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Lasker Laurels for Telomerase

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This year the Lasker Foundation pays tribute to telomerase, a medically important enzyme required for chromosome stability and long-term cell proliferation.

On September 29, the 2006 Albert Lasker Award for Basic Medical Research will be conferred on Elizabeth Blackburn, Carol Greider, and Jack Szostak. They are recognized for the discovery of telomerase, the enzyme that maintains telomeres, and the demonstration that immortal growth of eukaryotic cells requires the maintenance of telomeric DNA. Telomerase has achieved biomedical fame for its ability to propel cancer cells beyond their original expiration date. Telomerase activity, or lack thereof, has been used as a diagnostic tool for malignancies, and its inhibitors hold therapeutic promise for treating human cancer. Enthusiasm for telomerase is also fueled by its ability to endow normal human cells with infinite proliferative potential, an attribute relevant to the manipulation of stem cells and attempts at tissue regeneration. These biomedical merits clearly warrant recognition of telomerase and its discoverers. This enzyme did not emerge from research on cancer or aging, however. It was found as a solution to one of the most basic problems in cell biology, and the story of its discovery is worth telling.

Despite its medical importance, telomerase emerged from work on two organisms that pose no threat to human health: baker's yeast and the harmless pond-dwelling ciliate, *Tetrahymena thermophila*. In the mid-1970s, Elizabeth Blackburn learned about *Tetrahymena*'s experimental virtues from Joseph Gall, her postdoctoral advisor and recipient of this year's Albert Lasker Special Achievement Award. Working with Gall, Blackburn encountered the first eukaryotic telomeric sequences while studying the ciliate's ribosomal DNA (rDNA), a small linear DNA that codes for the RNA components of ribosomes. Like rDNA in other eukaryotes, the rDNA of *Tetrahymena* is present at high copy number. This allowed Blackburn and Gall to isolate large amounts of this DNA without having to resort to the nascent recombinant DNA technology of those days. Despite experimental hurdles, they deduced the nucleotide sequence of the ends of this minute chromosome and concluded that they were made up of 20–70 tandem copies of the sequence TTGGGG (Blackburn and Gall, 1978). No other chromosome end sequence was available at that time, so Blackburn and Gall could not anticipate that their findings on rDNA ends would be repeated for most eukaryotic telomeres.

In parallel with this early work by Blackburn and Gall, a theoretical discussion about the consequences of DNA replication was in progress. Jim Watson pointed out that the canonical DNA replication strategy could not account for duplication of the ends of linear genomes (Watson, 1972). Theoretical solutions for this "end-replication problem" either invoked recombination between the repetitive sequences or a hairpin loop that would allow polymerases to turn around and complete their task. Watson's considerations, and a related discussion by Olovnikov (Olovnikov, 1973), were a preview of experimental observations about to unfold.

In 1981, Jack Szostak reported on a different problem associated with DNA ends. Working with his student, Terry Orr-Weaver, and a collaborator, Rodney Rothstein, he described the fate of linear DNAs introduced into budding yeast (Orr-Weaver et al., 1981). They found that the ends of newly introduced linear DNAs were highly recombinogenic, such that the plasmids invariably integrated into homologous sequences in the yeast genome. In some cases, however, the ends were degraded or ligated in a recombination-independent manner. These findings echoed observations made by Barbara McClintock and Hermann Muller half a century earlier. They had surmised that broken chromosome ends were unstable and differed from natural chromosome ends, which carried what Muller termed a telomere (for the Greek word for end, telos). The nature of this telomere was not known then and is only partially understood today. A harbinger of future findings on human telomerase was Barbara McClintock's discovery that new telomeres could be synthesized in maize embryos but not in mature plants. We now understand that this is probably due to differential expression of telomerase.

Whereas the recombination of linear plasmids introduced into yeast (and mouse embryonic stem cells, as it later turned out) opened the way for gene targeting, the integration of the introduced DNAs thwarted Szostak's attempts to generate artificial chromosomes. Upon learning about the structure of *Tetrahymena* rDNA ends, Szostak hoped that they might block recombination and thus stabilize introduced plasmids in linear form. Accordingly, Szostak collaborated with Blackburn to force a union between *Tetrahymena* rDNA ends and a linear yeast plasmid. Capped with *Tetrahymena* repeats, the plas-

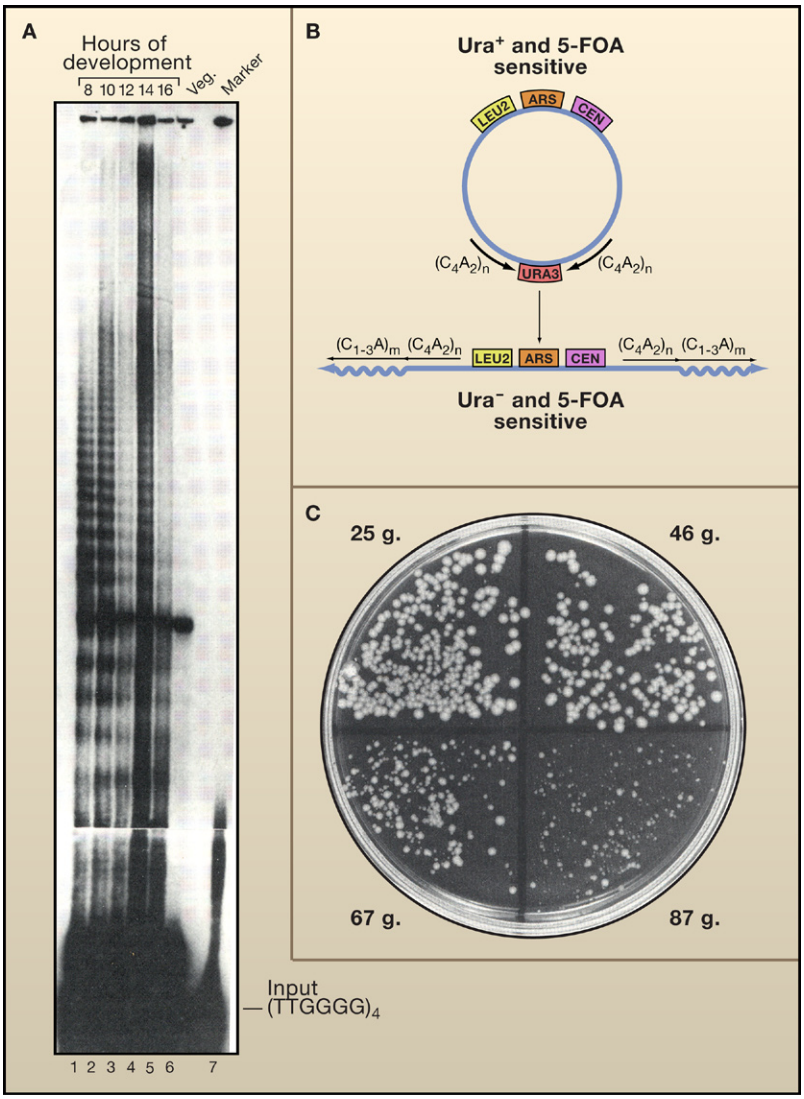

Figure 1. The Discovery of Telomerase and Its Role in Cellular Proliferation

(A) The telomerase assay performed by Greider and Blackburn, using extracts of the ciliate *Tetrahymena* at various time points after mating, showed telomerase activity at the time of extensive de novo telomere synthesis (Greider and Blackburn, 1985).

(B) Plasmid resolution assay used by Lundblad and Szostak to screen for yeast mutants deficient in telomere synthesis (Lundblad and Szostak, 1989).

(C) Plate showing the delayed senescence phenotype of the *est1-1* ("ever shortening telomeres") yeast mutant that has a defect in telomerase (Lundblad and Szostak, 1989).

mid ends no longer recombined and the DNAs were maintained as linear replicating yeast episomes (Szostak and Blackburn, 1982). Szostak and Blackburn then asked whether they could capture a yeast telomere if they capped only one end of the plasmid with rDNA and then ligated yeast DNA to the other end. The yeast telomeres they cloned in this way were made up of short irregular repeats that, like *Tetrahymena* telomeric repeats, had TG sequences in the strand running out to the 3′ end of the chromosome (Shampay et al., 1984). They also found that these yeast telomeric repeats were added to the plasmid ends capped with *Tetrahymena* repeats. In a remarkable reaction that lacked an apparent template for DNA polymerization, budding yeast had added approximately 200 basepairs of its own telomeric DNA to the ends, and this addition was ultimately responsible for the stability of the linear DNAs.

What was the nature of this untemplated telomere repeat addition reaction? Szostak and Blackburn realized that the "hairpin" model of end replication, in vogue at that time, was in discordance with their findings; nor could recombination explain the sequence addition they observed. Therefore, Szostak and Blackburn favored the idea of a terminal transferase-like enzyme that was dedicated to adding repeats onto chromosome ends. If there was such an enzyme, they speculated, its main role could be to fix Watson's end-replication problem. Although invoking an unknown enzyme might have seemed far-fetched, the proposal fit with Barbara McClintock's view of telomere synthesis. As McClintock explained in a 1983 letter to Blackburn (http://profiles.nlm.nih.gov/LL/), her finding of a maize stock that lacked the ability to heal the ends of broken chromosomes in the plant embryo had suggested to her that "this mutant affects the production or the action of an enzyme required for formation of a new telomere." A telomere-synthesizing enzyme could also explain the curious observation made by Piet Borst and colleagues that telomeres in the protozoan parasite *Trypanosoma brucei* grew steadily by ~6 basepairs with each cell division (Bernards et al., 1983), as well as the findings from several groups that new telomeric repeats were added to DNA fragments created during ciliate development.

Blackburn and Szostak went in different directions to try to find evidence for the hypothetical telomere-synthesizing enzyme. Blackburn took a biochemical approach using extracts from mating *Tetrahymena*, expecting that the proposed enzyme would be abundant at that stage. Ciliates have two nuclei, one of which, the micronucleus, is only used for meiosis and sexual conjugation. The macronucleus is the site of gene expression, and its genome is formed after mating by chromosome fragmentation and addition of

telomeres to the DNA ends. Joined in her efforts by her graduate student, Carol Greider, Blackburn set up various assays that might detect telomere synthesis in vitro. After trial and error, they detected an enzyme activity that added sequences to the 3′ end of an oligonucleotide containing TTGGGG repeats. The enzyme worked well on this telomeric primer and a similar oligonucleotide representing yeast telomeric DNA but ignored the C-rich telomeric sequences of the other strand. The product was a ladder with a 6 basepair periodicity composed of only Gs and Ts. As predicted, the enzyme activity was greater after mating (see Figure 1), and several control experiments argued that this was a new type of DNA polymerase activity. All the data pointed to the telomere-synthesizing activity hypothesized by Blackburn and Szostak; telomerase, as it later became known, had been found (Greider and Blackburn, 1985).

The enzymatic activity that Greider and Blackburn were characterizing had two remarkable features (Greider and Blackburn, 1987, 1989). First, the enzyme always added the correct sequence, the TTGGGG repeat of *Tetrahymena* telomeres. Second, the enzyme was able to discern the sequence of the DNA end that it was acting on. For instance, when they provided the enzyme with an oligonucleotide ending in TTGGG, the enzyme would first add a G, before adding two Ts. Greider and Blackburn correctly guessed that the enzyme used a nucleic acid as a template and showed that this guide was composed of RNA. When they isolated the telomerase RNA, they found that it contained the template for 1.5 telomeric repeats. This template region both specifies the telomeric DNA and helps the enzyme to recognize the phasing of the last repeat at the chromosome end. Blackburn's group eventually proved that the RNA specified the sequence of the telomeres by showing that mutated RNAs could reprogram telomerase to synthesize mutant telomeres (Yu et al., 1990). Thus, telomerase turned out to be a reverse transcriptase that copied part of its associated RNA using the 3′ end of the chromosome as a primer.

In 1984, Szostak and his postdoctoral fellow, Vicki Lundblad, had initiated their own hunt for genes involved in telomere synthesis. They developed a genetic screen based on the fate of plasmids carrying two *Tetrahymena* telomeric repeat arrays in opposite orientation (Lundblad and Szostak, 1989) (see Figure 1). They knew that yeast had the ability to spontaneously resolve these plasmids into linear DNAs, losing whatever sequence was inserted between the telomeres. Because this reaction required addition of yeast telomeres to the ends of the plasmid, Lundblad and Szostak predicted that a telomerase mutant would be defective in the linearization of the plasmid. To monitor the linearization reaction, they placed the *URA3* gene in between the telomeres. The *URA3* marker had just been developed as a gene one could select either for (on culture plates lacking uracil) or against (with 5-Fluorootic acid; 5-FOA). After mutagenesis with ethylmethanesulfonate, Lundblad and Szostak monitored yeast strains for their ability to lose the *URA3* gene while retaining the plasmid. One mutant out of 7000 strains examined not only had the expected behavior (diminished frequency of colonies resistant to 5-FOA) but also failed to maintain native telomeres. Examination of chromosome ends showed that this mutant gradually lost its telomeric DNA, an attribute referred to in the name of the gene, ever shorter telomeres 1 (*EST1*). Clearly, *EST1* was required for telomere maintenance as well as de novo telomere formation. Later work showed that the Est1 protein is a component of the yeast telomerase complex and is required for telomerase function in vivo (reviewed in Lundblad, 2003). The observation that *est1-1* cells had ever shorter telomeres represented experimental verification of the end replication problem.

Yeast strains lacking Est1 had two additional striking phenotypes. Although their initial growth was normal, the *est1-1* strain lost vigor after prolonged culture and eventually stopped growing altogether (see Figure 1). Lundblad and Szostak concluded that the telomere shortening of *est1-1* mutants induced senescence with delayed onset. The phenotypic lag could be explained if yeast telomeres were considerably longer than the minimum needed for chromosome end protection. Lundblad and Szostak also noted a high rate of chromosome loss that appeared after prolonged culture and was coincident with senescence. Thus, their data showed that loss of telomerase and the accompanying telomere attrition eventually resulted in genome instability and replicative senescence. It did not escape their attention that their findings could explain the fate of primary human cells, which undergo senescence after ~50 cell divisions and show chromosomal aberrations at the end of their replicative life span (Hayflick, 1965).

Epilogue

This tale of telomerase and telomeres ends in 1990, before the full implications of this discovery were realized (Cech, 2004). A few years later, the medical community took note of telomerase when a sensitive assay revealed that its activity is widespread in human cancer; normal human cells showed up negative in this assay. The absence of telomerase and the fact that telomeres of primary human cells continually shorten suggested that their replicative senescence could be due to telomere loss. To test this proposal, it was necessary to find a way to activate telomerase. With the identification of the telomerase reverse transcriptase (TERT), yet again from a ciliate (this time *Euplotes*) and from yeast, and the subsequent isolation of the human ortholog, hTERT, this crucial experiment became possible. When introduced into primary human cells, hTERT reconstituted robust telomerase activity and this abrogated telomere shortening. As predicted, such cells do not undergo replicative senescence; they are immor-

tal. This result not only proved the concept of a telomere-dependent mitotic clock but also solidified the view of telomere attrition as a tumor suppressor mechanism that can limit the progression of human malignancies. Given that most human cancers by-pass this block by activating telomerase, telomerase inhibitors are predicted to have broad efficacy and relatively mild side effects.

The question of whether telomere attrition contributes to aging phenotypes has proven much harder to address, in part because telomeres do not shorten naturally in the few animals amenable to genetic manipulation. However, patients with inherited deficiencies in telomerase develop severe hematological symptoms, suggesting that telomere shortening can wreak havoc in the bone marrow. The contribution of telomere attrition to blood disorders and other conditions in the aged is not yet understood, and it remains to be seen whether manipulation of telomerase can be beneficial in these and other contexts. Hopefully, the story of telomerase will not come to an end before its tremendous promise has paid off in the clinic. In the meantime, we also need to unravel a related puzzle: how do telomeres, the product of telomerase, protect chromosome ends?

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