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The Ends Have Arrived

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Abstract

The 2009 Nobel Prize in Physiology or Medicine has been awarded to Elizabeth Blackburn, Carol Greider, and Jack Szostak for their contributions to our understanding of how the ends of eukaryotic chromosomes, telomeres, are replicated by a specialized reverse transcriptase, telomerase. I present a personal view of the telomere field, putting the contributions of these three Nobel laureates into historical context.

The field of telomere biology began in the late 1930s with the work of Herman Muller, who was studying the effects of X-rays on *Drosophila* chromosomes (Muller, 1938). Although he could isolate many types of chromosome rearrangements, he was unable to isolate a terminally deleted chromosome, that is, one lacking its very end. These results led him to the conclusion that the end of the chromosome is a discrete structure that is essential for chromosome stability. Soon thereafter, Barbara McClintock working with corn concluded that one essential function of telomeres is to distinguish DNA breaks from natural chromosome ends (McClintock, 1939). She found that a broken meiotic chromosome frequently fused with another broken end to produce an unstable dicentric chromosome. As she did not detect fusions involving telomeres, she reasoned that they must protect the ends of chromosomes from these destabilizing events. Muller and McClintock both won Nobel prizes (in 1946 and 1983, respectively), although not for their telomere work.

Thirty years later, James Watson, who shared the 1962 Nobel prize for the structure of DNA, suggested another function for telomeres. The properties of DNA polymerases indicated that these enzymes cannot start DNA synthesis de novo (they need a primer, which is typically made of RNA) and synthesize DNA only in the 5′ to 3′ direction. From these facts, Watson reasoned that the complete replication of the ends of linear genomes presents a problem that is not faced by circular DNA molecules and suggested that special structures at DNA ends might promote their replication by a nonstandard mechanism (Watson, 1972).

The molecular era of telomere biology began when Elizabeth (Liz) Blackburn, fresh from PhD research with Fred Sanger at Cambridge University, joined Joseph (Joe) Gall's lab at Yale University as a postdoctoral fellow. In 1980, Sanger won one of his two Nobel prizes for developing methods for sequencing DNA. Joe suggested to Liz that she apply the sequencing expertise she had acquired in Sanger's lab to the ends of the ribosomal DNA (rDNA) from the ciliated protozoan *Tetrahymena*. Joe's lab had been working on the macronuclear rDNA of *Tetrahymena* for several years, having previously shown that it is a high-copy linear, palindromic episome. Moreover, the rDNA can be isolated in reasonably pure form by its differential buoyant density in cesium chloride, an important consideration as Liz's sequencing of rDNA telomeres was done prior to DNA cloning. These experiments revealed that the ends

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of *Tetrahymena* rDNA consisted of an irregular number of precise C4A2/T2G4 repeats with an average of 300 basepairs per end (Blackburn and Gall, 1978).

The next critical step in telomere research was the sequencing of DNA ends in another class of ciliated protozoans that includes the various *Oxytricha* species (Klobutcher et al., 1981). This work, done in David Prescott's lab at the University of Colorado, revealed intriguing similarities and differences between the ends of DNA molecules in two distantly related ciliates. As in *Tetrahymena*, the ends of *Oxytricha* DNA molecules are simple repeats. Although not identical, the *Oxytricha* telomeric sequence, C4A4/T4G4, is clearly related to the sequence of *Tetrahymena* rDNA termini. However, *Oxytricha* telomeres are short and precise, only 20 basepairs per end. Moreover, the G4T4 strand of the *Oxytricha* terminus is extended to form a sixteen base 3′ single-strand "G-tail," a structure later found to be a virtually universal and essential feature of eukaryotic telomeres.

At face value, it is perhaps surprising that ciliates have played a critical role in telomere studies. Their prominence stems from the unusual nuclear dimorphism that characterizes these organisms. Each ciliate has two types of nuclei that are distinct in both structure and function. The small micronucleus is diploid and contains conventional chromosomes, but these chromosomes are transcriptionally inert and participate only in meiosis. Transcription occurs almost exclusively in the large poly-ploid macronucleus, which is derived after mating and meiosis from a new micronucleus. During the process of macronucleus formation, micronuclear chromosomes undergo massive rearrangements involving deletion, ligation, telomere addition, and amplification. In organisms like *Oxytricha*, this genome rearrangement is so extreme that macronuclear DNA molecules are, on average, only ~2-3 kilobasepairs in size, resulting in an amazing density of telomeres per microgram of DNA. In *Tetrahymena*, genome rearrangements are more modest. However, this process generates ~20,000 copies of the episomal rDNA from a single micronuclear copy, which provided a rich source of telomeric DNA for Blackburn's sequencing studies. The remarkably high concentration of telomeres in ciliate macronuclear DNA explains why these somewhat obscure organisms are a gold mine for telomere biologists.

As described above, ciliates have an unusual genome organization. Thus, the generality of the emerging model for telomeric DNA was not clear until it could be extended to an organism with conventional chromosomes. Liz Blackburn and Jack Szostak collaborated on a set of experiments (Szostak and Blackburn, 1982) that made it possible to characterize telomeric DNA in the budding yeast *Saccharomyces cerevisiae* (Shampay et al., 1984). For these experiments, Szostak and Blackburn ligated *Tetrahymena* rDNA telomeres onto both ends of a yeast plasmid, introduced this plasmid into yeast, and found that it was maintained in linear form. Moreover, by digesting cellular DNA from a yeast transformant carrying this plasmid with an enzyme that lopped off one of the *Tetrahymena* ends, ligating the mixture, and reintroducing it into yeast, they obtained plasmids with *Tetrahymena* telomeric repeats on one end and a yeast telomeric fragment on the other. After the yeast ends were sequenced (Shampay et al., 1984), it was clear that *S. cerevisiae* and *Tetrahymena* telomeric DNAs are remarkably similar. Although the *S. cerevisiae* sequence is internally heterogeneous, C1-3A/TG1-3, it too consists of a variable number of simple repeats with the GT-rich strand forming the 3′ end of the telomere.

The identification of yeast telomeres was also important because it paved the way for telomere studies in an organism with superb genetics. Vicki Lundblad, while a postdoc in the Szostak lab, carried out a screen to identify genes involved in telomere maintenance (Lundblad and Szostak, 1989). This work identified Est1, later shown to be a subunit of yeast telomerase, and found that in its absence, cells were viable for 50-100 cell divisions during which time they slowly lost telomeric DNA, the so-called *EST* (ever shorter telomeres) phenotype. When

telomeres become critically short, chromosome loss increases dramatically and most *est* cells die.

After the discovery of ciliate telomeres, telomeric DNA was sequenced and studied in multiple, diverse organisms (many of these sequencing studies were done in the Blackburn lab). From various studies, there were hints that telomere replication might occur by a nonstandard mechanism. As first shown in *Tetrahymena*, telomeric restriction fragments usually form "fuzzy" rather than discrete bands when analyzed in agarose gels, reflecting the variability in the number of telomeric repeats at individual telomeres. Second, analysis of the generation of *Tetrahymena* macronuclear rDNA revealed that only one telomeric repeat is encoded in the micronuclear DNA (Yao and Gall, 1977). Third, *Trypanosoma* telomeres actually increase in size with each cell division (Vander Ploeg et al., 1984). Fourth, when ciliate telomeres are used to generate linear plasmids in yeast, the ciliate telomeres are lengthened by the addition of yeast telomeric DNA (Pluta et al., 1984; Shampay et al., 1984). All of these data suggested that telomeric DNA is not templated by an existing telomere.

Even though there were hints that telomeric DNA was not replicated by standard semiconservative DNA replication, it is my opinion that the discovery of telomerase was not a finding "whose time had come." That is, unlike discoveries such as splicing, it was not discovered simultaneously in multiple labs. Rather, the search for a biochemical activity that could lengthen telomeric primers in vitro was not an obvious experiment, and it was certainly high risk and gutsy. Carol Greider, a graduate student with Liz Blackburn at Berkeley, wisely used extracts from post-mating *Tetrahymena* cells undergoing macronucleus formation for the experiments. They found an activity that could lengthen a (T2G4) n oligonucleotide but not nontelomeric or C-strand oligonucleotides. However, consistent with the biology revealed during the earlier yeast experiments, the *Tetrahymena* extract could add T2G4 repeats to a yeast G-strand oligonucleotide (Greider and Blackburn, 1985).

Greider and Blackburn extended these initial findings in several critical ways, resulting in an extremely impressive PhD thesis for Greider. In addition to detecting telomerase, Greider and Blackburn showed that the in vitro activity was RNA dependent (Greider and Blackburn, 1987). Using a biochemical approach, they isolated the *Tetrahymena* telomerase RNA, a technically challenging experiment, and showed that its sequence contains a short segment that could provide a template for T2G4 repeats (Greider and Blackburn, 1989). Later, the Blackburn lab mutated the putative template region of the *Tetrahymena* telomerase RNA, reintroduced it into cells, and demonstrated that mutant repeats were incorporated into telomeric DNA. This result provided definitive proof that telomerase uses its integral RNA component as the template for making telomeric DNA.

After starting her own lab, Greider continued to work on telomerase, although much of her focus switched to mammalian systems. Maria Blasco and others in her lab cloned mouse telomerase RNA and in collaboration with the dePinho and Lansdorp labs used its sequence to generate a telomerase knockout mouse (Blasco et al., 1997). In many ways, the phenotype of these telomerase-deficient mice is similar to that of *est* yeast strains: mice lacking telomerase are initially viable but their chromosomes exhibit progressive loss of telomeric DNA with each cell division and ultimately a plethora of chromosome abnormalities.

Telomerase is a fascinating solution to an important biological question. However, the quest for the answer to how DNA ends are replicated, a quintessential basic science question, turned out to have considerable relevance for human health. In human cells, unlike ciliates or yeast, telomerase is not expressed in most cells, and thus human telomeres slowly shorten with replicative age. However, the vast majority of human tumors express telomerase, and this expression contributes to their ability to divide without limits. Thus, telomerase is compelling

as a virtually universal antitumor target, and considerable current research, including clinical trials, is testing this possibility. In addition, it has become increasingly clear that telomerase is limiting in human stem cells and that several inherited human diseases, such as dyskeratosis congenita, that result in stem cell failure and reduced life expectancy are due to mutations in telomerase subunits. Thus, the telomere story, fascinating from the perspective of biology, is also a ringing endorsement for support of untargeted basic research by the National Institutes of Health and other agencies interested in medical breakthroughs.

Congratulations to Liz, Carol, and Jack.

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