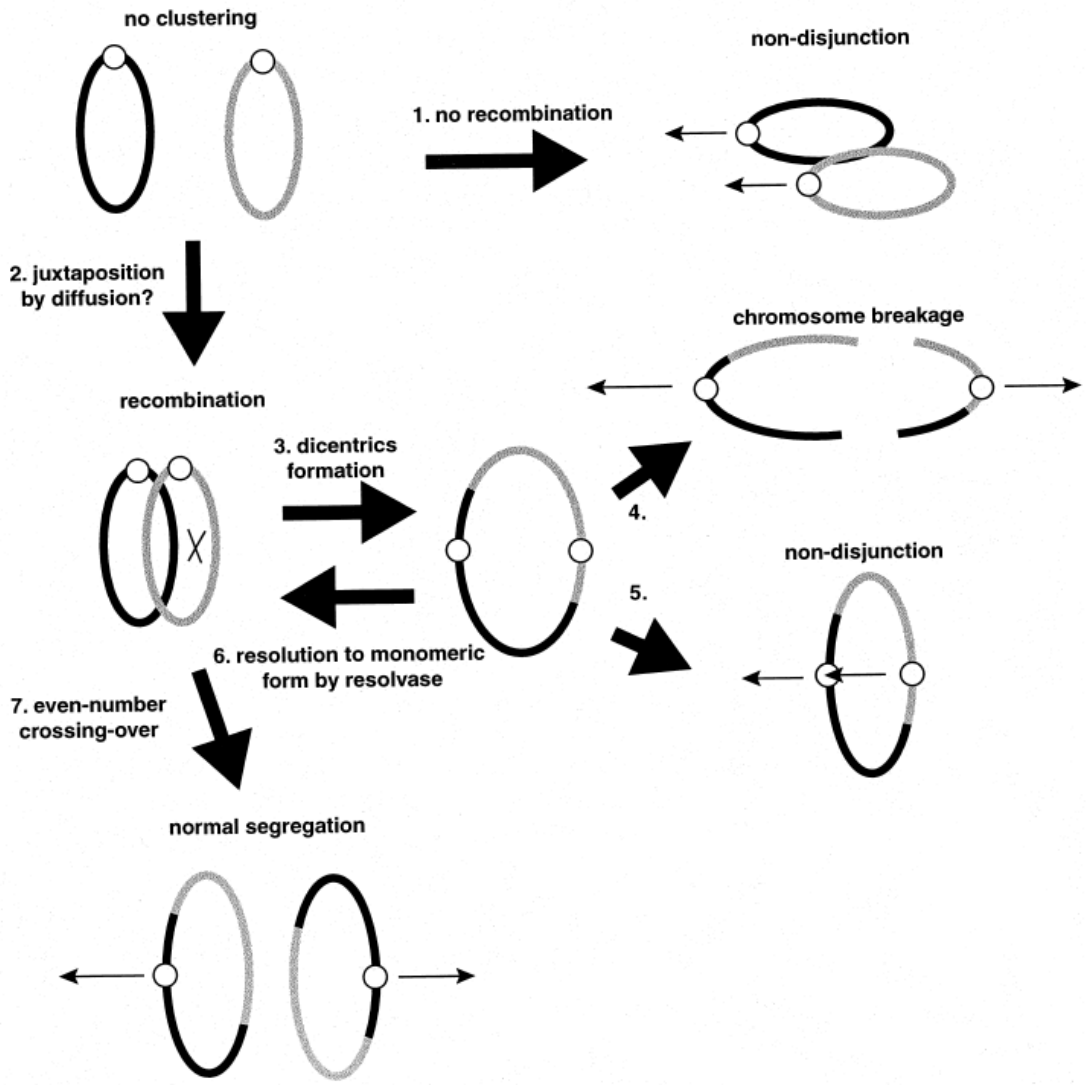



Chromosomes are fused and damaged

# Telomere Length Determines Cellular Longevity

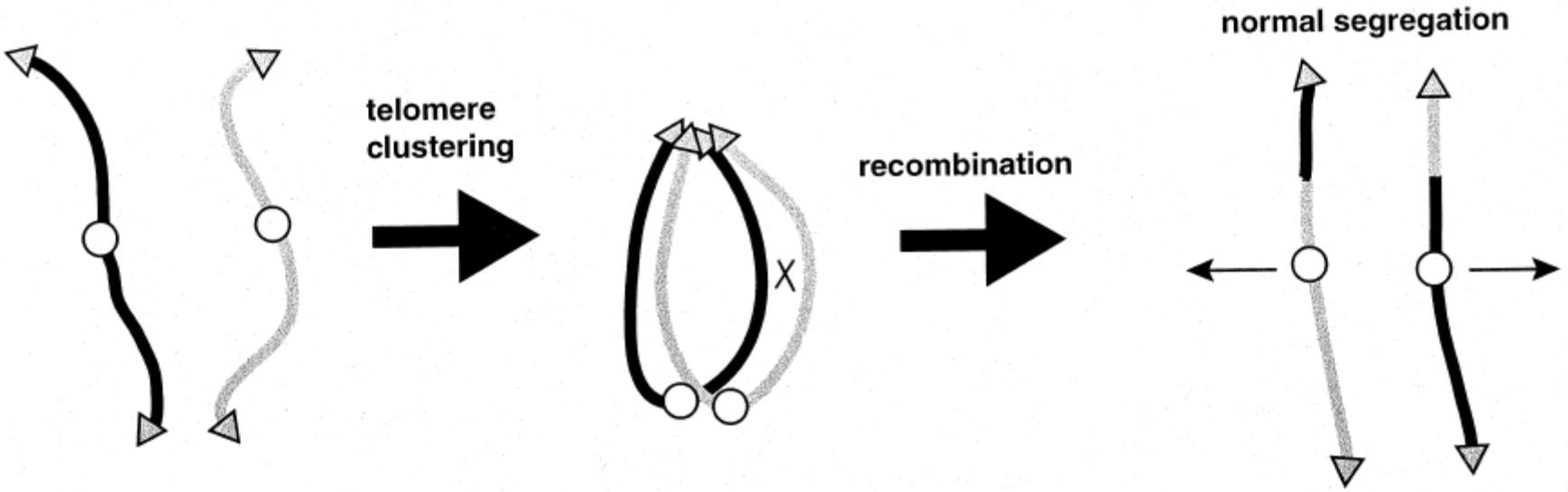


# Why Have Linear Chromosomes Anyway? Meioses with a Circular Chromosome.




Ishikawa, F; Naito T. Mutation Research 434 1999. 99–107

# Why Have Linear Chromosomes Anyway?



# Telomere Maintenance and Human Health

Normal aging, stress, and the environment can all reduce telomere length

- Stem cells senescence
- wound healing rate decreases
- tissue regeneration ability decreases

Aging disorders: deficiencies in telomere maintenance

- Mutations in hTR, hTERT and DKC1 gene
- Haplodefeciency in telomerase activity

# Telomere Maintenance and Human Health

Cancer and telomere maintenance

- Cancer cells require proper telomere maintenance
- Telomerase positive (85%), ALT (15%)
- Telomere length and telomerase correlations to prognosis

Stem cell technology

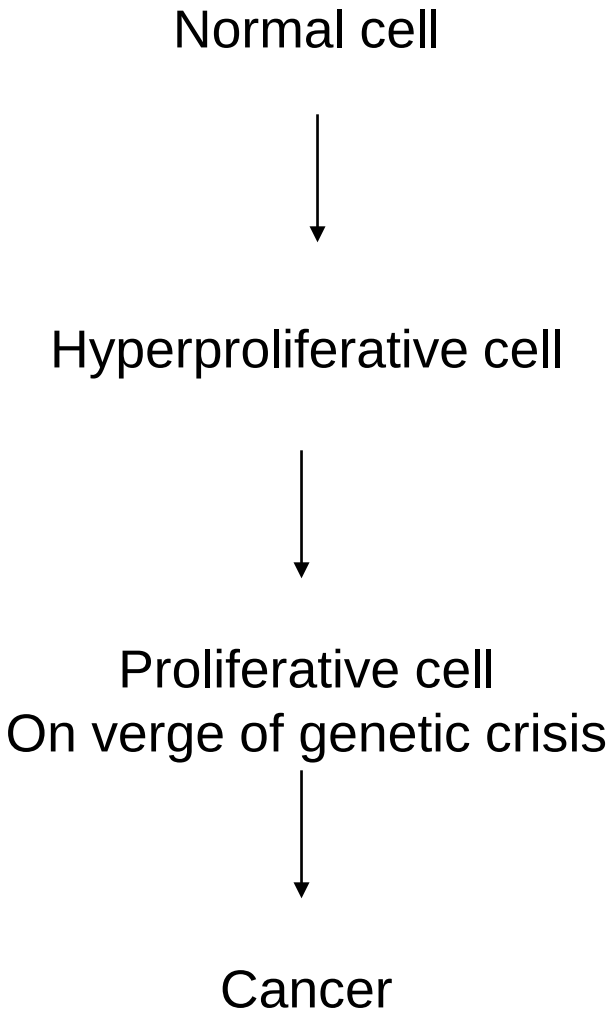
- Attempts to maintain cells in culture without senescence

# Cancer Requirements

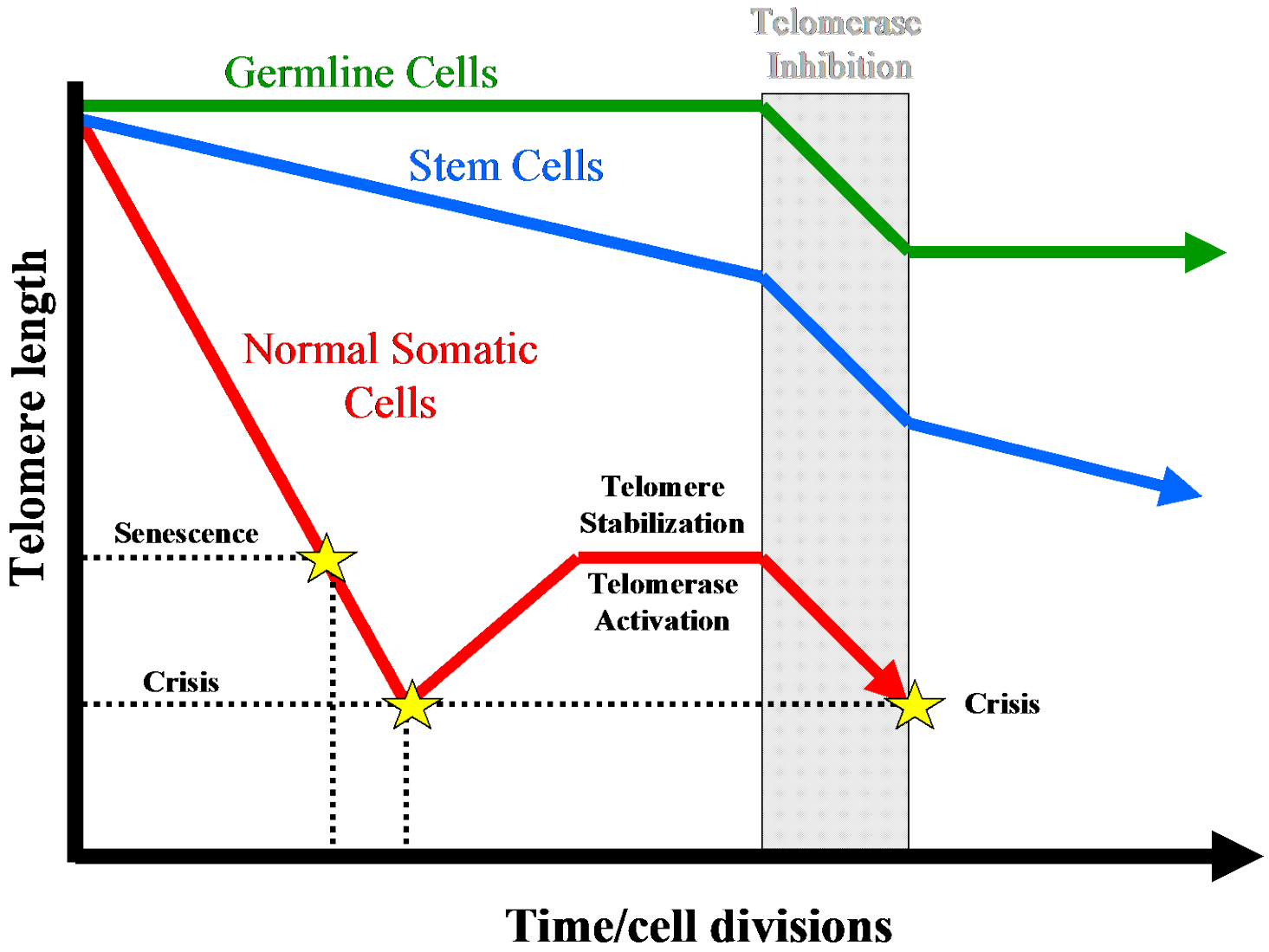
Continuous growth
*ras*

Overcome replicative senescence
*Rb, p53*

Maintain telomeres
*telomerase, ALT*



# Telomere Length Determines Cellular Longevity



# Telomerase: Functions Away From the Telomere

Inhibits apoptosis

Increases tumorigenicity

Promotes proliferation

Promotes healing

Promotes hair growth (in mice)

# Therapeutic Approaches

Direct telomerase inhibition as anti-cancer modality
    Delayed effect

*In situ* generation of a dominant negative complex
    Unknown effects

Combination therapy: Telomerase and another target
    Maybe useful for preventing remission and metastasis

Immunotherapy using anti-TERT antibodies: phase II clinical

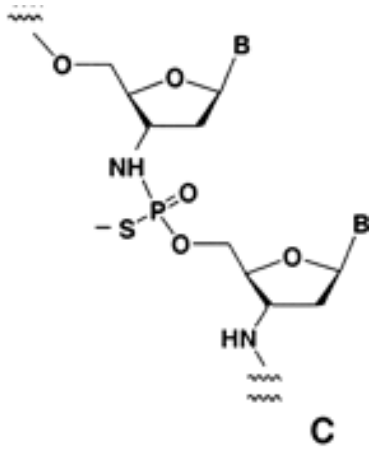
Anticancer therapy by changing telomere sequence:
    Instantaneous effect, but is it plausible?

Diagnostics and prognosis testing: TERT expression related
    to cancer progression

# GRN163L Enters the Clinic

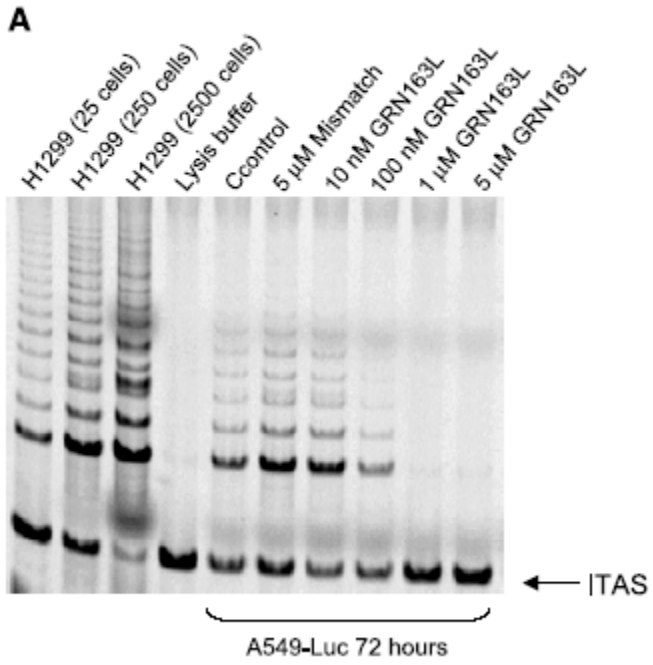
## 5’-Palm-TAGGGTTAGACAA-$NH_2$

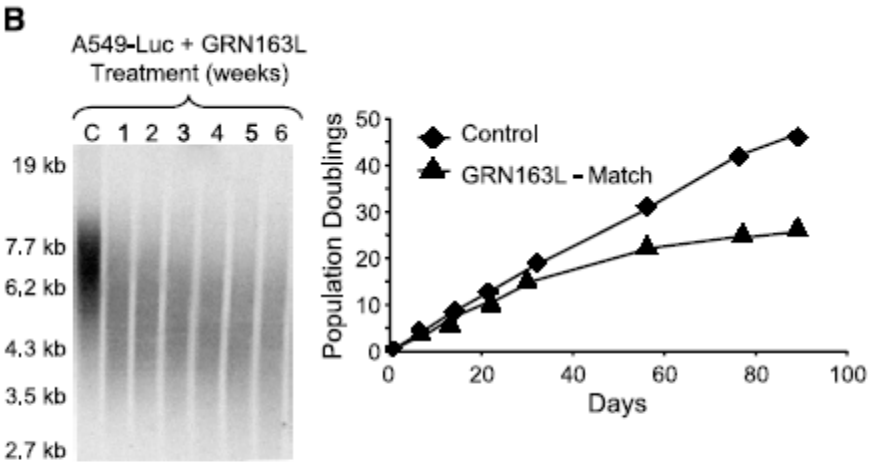
Palm = Palmitoyl



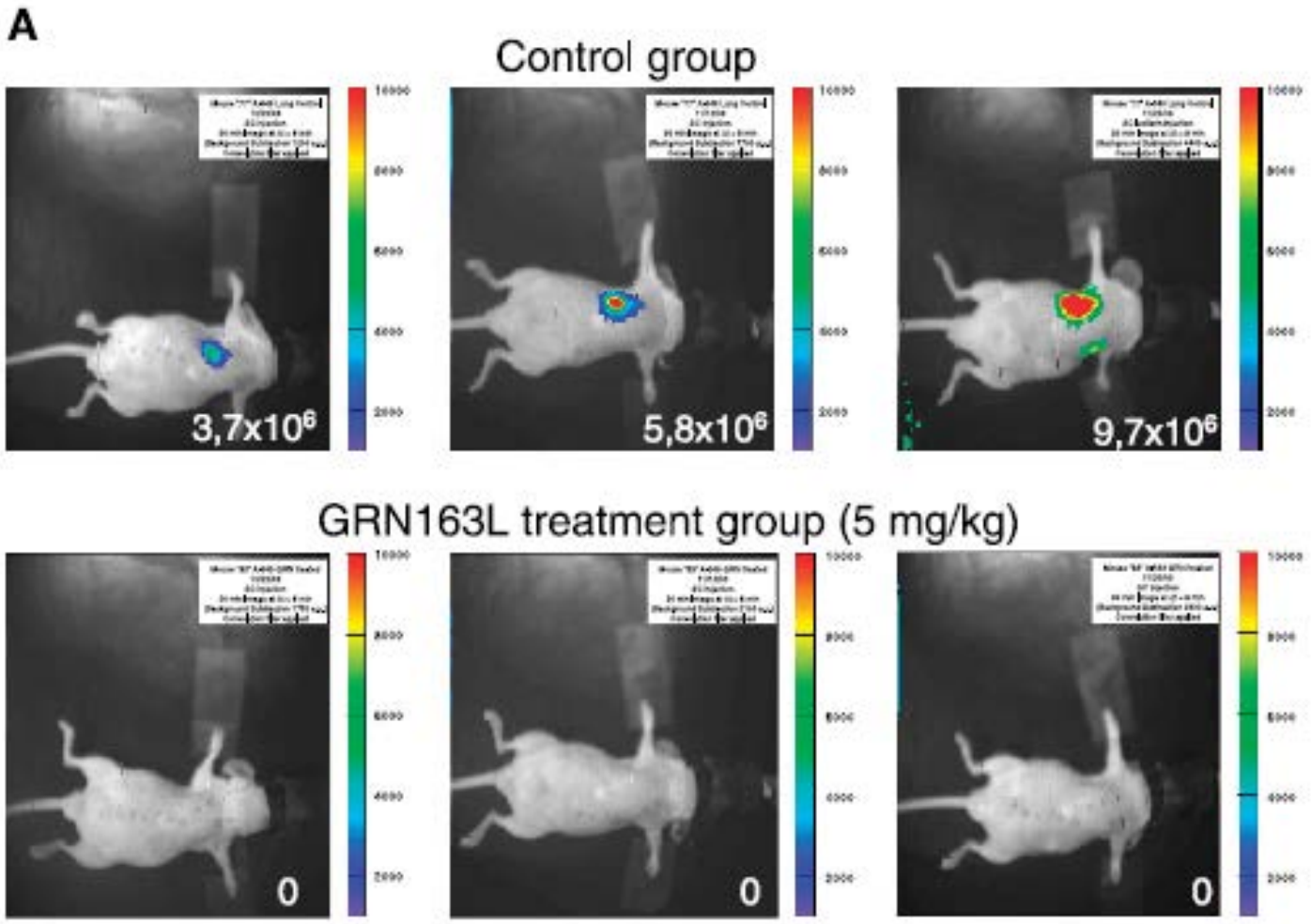

NPS

# GRN163L Inhibits Telomerase, Telomere Synthesis, and Cell Growth

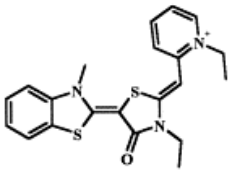


# GRN163L Inhibits Tumor Growth

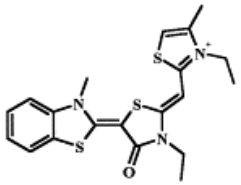


# Enzyme Inhibitors

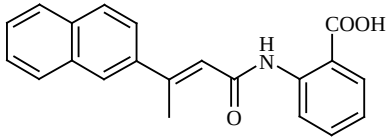


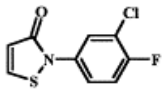
MKT 077 (XIV)

Fuji compounds



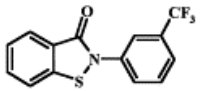
FJ 5002 (XV)



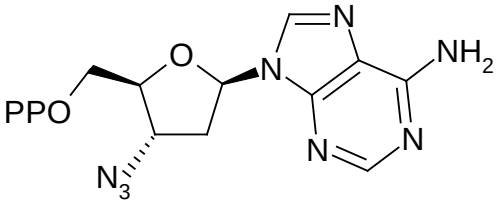
(XVI)

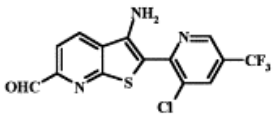
Berlex compounds



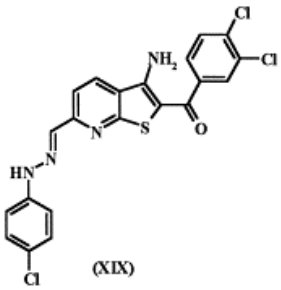
(XVII)

BIBR1532, from Boehringer Ingelheim

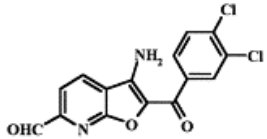


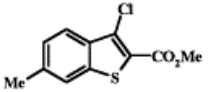
(XVIII)



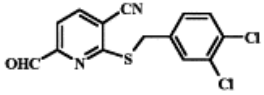
(XIX)



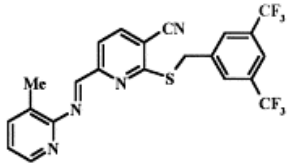
(XX)



(XXI)



(XXII)



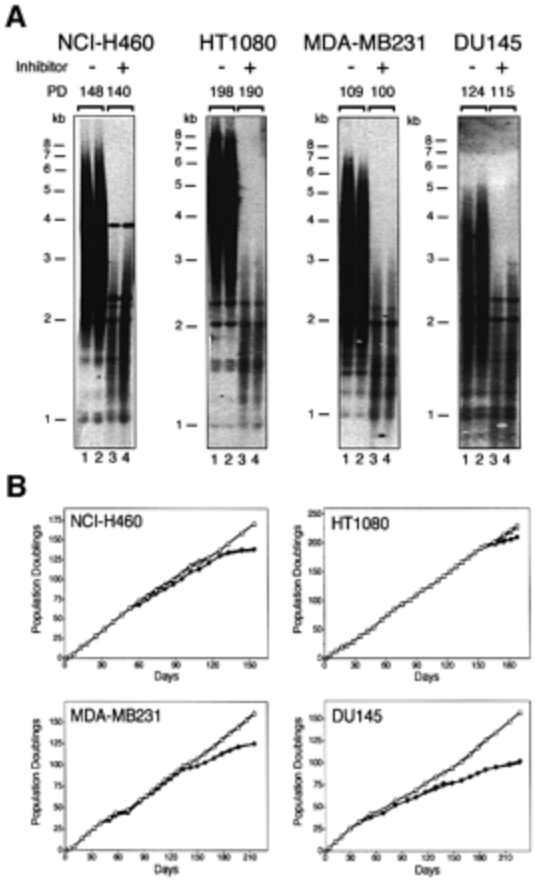
(XXIII)

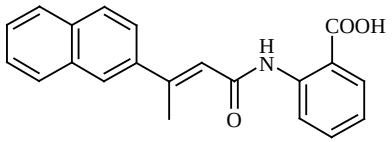
Geron compounds

Lavelle et al. *Crit Rev Onc/Hem* **2000**, *34*, 111-126

# Inhibition of Telomerase Causes Telomere Shortening, which can be Reversed

Shortening upon treatment



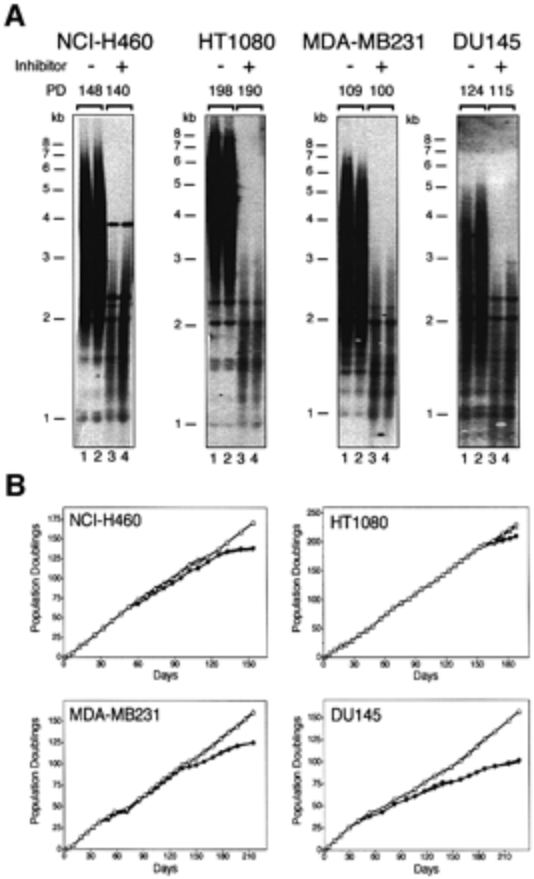




BIBR1532, from Boehringer Ingelheim

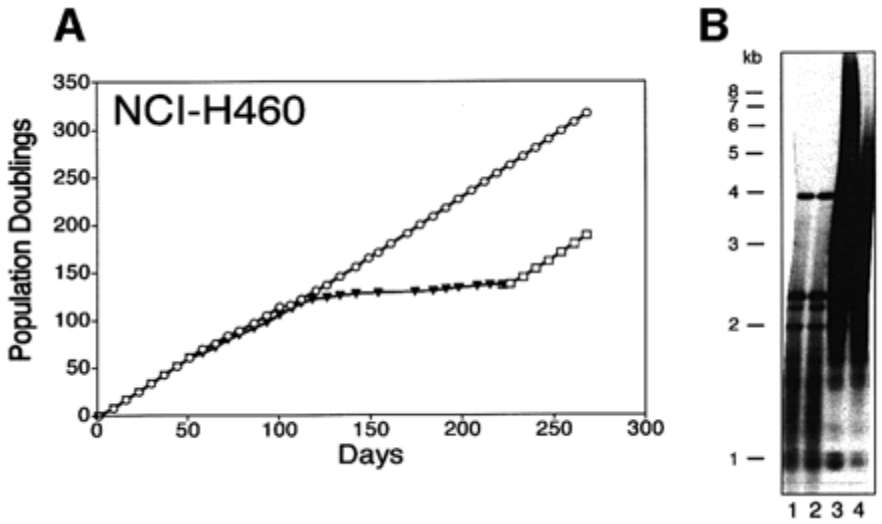
Damm et al. *EMBO J* **2001**, *20*, 6958-6968; Pascolo et al. *J. Biol. Chem.* **2002**,

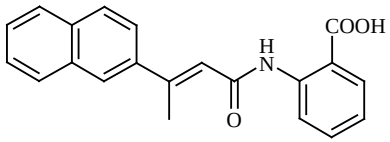
# Inhibition of Telomerase Causes Telomere Shortening, which can be Reversed

Shortening upon treatment




Halting treatment: regain telomeres




BIBR1532, from Boehringer Ingelheim

Damm et al. *EMBO J* **2001**, *20*, 6958-6968; Pascolo et al. *J. Biol. Chem.* **2002**,

# Combination Therapy