

HISTORICAL PERSPECTIVE ESSAY

Plant Telomere Biology

Analysis of telomeres, the nucleoprotein complexes that physically cap and protect the ends of eukaryotic chromosomes, has a long and intriguing history. The recent resurgence of plant telomere biology prompted us to recap this history to provide background and context for current investigations addressing how plants maintain a stable genome. Although many of the fundamentals of telomere biology were first uncovered in ciliates or fungi, telomere research in *Arabidopsis* allows us to ask basic questions in a multicellular organism with complex development and excellent genetic tools. This powerful combination of advantages is unsurpassed in other organisms widely used in telomere biology. An important, but less tangible, benefit is a sense of continuity from extending work in a field started nearly 70 years ago by the visionary Barbara McClintock, a field that is still challenging, fruitful, and rewarding.

TELOMERES: THE EARLY YEARS

When condensed eukaryotic chromosomes are viewed in a light microscope, they are essentially linear structures with nothing to distinguish the ends from the rest of the chromosome. Therefore, early cytologists had no need for a specialized name for this part of the chromosome. The initial hint that the ends of chromosomes had special features came from analysis of the first artificially created mutants, *Drosophila* that had been treated with x-rays by Herman Muller in the late 1920s. Muller recovered many flies with a wide range of genetic abnormalities, including inversions, deletions, translocations, and other rearrangements resulting from the breakage and fusion of chromosomes, but he never found mutants with deletions or inversions involving the natural ends of the chromosomes. Muller summarized nearly a decade of his work in a classic lecture at the Woods Hole Marine Biological Laboratory (Muller,

1938) and concluded that "... the terminal gene must have a special function, that of sealing the end of the chromosome, so to speak, and that for some reason a chromosome cannot persist indefinitely without having its ends thus sealed." After explaining that one difference between the terminal gene and the others is that it is unipolar, with genes on only one side of it, Muller deduced that the bipolar genes in the interior of the chromosome "... cannot be made into properly functioning unipolar ones by the simple expedient of tearing them loose from their connections on one side" (Muller, 1938). Muller coined the term telomere for this terminal gene from the Greek, meaning simply "end part," but the fact that this region of the chromosome deserved a specific name was a recognition that something unusual was going on there.

In the 1930s, at the height of the Great Depression, Barbara McClintock was working as a cytogeneticist in the Department of Botany at the University of Missouri, where she was interested in characterizing maize chromosomes that had been broken in vivo. To create a system in which she could examine many broken chromosomes, McClintock crossed a line containing a rearranged chromosome 9 with the wild type. The mutant chromosome, which contained a segment translocated from one side of the centromeres to the other, was induced by x-rays and originally isolated by Harriet Creighton, McClintock's former graduate student at Cornell. A crossover between the mutant and wild-type chromosomes in the translocated region during prophase I of meiosis would lead to a dicentric chromosome and an acentric fragment (Figure 1). The two centromeres on the dicentric chromosome could be pulled toward opposite spindle poles during anaphase and telophase, creating the appearance of a chromatin bridge across the growing divide between the two sets of chromosomes as they receded from each other.

McClintock knew from her previous studies that these chromatin bridges eventually snapped, and the sister chromatids then fused to recreate a dicentric chromosome (McClintock, 1938). The question she was asking now was whether this chromosome breakage-fusion-bridge cycle would continue indefinitely. To answer the question, McClintock placed her dicentric-generating chromosome 9 in a background with a normal chromosome 9 carrying recessive alleles for plant color (*yellow green*, *yg*) and seed characteristics (*waxy endosperm*, *wx*; *colored aleurone*, *c*; and *shrunkened endosperm*, *sh*) in the region between the two centromeres. Any breakpoint in this part of the chromatid would lead to the loss of the dominant allele in one of the daughter cells, uncovering the recessive allele (Figure 2). In this way, McClintock was able to follow the breakage-fusion-bridge cycle visibly in many seeds and plants (McClintock, 1939). She found that the endosperm contained sectors of various sizes where the recessive alleles were expressed, suggesting that the breakage-fusion-bridge cycle continued throughout development of this tissue. By contrast, neither the embryos nor the plants that grew from them ever displayed any variegation for the *yg* allele. Some seedlings did express the *yg* allele, indicating that the dominant allele had been lost early in development, but none of the fully green plants ever showed *yg* sectors. McClintock interpreted this lack of variegation to mean that chromosome breakage and loss of the dominant allele occurred in the gametophyte, but if the zygote inherited the dominant allele, it was never lost during subsequent development of the embryo or sporophyte. Although the zygote initially contained the dicentric-generating chromosome, once it broke after fertilization, the newly formed ends behaved like the natural chromosome ends that were never joined together. McClintock called the phenomenon that halted the breakage-fusion-bridge

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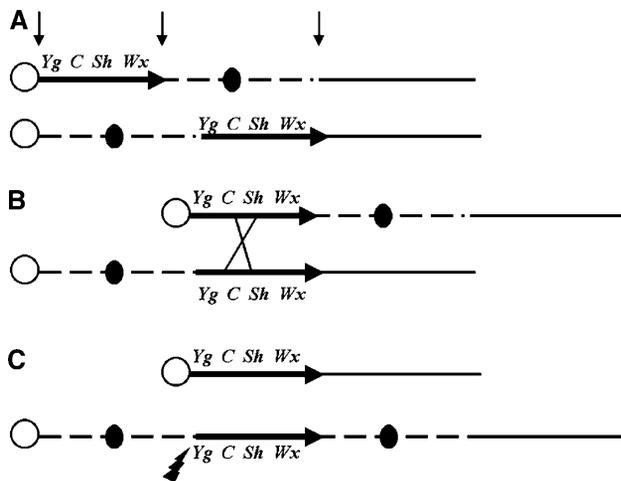


Figure 1. McClintock's Generation of Dicentric Chromosome 9 in Maize.

(A) The top line represents wild-type chromosome 9 with relevant genes indicated near the left end. The open circle represents a terminal heterochromatic knob, the closed oval represents the centromere, and the three arrows indicate sites of breakage by x-rays. The bottom line represents the rearranged chromosome after breakage and ligation of the three segments. The horizontal arrows and dashed lines are used here to mark rearranged segments and do not correspond to cytological features indicated in McClintock's original drawings.

(B) Alignment and crossing over of wild-type and mutant chromosome 9.

(C) Acentric fragment and dicentric chromosome resulting from the recombination shown in **(B)**. The centromeres on the dicentric chromosome would be pulled to opposite spindle poles during anaphase. Breakage at the position marked on the lower chromosome will produce the chromosome shown at the top of Figure 2.

cycle in the embryo "chromosome healing" (McClintock, 1941).

In 1944, McClintock was elected to the National Academy of Sciences for her pioneering cytogenetic work, including confirmation of the chromosomal basis of heredity by demonstrating a direct relation between chromosomal crossing over and genetic recombination (Creighton and McClintock, 1935). In the summer of 1944, McClintock decided to use repeated breakage-fusion-bridge cycles to create deletions throughout the short arm of chromosome 9 (McClintock, 1987). She knew that chromosome breakage and loss of genes would occur in the gametophytes, and if the resulting mutants were viable, the chromosome ends would be healed in the embryo. Expression of genes on these healed chromosomes should be stable in the plant, at least until the next gametophytic generation. McClintock did recover many such stable mutants, but in contrast

with predictions from known patterns of chromosome healing, a few of the mutant seedlings showed variegation similar to what she had previously seen only in the endosperm (McClintock, 1946). These unexpected pigment sectors in the sporophyte intrigued McClintock, and she spent the rest of her career analyzing this curious form of genetic instability. Although this new area of research eventually led to the discovery of transposable elements, for which she was awarded the Nobel Prize 40 years later, her talents were lost to telomere biology.

Barbara McClintock's life and career are documented in the recent biography *The Tangled Field* (Comfort, 2001). Many of McClintock's papers have been collected from sometimes obscure sources and reprinted in *The Discovery and Characterization of Transposable Elements* (McClintock, 1987), a compendium that charts the amazing progress she was able to make using

only simple techniques and a complex mind.

THE CRUCIAL QUESTION

The year 1944, when McClintock became distracted by transposable elements and abandoned telomeres, was the same year that Avery, MacLeod, and McCarty demonstrated that DNA was the genetic material. It would be a very long time before questions of telomere biology could be addressed at the molecular level, so perhaps it was just as well that McClintock left telomeres behind. Very little research on telomeres was done for the next three decades. Nevertheless, all of the great strides in molecular biology during this time—confirmation of DNA as the genetic material, discovery of DNA structure, elucidation of the mechanisms of semiconservative replication, development of DNA cloning techniques, and toward the end of this period, invention of methods for rapidly sequencing DNA by Fred Sanger and independently by Walter Gilbert and Alan Maxam—set the stage for the next, explosive era of telomere biology.

However, there was one key development in telomere biology in this otherwise quiescent period. This development was not an answer to a problem, but rather the definition of what was to become the crucial problem for the field. In the early 1970s, after mechanisms of DNA replication had been elucidated, both Alexey Olovnikov (1971, 1973) and Jim Watson (1972) independently described the end replication problem. The problem is that semiconservative DNA replication requires an RNA primer for initiation, and the lagging strand requires one primer to start each Okazaki fragment. Even if an RNA primer can be placed at the very 3' end of the chromosome replicated as the lagging strand, this primer eventually will be lost because no distal Okazaki fragment can be extended through it. The loss of 10 or 12 nucleotides (or more, if the last primer is not positioned at the very end) at each cell division should pose major problems for long-lived multicellular eukaryotes, such as humans, and for all eukaryotes over the course of a few generations. Indeed,

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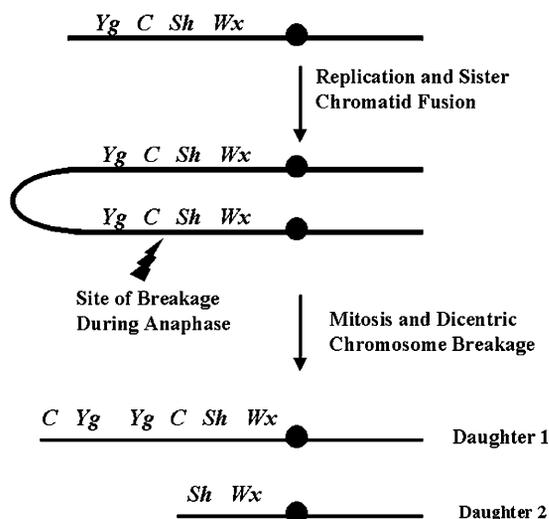


Figure 2. Loss (and Duplication) of Genetic Material during the Breakage-Fusion-Bridge Cycle.

The chromosome at the top of the figure resulted from breakage of a dicentric chromosome 9 as indicated in Figure 1. Replication of this chromosome, followed by sister chromatid fusion, will produce the dicentric chromosome in the middle of the panel. Breakage at the position indicated will cause the loss of dominant *Yg* and *C* alleles in one of the daughter cells, uncovering expression of recessive alleles carried on a normal chromosome 9. The other daughter cell will inherit a chromosome where the *Yg* and *C* alleles are further duplicated. McClintock followed propagation or cessation of the breakage-fusion-bridge cycle by analyzing expression of recessive alleles in seeds (*c*, *sh*, and *wx*) or plants (*yg*).

Olovnikov's "A Theory of Margintomy" made the remarkably prescient prediction that the loss of terminal sequences resulting from the end replication problem would lead to senescence (Olovnikov, 1971, 1973).

There are various solutions to the end replication problem. For example, bacteria eliminate DNA ends using circular chromosomes, and many bacteriophages concatenate their genomes so only the copies at the end face the problem. Clearly, eukaryotes also had solved the end replication problem to fully duplicate their linear chromosomes, but how?

TELOMERE DNA

The answer to this question originated in a serendipitous finding on an unrelated project. Elizabeth Blackburn learned how to sequence DNA as a graduate student working with Fred Sanger at Cambridge in the mid 1970s. She then joined Joe Gall's group at Yale as a postdoc where her project was to sequence the rRNA genes

from the macronucleus of *Tetrahymena*. Like other ciliates, *Tetrahymena* possesses two nuclei, a small diploid micronucleus and a highly polyploid macronucleus. The *Tetrahymena* macronuclear genome contains $\sim 10^5$ subchromosomal linear DNA molecules, with each gene amplified to ~ 50 copies. The rDNA, encoding rRNAs, is amplified even further to 10,000 copies. A relatively pure population of macronuclear rDNA molecules can be isolated as a satellite band by buoyant-density centrifugation in CsCl, a major advantage in the days before molecular cloning techniques were widespread. When Blackburn applied the newly developed DNA sequencing techniques to the rDNA fragments, she found that they ended in tandem copies of the 6-nucleotide sequence TTGGGG (Blackburn and Gall, 1978). Other reports rapidly followed, showing that macronuclear DNA fragments from hypotrichous ciliates also end in copies of the related sequence TTTTGGGG (Oka et al., 1980; Klobutcher et al., 1981). The discovery of simple repeats at the ends of yeast chromosomes

(Shampay et al., 1984) confirmed that the natural ends of larger eukaryotic chromosomes had a similar structure. Presumably, these short simple repeats somehow defended chromosomes against the end replication problem and other assaults on their integrity.

In 1988, Eric Richards, then a graduate with Fred Ausubel at Harvard, cloned telomeric DNA from *Arabidopsis* and found that it consisted of TTTAGGG repeats (Richards and Ausubel, 1988). Later that same year, the related hexanucleotide TTAGGG was identified as the human telomere repeat (Moyzis et al., 1988). The *Arabidopsis*-type telomere has since been found in most angiosperms, but several reports indicated that this sequence is absent in onion (*Allium cepa*) and related species (Pich et al., 1996). Apparently, this sequence was altered or lost during the rise of the Asparagales, a large monocot order including onions and >10% of all angiosperms. Many species within Asparagales possess the 6-base repeat TTAGGG (coincidentally the same sequence found in mammalian telomeres) rather than the *Arabidopsis*-type 7-base repeat TTTAGGG (Adams et al., 2001). Telomeres of other Asparagales species may possess other variant repeats, including TTGGGG, as judged from slot blots of total genomic DNA, but onion DNA did not hybridize to any of the probes tested, leaving the sequence of their telomeric DNA still unknown (Sykorova et al., 2003).

One of the more surprising developments in telomere structure was the discovery by collaborative efforts from Jack Griffith's and Titia de Lange's groups that mammalian telomeres looped back on themselves to form large lariat-like structures, called t-loops (Griffith et al., 1999). Although it was long known that the 3' end of the G-rich strand protrudes past the C-rich strand (Klobutcher et al., 1981; Henderson and Blackburn, 1989), electron micrographs demonstrated that this single-stranded region reaches back to invade the duplex portion of the telomere with the help of TRF2, a telomere DNA binding protein (Griffith et al., 1999). This structure may help to conceal the end of the molecule from DNA damage surveillance

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mechanisms. Since this initial report, t-loops have been visualized in other organisms, including plants (Figure 3) (Cesare et al., 2003).

Ironically, telomeric DNA of *Drosophila*, the organism that started it all, is not composed of simple repeats. Instead, telomeres in the fruit fly consist of two types of retrotransposons that preferentially insert themselves onto the ends of chromosomes (Biessmann et al., 1990).

TELOMERE BINDING PROTEINS

Telomeric DNA is coated with specialized proteins that work in concert with the t-loop to protect the chromosome terminus and guard against recognition of the chromosome terminus as a double-strand break (Figure 4). Again, studies in ciliated protozoa opened this avenue of research. Telomere-specific proteins were identified from hypotrichous ciliates in the 1980s, where their remarkable abundance and highly salt-stable interaction with DNA allowed them to be directly purified while still associated with the single-stranded terminus of the telomere (Gottschling and Zakian, 1986; Price and Cech, 1987). Cdc-13, a protein with similar properties, was subsequently found in budding yeast by Vicki Lundblad's group (Nugent et al., 1996). Almost 15 years after characteriza-

tion of end binding proteins in ciliates, Peter Baumann and Tom Cech identified homologs from multicellular eukaryotes, including *Arabidopsis* (Baumann and Cech, 2001; Baumann et al., 2002). This delay was a consequence of the poor sequence conservation of single-strand binding telomere proteins: only with the advent of genome sequencing projects could they be discerned.

In yeast, RAP1p was also found to bind the double-strand region of the telomere (Berman et al., 1986; Longtine et al., 1989). The role of RAP1p was initially quite ambiguous because it was shown to function both in transcriptional regulation and in telomere biology. In the early 1990s, Titia de Lange's group used a biochemical approach to purify TRF1, a mammalian protein specifically associated with double-stranded telomeric DNA (Zhong et al., 1992). The myb-like DNA binding motif that defines this class of molecules was later exploited to find additional telomere binding proteins (König et al., 1996). Over the past few years, a variety of different activities from plant extracts have been reported to bind double-stranded telomeric DNA (reviewed in McKnight et al., 2002). However, no studies have been conducted to confirm the role of these proteins in vivo. Furthermore, because the *Arabidopsis* genome harbors >100 genes that encode

proteins with myb-like domains (Jin and Martin, 1999), a bioinformatics approach to finding functional homologs will be challenging.

Recently, a host of DNA repair proteins were shown to be associated with telomeres in humans and yeast (reviewed in Williams and Lustig, 2003). Of particular interest are proteins such as the Ku70/80 heterodimer and the MRX complex [Mre11/Rad50/Xrs2(Nbs1)] (Figure 4). Both of these complexes are involved in double-strand break repair. Because a key function of telomeres is to prevent the natural ends of chromosomes from forming end-to-end associations, the presence of such machinery at telomeres was quite unexpected. Perhaps these DNA repair proteins contribute to genome stability by acting as sentinels to monitor the integrity of the telomere cap. Alternatively, DNA repair proteins may be recruited to telomeres because of their resemblance to double-stranded breaks, but the proteins are modified there so that they function to inhibit repair pathways, rather than activate them.

DISCOVERY OF TELOMERASE

With the important exception of dipteran insects, most organisms were found to have telomeres composed of many copies of short simple repeats, with one strand rich in Ts and Gs. Although the exact sequence varied from organism to organism, the general features were the same, and telomeric DNA from ciliates could even function as a telomere in yeast, stabilizing linear plasmids (Szostak and Blackburn, 1982). When these plasmids were isolated from the yeast cells, the ciliate sequences were retained, but typical yeast telomeric DNA had been incorporated onto the ends. Acquisition of the yeast sequences did not require recombination functions, suggesting that the cells possessed a terminal transferase-like activity capable of adding host-specific telomere repeats (Shampay et al., 1984).

To find this postulated telomere terminal transferase, Carol Greider, as a graduate student working with Blackburn, returned to *Tetrahymena*. They reasoned that a telomere terminal transferase would be

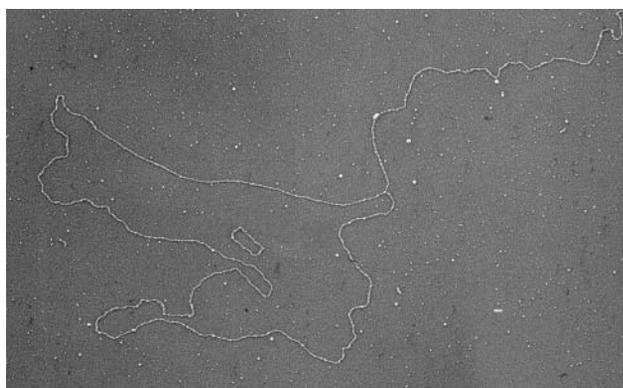


Figure 3. Plant Telomere Loop.

Scanning electron micrograph of telomere loop from *Pisum sativum*. The small circle of DNA inside the loop is pGEM, a 3-kb plasmid used as a size marker. The average size of t-loops in *P. sativum* is 22 kb, close to the 25 kb average length of telomeric DNA measured by gel electrophoresis (Cesare et al., 2003). (Figure courtesy of Jack Griffith.)

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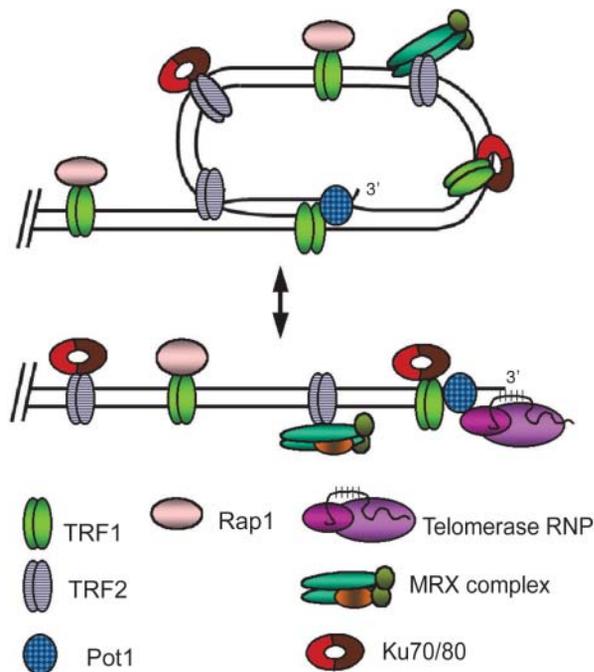


Figure 4. Schematic Diagram of Telomeres Modeled after the Human Architecture.

The top of the panel shows the telomeric DNA with associated proteins in the t-loop confirmation. Upon shortening or during replication, unfolding of the t-loop allows the telomerase holoenzyme to access the ends and extend them if necessary, as shown in the bottom of the panel. Although plant homologs for many of these proteins have been identified in databases, a direct association with telomeres has yet to be demonstrated in most cases.

abundant in an organism that harbors 20,000 telomeres. Greider developed an assay in which she incubated nuclear extracts with labeled nucleotides and a single-stranded oligonucleotide primer consisting of several repeats of the G-rich strand of *Tetrahymena* telomeric DNA. She detected an activity that extended the original primer and produced a pattern of bands with a 6-base periodicity on DNA sequencing gels. Incorporation of dideoxynucleotides allowed her to determine that this activity was adding TTGGGG, the *Tetrahymena* repeat, to the primer. The activity was not a standard DNA polymerase because it was insensitive to the usual inhibitors and did not extend primers based on the C-strand telomere repeat (Greider and Blackburn, 1985). In addition, a primer corresponding to the telomere repeat of yeast was elongated by the addition of the *Tetrahymena* telomere repeat

sequence. Thus, the primer itself was not being ligated into concatamers. Instead, information to guide the *de novo* synthesis of *Tetrahymena* repeats was inherent to the nuclear extract. This unusual terminal transferase activity was subsequently renamed telomerase.

TELOMERASE IS A REVERSE TRANSCRIPTASE

How was the specificity of the telomere synthesis reaction provided by telomerase? Greider found that treating the partially purified extracts with RNase destroyed its activity, implying that the enzyme was a ribonucleoprotein. Indeed, several small RNAs copurified with the activity through multiple purification steps (Greider and Blackburn, 1987). Perhaps one of these RNAs provided the specificity to enzyme, although the mechanism by

which this could be accomplished was not apparent until the RNA subunit of telomerase was cloned and sequenced (Greider and Blackburn, 1989). Near the 5' end of 159-nucleotide *Tetrahymena* telomerase RNA subunit was the sequence CAACCC-CAA, complementary to the TTGGGG repeat. Greider specifically destroyed this RNA in enzyme preparations by adding a DNA oligonucleotide complementary to this region and then treating with RNaseH. Cleavage in this region, but not another region of the RNA, eliminated telomerase activity. These and other results led to the hypothesis that the essential RNA subunit, specifically the region complementary to the telomeric repeat, provided a template for the DNA polymerizing activity of telomerase (Greider and Blackburn, 1989).

One major prediction of this model was that organisms with different telomeric repeats should contain species-specific templates in their telomerase RNA subunits. This prediction was verified by Dorothy Shippen, then a postdoc in Blackburn's group, who cloned and sequenced the RNA subunit from *Euplotes crassus*, another ciliate. The *Euplotes* telomere repeat is TTTTGGGG, and the corresponding region in the telomerase RNA is CAAAACCC-CAAAACC, or about one and a half copies of the DNA repeat. Using primers that extended across this region and beyond, Shippen defined the boundaries of the template domain *in vitro* (Shippen-Lentz and Blackburn, 1990). Further proof for the templating function of the telomerase RNA came with the demonstration that *Tetrahymena* cells transformed with telomerase RNAs mutated in the template region produced correspondingly mutated telomeric DNA (Yu et al., 1990).

Together, these findings supported the conclusion that telomerase was a specialized reverse transcriptase that carried its own template to direct the addition of telomeric repeats onto the chromosome terminus. In 1996, cloning and sequencing of the gene encoding the telomerase catalytic subunit TERT (telomerase reverse transcriptase) from several species, including *E. aedicularis*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and humans (Lingner et al., 1997; Nakamura

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et al., 1997), confirmed that the predicted protein contained all essential residues and common motifs found in typical reverse transcriptases.

The advent of plant genome projects made it possible to identify the TERT gene in *Arabidopsis* (Fitzgerald et al., 1999; Oguchi et al., 1999) and rice (Heller-Uszynska et al., 2002). The gene encoding the telomerase RNA template has not been identified yet in any plant. This RNA must contain some permutation of the TTTA-GGG repeat, but there appears to be little conservation of primary nucleotide sequence in telomerase RNAs, even among closely related organisms (Lingner et al., 1994). Some features of secondary structure, including a pseudoknot, appear to be conserved, so perhaps the *Arabidopsis* telomerase RNA can be identified by a concerted bioinformatics approach. Although several other subunits copurify with telomerase activity from a variety of sources (reviewed in Harrington, 2003), only TERT and the telomerase RNA are required to reconstitute telomerase activity *in vitro* (Weinrich et al., 1997; Beattie et al., 1998).

As mentioned above, *Drosophila* and related insects solve the end replication problem by using two types of retrotransposons to add DNA onto the chromosome ends. Despite the differences in telomeric DNA, telomere maintenance in *Drosophila* exhibits similarities to the telomerase-based mechanism. Both mechanisms employ a reverse transcriptase whose action is primed by a 3' OH on the chromosome terminus. In addition, the DNA strand that runs 5' to 3' toward the end of *Drosophila* chromosomes is G/T rich, implying that the RNA template used for retrotransposition is C/A rich because it is in the telomerase RNA template (reviewed in Pardue and DeBaryshe, 2003). Whether the similarities between these two approaches to telomere maintenance reflect a common origin or a convergence of mechanisms is not clear.

THE SENESCENCE CONNECTION

Another important line of research that eventually connected to telomere biology began in the early 1960s with Leonard Hayflick's observation that normal, diploid

human cells had a finite lifetime in tissue culture (Hayflick and Moorhead, 1961; Hayflick, 1965). After ~50 cell divisions, the population enters a senescence phase where mitosis stops. As mentioned above, Olovnikov (1973) suggested that this finite life span could result from genetic material being lost to the end replication problem. The subsequent discovery of telomerase and its ability to replicate the very ends of the chromosome suggested that the enzyme was not active in these mortal cells. This conjecture was eventually validated by a survey of 22 mortal human cell populations that found that none of them had detectable telomerase activity, whereas 98 of 100 immortal lines expressed telomerase (Kim et al., 1994).

Work led by Cal Harley at McMaster University also supported the connection between telomere length and cellular ageing, first by demonstrating that telomeric DNA shortens as human fibroblasts age *in vitro* (Harley et al., 1990) and then by showing that the length of telomeres in fibroblasts is a good predictor of their capacity to replicate *in vitro* (Allsopp et al., 1992). This telomere hypothesis of ageing (for isolated cells, at least) received further support a few years later by the demonstration that constitutively expressing the TERT gene in normal human cells was sufficient to confer a greatly extended capacity for cell division and possibly immortality (Bodnar et al., 1998). The activation of telomerase in most cancer cells (Kim et al., 1994) and the limited capacity for cellular proliferation without this enzyme suggested that telomerase might be a useful target for anticancer drugs.

In human organs that depend on the continuous ability to proliferate throughout our lives (skin epithelium, intestinal lining, etc.), telomerase is expressed. In most somatic tissues, however, telomerase is generally undetectable (Kim et al., 1994), and telomeres shorten as we age (Allsopp et al., 1992). Correlations between short telomeres in white blood cells and increased human mortality (Cawthon et al., 2003) and cardiovascular disease (Samani et al., 2001) have been found, but whether short telomeres are a cause or an effect (or neither) is not yet clear. Our survival as

a species is vitally dependent on telomerase, however, and the enzyme is active in the germline (Kim et al., 1994), ensuring that our progeny inherit a complete genome.

Plants develop along a much different pathway than humans. Plants do not have a typical germline, their cells are totipotent, and they produce new organs throughout their lives. Although this flexible developmental program could require a much broader pattern of telomerase expression, analysis of telomerase expression in plants reveals a pattern of expression similar to that seen in humans. Telomerase is not expressed in most vegetative tissues, but it is expressed in reproductive structures and immortalized dedifferentiated cells grown in culture (Fajkus et al., 1996; Fitzgerald et al., 1996; Heller et al., 1996). Despite the fact that plants and humans evolved into multicellular organisms along completely separate pathways, they appear to have developed strongly parallel patterns of telomerase expression.

The development of telomerase assays for plants allowed Andris Kleinhofs' group to revisit some of McClintock's early findings on maize telomeres. They demonstrated that although both endosperm and embryo contain telomerase activity during very early stages of seed development, activity in the endosperm is much lower and disappears at later stages, whereas activity in the embryo remains high throughout much of its development (Kilian et al., 1998). These results imply that McClintock's original observations of chromosome healing in the embryo but not the endosperm can be explained by differential activity of telomerase and *de novo* acquisition of telomeric DNA.

LIFE WITHOUT TELOMERASE

If telomerase is essential for fully replicating chromosomes, then the loss of this activity should have disastrous consequences for both genome and organism. This prediction was verified by knocking out the telomerase RNA gene in mice (Blasco et al., 1997; Lee et al., 1998). In the absence of telomerase, telomeres shortened by about 5 kb per mouse generation. The first few

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generations were relatively healthy and fertile, but after five generations, developmental defects were obvious in organs with a high capacity for cellular regeneration (Lee et al., 1998). Beginning in the fifth generation, male germ cells were less abundant, and by the sixth generation, there was a complete loss of spermatogenesis. Testes in the sixth generation mice weighed only 20% of age-matched wild-type testes, with most of the decline coming from a loss of highly proliferative spermatogenic cells; less prolific support cells were not noticeably affected. The female germline was functional for one more generation, but it too eventually collapsed, and no seventh generation telomerase-null mice were produced. The late-generation knockout mice also displayed problems with hematopoiesis, increased apoptosis of splenocytes following mitogenic stimulation (Lee et al., 1998), and a reduced capacity to respond to stresses such as wound healing (Rudolph et al., 1999). Sixth generation knockouts possessed cells with higher proportions of chromosome fusions and aneuploidy, consistent with McClintock's and Muller's original observations on telomere function (Blasco et al., 1997; Lee et al., 1998). Together, data from the knockout mice indicate that telomerase is dispensable for a few generations, but the inexorable erosion of telomeric DNA in its absence cannot be tolerated indefinitely.

We analyzed the consequences of life without telomerase in an Arabidopsis line carrying a T-DNA disruption of the *AtTERT* gene (Riha et al., 2001). It was not clear whether the homozygous *tert* mutants would be viable, given that telomerase-null mice lose 5 kb of telomeric DNA per generation, and telomeres in the Columbia ecotype of Arabidopsis are only 2 to 5 kb to begin with (Fitzgerald et al., 1999). The mutants were viable because they lost only 250 to 500 bp of telomeric DNA per generation. Similar to knockout mice, the first few generations of Arabidopsis *tert* mutants had a normal phenotype. Beginning in the sixth generation, however, developmental abnormalities appeared in the form of altered phyllotaxy, abnormal leaf shape, and reduced fertility (Riha et al., 2001). The

vegetative defects resulted from loss of cell proliferation potential in the shoot apical meristem. As in telomerase-null mice, the onset of these abnormalities was well correlated with the appearance of chromosome fusions. Problems with development and fertility were exacerbated with each subsequent generation until the plants reached a terminal phenotype where they were small, misshapen, and sterile. Only a few *tert* mutants survived into the ninth generation, and none survived >10 generations (Riha et al., 2001). Cytogenetic analysis of these late generation *TERT* knockouts revealed massive genome instability, apparently triggered by end-to-end fusion of chromosomes and perpetuated by multiple rounds of breakage-fusion-bridge cycles (Riha et al., 2001; Siroky et al., 2003).

One major difference between plant and animal responses to the immense genome instability resulting from dysfunctional telomeres is that animal cells can detect and react to this genetic catastrophe through p53-mediated pathways, frequently resulting in apoptosis. Double mutants in mice lacking both telomerase and p53 survive longer than single telomerase-null mutants, but the absence of p53 merely postpones inevitable cell death (Chin et al., 1999). By contrast, late-generation telomerase-null Arabidopsis live much longer than wild-type plants grown under identical conditions. The sterility of these plants may contribute to their longevity because their inability to flower and set seed apparently prevents generation of signals that would trigger senescence of vegetative organs. Although late-generation, telomerase-null Arabidopsis plants are small and abnormal, their cells are capable of undergoing multiple rounds of the breakage-fusion-bridge cycle and hence possess an amazing capacity to withstand raging genomic instability (Siroky et al., 2003). The molecular basis for this resistance is unclear. One possibility is that the absence of a p53 homolog from the Arabidopsis genome may allow continued survival under conditions that animals cannot endure. Moreover, because a significant portion of the Arabidopsis genome has been duplicated, a limited degree of sequence loss may be

tolerated. Finally, the plastic nature of plant development is also likely to contribute to organismal survival because cells that reach the damage threshold can be replaced by those with more intact genomes.

CURRENT QUESTIONS AND FUTURE DIRECTIONS

Recent investigations of plant telomere biology have been reviewed in detail elsewhere (McKnight et al., 2002; Riha and Shippen, 2003a). Current research is largely focused on interactions between telomeres and components of the DNA replication, repair, and recombination machinery. The roles of telomere-associated DNA repair proteins vary considerably among different eukaryotes. For example, the Ku70/80 heterodimer is a negative regulator of telomere length in Arabidopsis (Bundock et al., 2002; Riha et al., 2002) but a positive regulator in yeast (Boulton and Jackson, 1996; Gravel et al., 1998). Furthermore, the absence of Ku leads to chromosome fusions in mammalian cells (Bailey et al., 1999) but not in Arabidopsis (Riha and Shippen, 2003b). The biochemical basis for this variation in Ku function among the kingdoms is unknown and may reflect fundamental differences in the architecture of the telomere complex.

An even greater challenge will be to understand how Arabidopsis tolerates the severe genome instability associated with telomere dysfunction. Do cells with uncapped telomere escape detection by the DNA damage checkpoint machinery, or is a subset of the checkpoints described in animals simply absent in plants? Likewise, how can Arabidopsis tolerate the large number of unviable cells that must inevitably arise from breakage of fused dicentric chromosomes? Does the totipotency of plant cells allow them to replace damaged cells by a mechanism that is simply unavailable in animals?

Despite recent advances in plant telomere biology, many fundamental aspects of genome maintenance remain obscure. However, the increased interest in this area coupled with the ever-growing arsenal of molecular tools now available for analysis

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of Arabidopsis may ultimately reveal the molecular foundation for phenomena described by Barbara McClintock more than 60 years ago.

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Thomas D. McKnight
 Department of Biology
 Texas A&M University
 College Station, TX 77843-3258
 mcknight@bio.tamu.edu

Dorothy E. Shippen
 Department of Biochemistry and
 Biophysics Texas A&M University
 College Station, TX 77843-2128

REFERENCES

- Adams, S.P., Hartman, T.P., Lim, K.Y., Chase, M.W., Bennett, M.D., Leitch, I.J., and Leitch, A.R.** (2001). Loss and recovery of Arabidopsis-type telomere repeat sequences 5'-(TTTAGGG)(n)-3' in the evolution of a major radiation of flowering plants. *Proc. R. Soc. Lond. B Biol. Sci.* **268**, 1541–1546.
- Allsopp, R.C., Vaziri, H., Patterson, C., Goldestein, S., Younglai, E.V., Futcher, A.B., Greider, C.W., and Harley, C.B.** (1992). Telomere length predicts replicative capacity of human fibroblasts. *Proc. Natl. Acad. Sci. USA* **89**, 10114–10118.
- Bailey, S.M., Meyne, J., Chen, D.J., Kurimasa, A., Li, G.C., Lehnert, B.E., and Goodwin, E.H.** (1999). DNA double-strand break repair proteins are required to cap the ends of mammalian chromosomes. *Proc. Natl. Acad. Sci. USA* **96**, 14899–14904.
- Baumann, P., and Cech, T.R.** (2001). Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* **292**, 1171–1175.
- Baumann, P., Podell, E., and Cech, T.R.** (2002). Human Pot1 (protection of telomeres) protein: Cytolocalization, gene structure, and alternative splicing. *Mol. Cell. Biol.* **22**, 8079–8087.
- Beattie, T.L., Zhou, W., Robinson, M.O., and Harrington, L.** (1998). Reconstitution of human telomerase activity in vitro. *Curr. Biol.* **29**, 177–180.
- Berman, J., Tachibana, C.Y., and Tye, B.K.** (1986). Identification of a telomere-binding activity from yeast. *Proc. Natl. Acad. Sci. USA* **83**, 3713–3717.
- Biessmann, H., Mason, J.M., Ferry, K., d'Hulst, M., Valgeirsdottir, K., Traverse, K.L., and Pardue, M.L.** (1990). Addition of telomere associated Het DNA sequences 'heals' broken chromosome ends in *Drosophila*. *Cell* **61**, 633–673.
- Blackburn, E.H., and Gall, J.G.** (1978). A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. *J. Mol. Biol.* **120**, 33–53.
- Blasco, M.A., Lee, H.-W., Hande, M.P., Samper, E., Lansdorp, P.M., DePinho, R.A., and Geider, C.W.** (1997). Telomere shortening and tumor formation in mouse cells lacking telomerase RNA. *Cell* **91**, 25–34.
- Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.-P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., and Wright, W.E.** (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**, 349–352.
- Boulton, S.J., and Jackson, S.P.** (1996). Identification of a *Saccharomyces cerevisiae* Ku80 homologue: Roles in DNA double strand break rejoining and in telomeric maintenance. *Nucleic Acids Res.* **24**, 4639–4648.
- Bundock, P., van Attikum, H., and Hooykaas, P.** (2002). Increased telomere length and hypersensitivity to DNA damaging agents in an Arabidopsis KU70 mutant. *Nucleic Acids Res.* **30**, 3395–3400.
- Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., and Kerber, R.A.** (2003). Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* **361**, 393–395.
- Cesare, A.J., Quinney, N., Wilcox, S., Subramanian, D., and Griffith, J.D.** (2003). Telomere looping in *P. sativum* (common garden pea). *Plant J.* **36**, 271–279.
- Chin, L., Artandi, S.E., Shen, Q., Tam, A., Lee, S.L., Gottlieb, G.J., Greider, C.W., and DePinho, R.A.** (1999). p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* **97**, 527–538.
- Comfort, N.C.** (2001). *The Tangled Field: Barbara McClintock's Search for the Patterns of Genetic Control.* (Cambridge, MA: Harvard University Press).
- Creighton, H.B., and McClintock, B.** (1935). The correlation of cytological and genetical crossing over in *Zea mays*: A corroboration. *Proc. Natl. Acad. Sci. USA* **21**, 148–150.
- Fajkus, J., Kovarik, A., and Kralovics, R.** (1996). Telomerase activity in plant cells. *FEBS Lett.* **391**, 307–309.
- Fitzgerald, M.S., McKnight, T.D., and Shippen, D.E.** (1996). Characterization and developmental patterns of telomerase expression in plants. *Proc. Natl. Acad. Sci. USA* **93**, 14422–14427.
- Fitzgerald, M.S., Riha, K., Gao, F., Ren, S., McKnight, T.D., and Shippen, D.E.** (1999). Disruption of the telomerase catalytic subunit gene from Arabidopsis inactivates telomerase and leads to a slow loss of telomeric DNA. *Proc. Natl. Acad. Sci. USA* **96**, 14813–14818.
- Gottschling, D.E., and Zakian, V.A.** (1986). Telomere proteins: Specific recognition and protection of the natural termini of Oxytricha macronuclear DNA. *Cell* **47**, 195–205.
- Gravel, S., Larrivee, M., Labrecque, P., and Wellinger, R.J.** (1998). Yeast Ku as a regulator of chromosomal DNA end structure. *Science* **280**, 741–744.
- Greider, C.W., and Blackburn, E.H.** (1985). Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* **43**, 405–413.
- Greider, C.W., and Blackburn, E.H.** (1987). The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* **51**, 887–898.
- Greider, C.W., and Blackburn, E.H.** (1989). A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. *Nature* **337**, 331–337.
- Griffith, J.D., Comeau, L., Rosenfeld, S., Stansel, R.M., Bianchi, A., Moss, H., and de Lange, T.** (1999). Mammalian telomeres end in a large duplex loop. *Cell* **97**, 503–514.
- Harley, C.B., Futcher, A.B., and Greider, C.W.** (1990). Telomeres shorten during ageing of human fibroblasts. *Nature* **345**, 458–460.
- Harrington, L.** (2003). Biochemical aspects of telomerase function. *Cancer Lett.* **194**, 139–154.
- Hayflick, L.** (1965). The limited in vitro lifetime of human diploid cell strains. *Exp. Cell Res.* **37**, 614–636.
- Hayflick, L., and Moorhead, P.S.** (1961). The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **25**, 585–621.

HISTORICAL PERSPECTIVE ESSAY

- Heller, K., Kilian, A., Piatyszek, M.A., and Kleinhofs, A. (1996). Telomerase activity in plant extracts. *Mol. Gen. Genet.* **252**, 342–345.
- Heller-Uszynska, K., Schnippenkoetter, W., and Kilian, A. (2002). Cloning and characterization of rice (*Oryza sativa* L) telomerase reverse transcriptase, which reveals complex splicing patterns. *Plant J.* **31**, 75–86.
- Henderson, E.R., and Blackburn, E.H. (1989). An overhanging 3' terminus is a conserved feature of telomeres. *Mol. Cell. Biol.* **9**, 345–348.
- Jin, H., and Martin, C. (1999). Multifunctionality and diversity within the plant MYB-gene family. *Plant Mol. Biol.* **41**, 577–585.
- Kilian, A., Heller, K., and Kleinhofs, A. (1998). Development patterns of telomerase activity in barley and maize. *Plant Mol. Biol.* **37**, 621–628.
- Kim, N.W., Piatyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L., Coviello, G.M., Wright, W.E., Weinrich, S.L., and Shay, J.W. (1994). Specific association of human telomerase activity with immortal cells and cancer. *Science* **266**, 2011–2015.
- Klobutcher, L.A., Swanton, M.T., Donini, P., and Prescott, D.M. (1981). All gene-sized DNA molecules in four species of hypotrachs have the same terminal sequence and an unusual 3' terminus. *Proc. Natl. Acad. Sci. USA* **78**, 3015–3019.
- Konig, P., Giraldo, R., Chapman, L., and Rhodes, D. (1996). The crystal structure of the DNA-binding domain of yeast RAP1 in complex with telomeric DNA. *Cell* **85**, 125–136.
- Lee, H.-W., Blasco, M.A., Gottlieb, G.J., Horner, J.W., Greider, C.W., and DePinho, R.A. (1998). Essential role of mouse telomerase in highly proliferative organs. *Nature* **392**, 569–574.
- Lingner, J., Hendrick, L.L., and Cech, T.R. (1994). Telomerase RNAs of different ciliates have a common secondary structure and a permuted template. *Genes Dev.* **8**, 1984–1998.
- Lingner, J., Hughes, T.R., Shevchenko, A., Mann, M., Lundblad, V., and Cech, T.R. (1997). Reverse transcriptase motifs in the catalytic subunit of telomerase. *Science* **276**, 561–567.
- Longtine, M.S., Wilson, N.M., Petracek, M.E., and Berman, J. (1989). A yeast telomere binding activity binds to two related telomere sequence motifs and is indistinguishable from RAP1. *Curr. Genet.* **16**, 225–239.
- McClintock, B. (1938). The fusion of broken chromosomes ends of sister half-chromatids following chromatid breakage at meiotic anaphases. *Missouri Agricultural Experiment Station Research Bulletin* **290**, 1–48. In *The Discovery and Characterization of Transposable Elements*, J.A. Moore, ed (New York: Garland Publishing, 1987).
- McClintock, B. (1939). The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proc. Natl. Acad. Sci. USA* **25**, 405–416.
- McClintock, B. (1941). The stability of broken ends of chromosomes in *Zea mays*. *Genetics* **26**, 234–282.
- McClintock, B. (1946). Maize Genetics. Carnegie Inst. Wash. Yearbook **46**, 146–152. In *The Discovery and Characterization of Transposable Elements*, J.A. Moore, ed (New York: Garland Publishing, 1987).
- McClintock, B. (1987). The Discovery and Characterization of Transposable Elements, J.A. Moore, ed (New York: Garland Publishing) pp. vii–xi.
- McKnight, T.D., Riha, K., and Shippen, D.E. (2002). Telomeres, telomerase, and stability of the plant genome. *Plant Mol. Biol.* **48**, 331–337.
- Moyzis, R.K., Buckingham, J.M., Cram, L.S., Dani, M., Deaven, L.L., Jones, M.D., Meyne, J., Ratliff, R.A., and Wu, J.-R. (1988). A highly conserved repetitive DNA sequence, (TTAGGG)_n, present at the telomeres of human chromosomes. *Proc. Natl. Acad. Sci. USA* **85**, 6622–6626.
- Muller, H.J. (1938). The remaking of chromosomes. *Collecting Net* **8**, 182–198.
- Nakamura, T.M., Morin, G.B., Chapman, K.B., Weinrich, S.L., Andrews, W.H., Lingner, J., Harley, C.B., and Cech, T.R. (1997). Telomerase catalytic subunit homologs from fission yeast and human. *Science* **277**, 955–959.
- Nugent, C.I., Hughes, T.R., Lue, N.F., and Lundblad, V. (1996). Cdc13p: A single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. *Science* **274**, 249–252.
- Oguchi, K., Liu, H., Tamura, K., and Takahashi, H. (1999). Molecular cloning and characterization of ATERT, a telomerase reverse transcriptase homolog in *Arabidopsis thaliana*. *FEBS Lett.* **457**, 465–469.
- Oka, Y.S., Shiota, S., Nakai, S., Nishida, Y., and Okubo, S. (1980). Inverted terminal repeat sequence in the macronuclear DNA of *Stylonychia pustulata*. *Gene* **10**, 301–306.
- Olovnikov, A.M. (1971). Principles of marginotomy in template synthesis of polynucleotides. *Dokl. Akad. Nauk SSSR* **201**, 1496–1499.
- Olovnikov, A.M. (1973). A theory of marginotomy. *J. Theor. Biol.* **41**, 181–190.
- Pardue, M.-L., and DeBaryshe, P.G. (2003). Retrotransposons provide an evolutionarily robust non-telomerase mechanism to maintain telomeres. *Annu. Rev. Genet.* **37**, 485–511.
- Pich, U., Fuchs, J., and Schubert, I. (1996). How do Alliaceae stabilize their chromosome ends in the absence of TTTAGGG sequences? *Chromosome Res.* **4**, 207–213.
- Price, C.M., and Cech, T.R. (1987). Telomeric DNA-protein interactions of *Oxytricha* macronuclear DNA. *Genes Dev.* **1**, 783–793.
- Richards, E.J., and Ausubel, F.M. (1988). Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. *Cell* **53**, 127–136.
- Riha, K., McKnight, T.D., Griffing, L.R., and Shippen, D.E. (2001). Living with genome instability: Plant responses to telomerase dysfunction. *Science* **291**, 1797–1800.
- Riha, K., and Shippen, D.E. (2003a). Telomere structure, function, and maintenance in *Arabidopsis*. *Chromosome Res.* **11**, 263–275.
- Riha, K., and Shippen, D.E. (2003b). Ku is required for telomeric C-rich strand maintenance but not for end-to-end chromosome fusions in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **100**, 611–615.
- Riha, K., Watson, J.M., Parkey, J., and Shippen, D.E. (2002). Telomere length de-regulation and enhanced sensitivity to genotoxic stress in *Arabidopsis* mutants deficient in Ku70. *EMBO J.* **21**, 2819–2826.
- Rudolph, K.L., Chang, S., Lee, H.W., Blasco, M., Gottlieb, G.J., Greider, C., and DePinho, R.A. (1999). Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* **96**, 701–712.
- Samani, N.J., Boulby, R., Butler, R., Thompson, J.R., and Goodall, A.H. (2001). Telomere shortening in atherosclerosis. *Lancet* **358**, 472–473.
- Shampay, J., Szostak, J.W., and Blackburn, E.H. (1984). DNA sequences of telomeres maintained in yeast. *Nature* **310**, 154–157.
- Shippen-Lentz, D.E., and Blackburn, E.H. (1990). Functional evidence for an RNA template in telomerase. *Science* **247**, 546–552.
- Siroky, J., Zluvova, J., Riha, K., Shippen, D.E., and Vyskot, B. (2003). Rearrangements of ribosomal DNA clusters in late generation telomerase-deficient *Arabidopsis*. *Chromosoma* **112**, 116–123.
- Sykorova, E., Lim, K.Y., Kunicka, Z., Chase, M.W., Bennett, M.D., Fajkus, J., and Leitch, A.R. (2003). Telomere variability in the monocotyledonous plant order Asparagales. *Proc. R. Soc. Lond. B Biol. Sci.* **270**, 1893–1904.

HISTORICAL PERSPECTIVE ESSAY

- Szostak, J.W., and Blackburn, E.H.** (1982). Cloning yeast telomeres on linear plasmids. *Cell* **29**, 245–255.
- Watson, J.D.** (1972). Origin of concatemeric T7 DNA. *Nat. New Biol.* **239**, 197–201.
- Weinrich, S.L., et al.** (1997). Reconstitution of human telomerase with the template RNA component hTR and the catalytic subunit hTRT. *Nat. Genet.* **17**, 489–502.
- Williams, B., and Lustig, A.J.** (2003). The paradoxical relationship between NHEJ and telomeric fusion. *Mol. Cell* **11**, 1125–1126.
- Yu, G.L., Bradley, J.D., Attardi, L.D., and Blackburn, E.H.** (1990). In vivo alteration of telomere sequences and senescence caused by mutated *Tetrahymena* telomerase RNAs. *Nature* **344**, 126–132.
- Zhong, Z., Shuie, L., Kaplan, S., and de Lange, T.** (1992). A mammalian factor that binds telomeric TTAGGG repeats in vitro. *Mol. Cell Biol.* **12**, 4834–4843.

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Thomas D. McKnight and Dorothy E. Shippen
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