

# DNA Ends: Just the Beginning

Nobel Lecture

Dec. 7, 2009

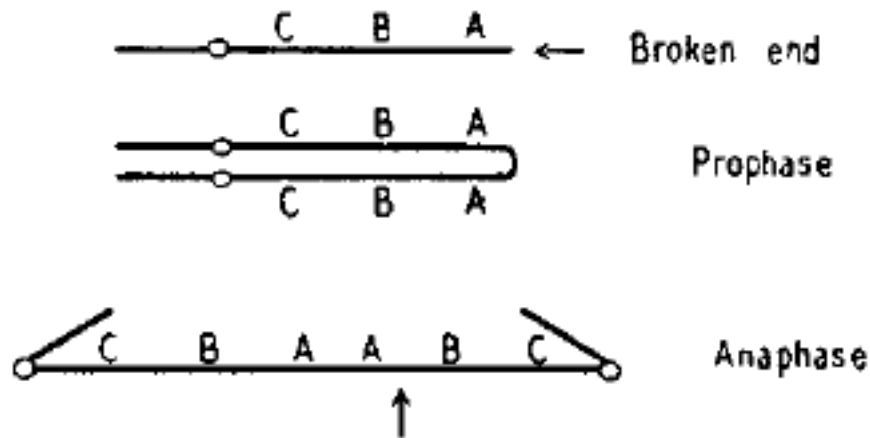
Jack W. Szostak

HHMI, MGH, HMS

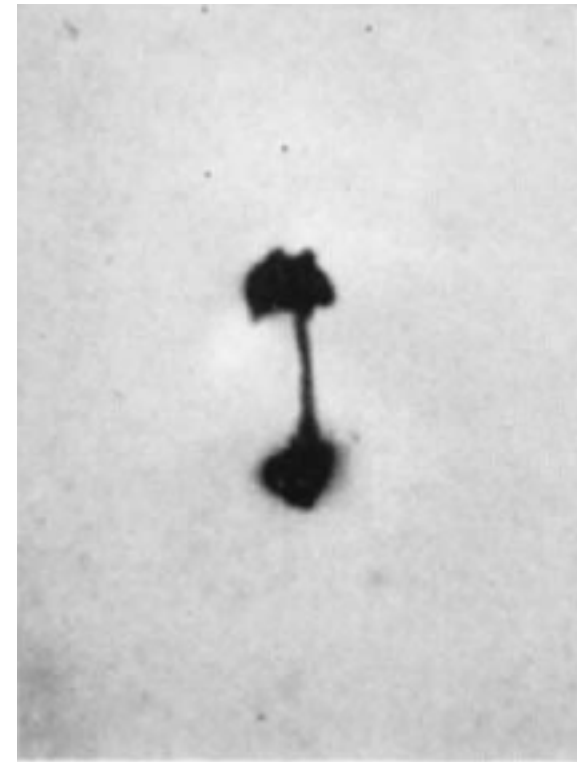
## Two Telomere Problems:

1. DNA ends are reactive
2. Incomplete Replication

# Telomeres have been known to be special since the 1930s



McClintock, Genetics 26:  
234-282 (1941)

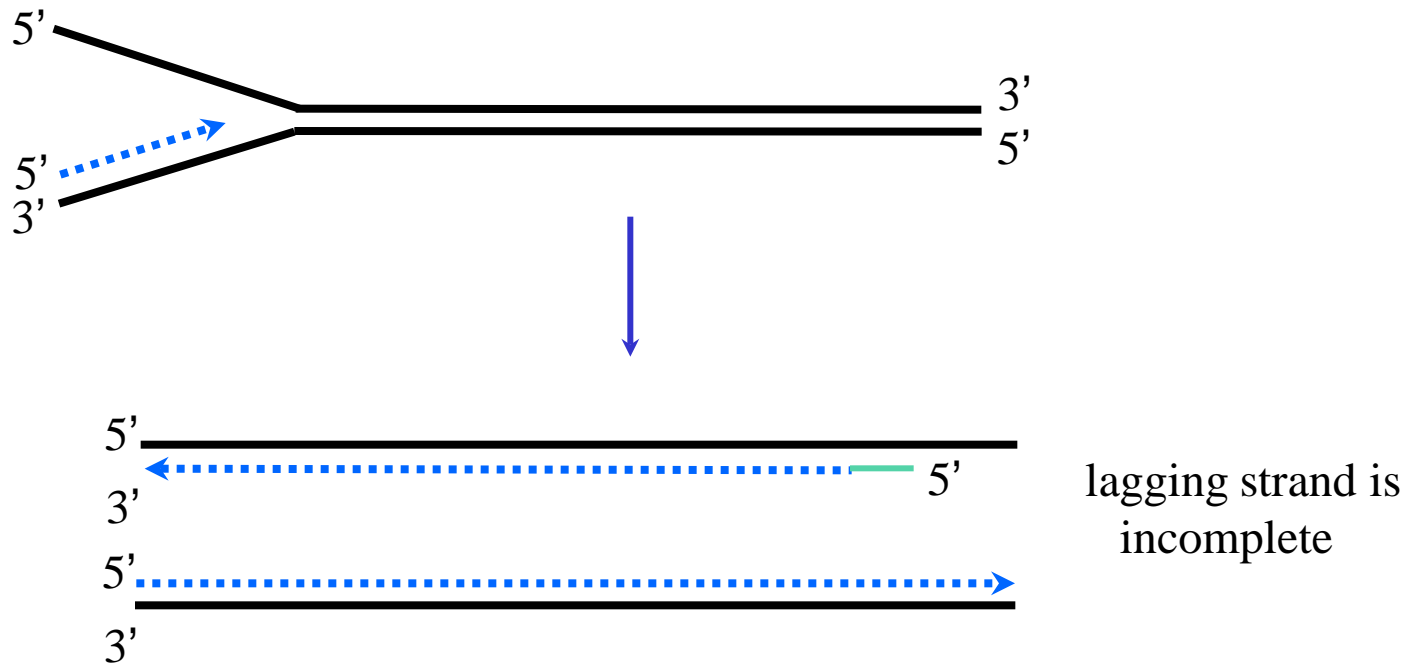


McClintock, Genetics 23:  
315-376 (1938)

“No case was found of the attachment of a piece of one chromosome to the end of another [intact chromosome]”

McClintock, Missouri Agr. Exp. Sta. Res. Bull. 163, 1-48 (1931)

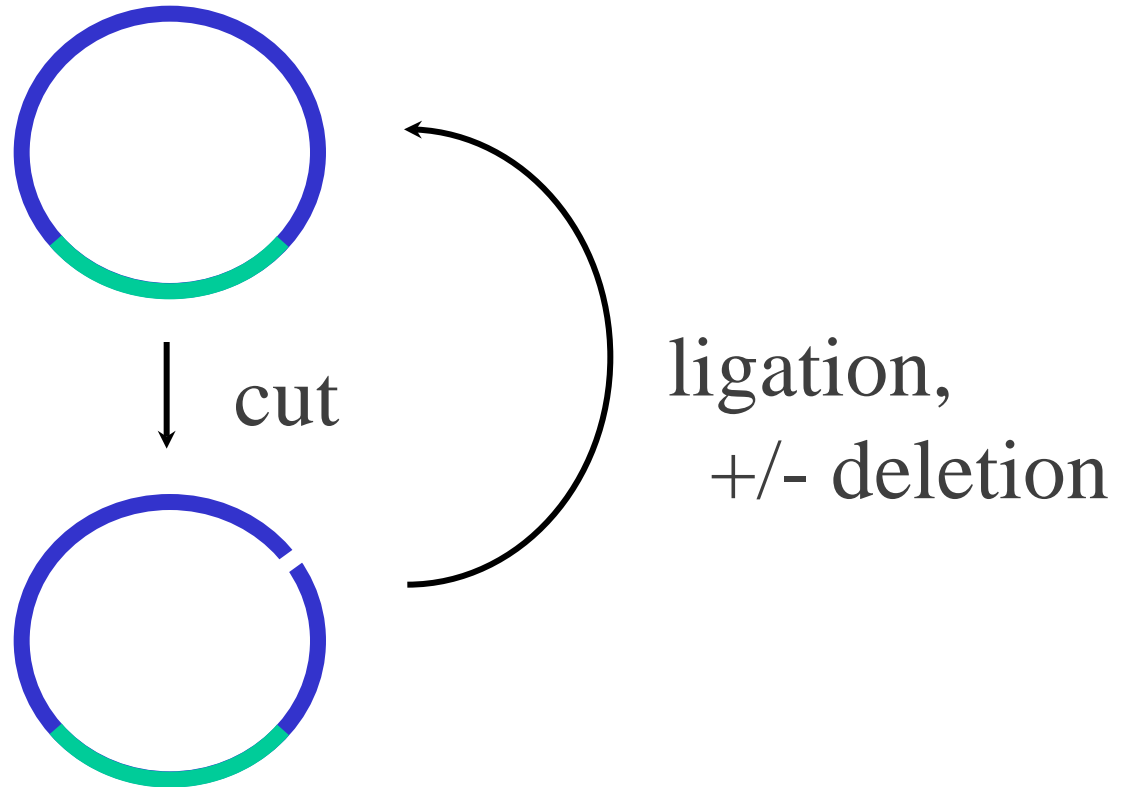
# Incomplete Replication of DNA Ends



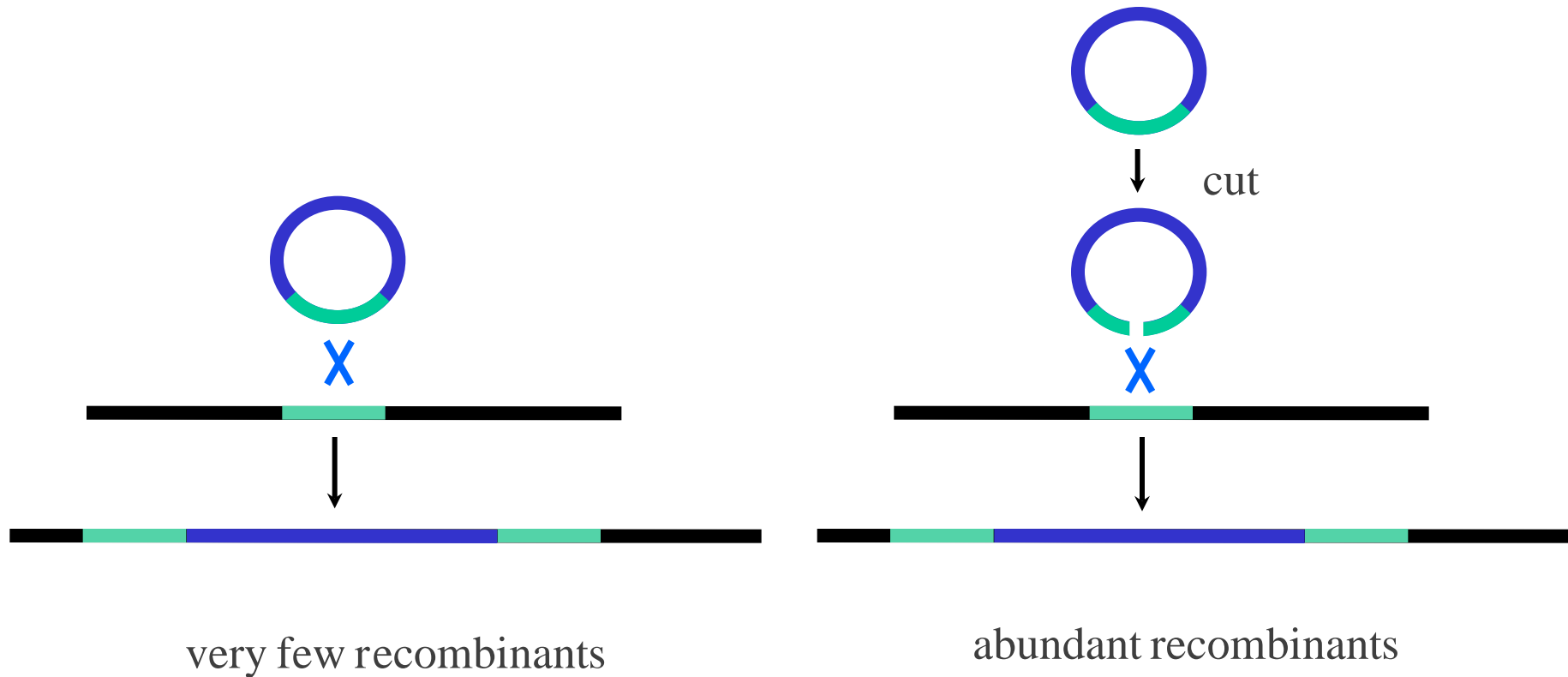
Setting the stage:

Molecular analysis of the  
reactions of DNA ends.

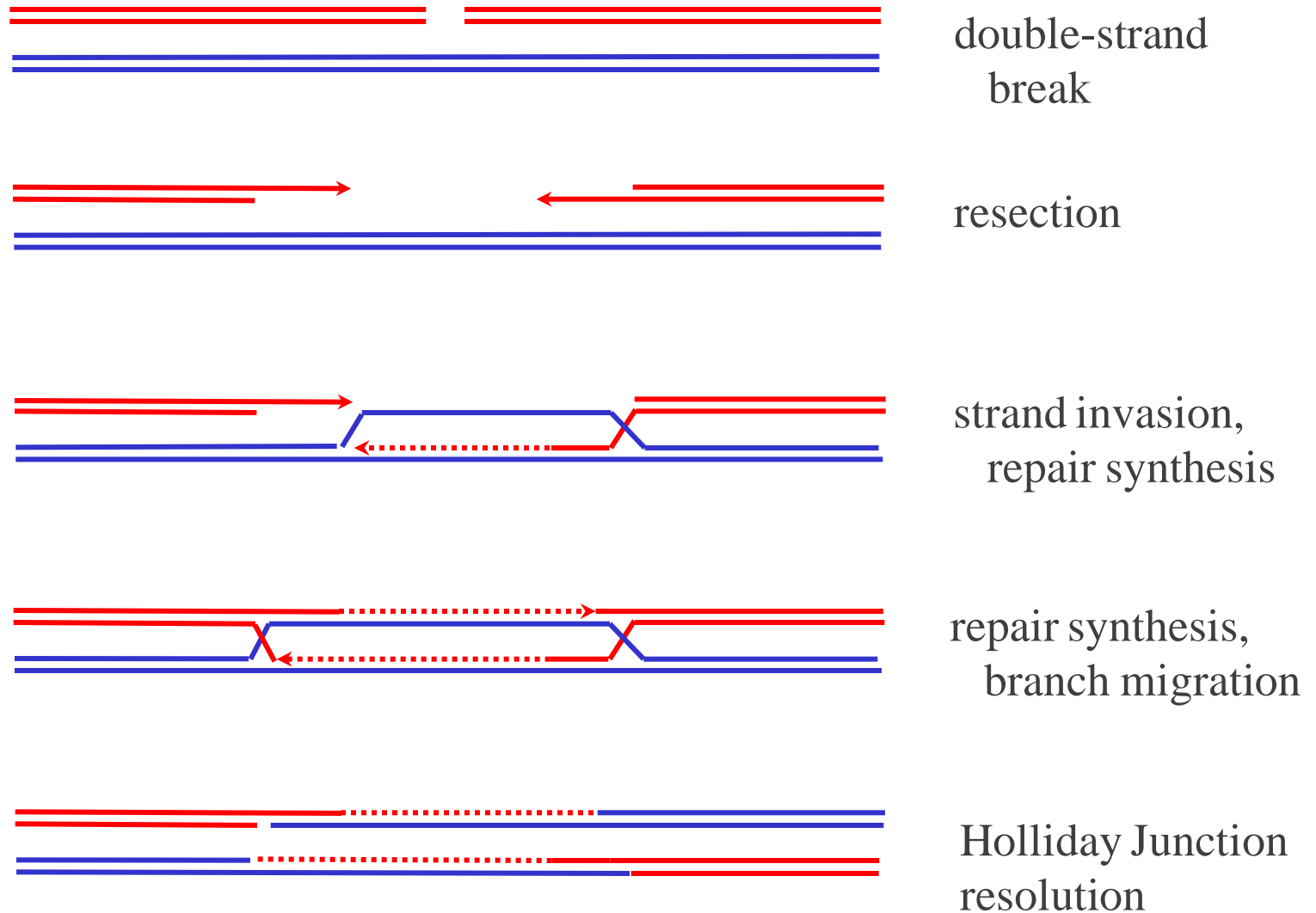
# Non-homologous end-joining in yeast



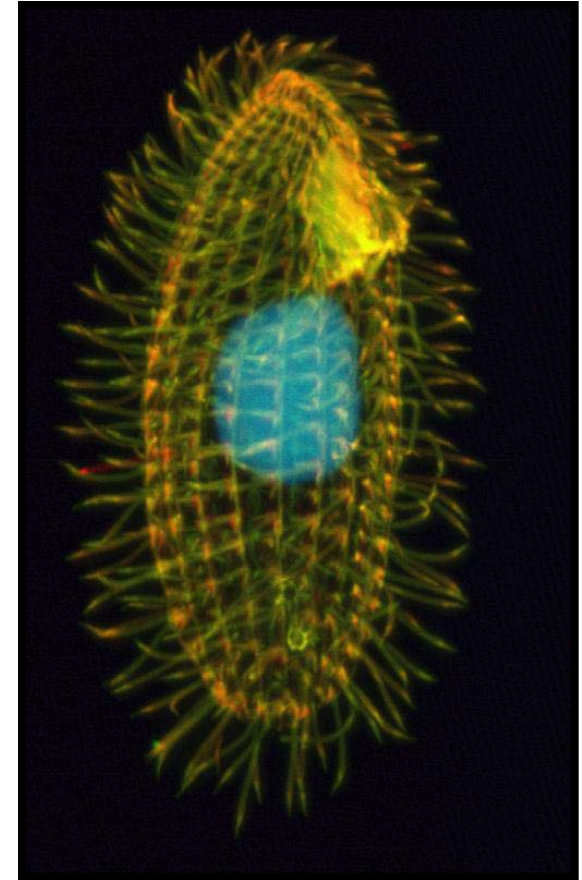
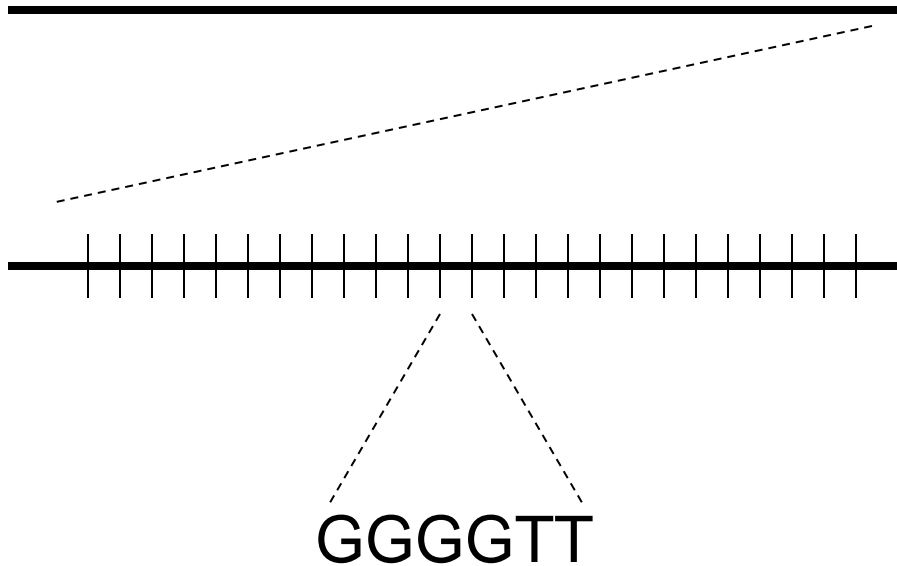
# Double-strand breaks in DNA stimulate recombination



# Double-strand break repair model for recombination



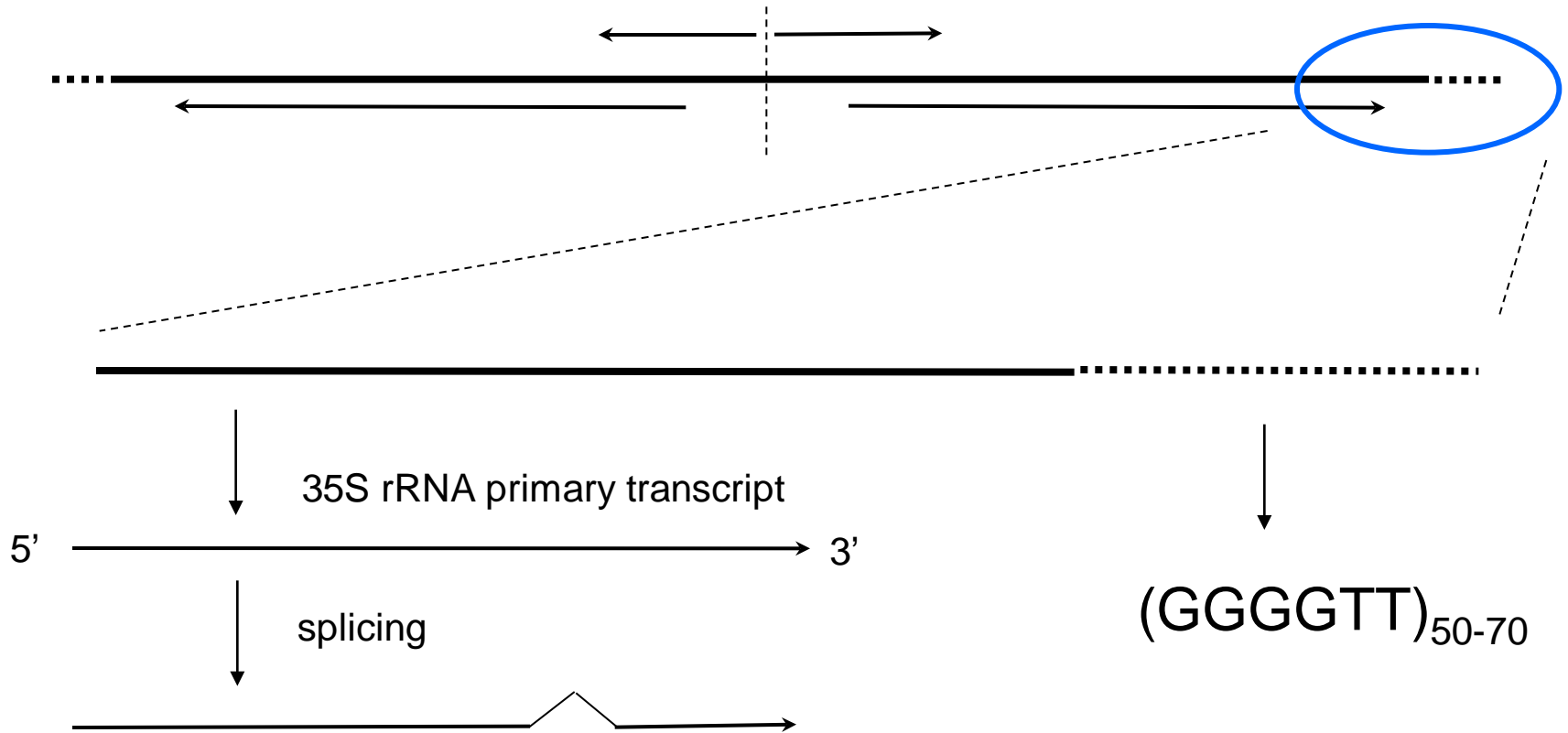
# Telomeres from *Tetrahymena*: stable DNA ends that are fully replicated



Blackburn and Gall, J. Mol. Biol. 120: 33-53 (1978)

# A very special piece of DNA:

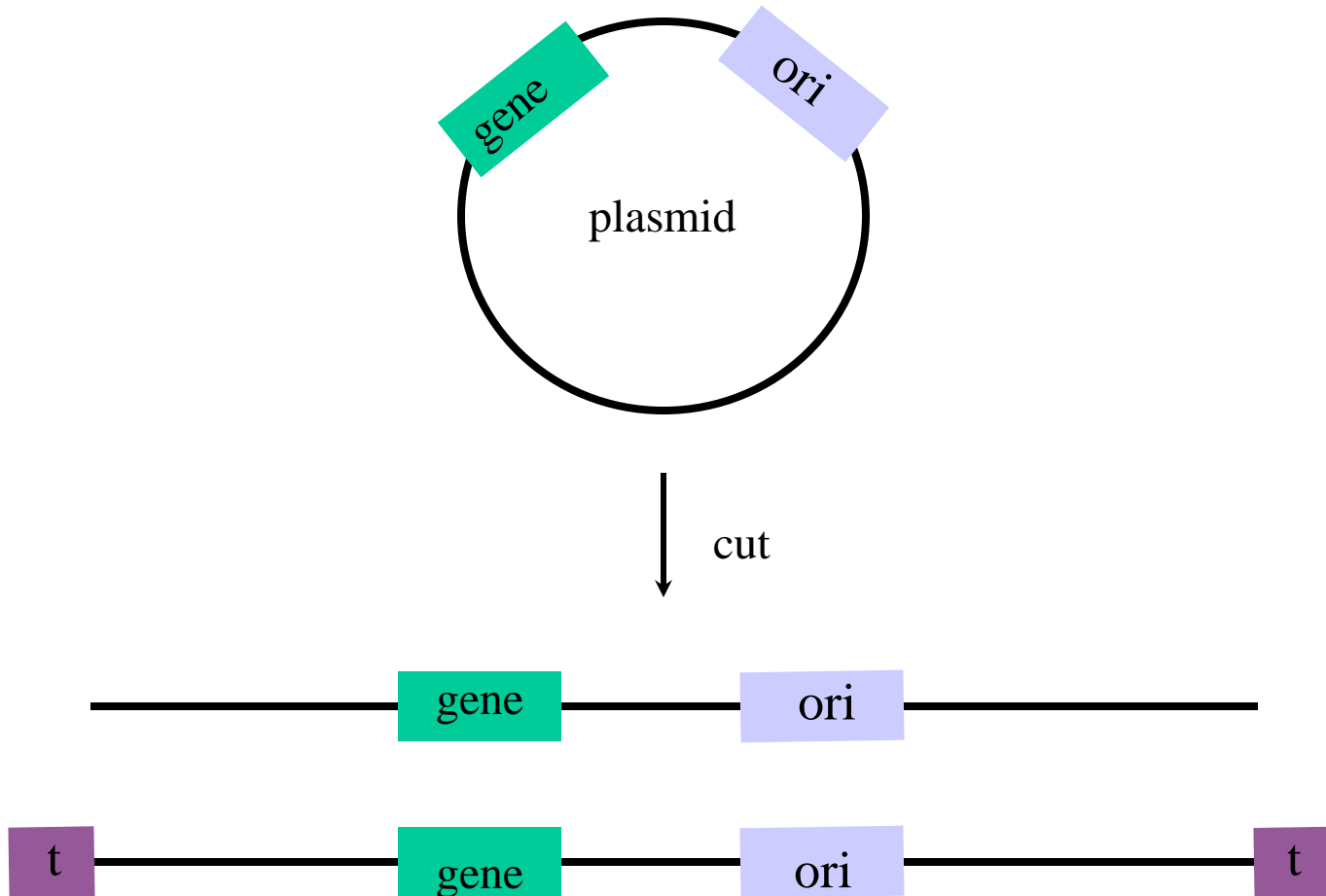
rDNA: high copy, symmetrical dimer



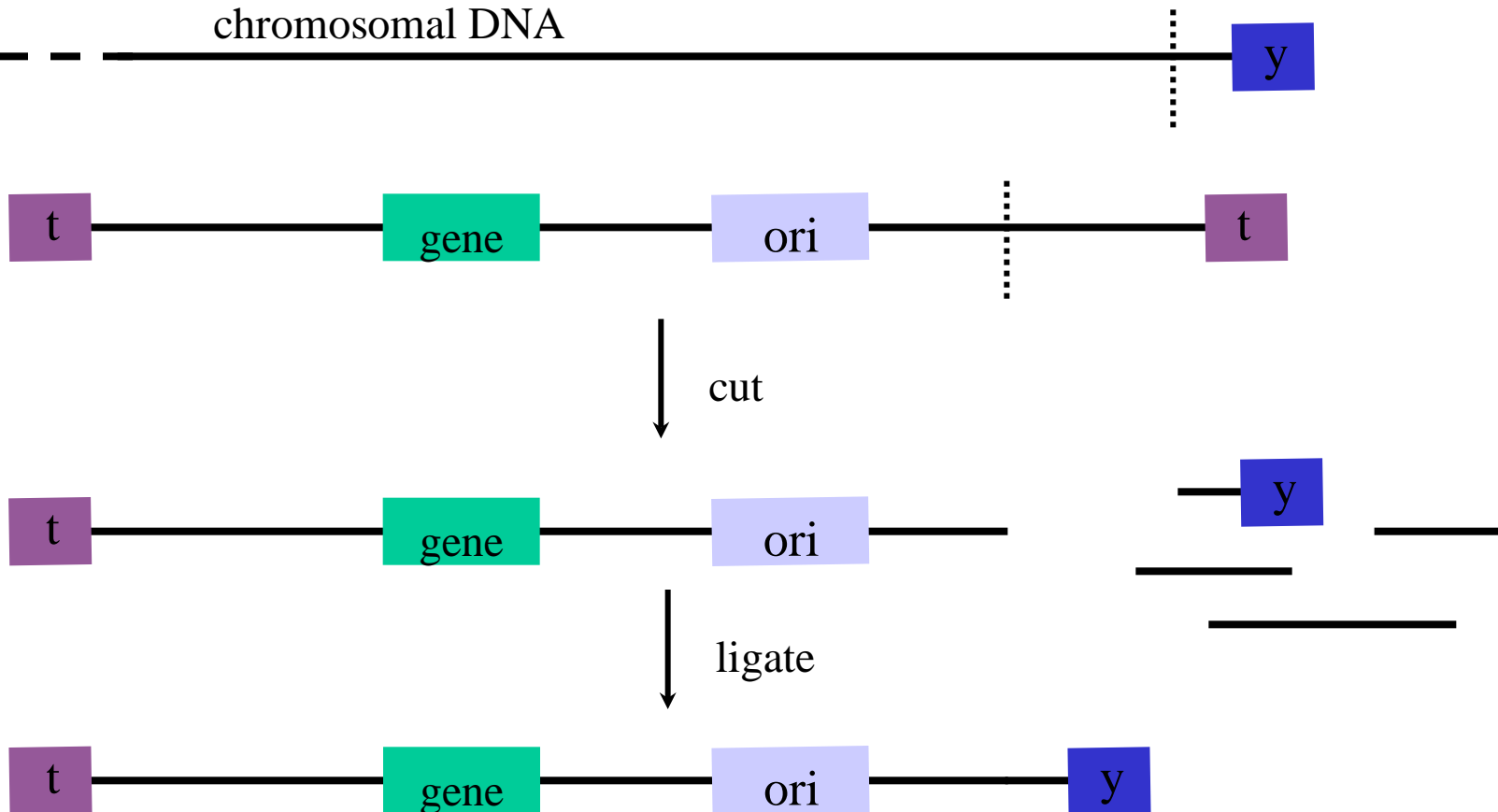
*Tetrahymena* telomeres in yeast:

A collaborative study to ask whether the biochemistry of telomeres is widely conserved.

# Moving *Tetrahymena* Telomeres into Yeast

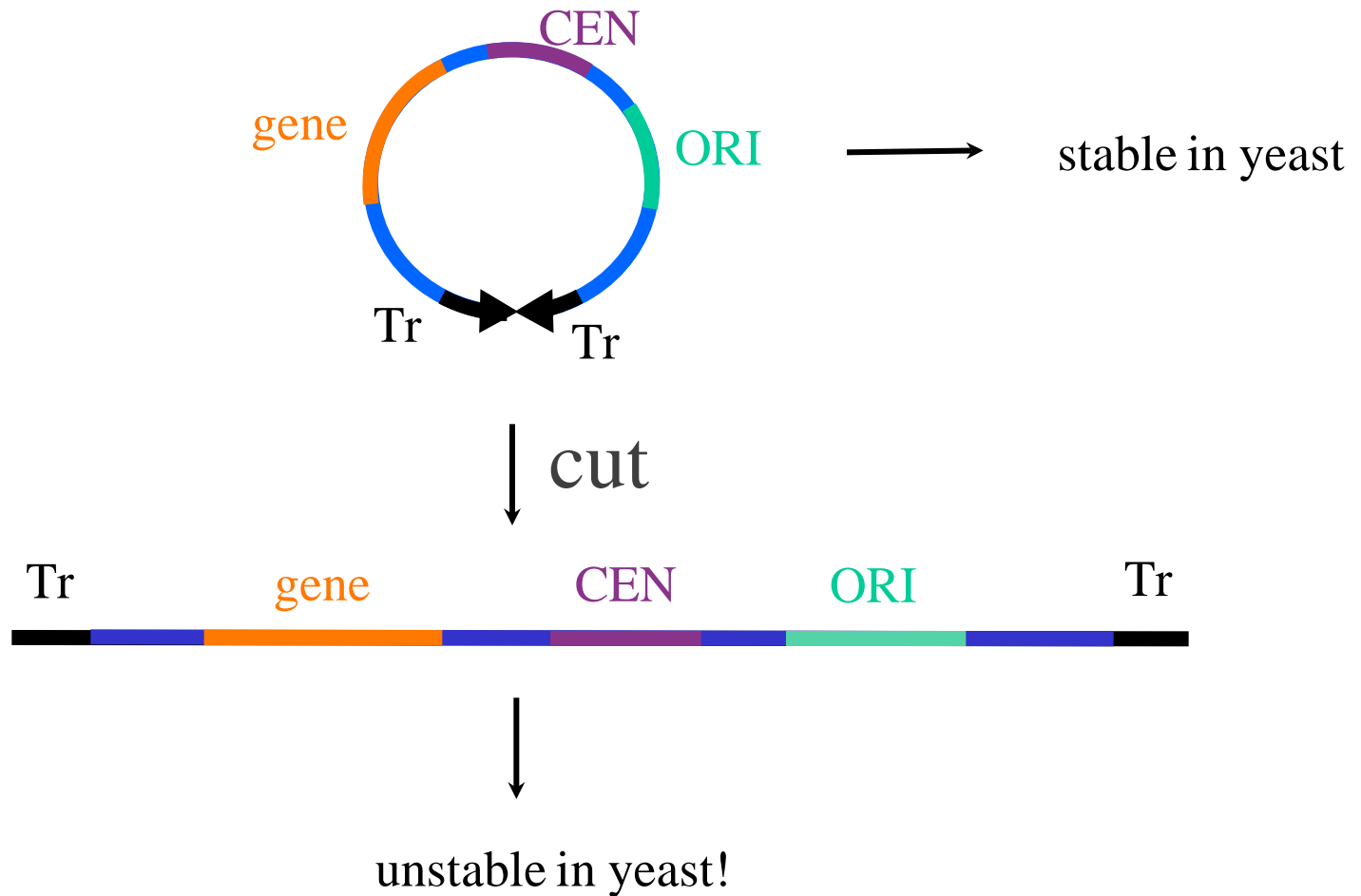


# Cloning Yeast Telomeres



# A digression: Yeast Artificial Chromosomes

# First attempt to make an artificial chromosome



# Successful attempt to make an artificial chromosome



unstable in yeast



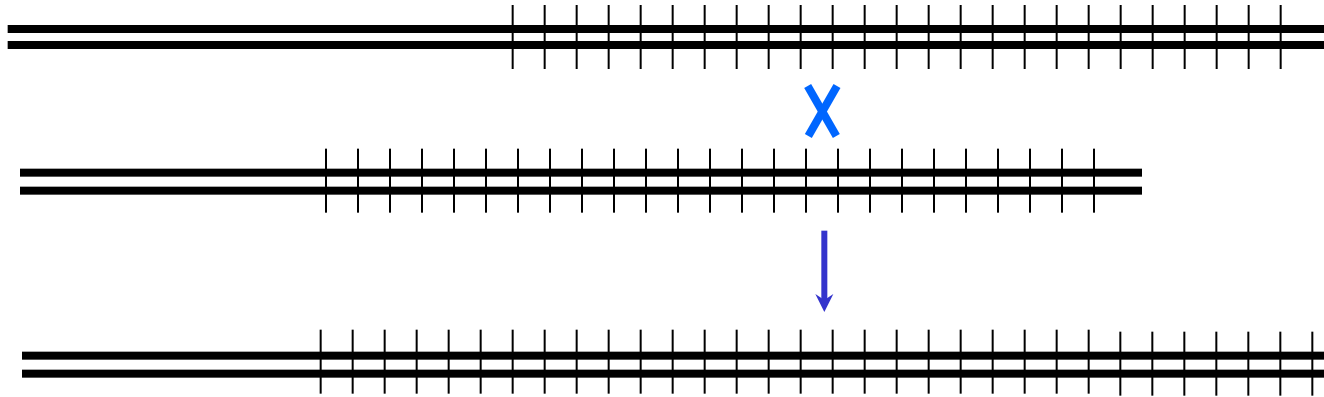
add extra DNA



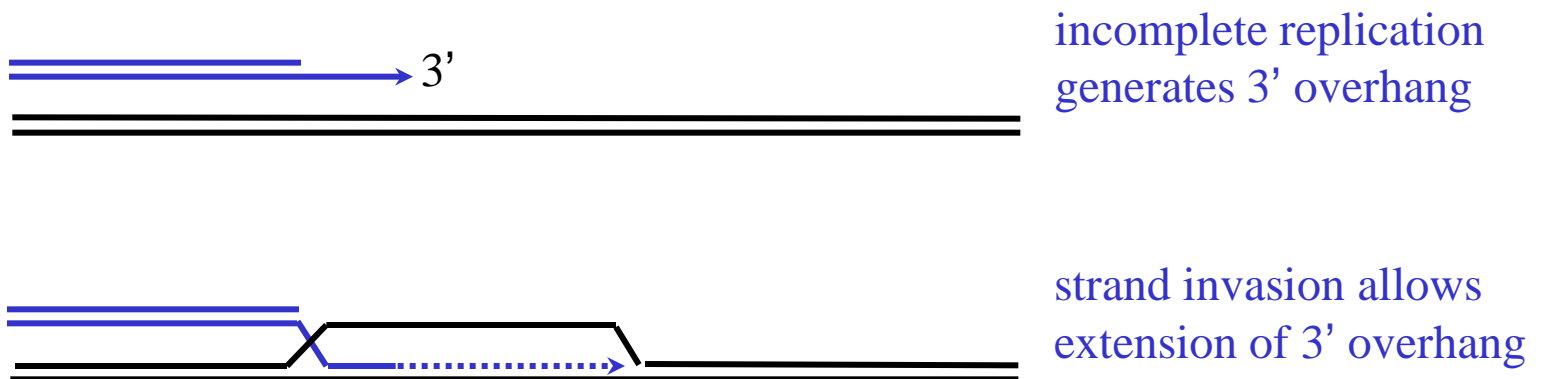
stable in yeast

# Recombination based models for telomere replication

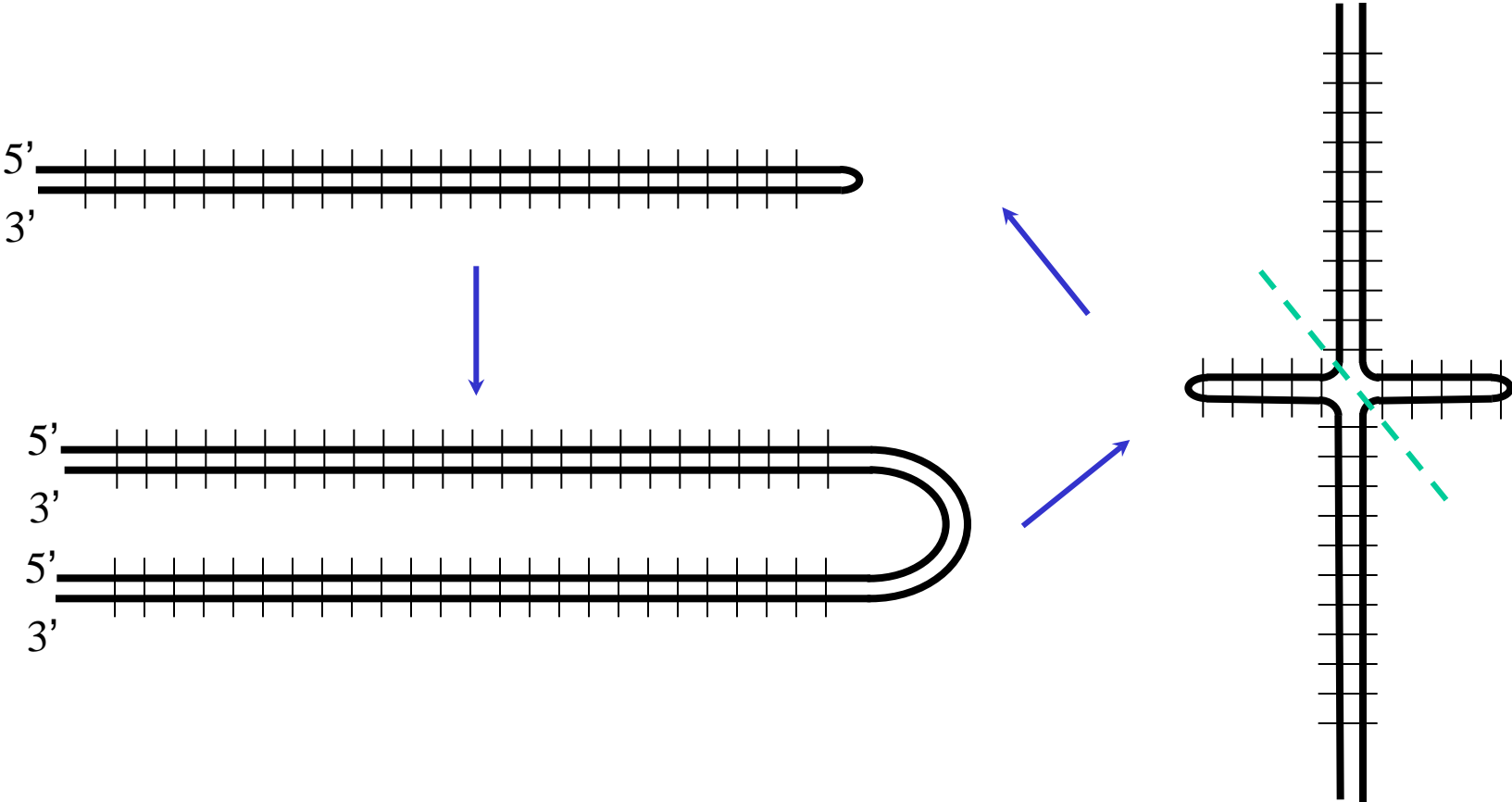
# Telomere Lengthening by Recombination



# Telomere Lengthening by Repair Synthesis

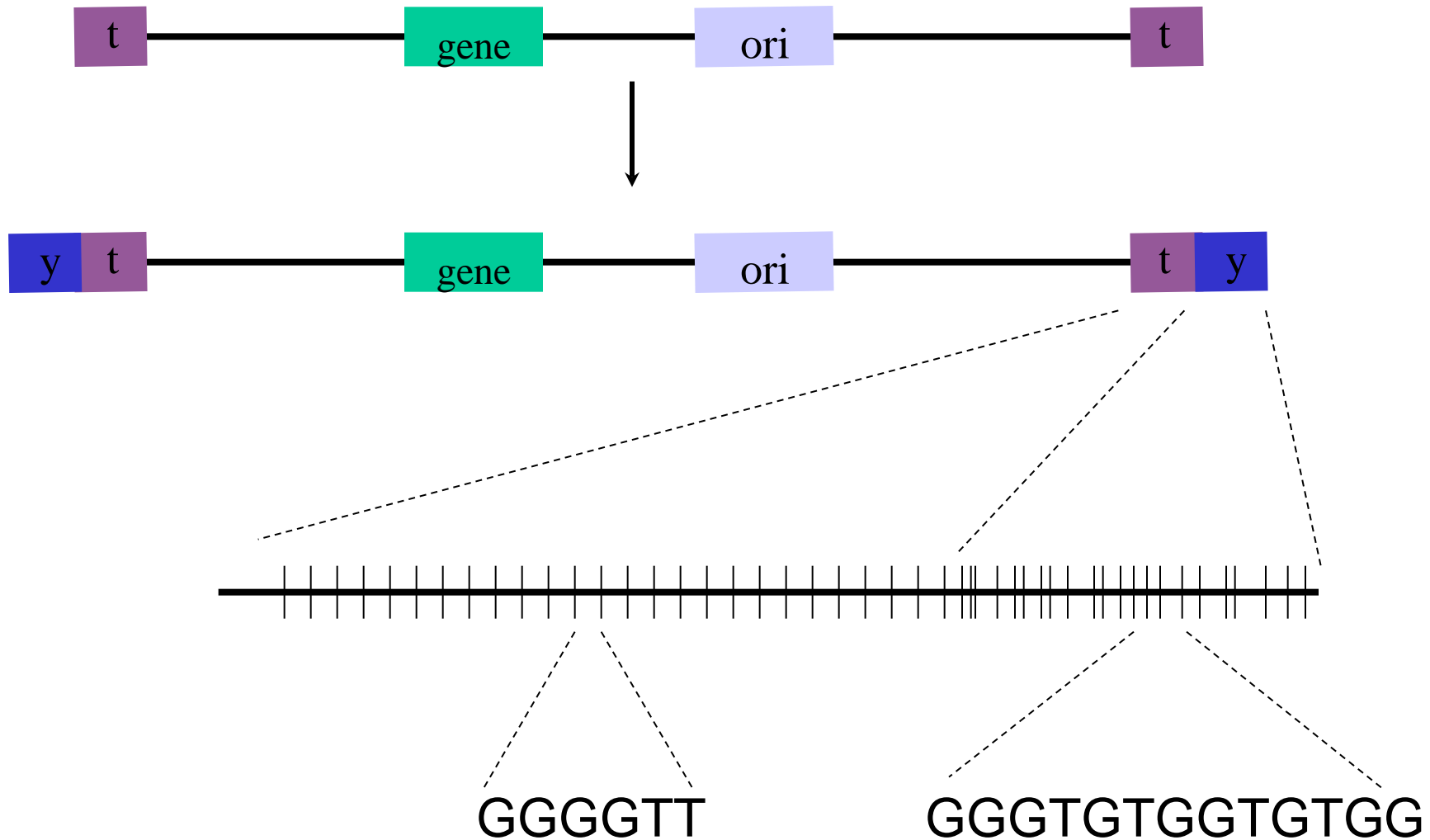


# Telomere Replication by Holliday Junction Resolution



telomere maintenance  
in yeast points to the  
correct solution

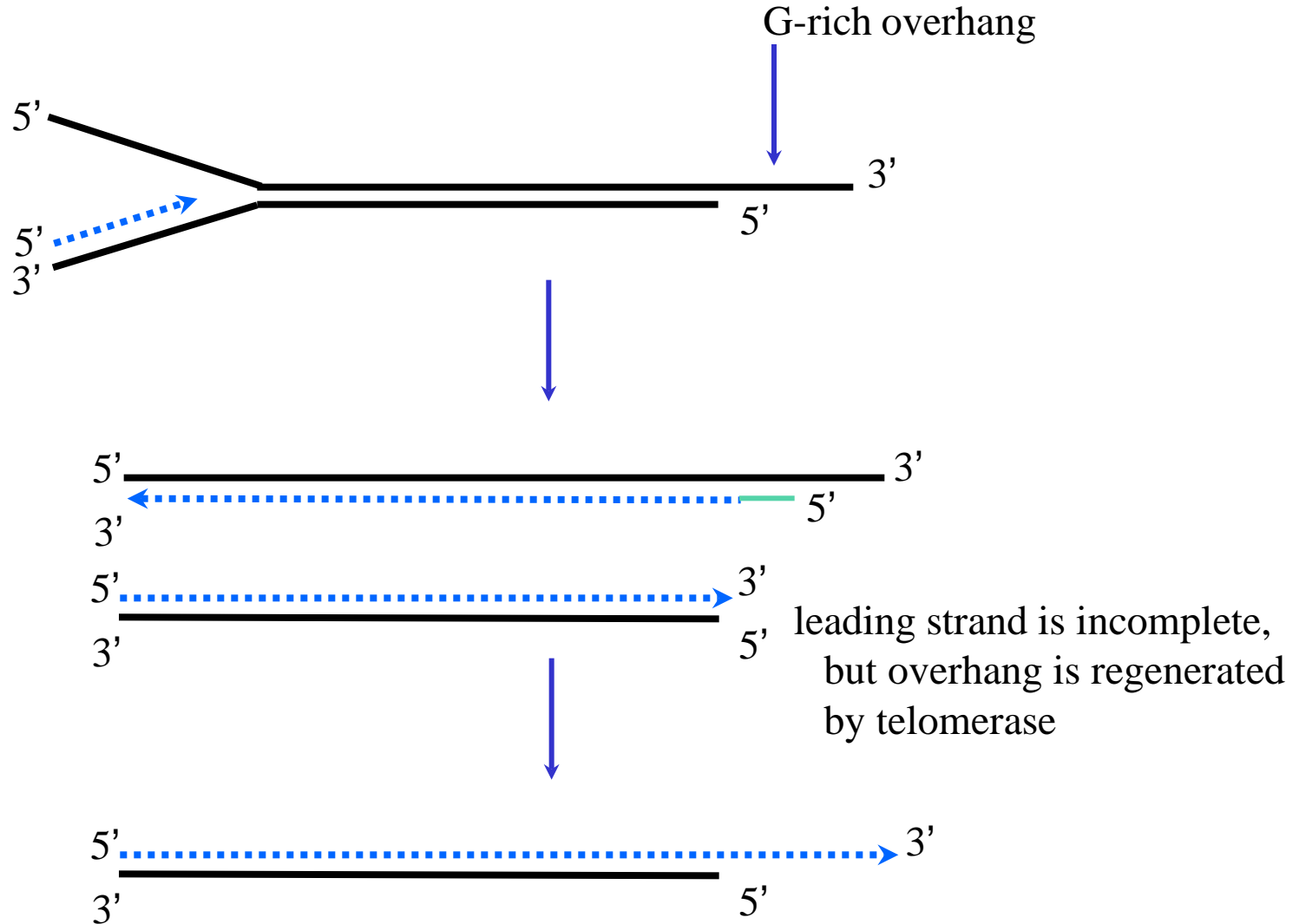
# Yeast adds new DNA to *Tetrahymena* Telomeres



# Correct Structure of Telomeric DNA Ends



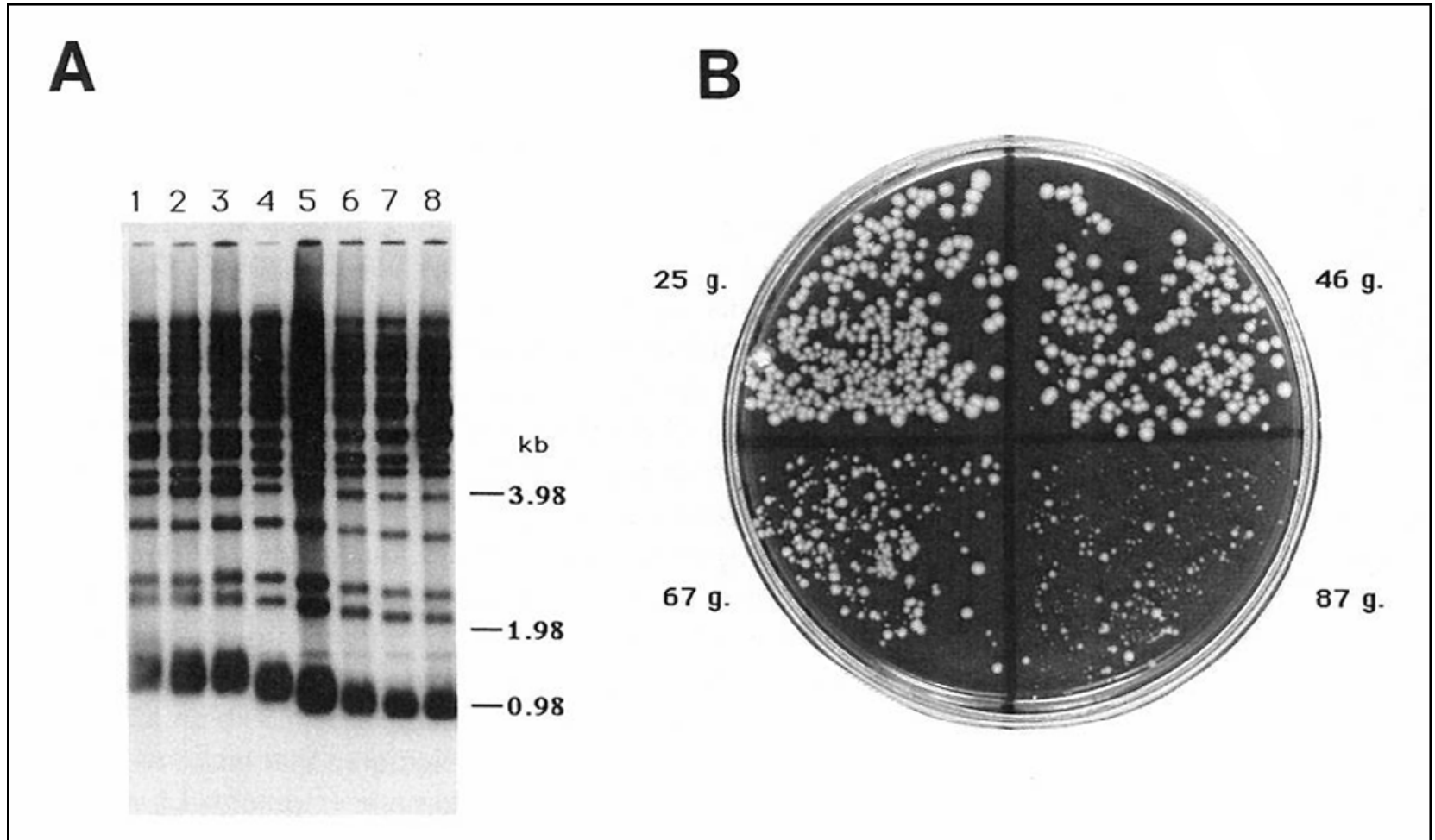
# New Model for Telomere Shortening, and the Role of Telomerase in Telomere Maintenance



Cells without telomerase  
have limited division potential,

Cells with telomerase can  
divide without limit.

# Senescence of Yeast EST-1 Cells



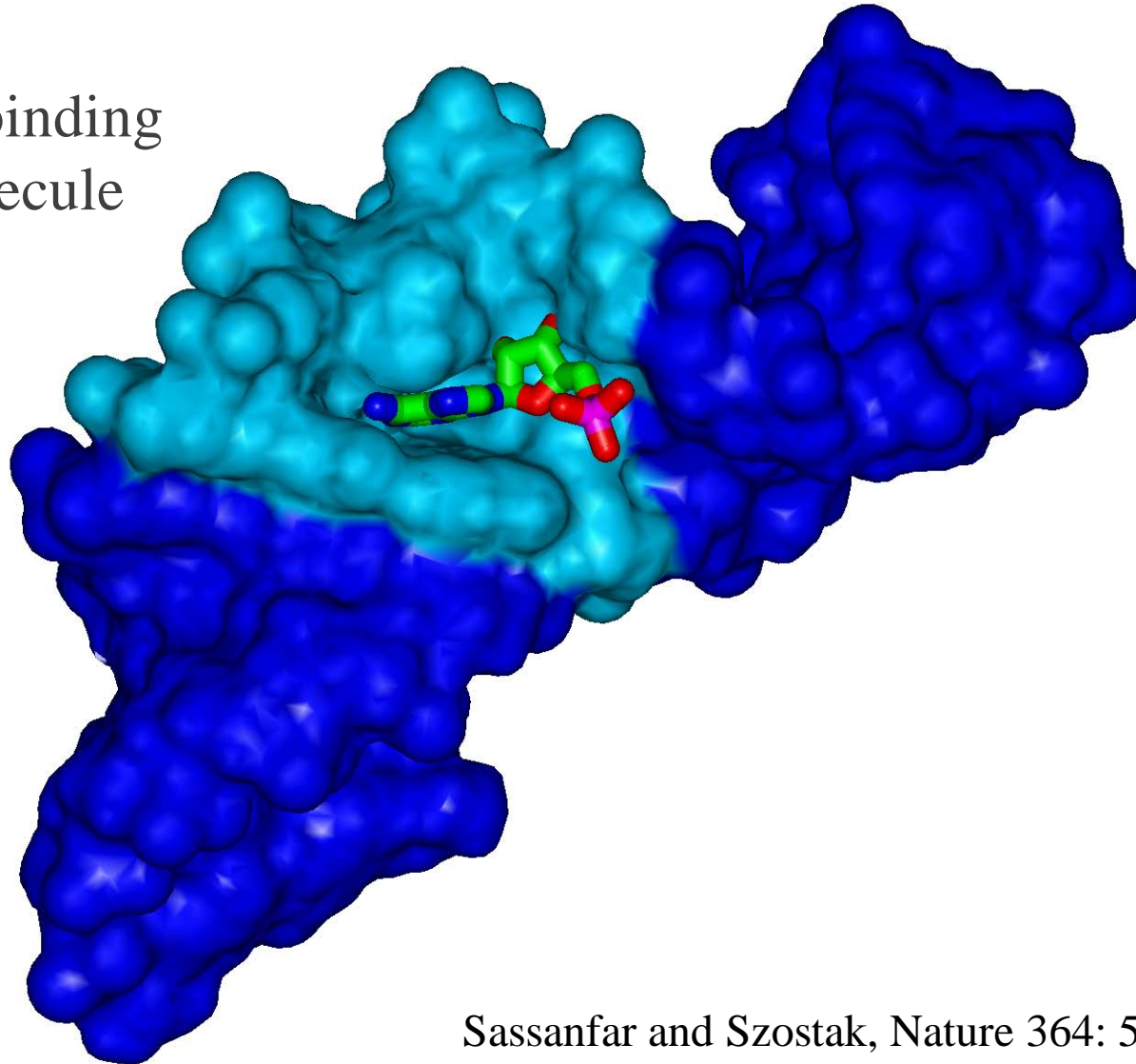
Lundblad and Szostak, Cell 57: 633-643 (1989)

After telomeres:

Directed Evolution of  
RNA and Protein

# Laboratory Evolution of Aptamers

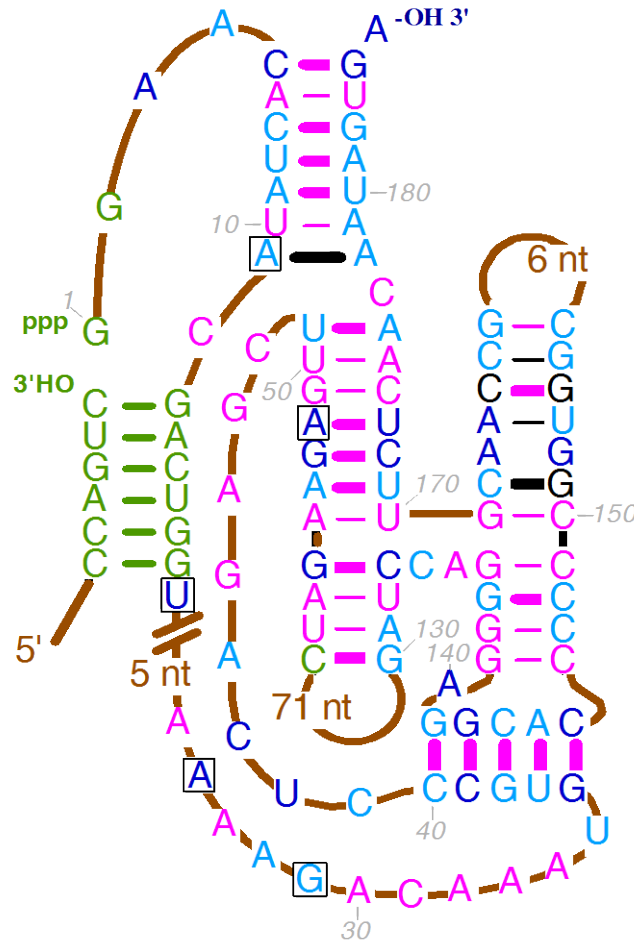
An ATP binding  
RNA molecule



Sassanfar and Szostak, Nature 364: 550-553 (1993)

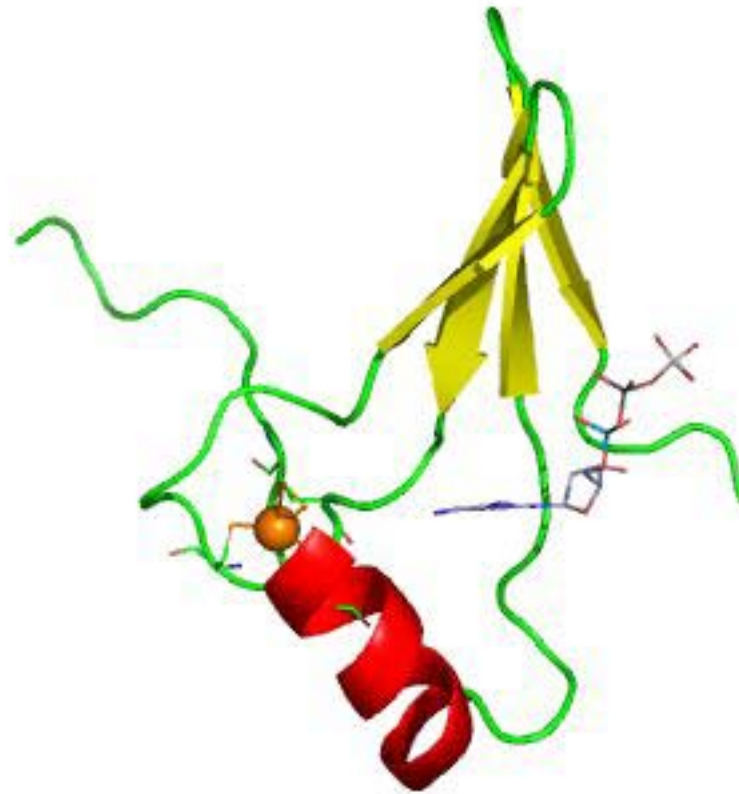
# Class I Ribozyme Ligase

Fig. 5





# ATP Binding Protein



Current focus:

Origin of Life

# Schematic Model of a Protocell

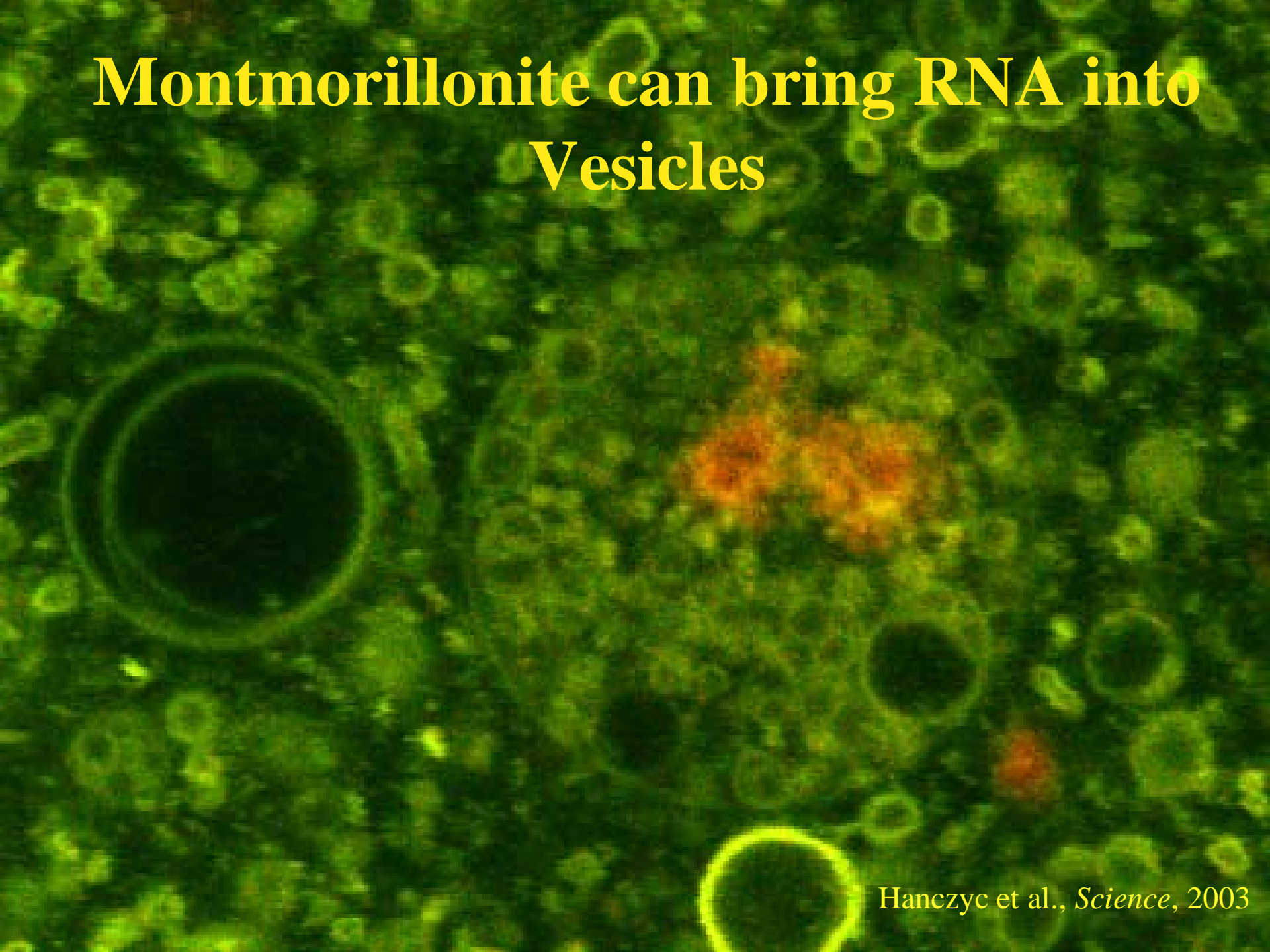
A simple cell might be based on a replicating vesicle for compartmentalization, and a replicating genome to encode heritable information. A complex environment provides nucleotides, lipids and various sources of energy.

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

Mechanical energy (for division), chemical energy (for nucleotide activation), phase transfer and osmotic gradient energy (for growth) may be used by the system.

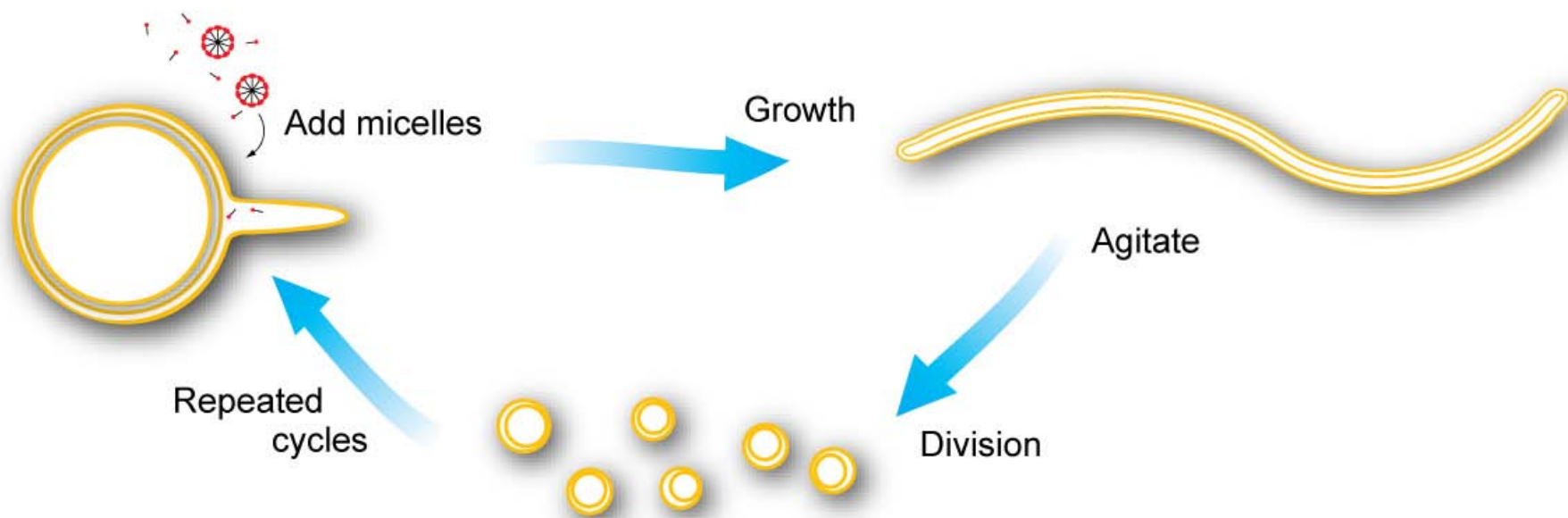
Mansy et al., *Nature*, 2008

# Montmorillonite can bring RNA into Vesicles



Hanczyc et al., *Science*, 2003

# Cycles of growth and division

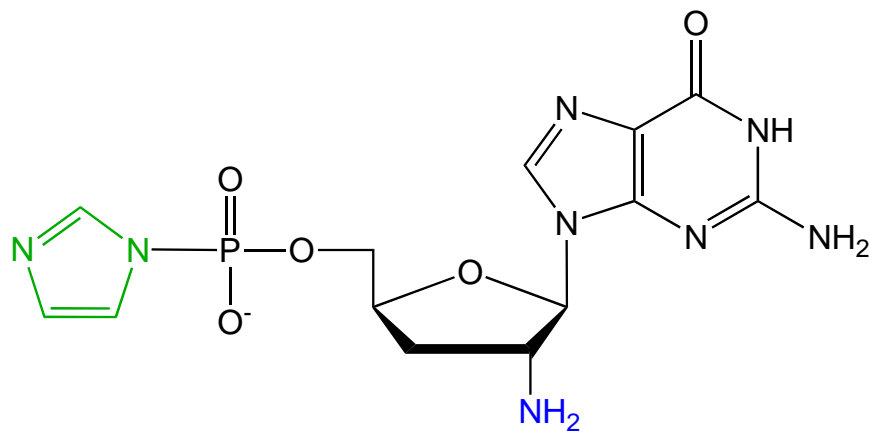


# Self-Replicating Genetic Polymers

*It seems likely that informational replication will be achieved in the next decade, and that it will throw new light on the origins of life.*

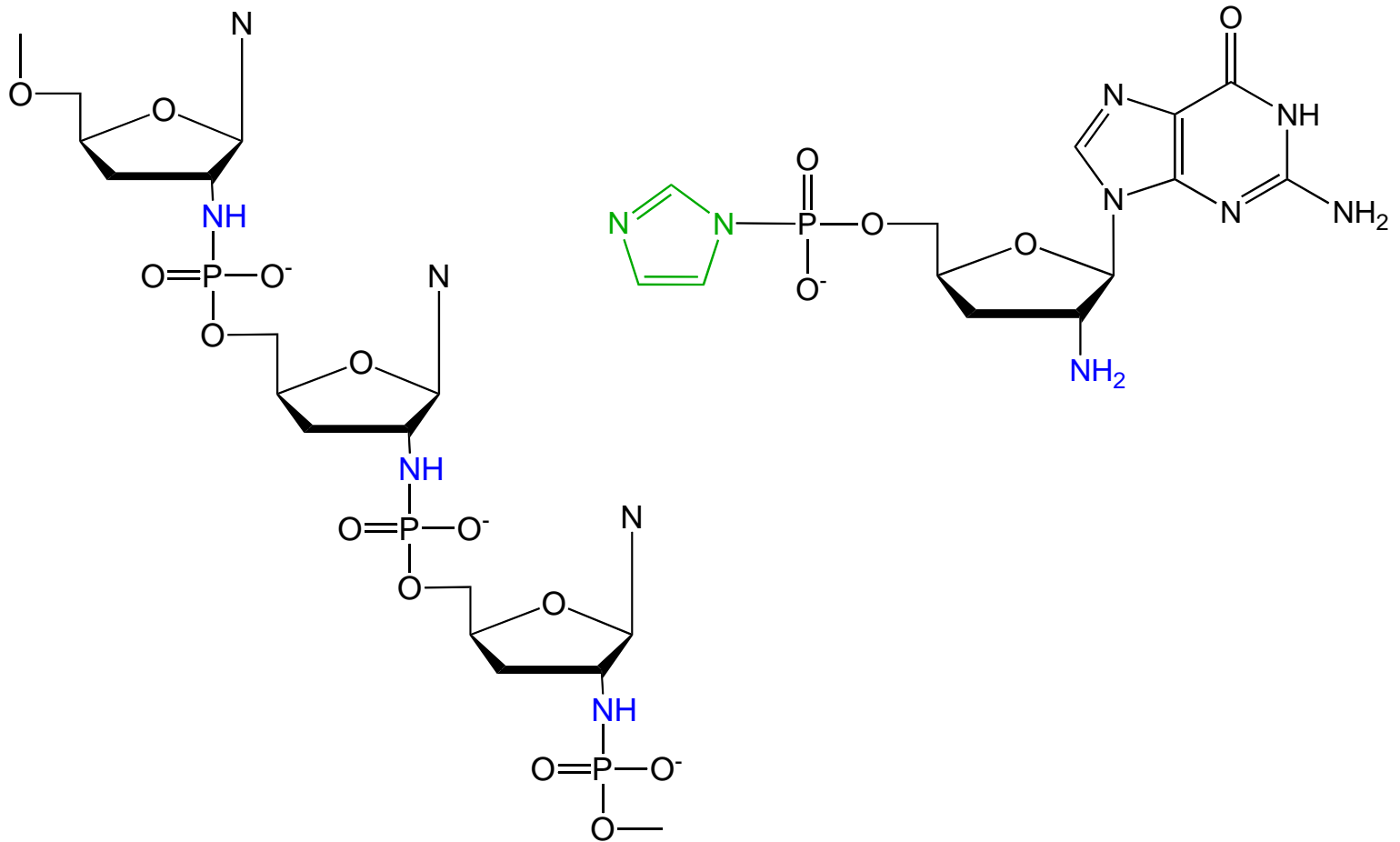
*Leslie Orgel, 1992*

# Typical monomer for spontaneous synthesis

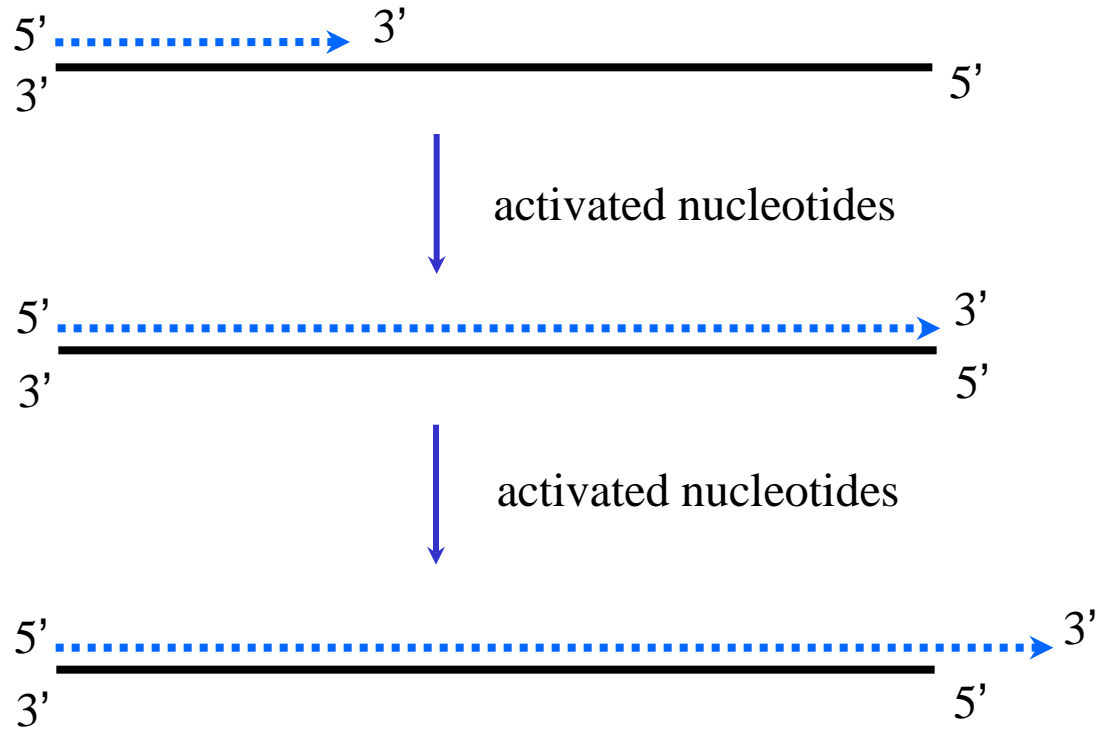


# Typical monomer for spontaneous synthesis and corresponding polymer

**2'-NP-DNA**



# Origin of Telomerase in Spontaneous Copying Chemistry?



...and thanks to the many  
students, postdocs, collaborators,  
colleagues and friends  
who made this work possible.