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How do the ends replicate?

Janis Shampay

Biology Department, Reed College, Portland, OR 97202, USA

Prologue

This year's Nobel Prize in Physiology or Medicine goes to Elizabeth Blackburn, Carol Greider, and Jack Szostak for demonstrating the function of telomeres and their maintenance by telomerase. This is a long-awaited and thrilling development; in the best tradition of research into fundamental biological questions, the impacts have reached far beyond the telomere field's humble, even obscure, beginnings.

A time capsule

I first faced this question in 1981, as a senior at Northwestern University. I was working in the yeast DNA replication lab of Larry Dumas, when a visiting seminar speaker inspired a drawing on the lab's chalkboard of a DNA double helix, with the end unwound and all the 5′s and 3′s labeled. At the bottom, in Larry's neat handwriting, was "How do the ends replicate?" Because it stayed up for some weeks, I readily remembered it when, ironically, I found myself in Liz Blackburn's lab as a first-year graduate student.

My project was to follow up on a remarkable experiment. Liz, who studied ciliate telomeres, and Jack Szostak, the yeast expert, had just put the terminal fragments of the *Tetrahymena* extrachromosomal rDNA on a yeast linear plasmid, and it was in press at *Cell*. Somehow, a piece of DNA with a simple repeating sequence, $(CCCCAA)_n$, could function as a stable end in an evolutionarily distant organism. Was there something about the DNA sequence that made a stable end, and how? Broken chromosomes fused – that much we'd known since the 1930s – and if you transformed yeast with a non-telomere-bearing linear plasmid, the few resulting colonies were due to rearrangements or integration into the chromosome. Jack and Liz used this knowledge to develop a powerful selection scheme to clone a yeast telomere. Finally, a chromosomal telomere was in hand, awaiting an explicit determination of its structure. To this day, Szostak and Blackburn resides in my VIP (Very Important Papers) folder [1].

Would this yeast telomere and the yeast-modified ciliate telomere help address the end replication problem? As one of the most basic questions about DNA since its double-helical structure was determined, it derives from the directional, anti-parallel DNA strands, and the requirement for a base-paired primer for DNA polymerase to proceed down its one-way street. The virus literature described baroque solutions to the problem: hairpin ends of vaccinia, palindromic sequences on parvovirus termini, a nucleotide covalently attached to an adenovirus end-associating protein. Not surprisingly, the theoretical models of the day also featured palindromes, transient fusions, and hairpin ends. In general, they attempted to explain how each nucleotide at the end could be replicated precisely.

But the experimental field centered on other observations. There was a cottage industry in cataloging telomeric DNA repeat units from any organism amenable to the techniques available: CCCCAA, CCCCAAAA, $C_{1\text{-}8}T$, typically on extrachromosomal rDNAs or other subchromosomal structures in peculiar, unicellular organisms [2]. The length heterogeneity exhibited by most DNA termini (a.k.a. fuzzy bands on gels) figured prominently in our thinking, as did the continuous, incremental addition of sequences onto trypanosome telomeres [3], soon to be observed in *Tetrahymena* [4]. It was difficult to reconcile slow DNA additions with a palindromic or hairpin model; I know because I tried, according to some unsatisfying diagrams in an old notebook. (Recombination-based mechanisms ultimately fared better, as we now know that recombination can and does take place at telomeres in wide-ranging species [5,6].) Just as importantly, other contemporary projects in the Blackburn lab – and other ciliate labs – focused on the developmentally programmed, extensive genomic rearrangements and mass addition of telomeric repeats onto the newly formed ends. Ciliate

Corresponding author: Shampay, J. (shampay@reed.edu).

rDNA termini were lengthened after propagation on yeast linear plasmids [1,7]. As we grappled with these conceptual issues, at the lab bench I was end-labeling restriction fragments with terminal deoxynucleotidyl transferase to sequence the ends of the precious linear plasmid that Jack and Liz built.

There had been whispers of the possibility of a terminal-transferase-like activity, even while those authors were presenting other mechanisms [2,7,8]. Determination of the DNA sequence of a *bona fide* chromosomal yeast telomere, and a precise look at the junction between ciliate telomeric repeats and the added yeast sequences, tipped the balance. The proposed solution, a telomere terminal transferase to add onto telomeric 3′ ends [9], was eminently logical in light of all the observations. If you want to be able to add DNA during replication: just do it!

A model, however, is just a model. Carol Greider entered the Blackburn lab at this auspicious time and embraced the idea of looking for this putative enzymatic activity. It was a long shot, a risk (would we do that in this day and age?), but she thoughtfully used a source most likely to have an abundance of the activity: a developmental stage of *Tetrahymena* at the peak of chromosome fragmentation and telomere addition [10]. With her success, recognition of a stable end, a telomere, was molecularly redefined as the ability of telomere terminal transferase to use a heterologous telomeric repeat sequence as a substrate for DNA addition, explaining how Jack and Liz's trans-kingdom experiment worked. And in a satisfying correction of the 1984 "untemplated" addition model, Carol identified an RNA template in what was now called telomerase [11]. Simultaneously, genetic evidence of such an activity sealed the deal [12]. Almost overnight, the literature shifted from describing telomeric repeats of different species as $(CCCCAA)_n$ or $(CCCTAA)_n$, to $(TTGGGG)_n$ and $(TTAGGG)_n$ – a focus that makes sense, since the enzymatic addition takes place on the 3′ end of the T-G strand.

A decade earlier, Olovnikov had proposed that normal somatic cell division is compromised by incomplete chromosome replication, but we were not aware of his work [13]. We were studying unicellular eukaryotes – as were most of the few labs interested in telomeres – and for these critters, reproducing vegetatively, dividing constantly, there just had to be a solution to the end-replication problem. That was the focus, not the absence of a solution under normal circumstances.

Telomere biology in contemporary research

As with most important scientific advances, the answer generates additional intriguing questions. The ensuing years have witnessed an explosion of activity, all made possible by acquiring this handle on telomere structure and metabolism. Wide-ranging phenomena in biology have been touched by this storm; a non-exhaustive list comes to mind.

In the 1980s, introductory paragraphs for telomere papers usually included the boilerplate statement that "telomeres must distinguish stable chromosome ends from unstable chromosome breaks." In a satisfying turn of events, a critically short telomere, or disruption of the telomere complex, makes telomeres resemble broken ends [14,15]. This leads to recruitment of DNA damage response factors, activation of cell-cycle checkpoints, and a variety of outcomes, depending on the cell type or the nature of the dysfunction. These include cell-cycle arrest, outright apoptosis, and some of the most dramatic examples of end-to-end chromosome fusions [16].

It is now well established that the cellular senescence exhibited by primary human cell lines is determined by telomere length. This is a fascinating finding in itself, linking the cell doubling limit to the appearance of apparent chromosome breaks due to telomere erosion in the absence of telomerase. Ectopic expression of the telomerase protein component, TERT, in combination with the ubiquitously expressed RNA template effectively immortalizes fibroblasts [17]. This provides a simple pragmatic benefit: research using cell culture models need no longer rely on tumor cell lines, with their aneuploid karyotypes and myriad genetic quirks.

In what is often described as the flip-side of cellular senescence, telomerase activity is present in most cancers, but it is low or undetectable in most normal human somatic tissues. This observation has led to one of the biggest explosions; indeed, the current PubMed tally with a search for "telomerase and cancer" exceeds 5000 entries. The targeting of telomerase, or of telomere structure more generally, has opened new avenues for the understanding of malignant transformation and new possibilities for its treatment [18]. An alternative view is that maintenance of telomere length by telomerase could be oncoprotective, by avoiding the genome instability that is a hallmark of cancer [19]. This lively debate continues.

Nevertheless, the yin and yang of cellular senescence/cellular immortality has led to a very *Homo sapiens*-centric view, that telomere maintenance is important only to organismal reproduction. Only dividing cells have an end-replication problem, so why not just shut it down in most differentiated tissue? Just because this seems logical does not make it universally true. In fact, this kind of telomerase regulation is simply one end of a spectrum of observed patterns: at the other end, the somatic tissues of fish [20,21] and frogs [22], and even sheep brains (A.S. Hayden, undergraduate thesis, Reed College, 2003) have readily detectable, even abundant telomerase. Whether this is due to telomerase activity within terminally differentiated cells, or a greater abundance of telomerase-positive stem cells in those species' organs is unknown. What does seem certain is that these differences are not insignificant and there are lessons yet to be learned from organisms other than the usual suspects.

When the first speculation arose that telomere erosion might be a cause of cellular senescence, I was skeptical that it might have anything to do with organismal aging. However, it is now clear that the physiological changes observed in telomerase-deficient mice resemble organ senescence, and that these changes can be ameliorated by constitutive expression of TERT [23]. Furthermore, a rare inherited condition known as dyskeratosis congenita, which has some features of premature aging, is linked to defects in telomerase, associated proteins and, most recently, to a component of the telomere protection complex [24,25]. The disorder even shows genetic anticipation, as would be expected for

a slowly eroding telomere. (This makes a great take-home exam subject for a human genetics class!) However, these two examples represent either extreme experimental manipulations or pathological conditions; whether I can blame my ordinary graying hair and aging skin on a short telomere or two remains to be seen.

Finally, in the telomere world as in others, it seems the more you learn, the more you discover that many proteins moonlight in other venues. There is growing evidence that TERT has cellular effects unrelated to its canonical telomere-extension job. Most recently, it has been shown to have RNA-dependent RNA polymerase activity [26], but my favorite observation is that it can act as a traditional terminal transferase [27], thus coming full circle with that first model.

Epilogue

As an embryonic scientist, I was not privy to the reasoning in Liz's grant proposals. But in the lab, I don't ever recall talking about cancer, or cellular senescence, or aging, at least not with respect to telomeres. Since the 5 October 2009 Nobel announcement, I have not been the first to point out that this award is a validation of the critical importance of basic research, and I won't be the last. It all started with a few labs working on quirky little organisms, wondering simply "how do the ends replicate?" And look where we are today.

It's all about asking a question.

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