DNA Ends: Just the Beginning

Nobel Lecture

Dec. 7, 2009

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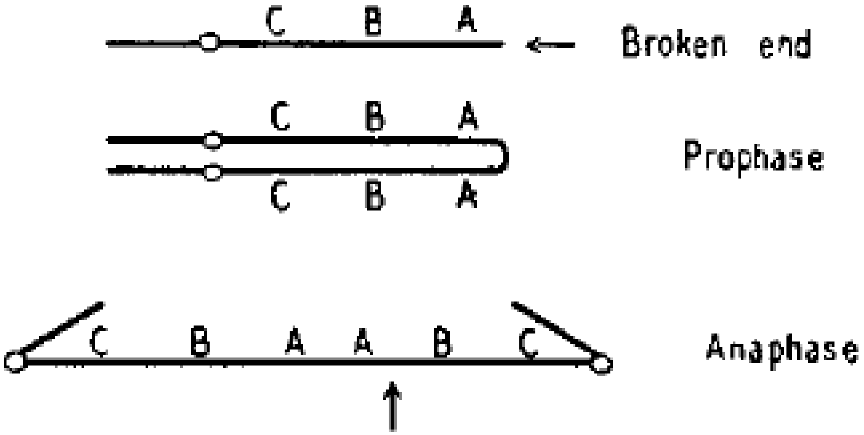
HHMI, MGH, HMS

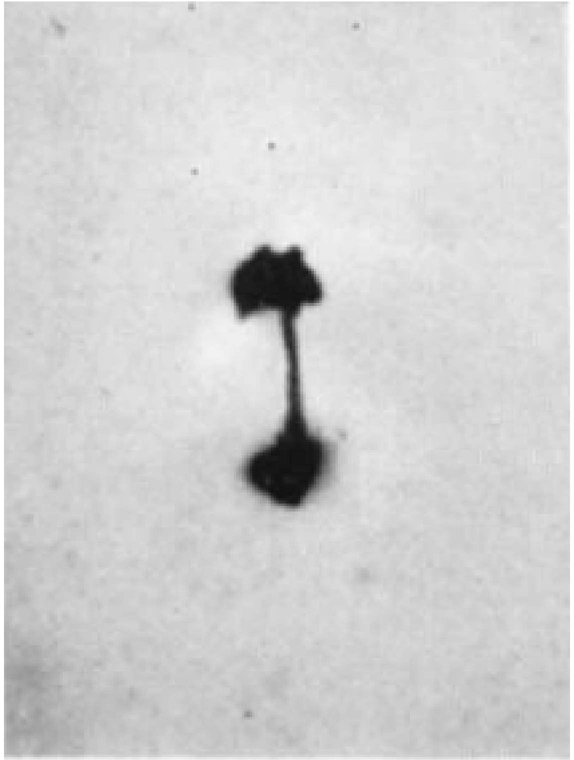
Two Telomere Problems:

1. DNA ends are reactive
2. Incomplete Replication

Telomeres have been known to be special since the 1930s



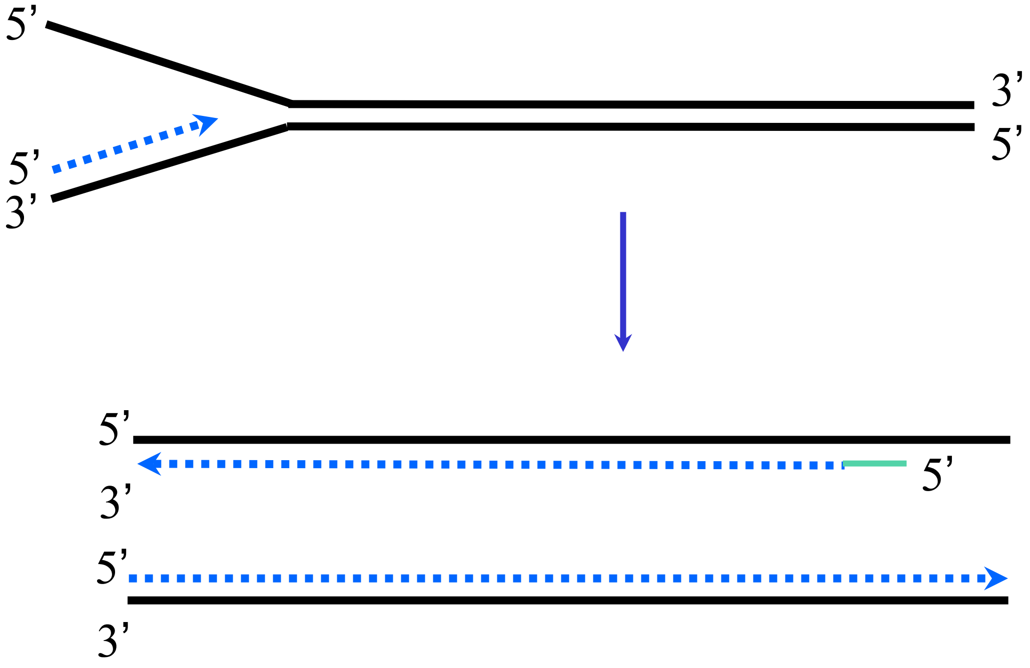
McClintock, Genetics 26: 234-282 (1941)



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

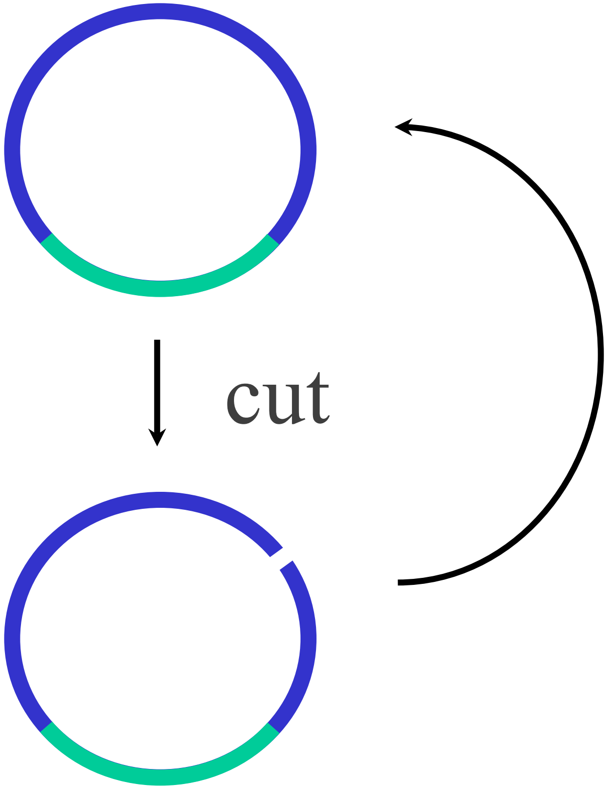


lagging strand is
incomplete

Setting the stage:

Molecular analysis of the reactions of DNA ends.

Non-homologous end-joining in yeast

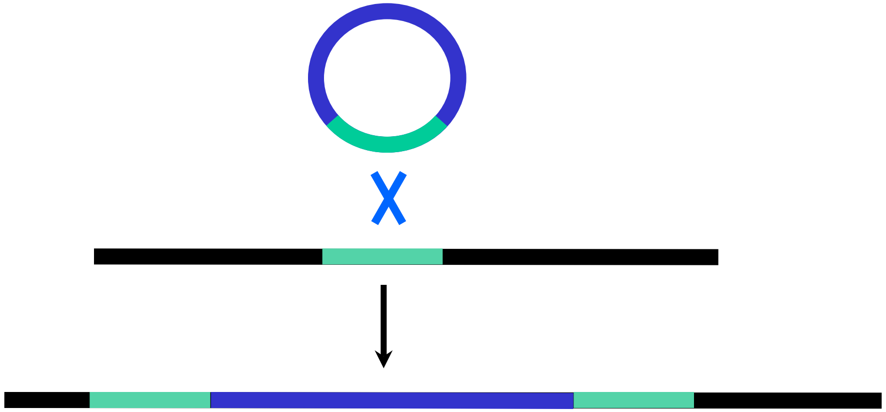


cut

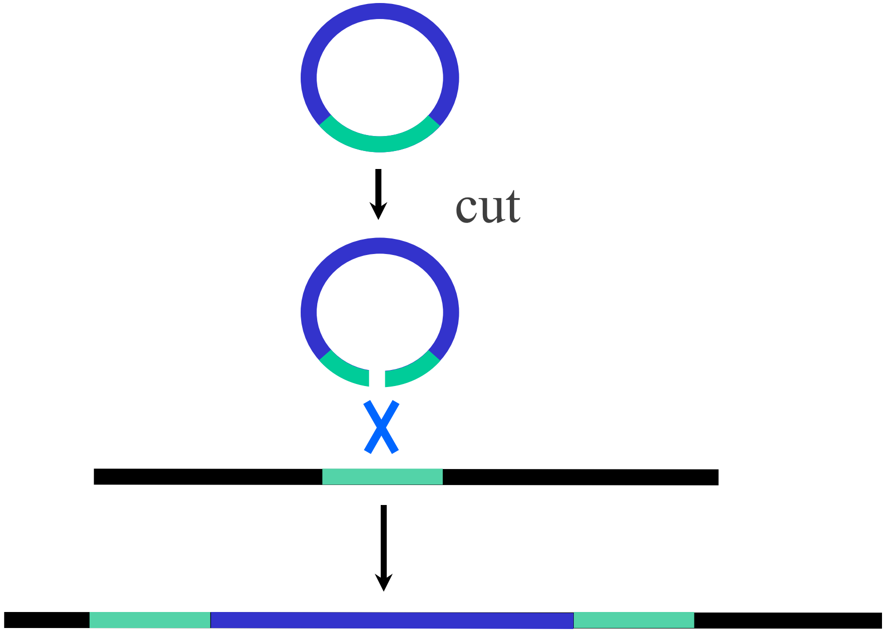
ligation,
+/- deletion

Orr-Weaver and Szostak, PNAS, 1983

Double-strand breaks in DNA stimulate recombination



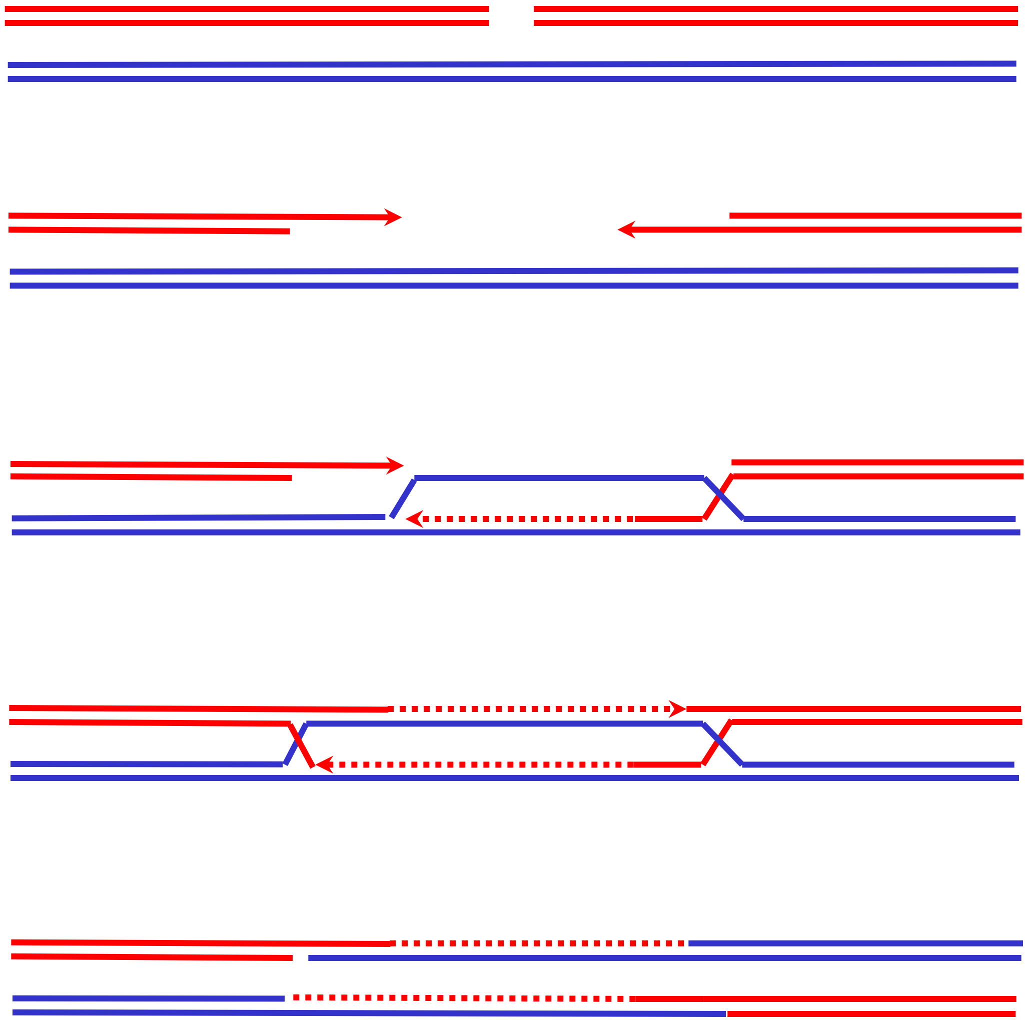
very few recombinants



abundant recombinants

Orr-Weaver et al., PNAS, 1981

Double-strand break repair model for recombination



double-strand
break

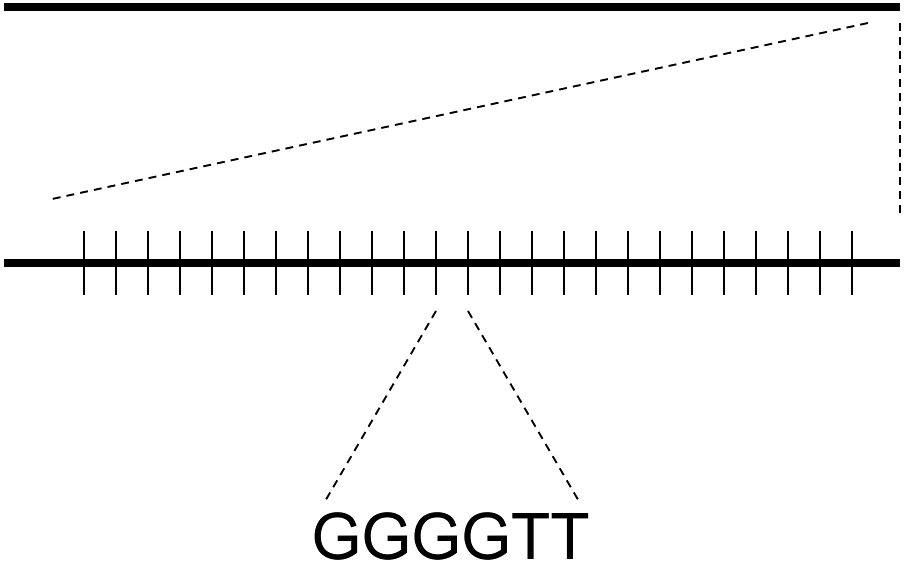
resection

strand invasion,
repair synthesis

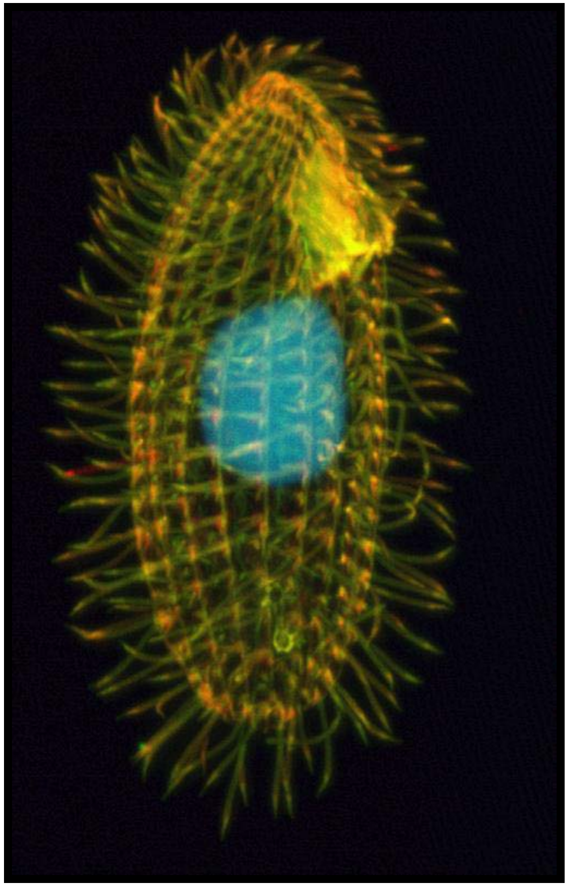
repair synthesis,
branch migration

Holliday Junction
resolution

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

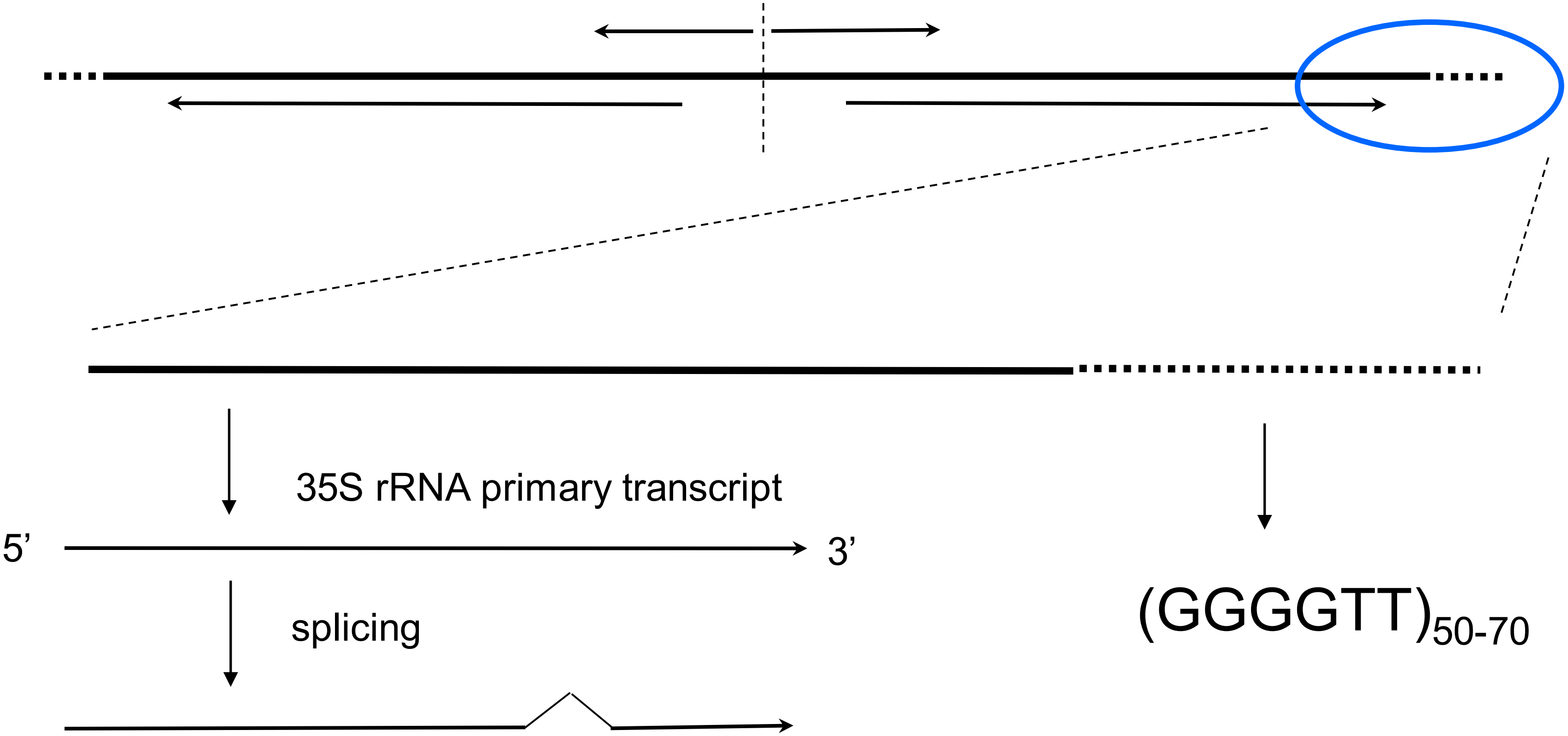


GGGGTT



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

A very special piece of DNA:



rDNA: high copy, symmetrical dimer

35S rRNA primary transcript

5’

3’

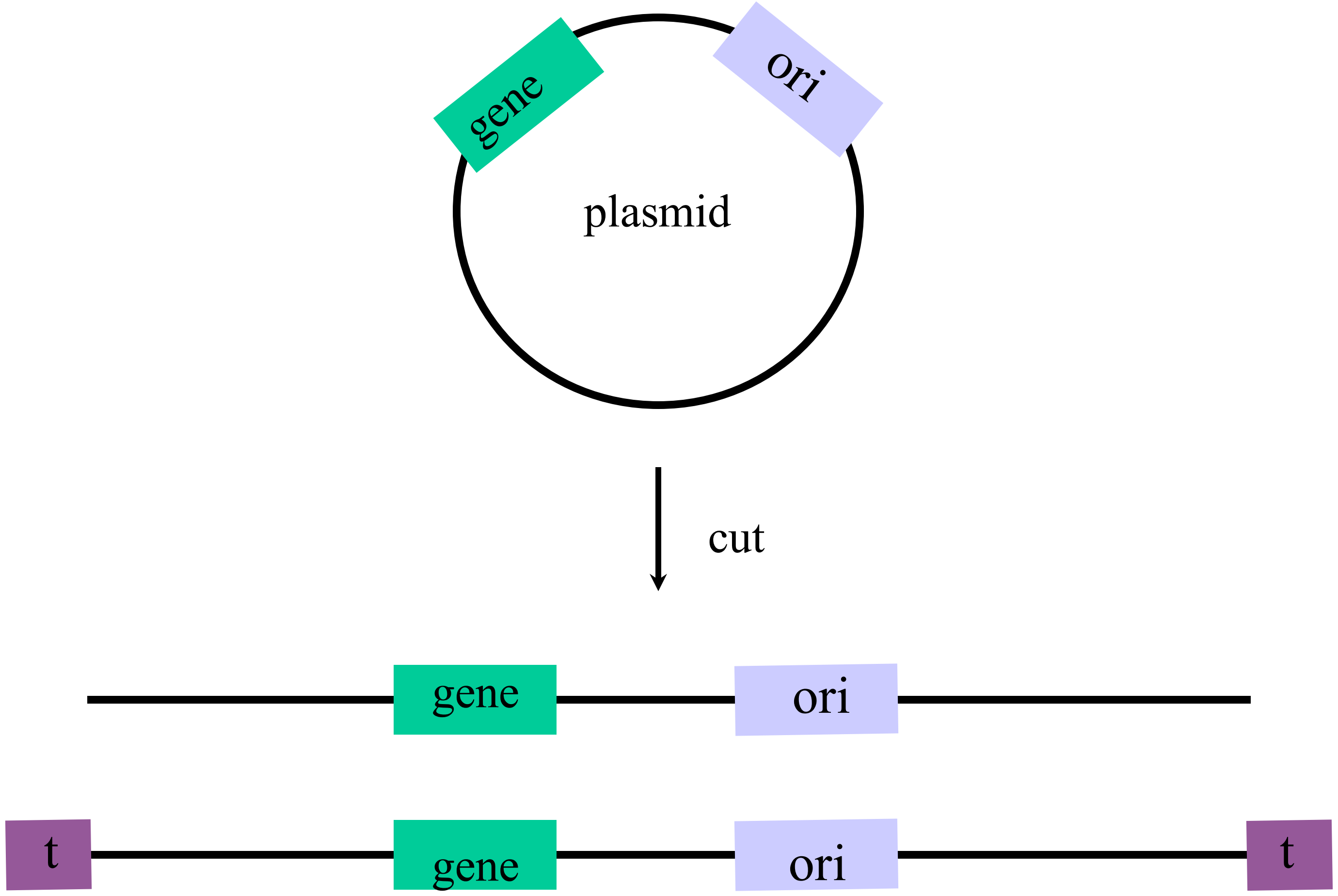
splicing

(GGGGTT)$_{50-70}$

Tetrahymena telomeres in yeast:

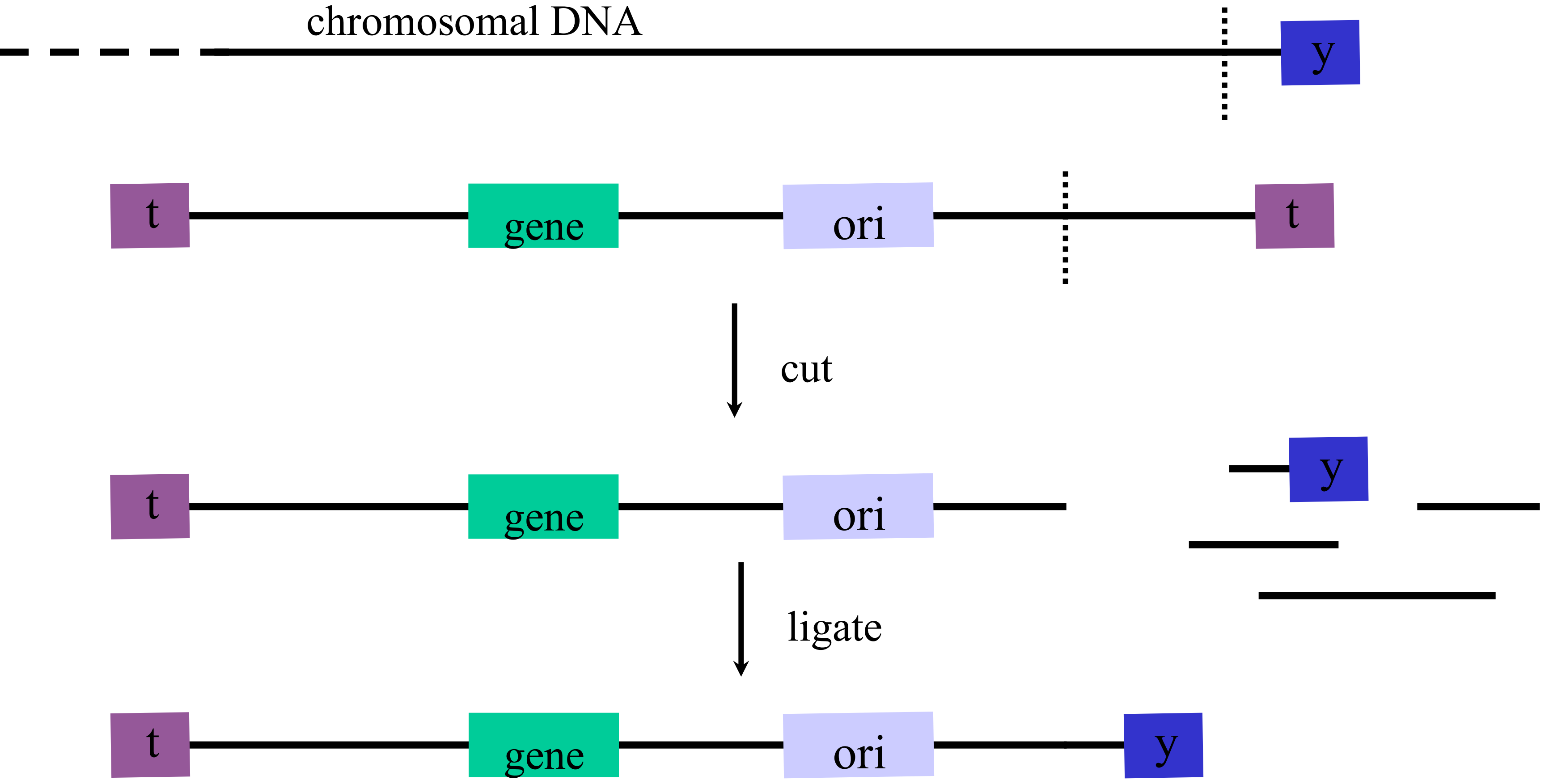
A collaborative study to ask whether the biochemistry of telomeres is widely conserved.

Moving *Tetrahymena* Telomeres into Yeast



Szostak and Blackburn, Cell 29: 245-255 (1982)

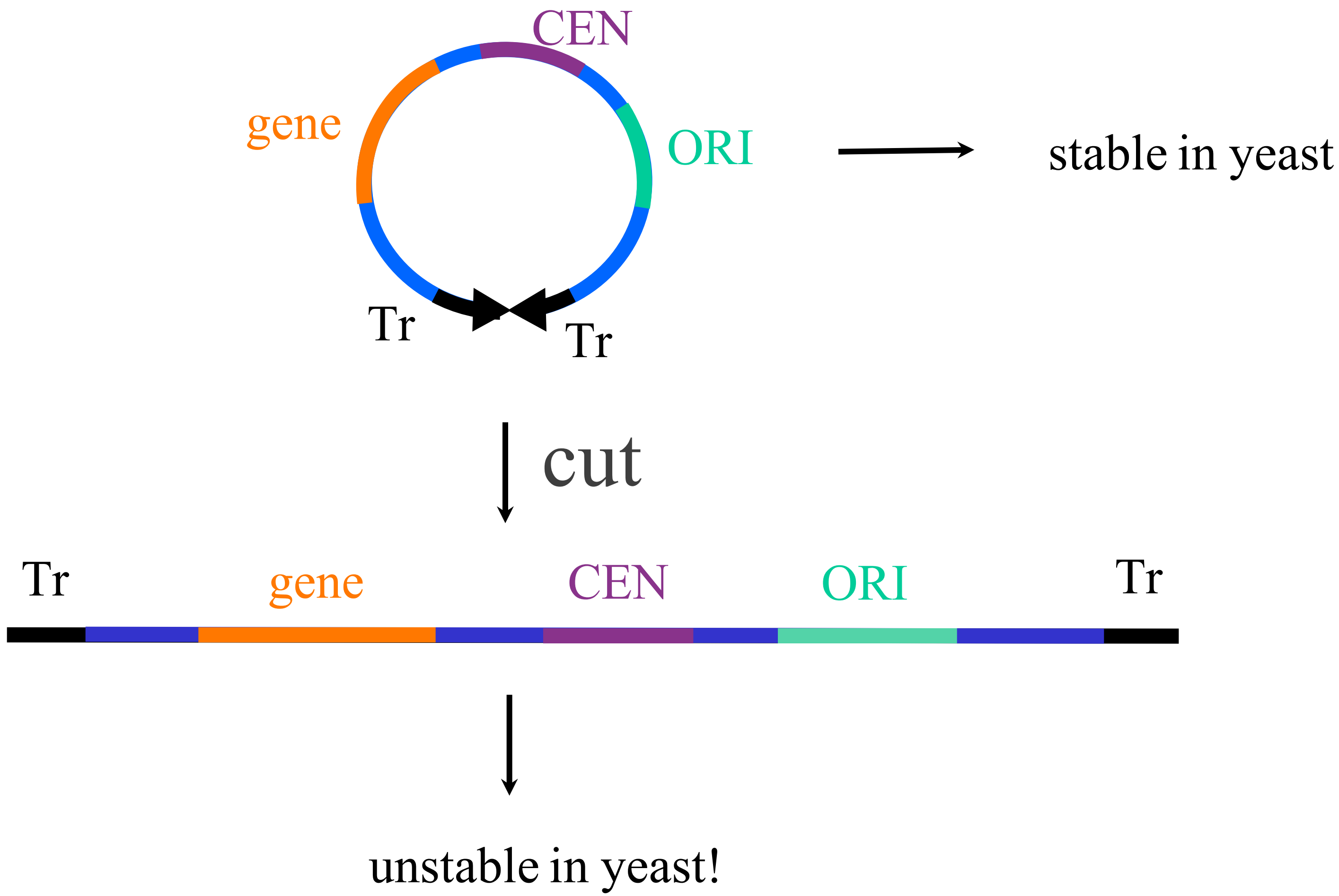
Cloning Yeast Telomeres



Szostak and Blackburn, Cell 29: 245-255 (1982)

A digression: Yeast Artificial Chromosomes

First attempt to make an artificial chromosome



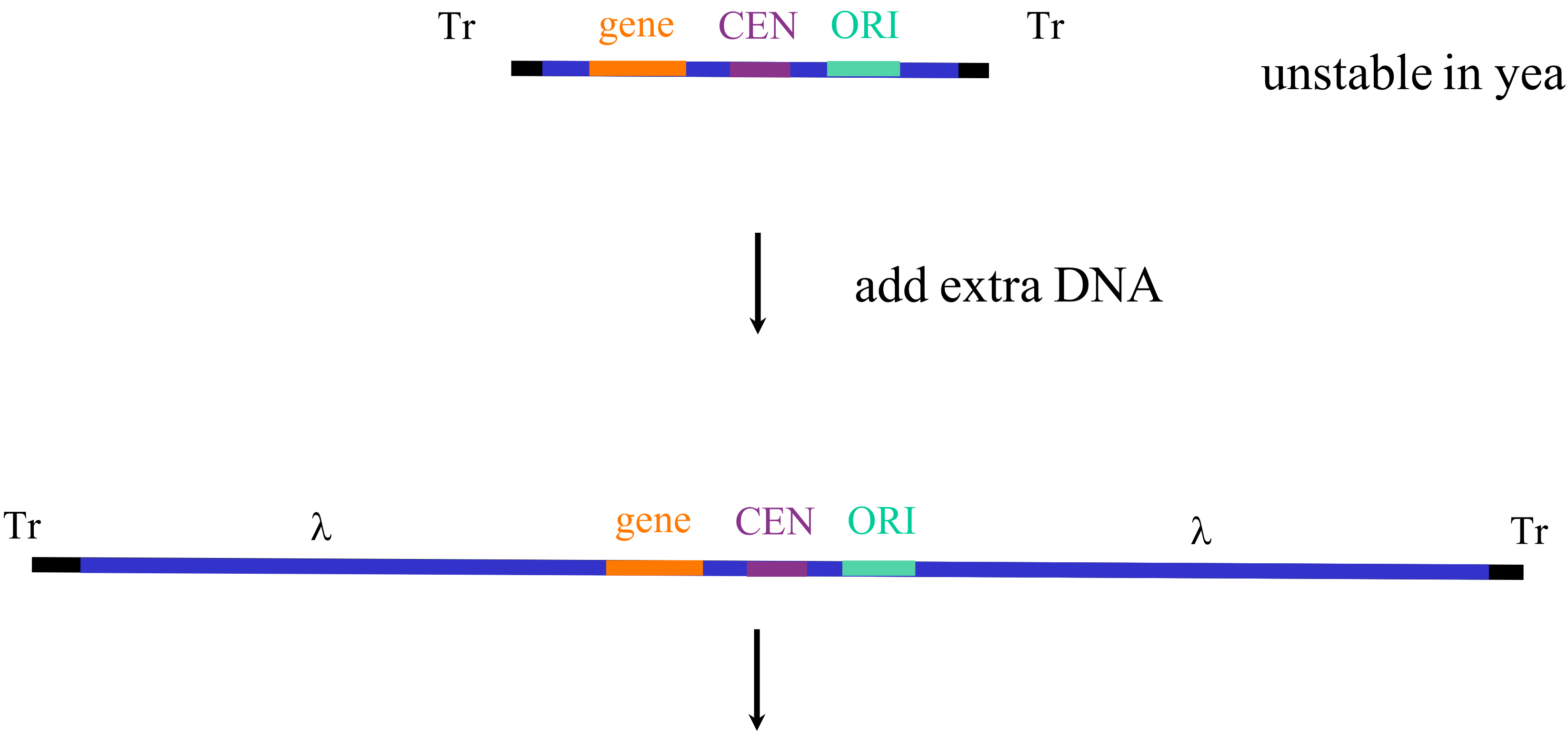
stable in yeast

cut

unstable in yeast!

Murray et al., Nature, 1983

Successful attempt to make an artificial chromosome



unstable in yeast

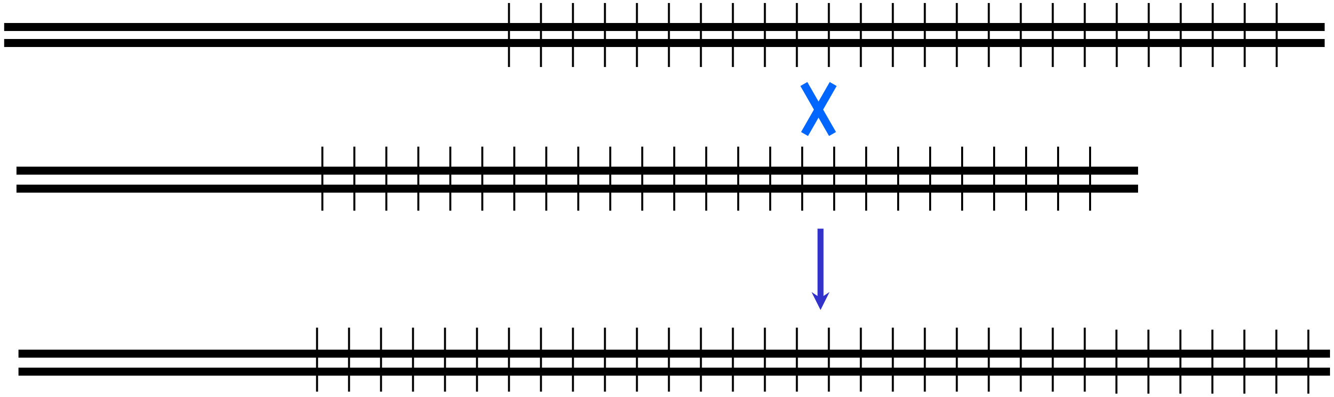
add extra DNA

stable in yeast

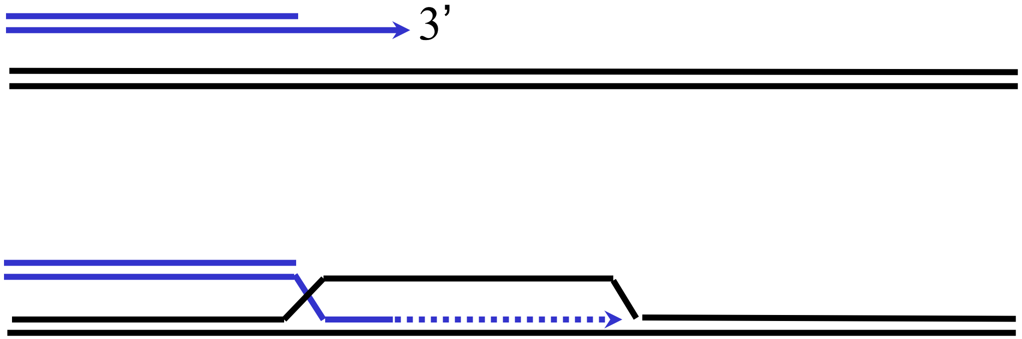
Murray et al., Nature, 1983

Recombination based models for telomere replication

Telomere Lengthening by Recombination



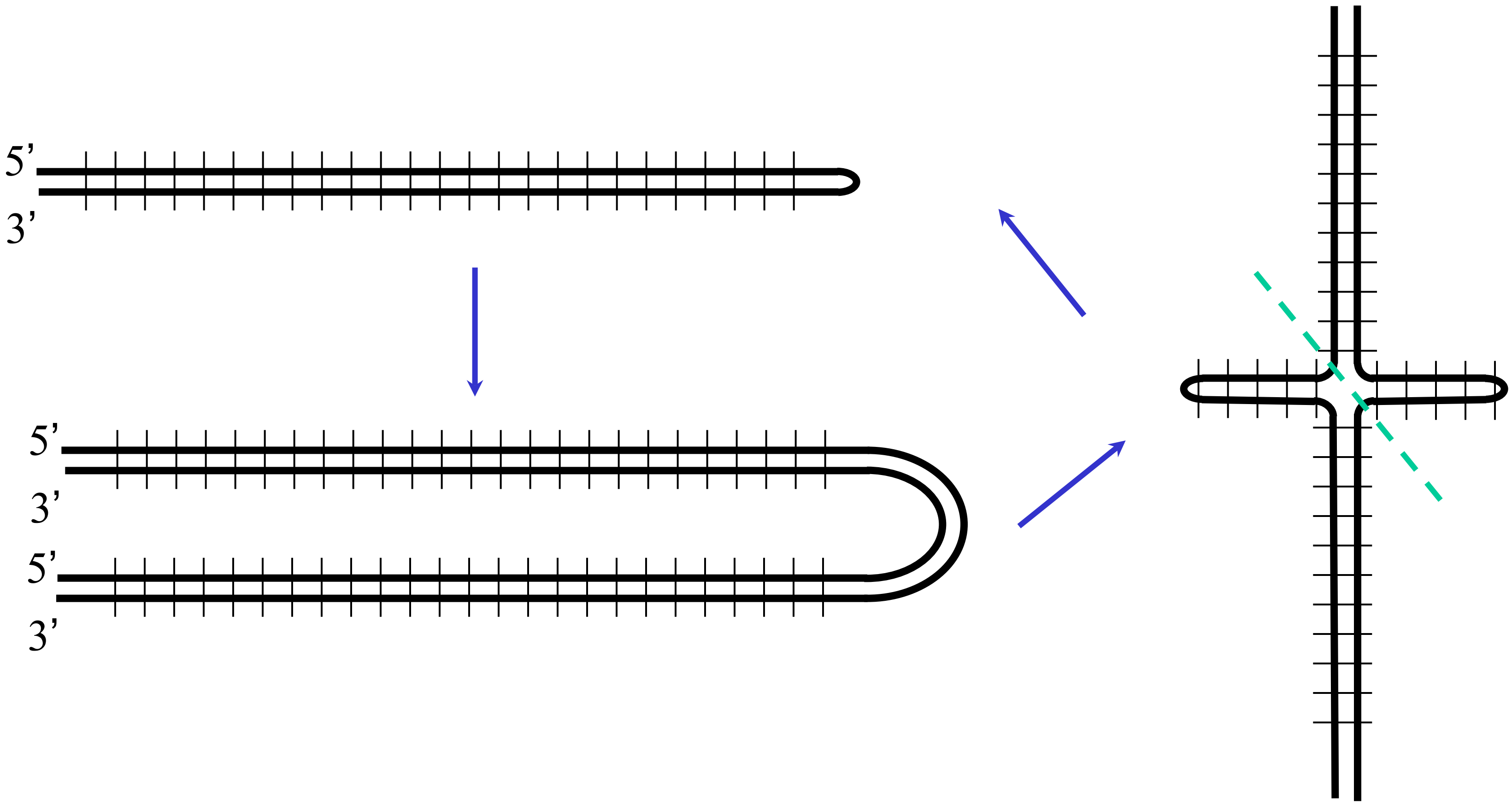
Telomere Lengthening by Repair Synthesis



incomplete replication
generates 3’ overhang

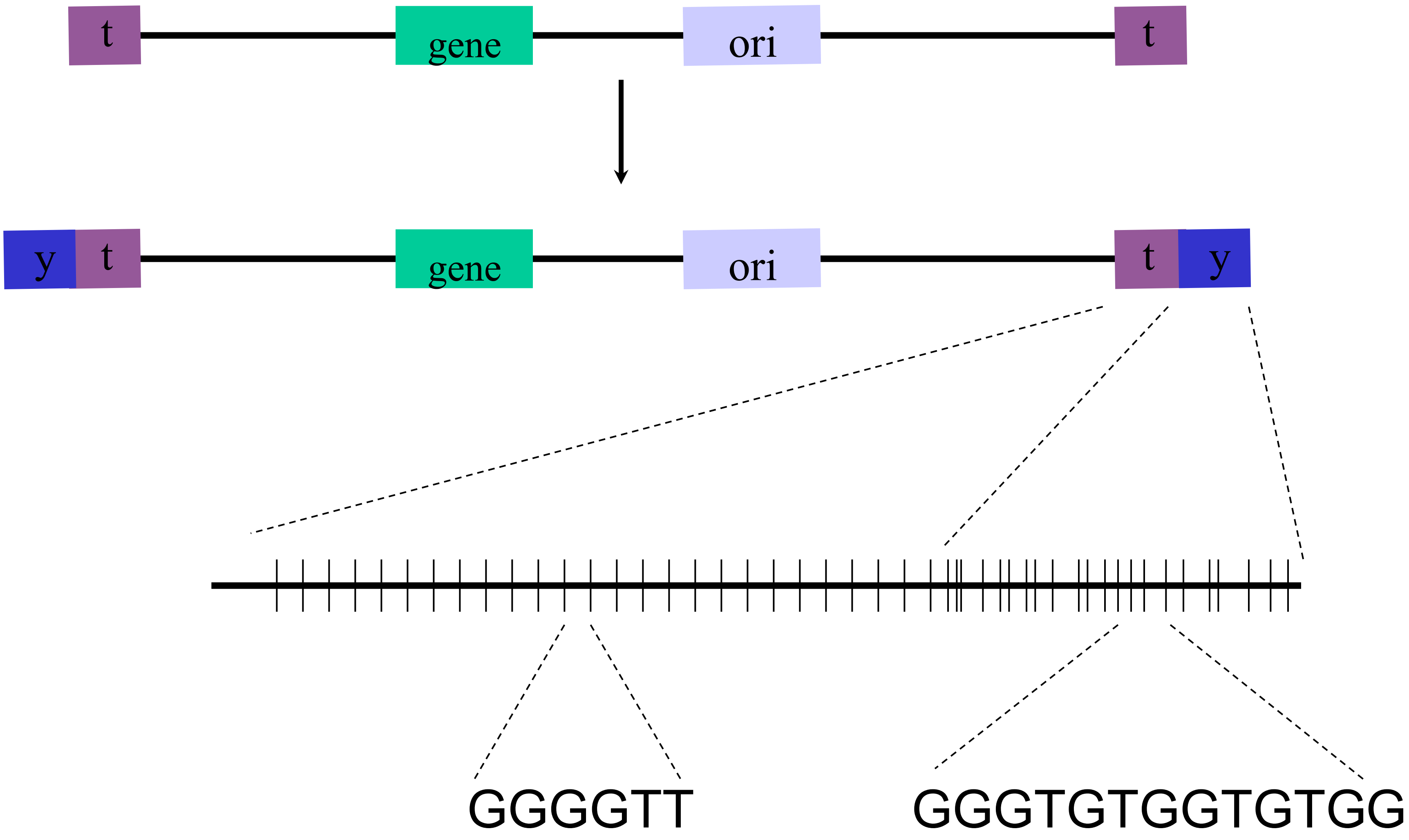
strand invasion allows
extension of 3’ overhang

Telomere Replication by Holliday Junction Resolution



telomere maintenance in yeast points to the correct solution

Yeast adds new DNA to *Tetrahymena* Telomeres



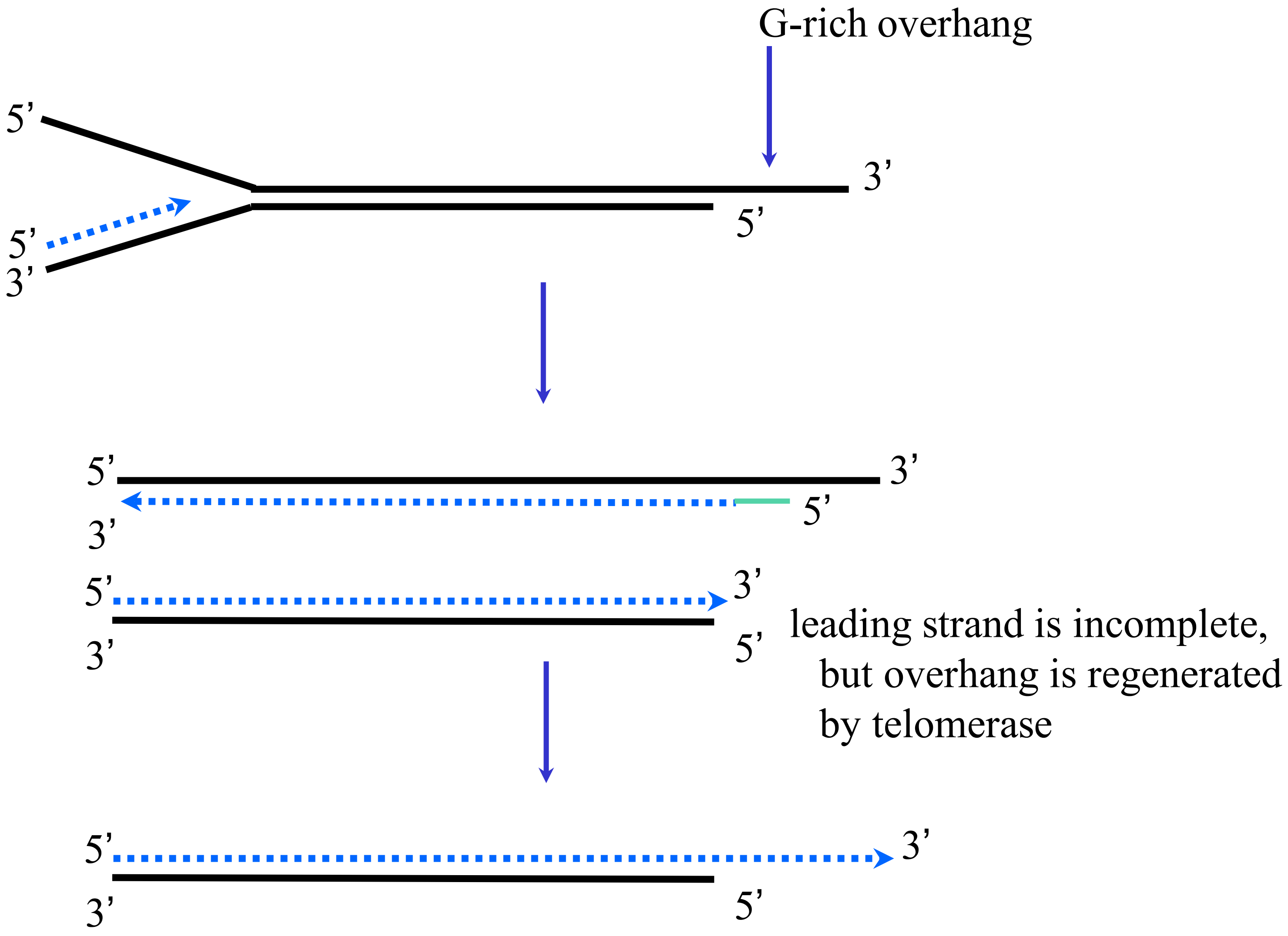
GGGGTT

GGGTGTGGTGTGG

Shampay et al., Nature 310: 154-157 (1984)

Correct Structure of Telomeric DNA Ends

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

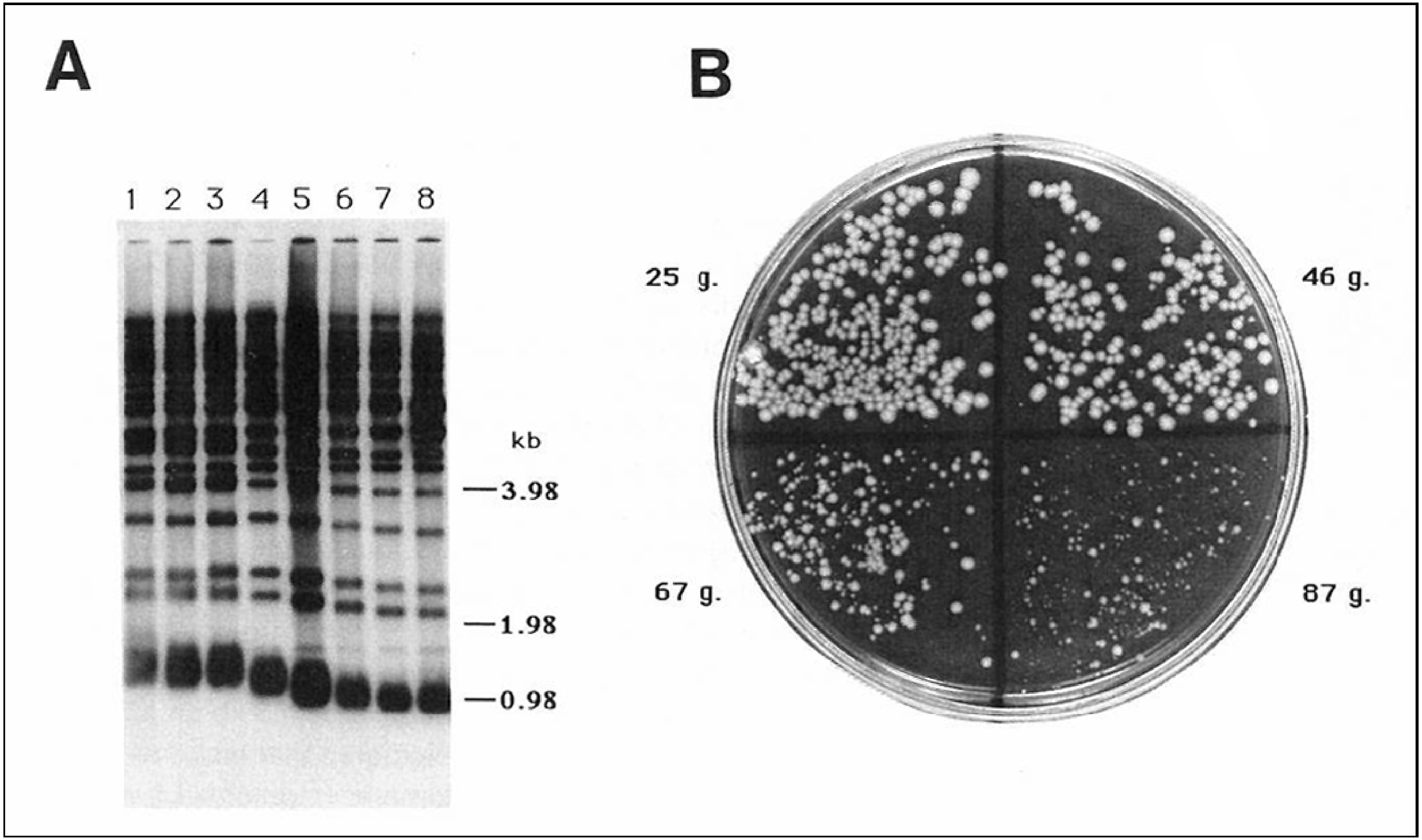


leading strand is incomplete,
but overhang is regenerated
by telomerase

Cells without telomerase
have limited division potential,

Cells with telomerase can
divide without limit.

Senescence of Yeast EST-1 Cells

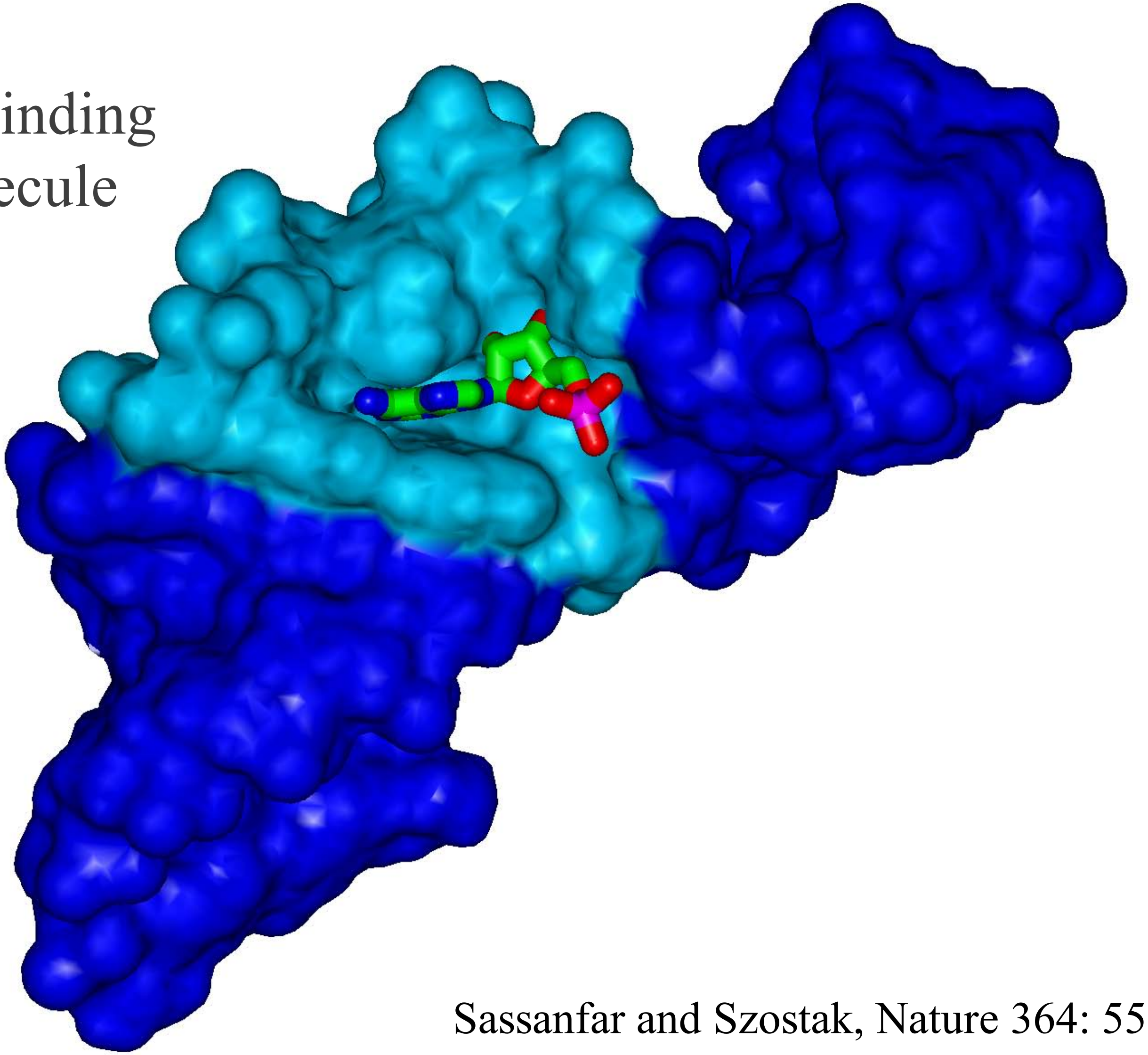


Lundblad and Szostak, Cell 57: 633-643 (1989)

After telomeres:

Directed Evolution of RNA and Protein

Laboratory Evolution of Aptamers

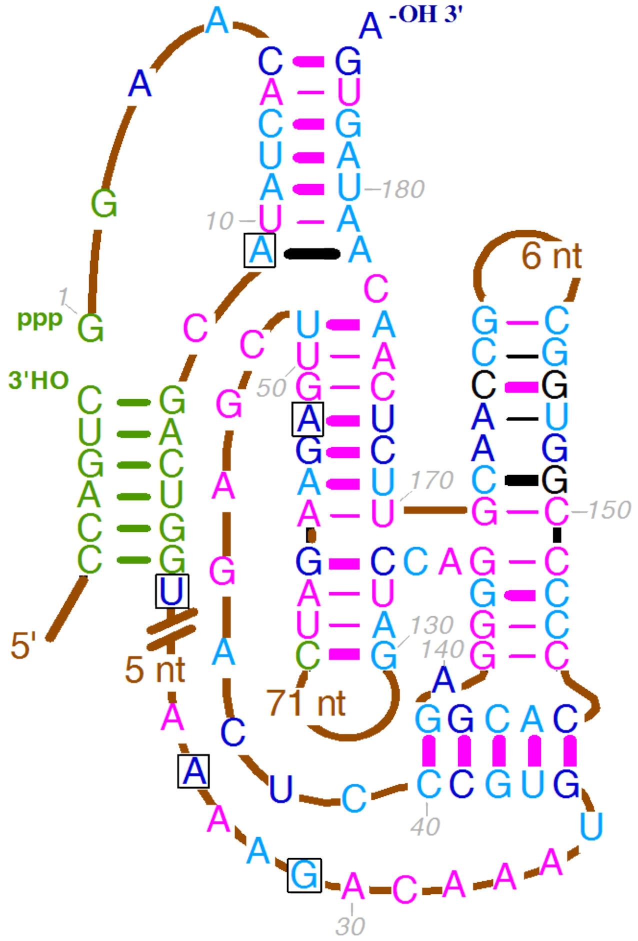


An ATP binding
RNA molecule

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

Class I Ribozyme Ligase

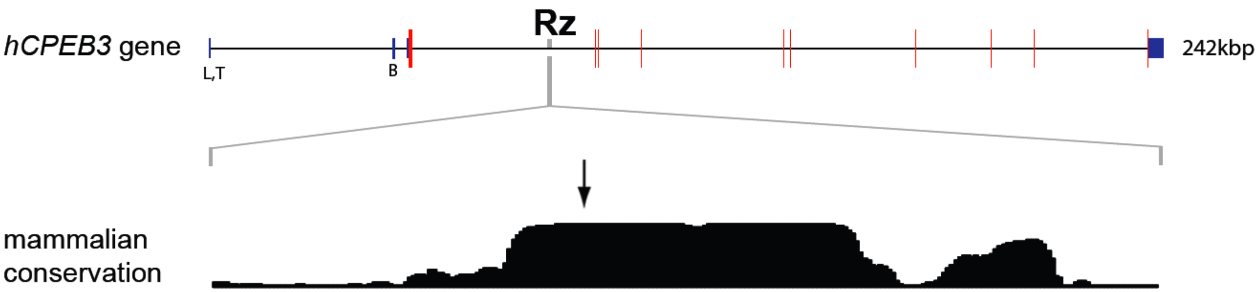
Fig. 5

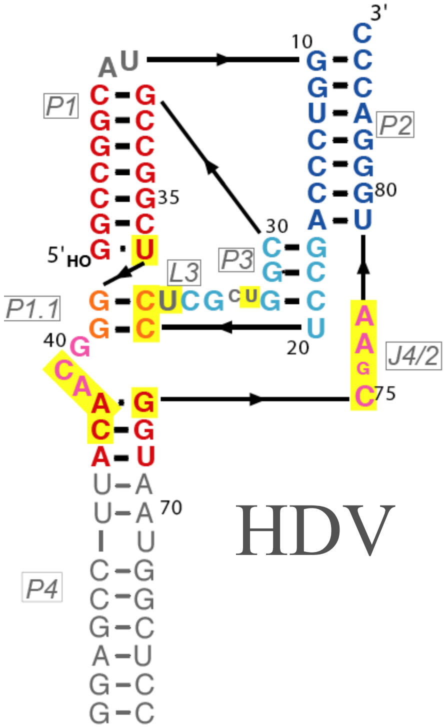


Bartel and Szostak, Science 261: 1411-1418 (1993)

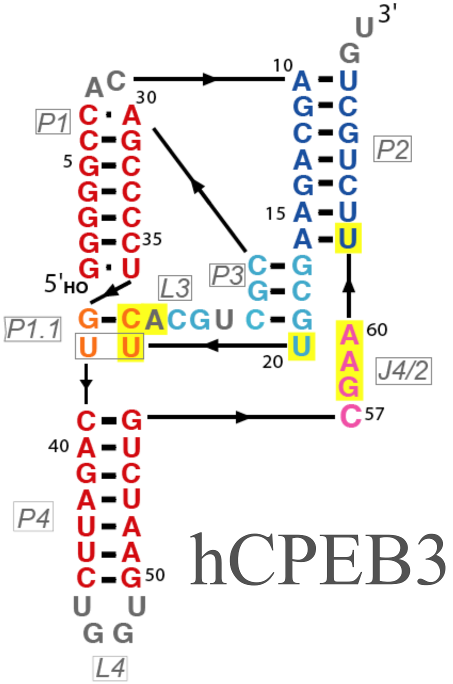
An HDV ribozyme in the human genome

CPEB3 ribozyme



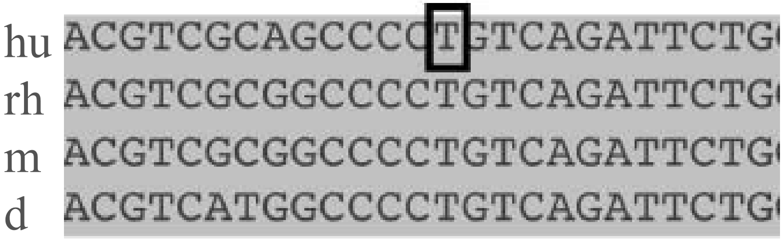


HDV



hCPEB3

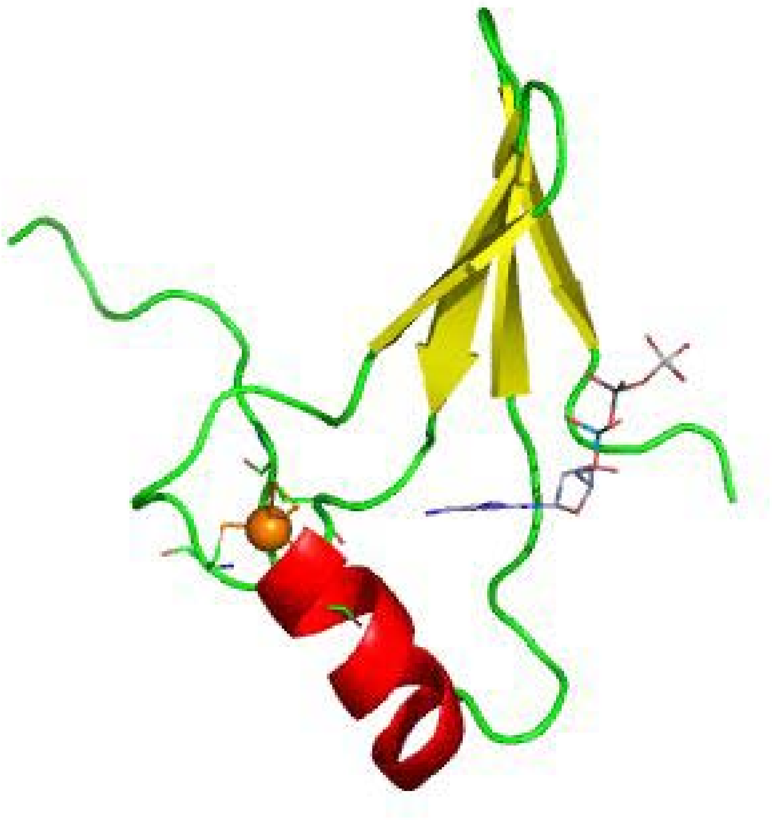
rs11186856
[T/C]



hu ACGTCGCAGCCCCTGTCAGATTCTG
rh ACGTCGCGGCCCCTGTCAGATTCTG
m ACGTCGCGGCCCCTGTCAGATTCTG
d ACGTCATGGCCCCTGTCAGATTCTG

Salehi-Ashtiani et al., *Science*, 2006

ATP Binding Protein



Current focus:

Origin of Life

Schematic Model of a Protocell

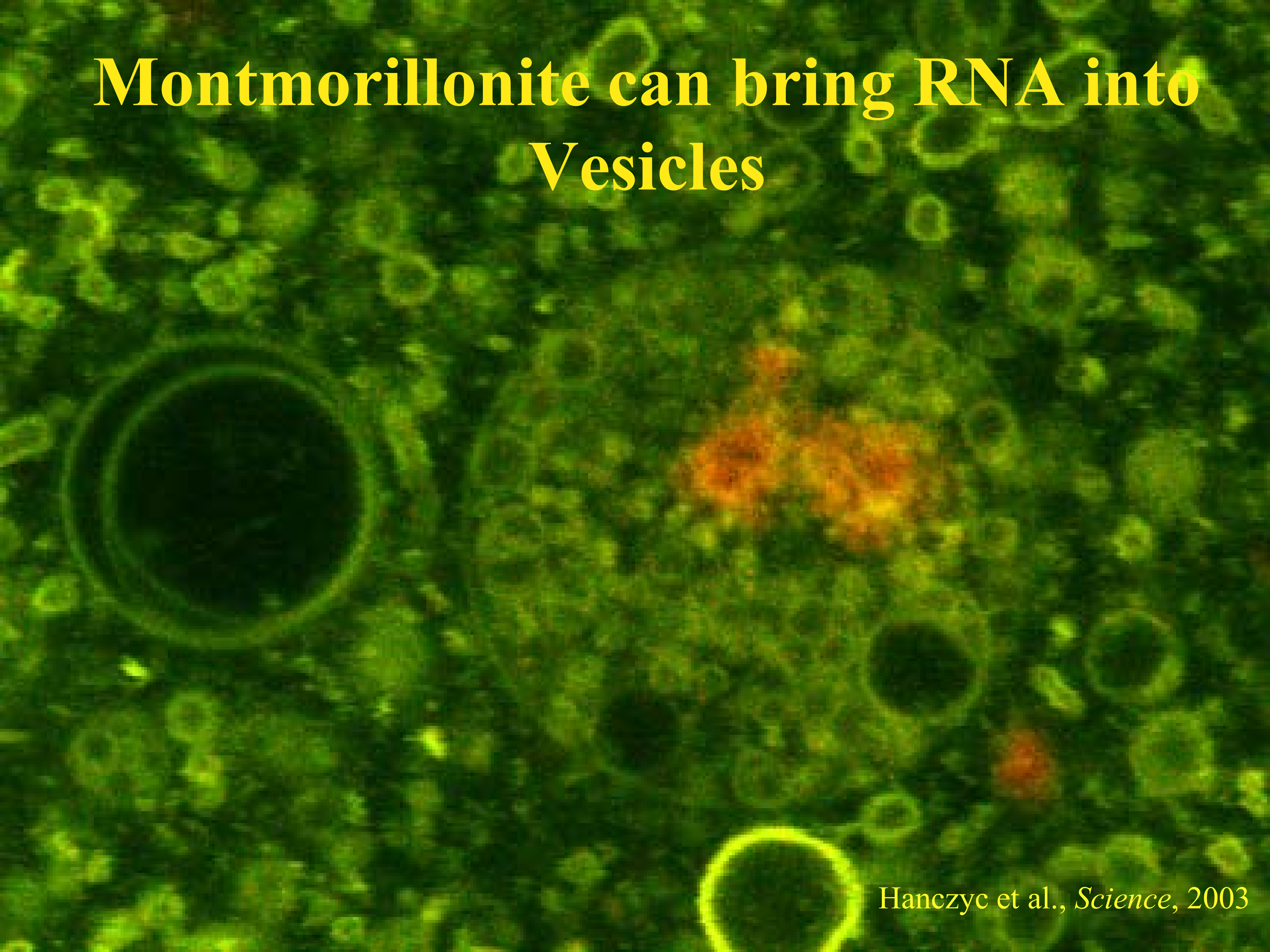
A simple cell might be based on a replicating vesicle for compartmentalization, and a replicating genome to encode heritable information. A complex environment provides nucleotides, lipids and various sources of energy.

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.

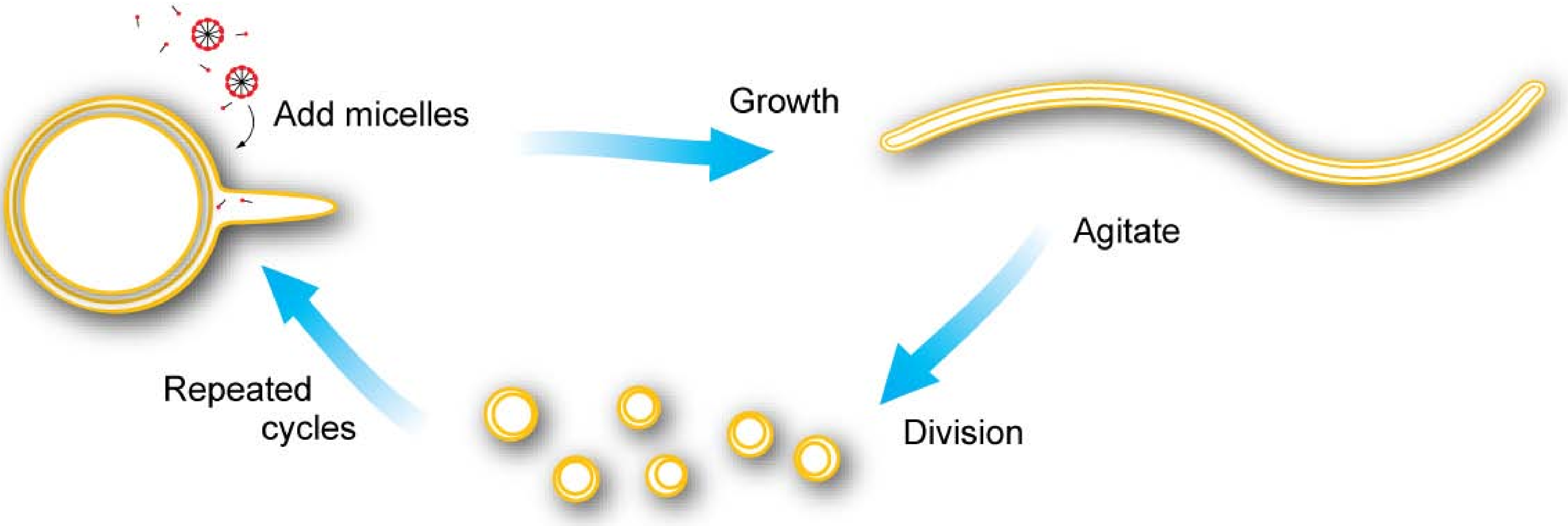
Mansy et al., *Nature*, 2008

Montmorillonite can bring RNA into Vesicles



Hanczyc et al., *Science*, 2003

Cycles of growth and division

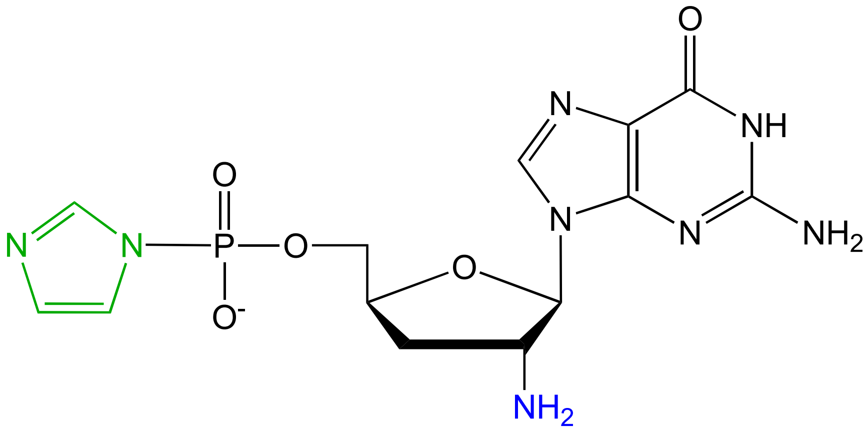


Zhu and Szostak, *JACS*, 2009

Self-Replicating Genetic Polymers

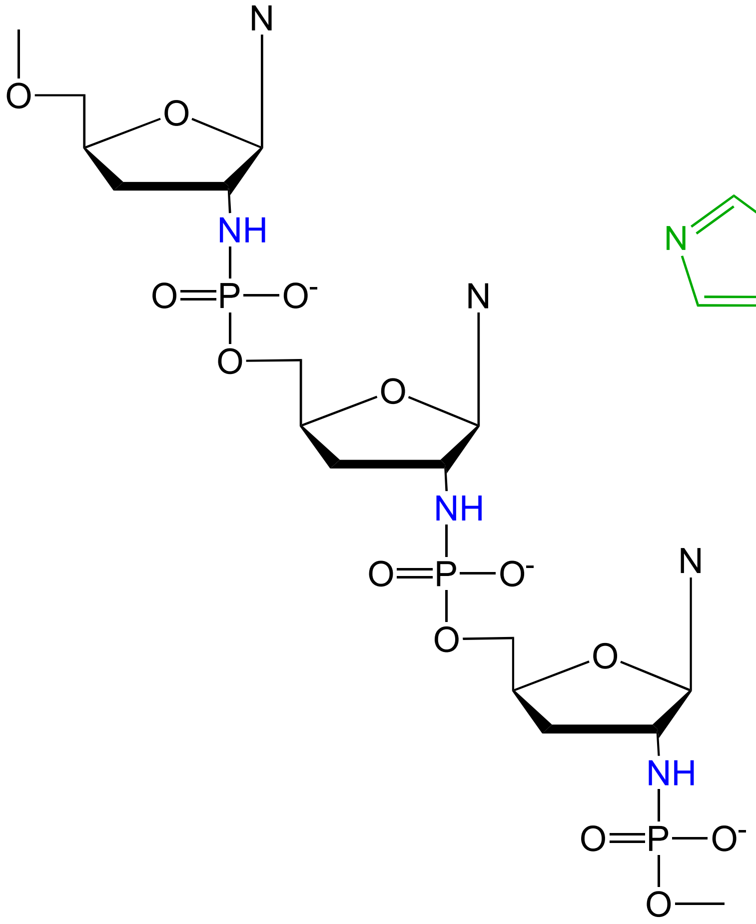
> *It seems likely that informational replication will be achieved in the next decade, and that it will throw new light on the origins of life.*
>
> *Leslie Orgel, 1992*

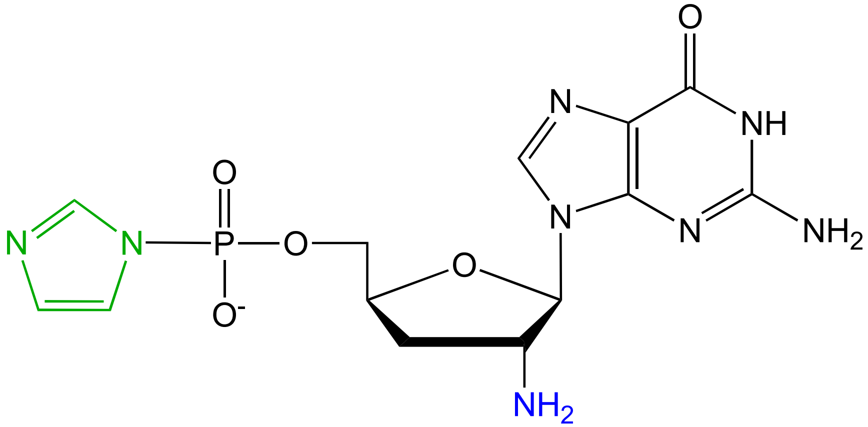
Typical monomer for spontaneous synthesis



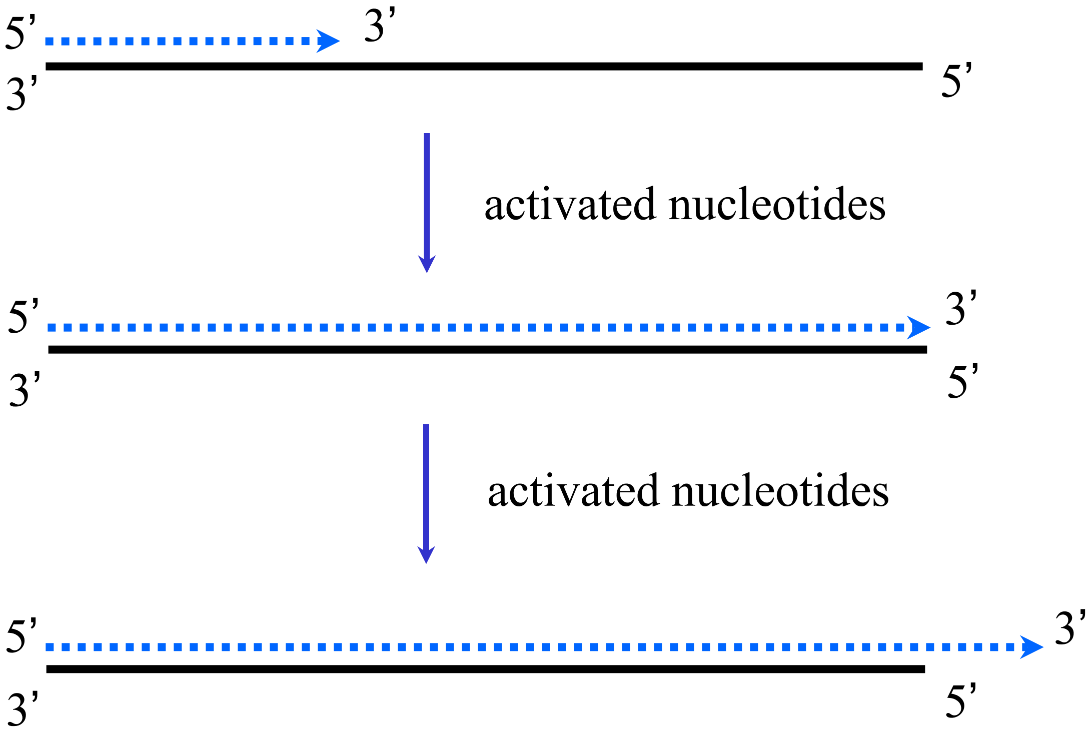
Typical monomer for spontaneous synthesis and corresponding polymer

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.