Angewandte International Edition Chemie

Telomeres/Telomerase

DOI: 10.1002/anie.201002387

# Telomeres and Telomerase: The Means to the End (Nobel Lecture)**

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DNA · Nobel lecture · telomerase · telomeres

## Autobiography

### Childhood

I was born in the small city of Hobart in Tasmania, Australia, in 1948. My parents were family physicians. My grandfather and great grandfather on my mother's side were geologists. My great-grandfather on my father's side, before coming to Australia as a minister of the Church of England, had lived for some time in Hawaii, where he had collected Coleoptera (beetles). He continued his collecting in Australia, eventually selling his collection to the British Museum of Natural History. My uncle and aunt (my father's sister and my mother's brother) were also both family physicians, who moved to England, married there and permanently settled there to practice medicine and raise their families.

I was the second child of eventually seven siblings. I spent my first 4 years living in the tiny town of Snug, by the sea near Hobart. Curious about animals, I would pick up ants in our backyard and jellyfish on the beach. Then my family moved to Launceston, a town in northern Tasmania. Our first house, at 120 Abbott Street, was a one-storied, verandahed house of typical Australian suburban architecture. I started kindergarten at a girls' school, Broadland House Girls Grammar School in Launceston.

Elizabeth Blackburn and her sister Katherine ready for Elizabeth's first day at school in Launceston, Tasmania. Circa 1953.

I kept tadpoles in rapidly-smelly-becoming glass jars in a back living room at home. When I was a preteen we moved to a larger house called Elphin House, which had a good-sized garden. Over the years we had many pets: at one stage I enumerated the family menagerie of the moment as consisting of budgerigars and canaries in an aviary in one corner of the garden, goldfish in a garden pond, chickens and pullets (for eggs and the occasional roast fowl) in a hen coop and henhouse, rabbits and guinea pigs in cages, and cats and a dog, who lived all over the house and garden. I was fond of all these animals, and of animals and nature in general.

All seven Blackburn family siblings in the garden at their home, 3 Olive Street, Launceston, Tasmania. From left to right, back row: Andrew, Elizabeth, Katherine, John, Barbara; front row: Caroline, Margaret. Circa 1965.

Perhaps arising from a fascination with animals, biology seemed the most interesting of sciences to me as a child. I was captivated by both the visual impact of science through science books written for young people, and an idea of the romance and nobility of the scientific quest. This latter was especially engendered by the biography of Marie Curie, written by her daughter, which I read and reread as a child. By the time I was in my late teens it was clear to me that I wanted to do science. I was educated at Broadland House Girls Grammar School, and received a generally excellent educa-

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tion. However, physics was not offered, so I took physics classes offered in the evenings at the local public high school. Latin and Greek were not taught at my girls' school either, a gap in my education that I rather regret later in life. But my school did provide an excellent piano teacher, Helen Roxburgh, by whom I was taught all throughout my school years in Launceston. I loved playing the piano, and even at one time wistfully hoped that I might become a musician. Fortunately I was also quite realistic about this, because I recognized that I was competent rather than greatly talented at piano playing, so I went in the direction of science.

My family moved, after some family disruptions, to the city of Melbourne, Australia, in time for me to complete my last year of high school at University High School. There I gained the confidence that I needed to apply for the undergraduate science degree at the University of Melbourne.

### *University Education*

I chose biochemistry as my major and graduated after 4 years with an Honours degree in Biochemistry. During that time, I had come to love biochemistry research, although I was just getting my feet wet in laboratory research.

The Chair of the Biochemistry Department, Frank Hird, then offered me a position as a Master's student in his research laboratory, where they investigated the biochemistry of amino acid metabolism. My undergraduate Honours thesis research advisors, Theo Dopheide and the late Barrie Davidson, had advised and encouraged me to do my Ph.D. abroad. Barrie in particular had urged me to consider going to the MRC Laboratory of Molecular Biology (LMB) in Cambridge, England, where he had done postdoctoral research. But in order to be accepted as a Cambridge Ph.D. student in biology, those from outside Britain were required to have done a year of research. The Master's degree with Frank Hird, studying the metabolism of glutamine in the rat liver, would constitute this required year.

Frank Hird taught his laboratory group members the joy and aesthetics of research. He said he thought each experiment should have the beauty and simplicity of a Mozart sonata. His laboratory group, dominated by his strong personality, was cohesive and we would sometimes drive to the hilly areas outside Melbourne, all piled into his car, Mozart playing loudly on the car radio, to have an outdoor lunch picnic among trees and wildflowers.

While I was still in Frank Hird's lab, Fred Sanger visited Melbourne. Frank Hird had done research in England on amino acids with Fred Sanger shortly after the Second World War, providing an introduction to Fred in which Frank encouraged me to tell Fred of my hope to study for my Ph.D. in Cambridge at the LMB. It was arranged that I would join Fred's lab in the LMB and I was admitted as a Cambridge Ph.D. student.

The adventure of setting off to England, away from home and family, was a huge step, but I felt ready. My aunt and uncle and their family in Cambridge, who lived close to the LMB, became my anchor of a family away from home. I loved the LMB, the science being done there, the atmosphere of being at the epicenter of molecular biology, the intensity of the scientists and the constant discussions about science. It was a world of complete immersion. For my Ph.D. thesis research, I carried out sequencing of regions of bacteriophage ΦX174, a small single stranded DNA bacteriophage. I transcribed fragments of the phage DNA into RNA and then used the methods that Fred had pioneered for piecing together RNA sequences. We combined the sequences derived by this method with the DNA sequencing that had been done by John Sedat and Ed Ziff and Francis Galibert, members of Fred's lab. All the sequences jibed. The first sequence of a 48-nucleotide fragment of this tiny bacteriophage DNA genome was a great excitement. I took it to show to my mathematically-talented Cambridge cousin, who was then about 12 years old, to see if any patterns emerged to his mathematically-inclined eye. He pointed out the repeats, but it was premature to think of analyzing DNA sequence patterns!

### *To the United States*

The world of discovering DNA sequences was opening up and I was entranced by its possibilities. I had planned to do a postdoctoral fellowship, beginning in 1975, with Howard Goodman and his close associate Herb Boyer of UCSF, a mutual decision made after an interview-cum-conversation Herb and I had walking through the garden of a monastery in Belgium at which we were attending a scientific conference, I still as a graduate student. But then love intervened: John Sedat and I decided to marry, and as John was going to Yale, I decided to see if I could change my postdoctoral research plans (for which I had obtained an Anna Fuller Fellowship to work at UCSF) to a laboratory at Yale. Howard Goodman wrote me a kind and understanding letter upon my letting him know the reasons for my change of plan, and I began inquiries into possibilities of a laboratory for my postdoctoral training at Yale.

Thus it was that love brought me to a most fortunate and influential choice: Joe Gall's lab at Yale. After a few hiccups engendered by misplaced international mail and other factors, at the beginning of 1975 Joe accepted me as a postdoctoral fellow in his lab, to which I was allowed to transfer my Anna Fuller Fellowship. I immediately began to work on finding ways to accomplish the sequencing the DNA found at the terminal regions of the abundant, short, linear ribosomal gene-carrying "minichromosomes" that Joe and his colleagues, in parallel with Jan Engberg of Denmark, had discovered in the somatic nucleus of the ciliated protozoan *Tetrahymena thermophila* (which was at that time called *Tetrahymena pyriformis*, shortly thereafter to be renamed *Tetrahymena thermophila*)

### *To the University of California*

After finishing my postdoctoral training in Joe Gall's lab at the end of 1977, John Sedat and I, having married in 1975,

moved to San Francisco, California. There John had accepted a position as Assistant Professor at the University of California San Francisco (UCSF). I had applied for several positions as an Assistant Professor in a variety of Universities and had been rejected from many of them, a discouraging experience. I had applied for such a position in the Department of Molecular Biology at the University of California Berkeley, but had not yet heard whether I was in the running for it. In the meantime, UCSF offered me a research track position and space in the Department of Biochemistry in the Genetics unit headed by Herb Boyer. My first NIH grant was the source of funding for my salary and research expenses. I had written this grant application with the encouragement of UCSF, in order to pursue my research on *Tetrahymena* telomeres and their associated proteins. This work grew out of that I had done in Joe Gall's laboratory at Yale. My grant was funded by the NIH General Medicine Institute. Unsure of my chances at obtaining funding, I had sent the same grant application to the National Institutes of Health, the National Science Foundation and the American Cancer Society, hoping for funding from any one of these. Reflecting the more informal scientific habits of the basic sciences community in those days, some time later one of the grant reviewers told me that he had been so intrigued by my photograph of the autoradiogram showing that telomeric DNA in *Tetrahymena* was mysteriously packaged as something other than nucleosomes that he had kept the photograph.

Then UC Berkeley offered me an Assistant Professor position in the Molecular Biology Department, which I immediately accepted. Once again, I transferred my funding from UCSF, this time to my own laboratory, at UC Berkeley.

Because research was, and still is, such a central part of my life, my autobiography would be incomplete without describing my research experiences. Thus, to convey a fuller flavour of them, here I describe some of the events of my early scientific research on the molecular nature of ends of chromosomes.

### *Early Work on the DNA at the Ends of Eukaryotic Chromosomes*

Very soon after arriving in Joe Gall's laboratory at Yale in early 1975, I started to apply methods for obtaining terminal DNA sequences to the *Tetrahymena* rDNA molecules. I had learned a collection of methods in Fred Sanger's laboratory in Cambridge, England, where I had just completed my Ph.D. Eager to sequence the end regions of these minichromosomes, with Joe's encouragement I set out right away early in 1975 to use end-labeling techniques on them. I incorporated $^{32}$P isotope-radiolabeled deoxynucleoside residues into the *Tetrahymena* rDNA molecules using commercially available DNA polymerases for in vitro DNA repair enzymatic reactions. The results were immediately promising. First, it became clear that the end regions of the rDNA were being selectively labeled by certain combinations of $^{32}$P isotope radiolabeled nucleoside substrates. And by June 1975, I had become tremendously excited: I had obtained my first autoradiogram of the two-dimensional separation of the $^{32}$P labeled depurination products. A strong signal of a run of 4 cytosine (C) residues was apparent. Furthermore, each such $C_4$ sequence was flanked by a purine residue (that is, an adenosine (A) or a guanosine (G) residue; this initial data did not show which). The way the depurination reaction worked was the following: Ken Burton, a New Zealander, had shown that a chemical reaction could be done that cleaves the DNA backbone on both sides of every purine nucleotide but leaves intact any runs of pyrimidine nucleotides (such as C residues) that are uninterrupted by purine nucleotides. This so-called depurination method, when applied to a complex sequence DNA like a whole bacteriophage genome, was further made useful by Vic Ling, when he was a postdoctoral fellow in Fred Sanger's lab in Cambridge, England. Vic had shown that the resulting short pyrimidine tracts (mono-, di-, tri-nucleotides, etc.) would yield a pattern of products like a grid when a 2D fractionation method was used (see 2009 Nobel Lecture by Elizabeth Blackburn, in this Volume). The most frequent products, on a random basis, of course are mono- and dinucleotides, with the longer tri- and tetranucleotide tracts being less and less abundant on a random basis. Thus this strong $C_4$ spot was interesting and informative.

It was also clear to me that the rDNA molecules were not simply lambda phage-like DNA. First, the terminal fragments were heterogeneous. In contrast, for any one type of lambdoid phage, in every viral particle the DNA molecule is just the same—a perfect carbon copy—as in every other viral particle. Second, on a per DNA molecule basis, much more incorporation of radiolabeled nucleotide precursor substrates occurred than expected for if rDNA molecule ends were like those of the sticky DNA ends of lambda phages. It was also fortunate that the $C_4$ repeat had such a regular nature and that it happened to have four Cs in a row. Even three Cs would have been striking, but harder to unravel. As it was, the $C_4$ spot consistently stood out like a beacon through my repeated productions of 2D fractionations ("homochromograms" as we called them in Fred Sanger's lab).

Next, I needed to validate independently that what I was radiolabeling in vitro validly reflected the rDNA sequence, and also try to get an closer estimate of the number of $C_4$ runs per rDNA molecule. I therefore decided I needed to $^{32}$P isotope-label the rDNA in vivo. The vast majority of the $^{32}$P isotope (chemically in the form of inorganic phosphate ions) taken into the cells would be incorporated into other molecules, including the much more abundant cellular RNA, with very little ending up in DNA, and even less in the rDNA (in *Tetrahymena*, only a percent or so at most of the DNA is rDNA). Therefore, with some trepidation I asked Joe Gall for permission to order sufficient $^{32}$P phosphate to be added to the cell growth medium for labeling the rDNA. This meant handling 2 milliCuries at once, which Joe's lab had not done before. But I knew that this was the only way currently available to get a sufficient amount of $^{32}$P into rDNA to detect the C4 spot in an autoradiogram, above background. Possibly with some trepidation on his side too, Joe agreed. I worked in the "hot room", a room set aside for doing work with radioactive isotopes. On October 9, 1975 the $^{32}$P was shipped to Yale. My laboratory notebook from that time reads: "$^{32}$P stored in refrig. until use—assayed for 10/14. 3 pm 10/14. Zeroed [the cell culture] on 1 % PPS medium blank.. Added 2

mCi 32p as h3 PO4 in water in 1 mL from syringe." For the next preparation I raised it to 5 milliCuries. Curious about this very radioactive departure from the more usual activities of the lab, my Gall labmates periodically looked in through the window set in the door of the hot room as I worked.

By October 22, 1975, I had the $^{32}$P rDNA purified. I triumphantly wrote down in my notebook that day my plans for this precious sample:

1) Depurination
2) denaturing gel after EcoRi treatment
3) 1.4% agarose gel

One by one, I inflicted various nucleases on the terminal region of the rDNA. I found it could be selectively radiolabeled using one triphosphate $^{32}$P labeled at a time. Then I carried out the battery of analyses possible at the time that would allow me to piece together the nucleotide sequence: I digested the radiolabeled end regions with Endonuclease IV nuclease or micrococcal nuclease, and performed depyrimidations and nucleotide nearest-neighbor analyses, and spleen phosphodiesteriase digestions.

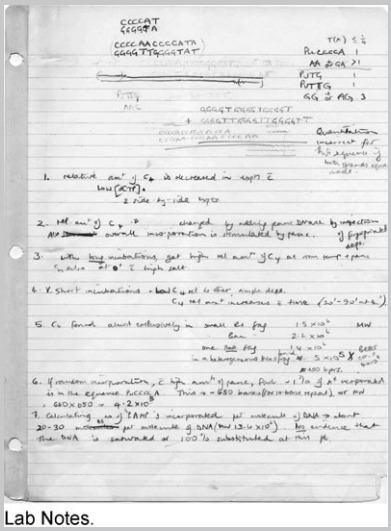
Lab Notes 02/12/1976.

Piecing together the rDNA end sequence was a matter of careful puzzle-solving. At an intermediate point, my notebooks of the time show that I had to consider two possibilities—CCCCAG and CCCCAA repeats. But is was apparent that there were a large number of repeated copies.

But by April 8, 1976, I was confident that the correct sequence was deduced, because the entry in my laboratory note-book page headed with that date reads:

Sequence data from:

1) Depurination
2) Endo IV
- deps [my abbreviation for depurinations]
- nearest neighbours
3) partial micrococcal
- deps
- nearest neighbours



Lab Notes.

- partial spdes [my abbreviation for spleen phosphodiesterase digestions]

4) Gel analysis of Endo IV digest of restriction fragments t ends of rDNA

→ AACCCC repeated

→ (AACCCCAACCCCAACCCC)etc.

Another laboratory note-book page, dated August 17 1976, shows I was already referring, in a routine way, to (CCCCAA)n sequence—by then in the course of experiments designed to see whether this same repeated sequence was also present in the other (much longer) chromosomal DNAs of *Tetrahymena*.

I put together a picture which tried to take into account all of my many observations. The deduced sequence consisted of a tandem array of CCCCAA repeats. One experiment done in 1979, radiolabeling the rDNA using just $^{32}$P-labeled dCTP, and unlabeled dATP, and separating the products on a denaturing gel electrophoresis, showed this visually as a beautiful ladder of tiger stripes extending up the gel. The size of every band in this regular ladder was 6 bases more than the band below it! This strikingly characteristic visible pattern of bands presaged the pattern that would later become important for our discovery of telomerase enzyme activity, as described in the Nobel Lecture by my co-Laureate, Carol Greider.

In the months following April 1976 much of my effort was also devoted to trying to understand the arrangement of DNA strand discontinuities along the tract of CCCAA repeat DNA. For this, I performed a great many experiments following the kinetics and specificity of radiolabeling the RNA end regions, using multiple different enzymes and protocols and analyses. This also was a matter of piecing things together—there was no template for me to work from as this was all uncharted territory.

In the late 1970s and early 1980s I did a variety of radiolabeling experiments trying to divine the structure right at the termini of telomeres of both ciliated protozoans and yeast linear plasmids. I could put together a composite but still incomplete picture. Some of the features, I realize in

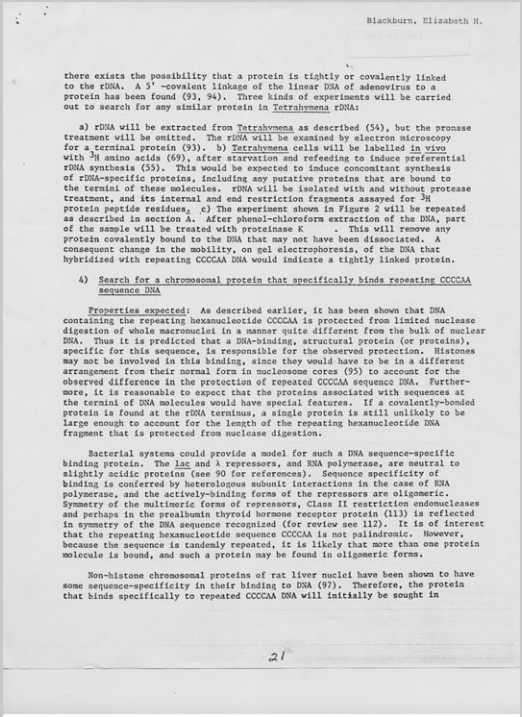
  

retrospect, might be attributed to the terminal G-strand sequence at the very ends of the telomeres assuming G–G paired or G-quartet structures. But other features are not so readily explainable. Why I was able to label the strands with DNA polymerase or a kinase to get the patterns of labeled strands and nucleotides I did has still not been completely fitted into a coherent view of the molecular structure of DNA ends. Various kinds of in vitro radio-labeling experiments of had suggested that, in both *Tetrahymena* rDNA and the macronuclear DNAs of hypotrichous ciliates, there is a short overhang of the G-rich strand consisting of a few telomeric repeat sequences. Currently the view is that in mammalian telomeres there is a long protruding G-rich strand. Yet this does not take into account the clear evidence for the short C-strand repeat oligonucleotides that I discovered can be readily melted off the telomeric DNA. This I found for both the *Tetrahymena* rDNA minichromosome molecules and linear plasmids purified from yeast. These tiny telomeric sequence oligonucleotides could be radiolabeled and clearly identified by two dimensional fractionations. However their significance is still unknown. To this day, aspects of the structure at the very terminal region of the telomeric DNA are enigmatic; the very ends of chromosomes remain as challenges.

### *Telomere Proteins: Earliest Attempts And Failures*

In my early work, my molecular views of telomeres were first focused on the DNA; not only because DNA was uppermost in my mind, but for several years DNA was also the only component of the telomeres that was identified. This was not for want of trying. I thought that DNA would not be entire story of chromosome ends and, by extension from work emerging about chromatin in general in the 1970s, that it was likely that the telomeric DNA repeats tract would be packaged with proteins. The 1970s had seen great interest in chromatin, and the discovery of nucleosomes as the basic packaging unit of eukaryotic DNA. Telomeric sequences in *Tetrahymena* looked very intriguing to me in that regard, and as soon as I had identified the telomeric DNA I wanted to get my hands on whatever packaged it. Therefore, while still a postdoctoral fellow in Joe Gall's laboratory, I performed micrococcal nuclease treatment on isolated *Tetrahymena* nuclei. I found that the CCCCAA$_n$ tracts of the telomeres were protected in chromatin as a heterogeneous class of DNA fragments very different from that expected for nucleosomal packaging.

Soon after moving from Joe Gall's lab at Yale, while still temporarily at the University of California, San Francisco in an independent research position (before I moved to the University of California, Berkeley as an Assistant Professor), on March 1, 1978, I wrote to Joe Gall: "I am getting quite excited about getting a CCCCAA$_n$-binding protein complex from the *Tetrahymena* macronuclei, so I've been busily making rDNA, the CCCCAA$_n$ probe, and macronuclear micrococcal nuclease digests. Results so far are that I've found a simple salt fractionation that enriches for CCCCAA$_n$ sequence plus putative protein(s). The plan at the moment is to purify this some more so I can get some structural characteristics of any such complex, that is, S value, and some identification of protein(s) in terms of 1-D and 2-D gel eletrophoretic properties […] The other aspect of course is to fish for something that will stick to a CCCCAA$_n$ column."



Blackburn, Elizabeth H.

there exists the possibility that a protein is tightly or covalently linked to the rDNA. A 5' -covalent linkage of the linear DNA of adenovirus to a protein has been found (93, 94). Three kinds of experiments will be carried out to search for any similar protein in Tetrahymena rDNA:

a) rDNA will be extracted from Tetrahymena as described (54), but the pronase treatment will be omitted. The rDNA will be examined by electron microscopy for a terminal protein (93). b) Tetrahymena cells will be labelled in vivo with $^{3}H$ amino acids (69), after starvation and refeeding to induce preferential rDNA synthesis (55). This would be expected to induce concomitant synthesis of rDNA-specific proteins, including any putative proteins that are bound to the termini of these molecules. rDNA will be isolated with and without protease treatment, and its internal and end restriction fragments assayed for $^{3}H$ protein peptide residues. c) The experiment shown in Figure 2 will be repeated as described in section A. After phenol-chloroform extraction of the DNA, part of the sample will be treated with proteinase K . This will remove any protein covalently bound to the DNA that may not have been dissociated. A consequent change in the mobility, on gel electrophoresis, of the DNA that hybridized with repeating CCCCAA DNA would indicate a tightly linked protein.

4) Search for a chromosomal protein that specifically binds repeating CCCCAA sequence DNA

Properties expected: As described earlier, it has been shown that DNA containing the repeating hexanucleotide CCCCAA is protected from limited nuclease digestion of whole macronuclei in a manner quite different from the bulk of nuclear DNA. Thus it is predicted that a DNA-binding, structural protein (or proteins), specific for this sequence, is responsible for the observed protection. Histones may not be involved in this binding, since they would have to be in a different arrangement from their normal form in nucleosome cores (95) to account for the observed difference in the protection of repeated CCCCAA sequence DNA. Furthermore, it is reasonable to expect that the proteins associated with sequences at the termini of DNA molecules would have special features. If a covalently-bonded protein is found at the rDNA terminus, a single protein is still unlikely to be large enough to account for the length of the repeating hexanucleotide DNA fragment that is protected from nuclease digestion.

Bacterial systems could provide a model for such a DNA sequence-specific binding protein. The lac and λ repressors, and RNA polymerase, are neutral to slightly acidic proteins (see 90 for references). Sequence specificity of binding is conferred by heterologous subunit interactions in the case of RNA polymerase, and the actively-binding forms of the repressors are oligomeric. Symmetry of the multimeric forms of repressors, Class II restriction endonucleases and perhaps in the prealbumin thyroid hormone receptor protein (113) is reflected in symmetry of the DNA sequence recognized (for review see 112). It is of interest that the repeating hexanucleotide sequence CCCCAA is not palindromic. However, because the sequence is tandemly repeated, it is likely that more than one protein molecule is bound, and such a protein may be found in oligomeric forms.

Non-histone chromosomal proteins of rat liver nuclei have been shown to have some sequence-specificity in their binding to DNA (97). Therefore, the protein that binds specifically to repeated CCCCAA DNA will initially be sought in

21

Page from grant application, 1977.

By 1980 I had done experiments to show that telomeric tracts of DNA in *Tetrahymena* were encapsulated in a protective sheath of protein that did not include nucleosomes. The vast majority of chromosomal DNA is packaged as nucleosomes: DNA-protein complexes. Each nucleosome is a flattened ball made up of histone proteins, around which the DNA is wrapped twice. The very basic (positively charged) histone proteins neutralize the negative charges of the phosphate chemical groups arrayed along the phosphodiester backbone of DNA and allow chromosomal DNA to become very closely packed and compactly folded in the nucleus. Nucleosomes in artificially stretched-out chromosomes are like beads on a strong, although mostly in the nucleus they are closely packed into shorter thicker fibers. If one clips up chromatin using an enzyme, micrococcal nuclease, that cuts across the two strands of the linker DNA between neighboring nucleosomes, after getting rid of the histones, one can see that there are nucleosome-sized fragments of DNA left—a fragment of about 142 base-pairs is protected by the histone core of the nucleosome, once the DNA linkers have been trimmed away. This kind of nuclease clipping behavior is a hallmark of a nucleosome. In contrast to nucleosomeal regions of chromosomes, special regions of DNA, for example promoters that must bind transcription initiation factors that control transcription, have proteins other than the histones on them. The telomeric repeat tract turned out to be such a non-

nucleosomal region. I found that if I clipped up chromatin using an enzyme that cuts the linker between neighboring nucleosomes, it cut up the bulk of the DNA into nucleosome-sized pieces but left the telomeric DNA tract as a single protected chunk. The resulting complex of the telomeric DNA tract plus its bound cargo of protective proteins behaved very differently, by various tests, from standard nucleosomal chromatin, and therefore I concluded that it had no histones or nucleosomes.

By 1977, it was known from work of Rekosh et al that adenovirus DNA has a covalently bonded terminal protein, presumably for viral genome replication. Thus, in 1979, Marcia Budarf, a postdoctoral fellow in my laboratory at UC Berkeley, began using used radioactive iodine procedures (the Bolton–Hunter reagent) to see if we could find any comparable protein at the ends of rDNA. Although Marcia found a covalently attached protein (that in hindsight may have been topoisomerase I) enriched toward the end of the rRNA transcribed region, it was not enriched in the terminal parts of the rDNA molecules. She was unable to detect any other covalently attached protein elsewhere on the rDNA. Any evidence for a protein on the bulk of the rDNA molecule ends, such as their behavior in gel electrophoresis and the appearance of the rDNA molecules under the electron microscope, was conspicuously lacking. This made me feel all the more confident that there was no covalently attached protein at the very ends of this minichoromosome. But what other proteins were at telomeres?

My lab was the first to try to identify these protective proteins. We used biochemical fractionations of *Tetrahymena* nuclear extracts. My 1979 notebooks record that, together with my technician San San Chiou in the Department of Molecular Biology at UC Berkekely, over and over I made attempts to purify the telomeric proteins from nucleoli. Nucleoli are the tiny bodies within the *Tetrahymena* nucleus that harbor the actively transcribed rDNA minichromosomes. Fractionations after fractionations, mostly using sucrose gradients, were patiently performed by San San. Then we scaled up the preparations—I purchased a huge industrial-sized Waring blendor that loomed like a leviathan on the laboratory bench. *Tetrahymena* cells were blended in order to disrupt them just enough to shake their nucleoli free from the rest of the nuclear contents. At one time my note-book laconically reported: " Respun only one-third of total […] Waring blender broke."

All these early efforts were to no avail. In retrospect, the experimental approach had been reasonable—to purify nucleoli, as being the most enriched form of telomeric chromatin known, then to digest them with micrococcal nuclease into fragments, the end ones containing the telomeric DNA terminal tracts and their bound proteins. Then, I would further fractionate these away from the rest of the chromatin by selective precipitation in potassium chloride solutions, or fractionate them by size on sucrose gradients. The goal was to see what protein(s) would co-purify, through these multiple fractionation steps, with the telomeric repeat tract DNA, which I followed through the multiple steps by its hybridization signal. But we were only able to obtain limited amounts of chromatin and binding factors, and we tried without success to get enough to identify any factors that might be specific to the rDNA ends. Looking back, I see that we were fighting against the numbers game—our detection methods were too frail, our preparation scale-ups too modest. Therefore, it was yeast genetics and approaches done by others that turned out to provide the next great leaps forward in understanding telomeric proteins. That I failed in this by my early attempts using *Tetrahymena* made me all the more determined, if anything, to use other approaches to try to understand the nature and biological significance of those strange-seeming repeated sequences at the ends of chromosomes.

I also recall that our failure to find telomeric proteins taught a lesson that became useful when it came to our work on *Tetrahymena* telomerase. As Carol Greider's Nobel lecture describes, at one point the value of scaling up the telomerase activity preparations became evident to her. Thus, when Carol proposed the purchase of a very large glass column for preparative gel filtration chromatography, I was very willing to make this expensive-seeming purchase, ruefully recalling the past history of my too-pusillanimous scale-ups of *Tetrahymena* chromatin preparations.

### *To the University of California San Francisco*

I became a Full Professor at UC Berkeley in 1986 (after 8 years on the faculty of UC Berkeley), and in the same year a mother (our son Benjamin David was born in December 1986). By around 1989, I decided that as the long drive to Berkeley each day from our home in San Francisco made it difficult to pursue both science and our family life optimally, it was time to begin investigating alternatives. I settled upon a professorship at UCSF, and the move of my laboratory to UCSF's Department of Microbiology and Immunology was accomplished in mid 1990. I have remained on the faculty of UCSF ever since. There, I have had the great good fortune to be able to keep delving into the nature and mechanisms of telomeres and telomerase. Together with colleagues in and out of UCSF and with my many talented students and postdoctoral fellows and technicians in my laboratory, I have

Mission Bay Laboratory Group BBQ, August 2007.

Julie Newdoll, *Bee Goddesses*.

been able to address the wondrous biological systems comprised of telomeres and telomerase. A fanciful depiction evoking both telomere dynamics and telomere researchers is shown in a painting, done by the artist Julie Newdoll in 2008. This painting elicits the idea of a telomere as an ancient Sumarian temple-like hive, tended by a swarm of ancient Sumarian Bee-goddesses against a background of clay tablets inscribed with DNA sequencing gel-like bands.

### *Out of the Laboratory*

In the 1990s my research's implications for humans began to intrigue me, but with scientific research, faculty and Department Chair duties, family and many associated commitments, I had little time to indulge in delving into the philosophical and policy questions that can arise as science opens new possibilities. I served as President of the American Society for Cell Biology in 1998 and become more cognizant of the world of national science policy. Thus it was that in late 2001, the request to consider becoming a member of a newly created U.S. Federal Commission, the President's Council on Bioethics, had a certain appeal. I felt that my knowledge of the relevant fields of science, and long experience in the world of research, would be useful contributions to the Council, a body that, as a Federal Commission, would be advisory on some matters of national science policy. A further appeal was the coincidence with my growing thinking about these issues. I reasoned that if I joined this Council, it would be an opportunity to contemplate some of these dimensions of research's ramifications, and the possible reverberations of my own area of research.

Time for quiet contemplation of these and related questions in the abstract was not forthcoming. I understood from the beginning that the Bioethics Council would be occupied with publicly debated topics including human somatic cell nuclear transfer and embryonic stem cell research, as well as other topics less clearly defined at the outset of the council's deliberations. I thought I should agree to serve on this Council because, as a seasoned scientist (particularly in cell and molecular biology), I might be able to offer perspectives that would be helpful in advising national scientific policy. I knew the topics upon which this Council, appointed by the George W. Bush administration, would advise would be politically charged ones. For this reason especially, I felt that a strong base of scientific fact and evidence would be particularly important, and useful advice in this vein was something that I could in fact offer to this advisory body.

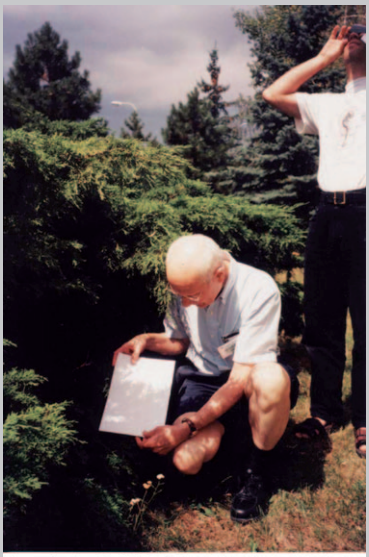
I publicly made clear my views on some of the council's recommendations, views that did not generally accord with those of the White House Administration or with those of the Council's Chair. After two years, I was informed by the Personnel Office of the George W. Bush White House that I would no longer be on this Council. This dismissal from the Council received quite a lot of public attention at the time. In the course of it, I was overwhelmed by the great many letters and communications I received. Almost without exception positive and supportive, they came from all over the United States and even from as far afield as a musician in London. His somewhat (to me) unexpected concern for science policy brought home to me how widespread is the wish among the public that science policy be informed by good scientific evidence. This entire episode was a broadening education. It reinforced my love of the searches for truth to which so many in research and academia aspire.

### *People Who Have Had Important Influences on My Life as a Scientist*

I am indebted to so many individuals that I can only describe a few of them here. Growing up, three of my schoolteachers in particular encouraged my interests in biology and chemistry and mathematics, not least by letting me know that they believed in my abilities to succeed in these areas—Nan Hughes, Jenny Phipps and Len Stuttard.

As I embarked on research in biological science, my teachers, advisors and mentors—notably Frank Hird in Australia, Fred Sanger in Cambridge, England, and Joe Gall in the USA—not only imparted their scientific knowledge, visions and wisdom, but also their examples of how to be a scientist. In particular, a photograph of Joe Gall from 1999, although taken several years after I had been in his lab, captures in a succinct visual way some of Joe's characteristics that influenced me when I was a member of his lab group. I took the photograph during a conference he was attending in Prague in the summer of 1999. During the conference a partial eclipse of the sun took place, and all the conference participants rushed out of the lecture hall to witness its progress. Joe is seen in the photograph demonstrating that it could be seen very simply and safely: All one had to do was hold a flat sheet of paper under a leafy bush so that the light, diffracted through the leaves onto the paper, caused to appear on the sheet of paper images of the "bite" being taken out of


Joseph G. Gall. Prague, 1999.

the disc of the sun by the moon passing in front of it. I recall that most of the conference participants had never seen this applied optics demonstration before. This photo evokes at once Joe Gall's desire and ability to teach—by his use of a very striking demonstration to teach something new to the conference participants—and, not least, one glimpse of his wide knowledge encompassing optics and natural science in general.

Like so many who are fascinated by chromosome behavior, I owe much to Barbara McClintock for her scientific findings. But in addition, Barbara McClintock also gave me a memorable lesson: in a conversation I had with her in 1977, during which I had told her about my unexpected findings with the rDNA end sequences, she urged me to trust my intuition about my scientific research results. This advice was surprising to me then, because intuitive thinking was not something that at the time I allowed myself to admit might be a valid aspect of being a biology researcher. I think her advice recognizes an important and sometimes overlooked aspect of the intellectual processes that underlie scientific research, and for me it had a liberating aspect to it. For this, also, I am very grateful to Barbara McClintock.

My husband, John Sedat, himself an accomplished scientist, has always urged me to dig deeper into myself and find the reserves of strength I might not have tapped—his encouragement in this way has helped me through years of doing science. Our son Ben inspired me to try to find ways of combining family and science, something that I have tried to convey to young scientists making their careers. Finally, my parents were both family physicians. From them I imbibed a sense of the importance of serving people kindly and as well as one can. I continue to believe that bioethics, done well and underpinned by the best available scientific evidence, can be an important part of our consideration, as a society, of the impact on people of scientific research in the biological sciences and medicine.


Elizabeth and her son, Ben, at the piano. Circa 1989.

## Nobel Lecture

### Introduction

DNA carries coding and noncoding sequences. Noncoding DNA both regulates and ensures the continued inheritance of DNA's coding information. In eukaryotes, by protecting the chromosome ends and thereby the chromosomes themselves, telomeric DNA is a class of noncoding DNA that ensures the stable inheritance of the genetic material. Research begun in the 1930s on the cytogenetics of telomeres was followed by a molecular understanding of telomeric DNA and its maintenance, which began in the 1970s and continues apace today. This fundamental, question-driven basic research has led into realms of human health and disease that have turned out to inform medicine in new ways.

### Beginning the Ends

> *"You corn kernels, ... may you succeed, may you be accurate."*
> Popul Vuh

Tracing the beginnings of the interwoven stories of science can be arbitrary, as beginnings are so often lost in the mists of time. For me, arguably the story of telomeres and telomerase began thousands of years ago, in the cornfields of the Maya Highlands of Central America. Today, under the brilliant, shifting sunlight of the Central American highlands, lush corn plants cover every inch of sloping land wherever they can gain a foothold. There, over millennia, agricultural breeding generated corn (maize) crops from the ancestral plant teocinte. Estimates place the early cultivation of corn in the Central American highlands to around 7000 years ago, and while early maize cobs dated from then were tiny, over millennia they progressively got bigger and bigger. Agricultural breeding is a process of consciously selecting the "best" plants. It was known that "like begets like", so that if one used the kernels from the biggest ears of corn, in the planting for

next year, a better crop would result. Intensely cultivated areas were carved out of the Central American rainforests and devoted to the production of corn. Maize came to occupy a central position in the agriculture and culture of the ancient Maya, and the Mayans had a maize goddess (Figure 1). Their ancient Council book, the Popul Vuh, includes many references to maize. The Popul Vuh even evokes genetic principles: "You corn kernels,… …may you succeed, may you be accurate."

***Figure 1.*** A form of the Mayan corn god.

As maize became important for human food worldwide, modern agricultural research on maize breeding continued the corn breeding begun thousands of years ago in the central American highlands.

### *The Telomere Concept*

> *"This is the beginning of the end."*
> Charles Maurice de Talleyrand, 1754–1838
> (announcing Napoleon's defeat at Borodino)

Perhaps another, more modern beginning to the story of telomere research is the discovery of X-rays by Roentgen. Hermann Muller, working on the fruit fly *Drosophila*, showed that X-rays could be used to produce mutations and chromosome fragmentation. By the end of the 1920s it was understood that the hereditary material was in chromosomes: Mendel's work, begun on heritable traits, had been integrated with findings showing that the inheritance patterns of genetic traits (or genes) corresponded with the regular movements of chromosomes in cells in meiosis and mitosis. By the 1930s, in the United States maize breeding research using such genetic principles came to be undertaken under government sponsorship, in agricultural research stations. And in one such station, the Missouri Agricultural Research Station, the geneticist and cytologist Barbara McClintock worked with maize, using the methods she had developed for examination of individual chromosomes. Genes were arrayed along chromosomes, as Muller's work showed. But in the 1930s there was no particular interest in what was at the ends of those arrays of genes, until it was noticed that the ends had some distinct properties (for a brief review, see ref. [1]) In the early 1930s McClintock concluded that "the natural ends" of chromosomes (McClintock's 1931 phrase[2]) were functionally different from experimentally-induced or accidental chromosomal breaks. A "stickiness" of broken ends of chromosomes (causing chromosomal fusions) was one of their defining features, while in contrast telomeres, the natural ends of chromosomes, had no such stickiness. This recognition arose from McClintock's research on broken ends of chromosomes and their behavior.[3] Independently, Muller reached the same conclusion from his fruitfly work, and in 1938 he named these ends "telomeres".[4] (Reviewed in ref. [1].)

### *Diving into Pond Water*

> *"Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."*
> Sir Winston Churchill, Speech in November 1942

On reading the insightful early cytogenetic work of McClintock and Muller from the 1930s and 1940s, it is sometimes hard to remember their deductions of the fundamental cytogenetic properties of the natural ends of chromosomes preceded any knowledge that the genetic material is DNA. The molecular mechanisms underlying the telomeric properties were completely unknown when, in the mid 1970s, I first began research using DNA purified from the ciliated protozoan *Tetrahymena*, as described below. From these molecular analyses emerged the nature of the specialized DNA-protein complex that comprises the telomere, distinguishing it from an accidental DNA break.

First, the sequence and structural features of telomeric DNA had to be understood. By the early to mid 1970s, viral and bacteriophage DNAs, and in some cases their ends, had been studied both biochemically and genetically. But what was the end of a cellular DNA in a eukaryotic nucleus—a chromosomal end—like? What was most daunting to a molecular biologist interested in that question in the 1970s, at the time just before the advent of DNA cloning methodologies, was the sheer length of typical chromosomal DNAs.

By the early 1970s Kavanoff and Zimm had carefully isolated chromosomal DNA in as intact a form as possible from fruitfly cells.[5] The kind of molecular weight range they deduced corresponded to long DNA molecules extending from one end of the chromosomes to the other. Thus the typically long chromosomal DNAs from a cellular nucleus were thousands of times longer than phage DNAs. This presented an enormous technical hurdle with respect to analyzing their telomeric regions. Answering the question of the molecular nature of telomeres meant going into pond water.

The specific pond water denizen in question was a single-celled ciliated protozoan, *Tetrahymena thermophila*. In the early 1970s Joe Gall, at Yale University, had discovered that *Tetrahymena* harbors a class of abundant, homogenous, short, linear chromosomes ("minichromosomes"). These were the

key to my being able to analyze telomeric DNA directly. I first encountered *Tetrahymena* when I joined Joe Gall's lab as a postdoctoral fellow at Yale. Although single-celled, a ciliated protozoan such as *Tetrahymena thermophila* contains two different types of nucleus. As Grell describes it,[6] "the majority of ciliates […] have generative nuclei capable of unlimited reproduction as well as somatic nuclei which perish sooner or later to be re-formed by descendents of the generative nuclei." Thus *Tetrahymena* economically combines both "soma" and "germline" into one cell. The abundant "minichromosomes" that Joe Gall discovered resided in the somatic nucleus. These linear DNA molecules bore the genes encoding ribosomal RNAs (rDNA), their high abundance ensuring sufficient expression for the large *Tetrahymena* cell. Kathleen Karrer, then a graduate student in Joe Gall's lab, had just discovered that the rDNA molecules consisted of two equal halves in a palindromic arrangement. This made them even more attractive: each telomeric end region would be the same as the other end region! The abundance and relative shortness (only 20000 base pairs) of these molecules would, I reasoned, make it feasible to apply methods on them like those that had been used by Ray Wu and colleagues, for example, to sequence nucleotides at the ends of bacteriophage DNA in the early 1970s.

It was with these rDNA molecules that, very soon after arriving at Yale in early 1975, I started to use methods for determining the DNA sequences at the rDNA ends. I describe my experiments that succeeded in piecing together the telomeric sequence in more detail in the accompanying autobiography. Briefly, initially, upon fractionation of depurination products of radiolabeled rDNA end regions, the strong CCCC sequence spot seen was particularly informative (Figure 2).

By August 1976 I was confident that the *Tetrahymena* rDNA molecules ended in $(CCCCAA)_n$ sequence, and I was looking to see whether this same repeated sequence was also present in the other (much longer) chromosomal DNAs of *Tetrahymena*, and exploring the molecular structures of the DNA end regions. The results describing tandemly repeated CCCCAA sequences at the rDNA ends were published in 1978.[7] One experiment done in 1979, radiolabeling the rDNA using just $^{32}$P-labeled dCTP, and unlabeled dATP, and separating the products on by denaturing gel electrophoresis, showed a beautiful ladder of tiger stripes extending up the gel. The size of every band in this regular ladder was 6 bases more than the band below it—a strikingly visual confirmation of the repeated hexameric sequence I had deduced! This arrestingly characteristic pattern of bands was the first example of the pattern that would later become important for our discovery of telomerase enzyme activity, as described in the Nobel Lecture by my co-Laureate, Carol Greider.

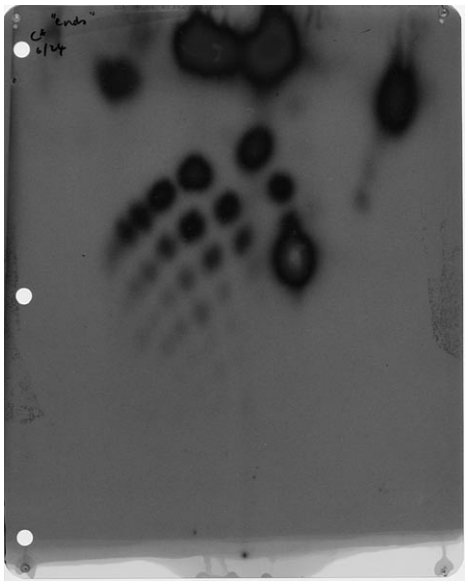


***Figure 2.*** First autoradiogram showing a prominent CCCC sequence.

In my early work, my molecular views of telomeres were first focused on the DNA. This was not only because DNA was so central to the problem of incomplete replication of linear DNAs, as had been recognized by the early 1970s (reviewed by Blackburn and Szostak[8]), but also for several years DNA was the only component of the telomeres that was identified. By 1980, DNA sequences were known for the ends of a few different eukaryotic nuclear DNAs. By then we had shown that DNA ends of *Tetrahymena* macronuclear DNAs in general, not only the rDNA molecules, consisted of long arrays of the same simple 6-nucleotide CCCCAA ($C_4A_2$) repeats.[9] Others showed that the ciliate *Oxytricha* and its relatives had very short tracts of 8-nucleotide ($C_4A_4$) repeats at their macronuclear DNA ends, and that the high copy-number linear rDNA minichromosomes in two different slime molds similarly had tracts of simple repeat sequences, CCCTAA and $C_{1-8}T$, respectively (reviewed in ref. [10–12]) at their DNA ends. These all resembled the sequence I had found for the *Tetrahymena* rDNA molecules. But how did this emerging common DNA sequence arrangement at the ends of the nuclear DNA molecules inform us about the special properties of telomeres? One approach was to see what proteins might be on the telomeric DNA. I describe my early unsuccessful efforts to identify telomeric proteins in the accompanying autobiography.

Now we know that the essential telomeric sequences sequences are surprisingly similar among phylogenetically widely divergent eukaryotes. Each end of a chromosome consists of a block of very simple telomeric sequences which are tandemly repeated over and over again, all the way to the molecular end of the chromosomal DNA. All of the chromosomes in a given organism have the same species-specific telomeric repeat sequence. However the same telomeric repeat sequence crops up in very diverse eukaryotes. For example, human telomeres consist of AGGGTT repeats, tandemly repeated for thousands of nucleotides at the ends of all of our chromosomes. The same repeated AGGGTT sequence is the telomeric sequence of the mold, Neurospora, the slime mold, Physarum, and the trypanosome protozoan parasites. (This makes telomeric DNA sequences possibly the world's worst sequences for deducing phylogenetic relationships!) Telomeric DNA generally has a strand composition asymmetry, resulting in a G-rich and a C-rich strand. It is the G rich strand that is always oriented in the 5′ to 3′ direction toward the end of the chromosome. This overall

structural conservation of telomeres suggests that this general arrangement and composition of DNA strands is of fundamental importance for telomere function.

### *The Lines of Evidence That Led to the Concept That Telomerase Activity Existed*

That telomeric DNA had certain molecular behaviors indicative of dynamic properties in vivo had emerged by the early 1980s. Four main lines of such molecular evidence were instrumental in spurring me to hunt for a new type of enzymatic activity might synthesize telomeric DNA and elongate telomeres. This evidence took the form of molecular observations on telomeric DNA not readily explainable by any of the knowledge then about DNA replication or recombination.

First, the telomeric CCCCAA repeat tracts (which we eventually ended up referring to by their sequence on the complementary DNA strand, GGGGTT repeats) in the ciliates *Tetrahymena* and *Glaucoma* were heterogeneous in length; that is, the DNA molecules in the population carried different numbers of repeats.[7,13] Perfect DNA replication of parental DNA to make two daughter DNAs was not predicted to produce such heterogeneity.

Second, during development of the somatic macronucleus in different *Tetrahymena* strains, telomeric GGGGTT repeat tracts were found to become joined, by then-mysterious means, to various sequences in the rDNA minichromosomes; that is, new telomeres were forming on macronuclear chromosomes. Meng-Chao Yao, continuing work he had started as a Ph.D. student in Martin Gorovsky's laboratory and then as a postdoctoral fellow Joe Gall's lab (at the same time I was there), had observed this first for *Tetrahymena* rDNA telomeres.[14] But a single TTGGGGTT sequence already present at this position in the precursor DNA sequence had made it conceivable that this sequence could itself somehow be a seed sequence for repeated unequal recombination events to generate multiple repeats, for example. However, then my lab at Berkeley made similar observations for other rDNAs and non-rDNA telomeres of the somatic nucleus, with the difference that in these cases the telomeric DNA sequences were found to be joined to sequences where there was no initial GGGGTT repeat at all.[15] Thus in 1982 I wrote about these observations: "..the sequences common to the macronuclear DNA termini must be acquired by these subchromosomal segments during their formation. Two types of routes can be envisaged: Telomeric sequences are transposed or recombined onto the developing macronuclear DNA termini, or the simple, repeating telomeric sequences are synthesized de novo onto these termini by specific synthetic machinery".[15] Simultaneously, David Prescott's group in Colorado had made the observation that $T_2G_4$ repeats similarly appeared to become joined onto the ends of the short chromosomes of the macronucleus in a hypotrichous ciliate.[16]

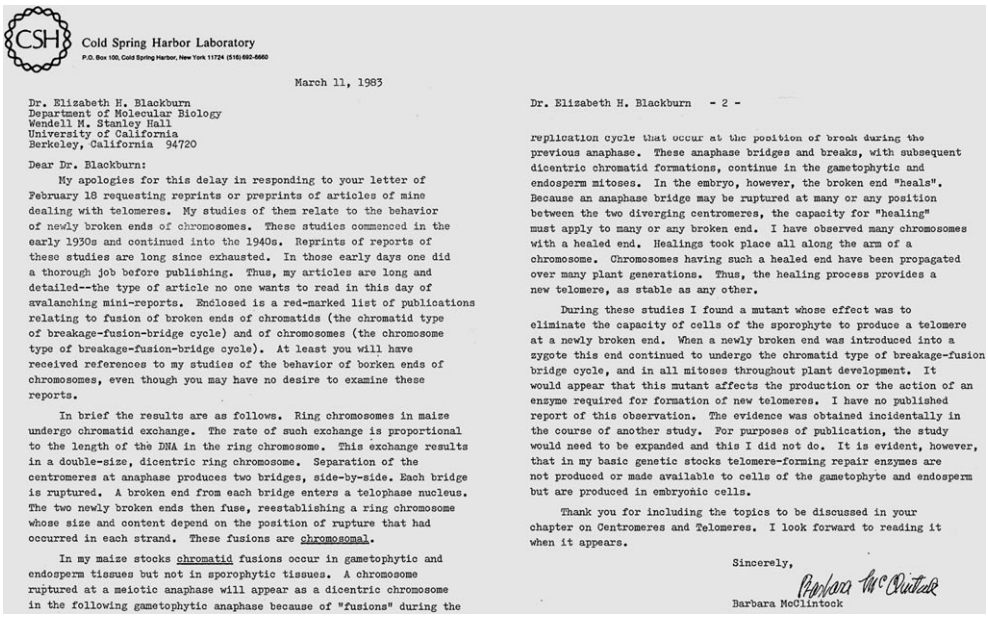
Third, as described in detail in my co-Laureate Jack Szostak's Nobel Lecture, we had discovered that yeast telomeric sequence DNA (irregular $TG_{1-3}$ repeats, which Janice Shampay, a graduate student in my lab at UC Berkeley, first sequenced as part of our collaboration with Jack) was added directly to the ends of *Tetrahymena* GGGGTT repeat telomeres maintained in yeast.[17,18] In this collaboration, we showed that a telomere from the ciliated protozoan *Tetrahymena*, consisting of GGGGTT repeats, was able to function as a telomere in the yeast *S. cerevisiae* in a particular sense: specifically, the *Tetrahymena* telomeric sequences, when put into a yeast cell on the ends of a linearlized plasmid DNA molecule, could stabilize the plasmid, such that now it was maintained indefinitely as an extrachromosomal, linear DNA molecule through many rounds of replication, mitosis and even meiosis. But in addition, something very interesting always happened to the introduced foreign (*Tetrahymena*) telomere in yeast. We found that yeast telomeric repeats, which Janice Shampay, the afore-mentioned graduate student in my lab at UC Berkeley, sequenced and found to be $TG_{1-3}$ repeats, were added to the distal end of the foreign, GGGGTT-repeat telomere after it had been maintained in dividing yeast cells. Other observations made soon after, in the course of following up these findings, further highlighted the dynamism of telomeric DNA in cells. For example, by the early 1980s Janice Shampay had also observed that if we introduced such a high-copy-number plasmid into yeast (thereby adding of the order of 100 extra telomeres into these cells), the telomeres of the chromosomes themselves, whose average length had heretofore remained steady for 300 generations of previous mitotic passaging, now underwent a slow shortening over the ensuing cell divisions (Elizabeth H. Blackburn and Janice Shampay, unpublished results).

Fourth, Piet Borst and his collaborators published an intriguing observation in 1983. They were monitoring the inheritance of a gene of trypanosomes (which cause sleeping-sickness) encoding a variant surface antigen. These antigens play important roles in the parasite's ability to evade the host's immune response. The gene they had found was located on a telomeric restriction fragment. In the course of passaging the trypanosome cells, this gene's restriction fragment steadily became progressively longer, implying that the telomeric DNA tract was growing.[19]

Finally, from yet another independent direction, a cytogenetic observation by Barbara McClintock reinforced my nascent notion that some undiscovered kind of developmentally-controlled cellular enzymatic activity might act on telomeres. In essence, first in a conversation, and later in a letter McClintock wrote to me in 1983, she described how, long ago, she had identified a maize mutant that had lost the normal capacity of maize to heal broken chromosome ends that specifically exists very early in plant development—just after fertilization (Figure 3):

Finding a mutant implied that there is a gene associated with the ability to heal—a gene that could be mutated to nonfunctionality. I was struck by the implication that in zygotes, a fully functional telomere ('healed end' in McClintock's terminology) was generated from a broken chromosome end not just by chance but rather, by an active, developmentally controlled process; a process, furthermore, occurring just after fertilization—the developmental stage equivalent to when ciliate chromosomes become broken



Cold Spring Harbor Laboratory
P.O. Box 100, Cold Spring Harbor, New York 11724 (516) 692-6800

March 11, 1983

Dr. Elizabeth H. Blackburn
Department of Molecular Biology
Wendell M. Stanley Hall
University of California
Berkeley, California 94720

Dear Dr. Blackburn:

My apologies for this delay in responding to your letter of February 18 requesting reprints or preprints of articles of mine dealing with telomeres. My studies of them relate to the behavior of newly broken ends of chromosomes. These studies commenced in the early 1930s and continued into the 1940s. Reprints of reports of these studies are long since exhausted. In those early days one did a thorough job before publishing. Thus, my articles are long and detailed--the type of article no one wants to read in this day of avalanching mini-reports. Enclosed is a red-marked list of publications relating to fusion of broken ends of chromatids (the chromatid type of breakage-fusion-bridge cycle) and of chromosomes (the chromosome type of breakage-fusion-bridge cycle). At least you will have received references to my studies of the behavior of borken ends of chromosomes, even though you may have no desire to examine these reports.

In brief the results are as follows. Ring chromosomes in maize undergo chromatid exchange. The rate of such exchange is proportional to the length of the DNA in the ring chromosome. This exchange results in a double-size, dicentric ring chromosome. Separation of the centromeres at anaphase produces two bridges, side-by-side. Each bridge is ruptured. A broken end from each bridge enters a telophase nucleus. The two newly broken ends then fuse, reestablishing a ring chromosome whose size and content depend on the position of rupture that had occurred in each strand. These fusions are chromosomal.

In my maize stocks chromatid fusions occur in gametophytic and endosperm tissues but not in sporophytic tissues. A chromosome ruptured at a meiotic anaphase will appear as a dicentric chromosome in the following gametophytic anaphase because of "fusions" during the

Dr. Elizabeth H. Blackburn - 2 -

replication cycle that occur at the position of break during the previous anaphase. These anaphase bridges and breaks, with subsequent dicentric chromatid formations, continue in the gametophytic and endosperm mitoses. In the embryo, however, the broken end "heals". Because an anaphase bridge may be ruptured at many or any position between the two diverging centromeres, the capacity for "healing" must apply to many or any broken end. I have observed many chromosomes with a healed end. Healings took place all along the arm of a chromosome. Chromosomes having such a healed end have been propagated over many plant generations. Thus, the healing process provides a new telomere, as stable as any other.

During these studies I found a mutant whose effect was to eliminate the capacity of cells of the sporophyte to produce a telomere at a newly broken end. When a newly broken end was introduced into a zygote this end continued to undergo the chromatid type of breakage-fusion bridge cycle, and in all mitoses throughout plant development. It would appear that this mutant affects the production or the action of an enzyme required for formation of new telomeres. I have no published report of this observation. The evidence was obtained incidentally in the course of another study. For purposes of publication, the study would need to be expanded and this I did not do. It is evident, however, that in my basic genetic stocks telomere-forming repair enzymes are not produced or made available to cells of the gametophyte and endosperm but are produced in embryonic cells.

Thank you for including the topics to be discussed in your chapter on Centromeres and Telomeres. I look forward to reading it when it appears.

Sincerely,

Barbara McClintock

***Figure 3.*** McClintock letter to Elizabeth H. Blackburn, 1983.

(albeit deliberately in their case) and telomeric DNA is efficiently added to the freshly produced DNA ends.

This remarkable information was another one of the reasons that I decided to look for telomerase. The capacity of ciliates to form de novo telomeres just after fertilization (equivalent to the zygote) was just too striking a parallel to ignore.

### *Tetrahymena* Cells by a Biochemical Approach

> *"If your knees aren't green by the end of the day, you ought to seriously re-examine your life."*
>
> Bill Watterson
>
> (American Author of the comic strip "Calvin & Hobbes", b. 1958)

*Tetrahymena* cells provided an attractive system to use to hunt for this putative telomeric DNA-adding enzymatic activity. My choice of approach was to prepare extracts from *Tetrahymena* cells. As Joe Gall had pointed out when I proposed sequencing DNA end regions, they could be grown in large quantities relatively inexpensively. Furthermore, their developmental time course could be synchronized, thanks to the efforts of several laboratories, notably those of David Nanney, Peter Bruns, Ed Orias, Sally Allen and their colleagues. Hence, one could make cellular extracts from a large population of cells all undergoing macronuclear development and, concomitantly, I hypothesized, the putative telomere addition reactions. This, therefore, seemed to me likely to be a developmental stage when any such activity would be in high demand by the cell and therefore, I reasoned, would allow the best chance of its being detectable. The big question was the choice of substrates to use. What DNA would be best to use to prime any telomeric DNA addition, and what nucleotide building-block precursors would be required? Would both strands of the telomeric DNA have to be synthesized together in a coupled reaction, or perhaps even in a reaction that had to be coupled to the production of the freshly-cut DNA ends? Would it be preferable to provide DNAs that resembled the freshly-cut ends in the developing macronucleus, or pre-existing telomeric repeat tracts that could be further elongated: both reactions would be expected to be performed in *Tetrahymena* cells at this stage. To make sure I did not miss any of these possibilities, I added a mixture of all four deoxynucleoside triphosphates and all four ribonucleoside triphosphates, an energy-generating (ATP-generating) enzyme system, and a mixture of cloned DNA fragments, purified from bacterial cells, that would present to any enzymes in the *Tetrahymena* extracts both telomeric and nontelomeric DNA termini. I prepared cell extracts from cells at this developmental stage, adapting a method that my graduate student Peter Challoner had in turn adapted from one used by Tom Cech and collaborators to examine rDNA gene expression (which led Tom Cech to the discovery of self-splicing RNA). Peter had found that incubating such extracts (for a different experimental goal) allowed him to detect changes in the DNA restriction fragments that he had added to his extracts. The changes had even hinted at some form of alteration specific to the telomeric DNA ends. As described elsewhere,[10] in early 1984 I was able to see increasing amounts of telomeric GGGGTT-hybridizing repeat sequences were somehow generated during the course of the reactions. The hunt was on!

### The Discovery of Telomerase

> *"... to make an end is to make a beginning."*
>
> T. S. Eliot, 1888–1965
>
> Four Quartets: "Little Gidding"

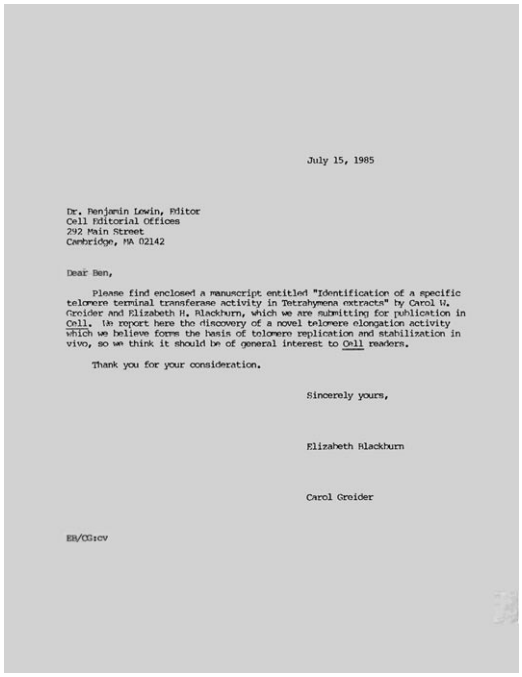
The next immediate need was to greatly simplify and refine the reaction conditions, in order to unravel what was actually occurring during the reactions that were being carried out by enzymes apparently present in the *Tetrahymena* cell extracts. In 1984 Carol Greider joined my lab at UC Berkeley as a Ph. D. student and was immediately interested in doing just this. This work, which led us to discover telomerase activity, is described in detail in Carol Greider's Nobel Lecture in this Volume, so I summarize only briefly some points here.

We discovered that short fragments of DNA, synthesized chemically as DNA oligonucleotides and therefore available in high concentrations, would get telomeric GGGGTT repeats added to their 3' ends when incubated with *Tetrahymena* extracts (Figure 4 and 5). This enzyme reaction was more efficient when the extracts were made from cells at the developmental stage when new telomeres are added during macronuclear development. Fortunately, the reaction, at least in the test tube, was not obligatorily or mechanistically coupled to synthesis of the complementary DNA strand or to DNA cleavage.[20]

Ribonuclease treatment abolished this telomeric DNA repeat addition capability of the extract. Hence the enzyme activity needed RNA. Protease treatment also destroyed the enzyme reaction, implicating required protein component(s) as well as RNA.[21]

The essential RNA component of telomerase was identified and found to contain a sequence, 5′-CAACCCCAA-3′ (Figure 6). This sequence is complementary to one and a half repeats of the 6-nucleotide repeat sequence that was synthesized in vitro. Starting with this powerful hint, we found that all the properties of the synthesis reaction in vitro, including its particular DNA and nucleoside triphosphate precursor



July 15, 1985

Dr. Benjamin Lewin, Editor
Cell Editorial Offices
292 Main Street
Cambridge, MA 02142

Dear Ben,

Please find enclosed a manuscript entitled "Identification of a specific telomere terminal transferase activity in Tetrahymena extracts" by Carol W. Greider and Elizabeth H. Blackburn, which we are submitting for publication in Cell. We report here the discovery of a novel telomere elongation activity which we believe forms the basis of telomere replication and stabilization in vivo, so we think it should be of general interest to Cell readers.

Thank you for your consideration.

Sincerely yours,

Elizabeth Blackburn

Carol Greider

EB/CG:cv

***Figure 4.*** Letter to Editor of Cell, 1985.

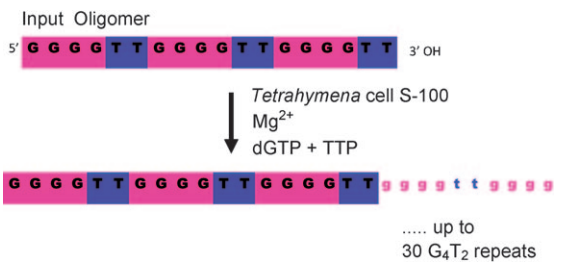



***Figure 5.*** The original telomerase assay.[20,21]

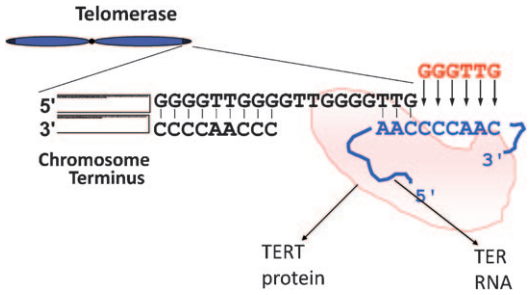



***Figure 6.*** A model for the action of the *Tetrahymena* telomerase.[20–22]

requirements, added up to a coherent model by which telomere synthesis by telomerase (as we eventually named the enzymatic activity) is templated by repeated rounds of copying of this short template sequence in the telomerase RNA. The synthesis is aided by alignment of the 3′ end of the DNA primer on the template, thereby positioning the primer for addition of the next templated repeat.[22]

We thus had discovered that *Tetrahymena* telomerase added tandem repeats of the *Tetrahymena* telomeric DNA sequence, TTGGGG, onto the 3′ end of a variety of G-rich telomeric DNA sequence oligonucleotide primers, independently of an exogenously added nucleic acid template. The cellular activity that carried out this reaction was both RNAse- and protease-sensitive. Similar experiments were then devised for telomerase activities from the ciliates Euplotes and Oxytricha,—by Dorothy Shippen-Lenz in my lab and by Alan Zahler in David Prescott's lab, respectively, and from human cells by Gregg Morin in Joan Steitz's lab at Yale. Each telomerase synthesized its own species-specific sequence—GGGGTTTT repeats (hypotrichous ciliates) or AGGGTT repeats (human cells). These other telomerase activities were very like the *Tetrahymena* telomerase in their primer recognition and other characteristics, including ribonuclease-sensitivity, arguing for the generality of this enzyme activity among eukaryotes. When Dorothy Shippen identified and sequenced the RNA moiety of the telomerase of the ciliate *Euplotes*, it gratifyingly contained the sequence 5′-CAAAACCCCAAAA-3′. Experiments indicated that this sequence was indeed the templating domain for synthesis of GGGGTTTT repeats, the telomeric sequence of Euplotes. Together, these findings established telomerase as a widespread specialized reverse transcriptase, unusual in being of cellular origin and in carrying its own internal RNA template for repeated DNA synthesis.

### *Demonstration of the Reverse Transcriptase Action of Telomerase In Vivo*

> *"They didn't have to walk around to see what was under the sky; they just stayed where they were. [And] as they looked, their knowledge became intense."*
> Popul Vuh, p. 165

We now had good evidence that the telomerase enzyme could synthesize the G-rich strand of telomeric DNA in vitro. Proving that the 5′-CAACCCCAA-3′ sequence in the *Tetra-*

*hymena* telomerase RNA gene was the template for telomere synthesis in vivo required site-directed mutagenesis of this sequence in the telomerase RNA gene (which we called the TER gene). Again, *Tetrahymena* proved the first key to being able to do these experiments. Its telomerase RNA gene had been recently cloned by Carol Greider, and we had devised a system in my lab for overexpression of such mutated genes in *Tetrahymena* cells. We inserted the engineered telomerase RNA genes into a self-replicating vector devised by Guo-Liang Yu, a graduate student in my lab. Guo-Liang then introduced them into *Tetrahymena* cells by microinjection of the DNA molecules. He analyzed and sequenced the telomeres in the cells expressing the mutated telomerase RNAs. These experiments, done with help from Guo-Liang's labmates Laura Attardi and John Bradley, established the in vivo role of telomerase in three ways. As predicted from the sequence of the telomerase RNA and from the in vitro experiments described in Carol Greider's Nobel Lecture, altered telomere repeats specified by the mutant gene appeared in the telomeres that he cloned out of the transformant cells. This proved that telomerase was the cellular reverse transcriptase enzyme that synthesizes telomeres in cells by copying its own internal RNA template within the TER moiety of the enzyme complex. The cells rapidly showed abnormal nuclei indicative of failure to segregate their DNA properly (Figure 7). This indicated that the correct DNA sequence was necessary for proper nuclear behavior.[23]

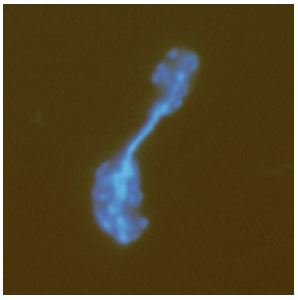


***Figure 7.*** A *Tetrahymena* cell expressing a telomerase RNA with mutated template attempts to divide.

### *Demonstration of the Need for Telomerase for Cell Growth*

> *"Like as the waves make towards the pebbled shore, so do our minutes hasten to their end."*
> William Shakespeare, 1564–1616, Sonnet 60

In addition to proving the templating role of telomerase RNA in *Tetrahymena*, we obtained a third, bonus result from these experiments. *Tetrahymena* cells are normally effectively immortal. With one particular template mutation, Guo-Liang found none of the predicted sequence DNA was added onto telomeric ends. Instead, the cells continued to grow for only about 20 to 25 more cell divisions. During that time their telomeres progressively shortened. The cells then ceased to divide. This result was the first demonstration that interference with normal telomerase function itself could limit cellular lifespan. It established that continuing action of

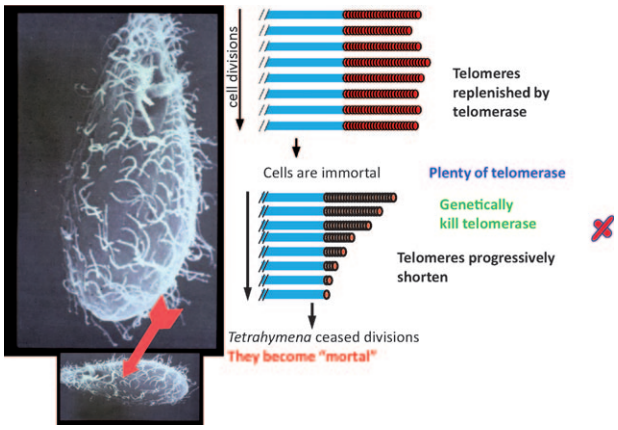



***Figure 8.*** *Tetrahymena* cells dependence on telomerase.[23]

telomerase itself was necessary for replicative immortality of cells (Figure 8).[23]

The action of telomerase thus could explain how replication of the 5′ ends of the chromosomal DNA can be completed, without the loss of terminal sequences that would result from normal semi-conservative DNA replication mechanisms: continuous addition of telomeric DNA to the chromosomal ends by telomerase could counterbalance this predicted terminal DNA attrition.

### *Telomeres as Protein–DNA Complexes*

> *"Having well polished the whole bow, he added a golden tip."*
> Homer, The Iliad (bk. IV, III)

As recounted in my autobiography in this Volume, soon after identifying the telomeric sequence, I found that in *Tetrahymena* chromatin, telomeric DNA tracts were protected by bound protein(s), distinct from nucleosomes. I tried to identify the proteins on *Tetrahymena* telomeres, but did not succeed in this. It was others' work, initially using yeast molecular genetic approaches, that unlocked the door to telomeric proteins. Now an extensive list of proteins associated with telomeres, from various eukaryotes, is known. Many of these have been characterized to varying extents with respect to biochemistry, structure, occupancy levels on telomeres and circumstances that lead to measurable changes in these occupancy levels. Many functions are deduced, often by looking at the consequences to cells of mutating or deleting the protein. But despite the extensive work that has built up the current molecular knowledge of the telomeric DNA-protein complex, the actual picture of a telomere is in some ways still a rather ghostly and partial image. This is almost certainly because telomeres are highly dynamic.

### *Telomeres as a Dynamic Homeostatic System*

> *"Stability is not immobility."*
> Klemens von Metternich, Austrian Statesman, 1773–1859

During the 1990s, the view of a telomere that emerged was that of a self-regulating entity, normally resilient to change

and buffered from it by a variety of molecular mechanisms. Mike McEachern, a postdoctoral fellow then in my laboratory, proposed a model for telomere dynamics based on his experiments with telomeres in *Kluyveromyces lactis*, a budding yeast. The essence of the model is that, first, the rate of shortening of a telomere in the absence of telomerase stays constant as that telomere shortens. But the probability of lengthening it by telomerase actually changes depending on the telomere length—the shorter the telomere, the more likely it is to be lengthened by telomerase action. Mike deduced this largely from a series of experiments in which he altered the telomerase RNA template sequence to direct the synthesis of various repeats. Anat Krauskopf, then concurrently a postdoctoral fellow with Mike in my lab, extended these findings: Telomere length regulation became altered in a way that tracked with altered binding of a yeast telomeric protein to the mutated telomeric sequence. Together, Mike's and Anat's experiments showed that a major contributor to such negative regulation of telomerase action on telomeres is the telomere protein-DNA complex structure itself. Thus the telomere itself was a like a gatekeeper, regulating access of telomerase onto the telomere, even in the presence of excess telomerase in the cells. Over subsequent years much more has been learned about the details of the proteins involved, but this general model has stood the test of time.

A general and important corollary concept is that telomeres can exist in two states: capped or uncapped. Capped telomeres signal the cells to keep on proliferating, all other things being well. But uncapped telomeres in the cell signal the cell; if uncapping is persistent, it signals the cell to arrest its divisions. Mike McEachern and Anat Krauskopf showed that one of the most striking properties of a telomere is how resilient it can be to molecular insults of a variety of types, and then, like the last straw, just one more molecular change is sufficient for the telomere to collapse catastrophically into disaster. Thus it emerged that cells have evolved elaborate and overlapping, redundant or mutually reinforcing mechanism to ensure that their telomeres stay functional.[24]

Currently, the combined picture from the results from many researchers is that the telomere in a cell is a highly dynamic structure. Rather than being a rock-stable complex, it is perhaps reminiscent of a swarm of bees: the size and shape of the swarm overall appears the same, but in reality its composition is constantly changing as the bees (the telomeric proteins) of the swarm constantly come off it and are replaced by other bees.

### *Similar Molecular Machineries: Different Life Histories*

> "*Have regard to the end.*"
> [Lat.: Finem respice (or Respice finem).]
> Chilo of Sparta (Chilon)

The structure and the function of telomeres are highly evolutionarily conserved among eukaryotes. This conservation underlies why in the early 1980s Jack Szostak and I were able to successfully propagate *Tetrahymena* telomeres in the distantly related organism baker's yeast. As described above, and in detail in Jack Szostak's Nobel Lecture, we found that yeast telomeric sequences were added to the introduced *Tetrahymena* telomeric ends on a plasmid, thereby stabilizing the plasmid and allowing it to replicate indefinitely, in linear form, as an extrachromosomal plasmid. Similar conservation applies to the telomerase mechanism for telomere maintenance: throughout the eukaryotes telomerase, a specialized ribonucleprotein reverse transcriptase, is used to maintain the ends of eukaryotic chromosomes, with relatively rare exceptions. Telomerase RNA and core protein of telomerase, TERT, each retain well-recognizable conserved features in even the most distantly related eukaryotes.

In the face of this widespread conservation of telomeres and telomerase, extending down to the deep roots of eukaryotic evolution, a fascinating finding is the great variety of telomere maintenance stories that play out during the lives of different eukaryotes. Among mammals alone, even under favorable living conditions species clearly differ in their maximal possible lifespans, implying that maximum lifespan has considerable genetic determination. Humans can have a life expectancy of about eighty years, and laboratory mice about two years. Thus, it is reasonable to contemplate the possibility that the rate-limiting steps causing aging and eventual death may differ between these two species, despite the common underlying cellular and molecular mechanisms they share. Even within the mammals, the qualitative and quantitative contributions of telomere maintenance to cellular proliferative lifespans seem to differ widely.[25] And, extending further out from mammals to invertebrates, despite much conservation of fundamental molecular and cellular mechanisms, it is possible that those that contribute to their aging and death from old age may be divergent from those that are quantitatively important or rate-limiting for aging and lifespan in humans. All of these considerations have raised the question of whether telomere maintenance is a quantitatively important determinant of normal human aging and lifespan.

### *Telomerase in Human Health and Disease*

#### *Telomerase in Cancer Cells*

> "*We ought to consider the end in everything.*"
> [Fr.: En toute chose il faut considerer la fin.]
> Jean de la Fontaine, Fables (III, 5)

One special and notable context in which telomerase plays a prominent role in humans is in human cancer cells. Hyperactive telomerase in the cancer cells is a prominent characteristic of the great majority of most types of malignant human tumors. In this setting of the cancer cell—which, importantly, has undergone multiple other genetic and epigenetic changes in its progression to tumorigenicity—telomerase plays cancer-promoting roles. Most clearly, it promotes cellular immortality by providing cancer tell telomeres with the means for continuous replenishment. The high level of telomerase that characterizes human cancer cells thus is a rational target for anti-cancer therapies.

*Telomere Maintenance and Human Life Histories*

> *"The end crowns all, and that old common arbitrator, Time, will one day end it."*
> William Shakespeare, 1564–1616, The History of Troilus and Cressida (Hector, act IV, v)

As described above, abrogating telomerase in otherwise effectively "immortal" single-celled species causes progressive telomere shortening over several cell generations followed by cessation of cell division ("senescence"). This naturally led to the question of whether the same progressive process operates to cause human aging and limit human lifespan. Rare genetic mutations in telomerase component genes leading to reduced telomerase levels and telomere shortening in humans clearly have adverse disease-causing effects and can prevent the affected individual from attaining an old age. Yet until recently, for the vast majority of people, who by definition are not "mutants", the contributions of insufficient telomere maintenance to aging and lifespan limitation was less clear. What does one observe in the human population in general?

Large amounts of epidemiological molecular data on humans and their in vivo telomere maintenance have now accumulated. From our present knowledge of telomeres, the picture that has emerged is that telomere maintenance is linked to human aging and diseases of aging. First, telomere shortness in white blood cells is linked to a large and impressive list of the major diseases of aging: in multiple cohorts, often involving hundreds to thousands of individuals, short telomere length has been found to be associated with risk of, and incidence of, cardiovascular disease, stroke, vascular dementia, osteoporosis and obesity and risks for diabetes and certain cancers. Longer mean white blood cell telomere length is not consistently linked to longer lifespan, but longer telomere length has been linked to more years of healthy life, in a cohort of people in their seventies.[26]

Second, telomerase activity is not only present in many normal human somatic cells but also, importantly, quantifiable[27–30] in adult (including elderly) humans, even in resting white blood cells, as well as in stem and proliferating progenitor cell types, telomerase is active. This means that telomere shortening in normal cell populations has the possibility of being counterbalanced, or even reversed, throughout life. While cross sectional studies show a slow loss of telomeric length across populations of humans in general, the datapoints are noticeably scattered: it is not uncommon for an 80-year old's telomeres to be as long as those in a 30-year old. What might account for such scatter? In fact, lengthening of telomeres in white blood cell populations is now found to be much more common than expected from the previous models of inexorable telomere shortening throughout life. However such models had been based almost solely on cross sectional studies and on the presumption of lack of telomerase in the normal cells of adult humans.

What determines and regulates the variation in long-term telomere maintenance in people? While genetic influences have been detected, non-genetic factors are also coming to the fore as significant influences on telomere length maintenance in human white blood cells. In summary, in humans, telomere maintenance status results from the integrated influences of many factors, genetic and non-genetic. The non-genetic influences include modifiable factors; notably, psychological stress (Figure 9), behavioral and even nutritional factors.

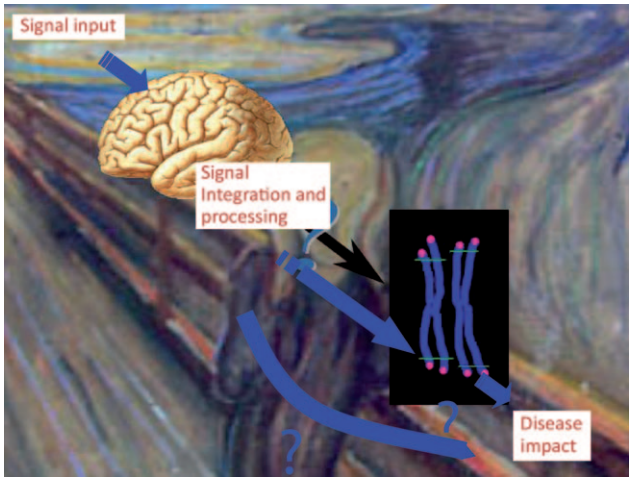



***Figure 9.*** Input of stress on telomeres and its disease impact: Chronic life stress wears down telomeres.

The major conditions and diseases or disease risks occurring with human aging have now been associated with shortness of blood cell telomeres (Figure 10): prominently, cardiovascular disease, cancers, diabetes and impaired immune system function in various forms. Thus, telomere shortness is not specifically associated with any one disease. Rather, this seeming non-specificity of telomere maintenance may instead be more usefully considered as reflecting—perhaps causing—aging more fundamentally. Telomere maintenance status may be a truer integrative measure of actual "biological age" than chronological age. Furthermore, human life conditions impact on telomere maintenance in humans. Perhaps telomere monitoring will become as common as regular weighing as an integrative indicator of health. Certainly, these findings and implications are taking the field of telomere and telomerase biology into realms far from

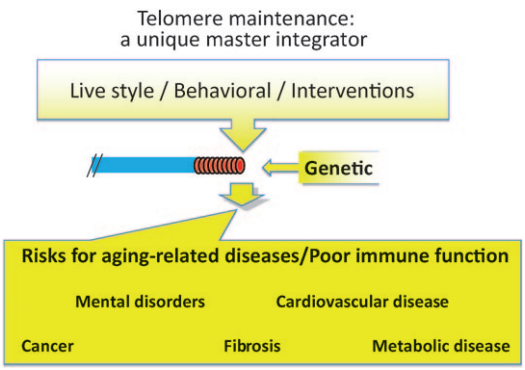



***Figure 10.*** The telomere as an integrator of many factors.

the single-celled pond microorganisms in which I began this work.

*I am indebted to my many valuable colleagues with whom I have been blessed over the years, without whom I would have done much less.*

Received: April 22, 2010
Published online: September 6, 2010

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