DNA Ends: Just the Beginning

Nobel Lecture

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Two Telomere Problems:

1. DNA ends are reactive
2. Incomplete Replication
Telomeres have been known to be special since the 1930s

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]"
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

Orr-Weaver and Szostak, PNAS, 1983
Double-strand breaks in DNA stimulate recombination.

Orr-Weaver et al., PNAS, 1981

Very few recombinants

Abundant recombinants

Orr-Weaver et al., PNAS, 1981
Double-strand break repair model for recombination

1. Double-strand break
2. Resection
3. Strand invasion, repair synthesis
4. Repair synthesis, branch migration
5. Holliday Junction resolution
Telomeres from *Tetrahymena*: stable DNA ends that are fully replicated

A very special piece of DNA:

rDNA: high copy, symmetrical dimer

35S rRNA primary transcript

(GGGGGTT)_{50-70}
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

chromosomal DNA

t gene ori t

cut

t gene ori

ligate

t gene ori y

A digression: Yeast Artificial Chromosomes
First attempt to make an artificial chromosome


stable in yeast

unstable in yeast!
Successful attempt to make an artificial chromosome

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

Correct Structure of Telomeric DNA Ends

G-rich 3’-overhang
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

After telomeres:

Directed Evolution of RNA and Protein
Laboratory Evolution of Aptamers

An ATP binding RNA molecule

An HDV ribozyme in the human genome

CPEB3 ribozyme

hCPEB3 gene

Rz

242 kbp

mammalian conservation

rs11186856 [T/C]

HDV

hCPEB3

Salehi-Ashtiani et al., Science, 2006
ATP Binding Protein
Current focus:

Origin of Life
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.

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

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?

activated nucleotides

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