Telomeres and telomerase: the path from maize, *Tetrahymena* and yeast to human cancer and aging

Elizabeth H Blackburn, Carol W Greider & Jack W Szostak

The telomere problem

Scientific discoveries are each individual and occur by their own unique path. However, there are key ingredients that set the stage for them. Many of these ingredients were important in the discovery of telomerase: talking with scientists from different fields, paying attention to unusual findings and taking the risks of doing crazy experiments. We will describe how a combination of these ingredients and productive collaborations led us to postulate and discover telomerase.

The earliest functional description of telomeres was by geneticist Hermann Muller when he used X-rays to fragment chromosomes. Muller, working with fruit flies, and Barbara McClintock, working with maize, converged on the same conclusion around the same time: that the natural ends of chromosomes are different from those created at the site of a chromosomal break. The natural ends were somehow protected from the frequent rearrangements that occur at broken ends. As McClintock wrote in a 1931

Elizabeth H. Blackburn is at the Department of Biochemistry and Biophysics, University of California, San Francisco, 600 16th Street, MC 2200, San Francisco, California 94158-2517, USA. Carol W. Greider is Daniel Nathans Professor and Director, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 603 PCTB, 725 North Wolfe Street, Baltimore, Maryland 21205, USA. Jack W. Szostak is Professor of Genetics at Harvard Medical School, Department of Molecular Biology and Center for Computational and Integrative Biology, Simches Research Center, 85 Cambridge Street, Boston, Massachusetts 02114, USA.
e-mails: elizabeth.blackburn@ucsf.edu, cgreider@jhmi.edu or szostak@molbio.mgh.harvard.edu.

research report that described the frequent joining of broken chromosome ends, "no case was found of the attachment of a piece of one chromosome to the end of another [intact chromosome]"[1]. In 1938, Muller named the natural ends of chromosomes 'telomeres'[2]. But neither Muller nor McClintock had the tools to understand the molecular nature of these chromosome ends.

The question of the molecular nature of the chromosome end only became meaningful in 1953, when the structure of DNA was described[3]. By the 1960s, Arthur Kornberg had discovered DNA polymerase and its mechanism had been determined[4]. This understanding posed yet another question about DNA ends—how was their complete replication ensured? Because DNA polymerase could only extend a preformed primer, it could not copy the very end of a linear DNA; this became known as the DNA end-replication problem[5]. By the early 1970s, studies of DNA bacteriophage genomes had shown that the answers to the DNA end-replication problem differed between one virus and another[6]. How, then, was the DNA at the very end of eukaryotic chromosomes arranged? In 1975, Liz Blackburn arrived at Yale in Joe Gall's lab to do postdoctoral research, having recently completed her graduate work in Fred Sanger's group in Cambridge, England, where DNA sequencing was being invented. Liz wanted to apply her knowledge from the Sanger lab to understanding the molecular nature of chromosome termini.

Telomeres go molecular: mysterious DNA termini

One of the most daunting aspects of addressing the question of the DNA at chromosomal ends was the enormous length of the chromosomal DNAs of eukaryotes. DNA cloning methods had not yet been invented, so to be able to study the ends, short chromosomes were needed. In

the 1970s, Joe Gall had been delving into the processes by which some organisms produce extra copies of the genes for ribosomal RNA (rRNA). This occurs, for example, early in development, when large amounts of protein synthesis must occur rapidly. Joe had discovered that the genes encoding rDNA are amplified on circular DNA molecules that are present in very high numbers in the developing oocyte in frogs. He then found that the same thing happened in a very different organism—the ciliated protozoan *Tetrahymena thermophila*—but this time, the rDNA was amplified into linear DNA molecules. *Tetrahymena* contained large numbers of nearly identical, relatively short minichromosomes. This was the material that Liz decided to use to analyze natural ends of chromosomes.

There was no road map for how to do this. But Joe Gall had already shown that a fraction of the molecules were circular when extracted from cells, a property reminiscent of phage lambda, the linear DNA of which circularizes to replicate. Because Liz had grown to believe that nature uses elegant and universal solutions, she thought that the lambda ends might be a possible model for the molecular nature of the *Tetrahymena* termini. Thus, she decided to use *in vitro* the DNA 'repair' reaction of DNA polymerase, which had been successfully used by Ray Wu and colleagues to sequence the cohesive ends of the lambda phage family genomic DNAs[7].

This was a fortunate choice, because the molecular ends of the rDNA turned out to have discontinuities (the significance of which remains mysterious to this day) within the telomeric repeat tract DNA that allowed DNA polymerase to label them readily *in vitro* using radiolabeled triphosphate substrates. Liz was then able to piece together the DNA sequence of the telomeres by combining a variety of

in vitro labeling and other analytical techniques, and she came up with a very un-lambda-like surprise. The rDNA minichromosome end sequence and the structure at the termini of these molecules were complex and unlike any previously described. At each end of the *Tetrahymena* rDNA molecules there were around 50 tandem repeats of the hexanucleotide unit CCCCAA—TTGGGG on the complementary strand—with the latter (G-rich) strand bearing the 3′ OH end at each end of the linear DNA. The C-rich CCCCAA-repeat strand had single-stranded discontinuities in at least a portion of the repeat array. Oddly, too, the number of tandem repeats per end was heterogeneous among the population of purified molecules, ranging from an estimated minimum of 20 to up to about 70 (ref. 8). Late in 1977, Liz gave a talk describing the unusual molecular features of the rDNA ends at the University of California, San Francisco to Herbert Boyer's group in the Biochemistry Department. In the discussion after her presentation, a member of the Boyer lab asked whether the heterogeneity in the number of CCCCAA repeats in the DNA population might arise by addition of repeats to the chromosome ends. Liz was intrigued but at the time could see no known way this could occur. Looking back, this conversation seems prescient, as later Liz would find that, in fact, this addition does occur.

New telomeres are added to fragmented *Tetrahymena* chromosomes

In collaboration with Meng-Chao Yao in Joe Gall's lab, Liz showed that the same terminal, heterogeneous array of CCCCAA repeats occurred at the ends of the other chromosomal DNA molecules of the somatic nucleus (the macronucleus) in *Tetrahymena*, but that these sequences were not present in the precursor DNAs in the germline nucleus (the micronucleus) from which the somatic nucleus is generated[9,10]. Soon after, the telomeric sequences of the linear rDNA minichromosomes from the slime molds *Physarum*[11] and *Dictyostelium*[12] were also determined. Liz continued her work on ciliate DNA termini when she set up her own lab in the Molecular Biology Department of the University of California, Berkeley in 1978. There she found that new telomere sequences were added to the ends of the linear rDNA minichromosomes by an unknown mechanism[13]. These subchromosomal macronuclear DNAs are generated by developmentally controlled DNA fragmentation of the germline nuclear chromosomes. Surprisingly, there was no invariant DNA sequence to which the telomeric repeats were joined[13–15]. At around the same time, David Prescott's group obtained similar results in a different group of

ciliates[16,17]. The question then became: how was the telomere repeat added?

In considering telomere formation in *Tetrahymena*, Liz wrote in 1982: "...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"[13]. This idea for *de novo* telomere addition was further supported by ongoing experiments in yeast.

Telomere function transcends kingdom boundaries

By 1980 we understood the molecular structure of *Tetrahymena* telomeres, but the connection between this structure and the special properties of telomeres remained obscure. Whether this surprising structure was unique to *Tetrahymena* and its ciliate relatives or was more broadly conserved was also unknown. The answers to these questions began to emerge from an unlikely collaboration between Jack Szostak and Liz. Jack had recently completed his graduate and postdoctoral work with Ray Wu at Cornell, where he had begun to study recombination in yeast. In 1979, he had set up his own lab at what was then the Sidney Farber Cancer Institute in Boston, and was investigating the highly recombinogenic nature of DNA ends in the budding yeast *Saccharomyces cerevisiae*. At that time, plasmid vectors for yeast transformation were all maintained as circular DNA molecules. Furthermore, linearization of these plasmids by restriction enzyme digestion led to DNA ends that were extremely reactive inside yeast cells: if the DNA ends were homologous to yeast DNA, recombination would result in integration of the plasmid into the chromosome[18]; otherwise, the DNA termini would be degraded, ligated or otherwise rearranged. Liz and Jack's collaboration began with an intense conversation in the summer of 1980 at a New Hampshire school, the site of that year's Gordon Research Conference on Nucleic Acids. After hearing Liz's description of the remarkable molecular biology of *Tetrahymena* telomeres, Jack asked Liz about testing whether *Tetrahymena* telomeres might function in yeast. The idea was so far-fetched that it seemed outlandish: to test whether the telomere replication mechanisms are conserved between such evolutionarily distant species.

Liz and Jack reasoned that if *Tetrahymena* telomeres retained their ability to stabilize DNA ends when transferred into yeast cells, they might be used to cap the ends of a linearized yeast plas-

mid, resulting in a linear replicating plasmid with stable DNA ends. The experiment was technically simple to perform, and the prediction—that linear plasmid molecules would be seen instead of the usual circles—would be trivial to confirm. Armed with a purified *Tetrahymena* telomeric DNA fragment supplied by Liz, Jack generated a few nanograms of a linearized yeast plasmid capped with *Tetrahymena* telomeres, and introduced the DNA into yeast cells. He obtained a dozen or so transformants that were analyzed by Southern blotting. The result was immediately clear: over half of the colonies maintained the introduced DNA in linear form. This result was confirmed by more detailed DNA analysis[19]. This result showed that telomeres could function across phylogenetic kingdoms, implying remarkable functional conservation.

Yeast telomeres reveal conservation of telomere structure

These linear plasmids then provided the ideal vector for cloning yeast telomeres. Jack removed one *Tetrahymena* telomere, generating a linear DNA fragment that could not be maintained in yeast. He then went fishing for functional yeast telomeres by joining random pieces of yeast genomic DNA onto this linear DNA fragment; only when the missing telomere was replaced with a yeast telomere could the DNA survive in yeast as a linear plasmid. Three of the expected linear plasmids were recovered, and Southern blots of genomic yeast DNA showed that the linear plasmids indeed carried a functional yeast telomere (**Fig. 1**). With yeast telomeres in hand, it was now possible to study the structure of telomeres of a eukaryotic chromosome capable of proper mitotic and meiotic segregation.

Closer examination of the initial linear plasmids revealed that the *Tetrahymena* telomeres had become longer and more heterogeneous in length during their maintenance in yeast. Janis Shampay in Liz's lab sequenced the subcloned yeast telomeres and the *Tetrahymena* telomeres maintained in yeast and found that yeast-specific TG_{1-3} repeats had been added onto the *Tetrahymena* TTGGGG repeats[20]. Liz labeled the yeast telomeres *in vitro* and found that they also had single-stranded discontinuities in the C-rich DNA strand, as seen in *Tetrahymena* telomeres. Janice then showed that yeast chromosomal telomeres also consisted of a variable number of terminal TG_{1-3} repeats. Together these results indicated that these telomeres were very similar in structure to the archetypal telomeres of *Tetrahymena*. The length heterogeneity of telomeric fragments reflected variable amounts of DNA at the very ends of the plasmid or chromosome. These results led Jack and Liz to propose the existence of a terminal
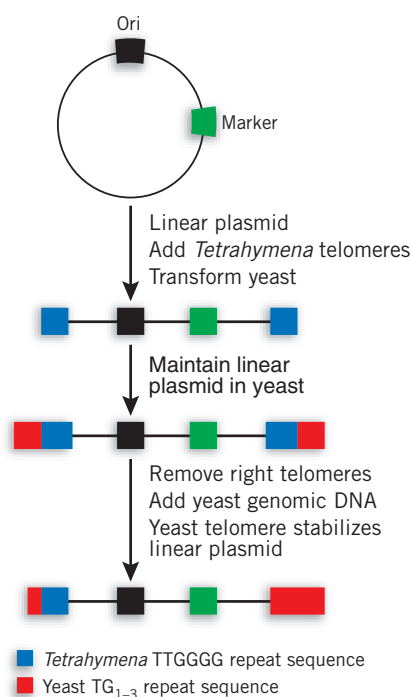
Figure 1 Yeast sequences are added to *Tetrahymena* telomeres *in vivo*. The cross-kingdom experiment that showed telomere function is conserved, and it allowed the cloning of yeast telomeres shown in the diagram. A circular yeast plasmid with an origin of replication (Ori, black) and a selectable marker (green) was linearized and *Tetrahymena* telomeres (blue) were ligated onto the ends. When this plasmid was transformed into and grown in yeast, the plasmid remained linear but yeast telomere sequence (red) was added to the end of the *Tetrahymena* telomere repeats. This plasmid was extracted from yeast, and the right end was removed and replaced by fragments of yeast DNA. A yeast telomere (red) captured by this method represented the first cloned telomere.

transferase–like enzyme that would add repeat units to telomeric DNA as a way of compensating for the erosion caused by incomplete terminal replication[20].

The availability of cloned telomeric fragments allowed Andrew Murray, then a graduate student in Jack's lab, to begin construction of the first artificial chromosomes. Andrew combined centromeres, replication origins, genetic markers and telomeres on a single yeast plasmid. Surprisingly, this and subsequent work showed that this full complement of known chromosomal elements was insufficient for proper mitotic segregation, and that a minimum length of DNA was also required. This initial work on artificial chromosomes[21] set the stage for later studies of highly engineered chromosomes as a path toward understanding the cellular mechanisms that ensure accurate chromosomal inheritance, in both mitosis and meiosis[22,23].

Artificial chromosomes were subsequently used to clone very long DNA fragments in yeast, and they became an essential tool early in the analysis of the human genome.

Some unusual goings-on at the ends
The addition of yeast sequences directly onto the *Tetrahymena* telomeres on the linear plasmid in yeast, the *de novo* telomere addition in *Tetrahymena* and the length heterogeneity of telomeres indicated that an unusual process might be involved in telomere maintenance. In addition, the curious fact that telomeres grew longer when trypanosomes were grown in culture also indicated something unusual was going on at the ends[24]. By 1983, there were two competing models for the molecular mechanism that generated the telomere length heterogeneity and maintained the telomere length. One model proposed that there was an unknown enzyme that synthesized the ends[20]. The second model proposed that the addition of telomere repeats occurred through a recombination-mediated process[24,25]. Recombination was a well established process and the mechanism proposed for telomere repeat addition seemed plausible. To distinguish between these mechanisms, direct experimental evidence was needed.

The discovery of telomerase
The biochemical evidence for telomerase came from a series of experiments carried out in Liz Blackburn's lab by Carol Greider. Carol joined Liz's lab as a graduate student in 1984, and she set out to study how telomeres replicated and to investigate what caused the sequence additions seen in *Tetrahymena*, yeast and trypanosomes. After preliminary experiments done by Liz and her graduate student Peter Challoner, Carol initiated biochemical experiments to look for evidence for a telomere-addition enzyme. Carol began by adding DNA restriction fragments and radiolabeled nucleotides to *Tetrahymena* cell extracts to determine whether a telomere sequence on one end of the restriction fragment would become preferentially labeled. These experiments initially showed promising results. However, closer examination showed that the labeling was due to known DNA polymerase activities. Carol therefore tried a variety of different substrates and reaction conditions to determine whether there was an activity that would act only on telomere sequences.

The breakthrough came in December of 1984, when she used a synthetic DNA oligonucleotide as a substrate in the reaction. This synthetic oligonucleotide represented a different molecular substrate that could also be added in higher concentration than a restriction fragment. She added the DNA oligonucleotide $(TTGGGG)_4$ to unfrac-

tionated cell extract containing radiolabeled dGTP. When the products were fractionated by size on a sequencing gel, a ladder of repeats with a six-base periodicity extended up the gel[26]. We now know that this experiment worked because the telomeric oligonucleotide substrate was present in a very high concentration compared with that in the earlier experiments using restriction fragments.

Yeast telomeres in *Tetrahymena* extracts: proof comes full circle
This first hint of an activity that generated a six-base repeating pattern was very exciting. Carol and Liz then set out to determine whether this repeating pattern was due to a new enzyme or to an established DNA polymerase that might be using the input oligonucleotide and copying endogenous telomere repeats in the cell extract. Carol used different approaches to rule out endogenous DNA as a template. First, she showed that the elongation activity was specific to telomeric sequences, as an oligonucleotide with an unrelated sequence was not extended in the reaction. The key experiment, however, came when Carol and Liz decided to do the inverse of the Szostak and Blackburn cross-kingdom telomere-function experiment[19]: this time in *Tetrahymena* cell extracts. They used a synthetic oligonucleotide consisting of the yeast telomeric sequence repeat. This 24-base oligonucleotide had the sequence TGGG at its 3′ end, but was otherwise not related in sequence to the *Tetrahymena* repeats. The experiment showed that not only was this yeast telomere efficiently elongated in the extracts, but the pattern of the repeats on the gel was shifted up by one base (**Fig. 2a**). This was the exciting breakthrough that finally convinced Carol and Liz that the activity was indeed a unique telomere-synthesizing enzyme. The one-base shift in the banding pattern was due to the correct synthesis of the TTGGGG sequences by the *Tetrahymena* enzyme (**Fig. 2b**). As the *Tetrahymena* substrate $(TTGGGG)_3$ has 4 G's at the end, and the yeast oligonucleotide had only 3 G's, it had to first be extended by an extra G before the TTGGGG repeat pattern could be continued. This resulted in an upward shift in the entire banding pattern—compelling evidence that the activity was a specific telomere terminal transferase activity[26]. In a later paper, the name of the enzyme was shortened to 'telomerase'[27].

Telomerase: how is the sequence specified?
Having a newly discovered telomere-synthesizing activity in hand, the next most important question was how the telomeric sequence is specified. One model was that there might be a nucleic acid component to specify telomere
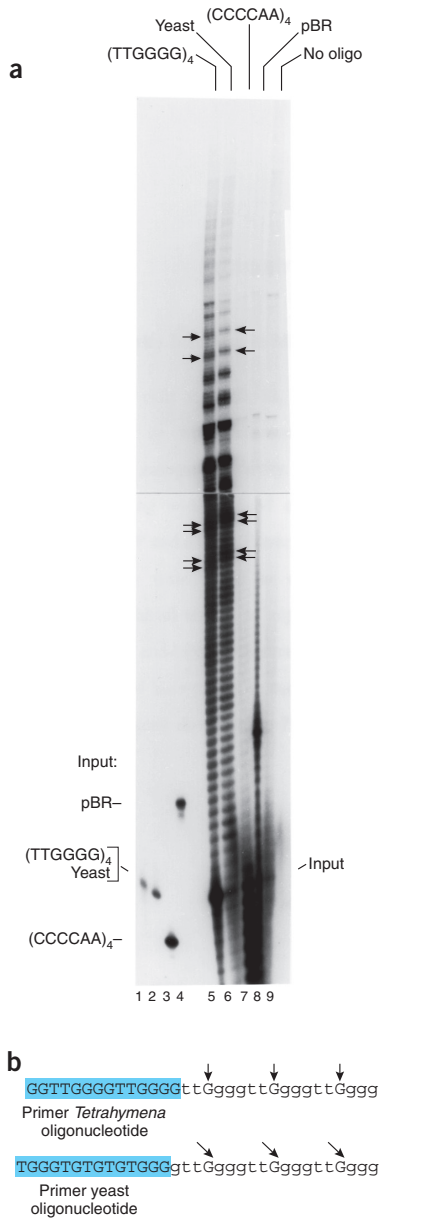
Figure 2 *Tetrahymena* sequences are added to yeast telomeres *in vitro*. (**a**) The autoradiogram shows the addition of *Tetrahymena* telomere repeats onto either a *Tetrahymena* telomere sequence primer (lane 5) or a yeast telomere sequence primer (lane 6). In both cases, a 6-base repeated sequence is added, but with the yeast primer the pattern is shifted up by one nucleotide (arrows). Lanes 1–4 show the size of the input primers, and lanes 7–9 show that oligonucleotides with unrelated sequences are not substrates for addition. (**b**) Diagram representing the sequence of the *Tetrahymena* primer (top, blue) and the yeast primer (bottom, blue). The sequence added onto the primer is dictated by the sequence at the 3′ end of the primer. Thus, the banding pattern is shifted up by one nucleotide for the yeast primer because a G must be added to complete a set of GGGG before the TT sequence follows. This extra G sets the phase for all the other repeats that are added, shifting the entire pattern for every repeat that follows (arrows).

repeats. Indeed, treatment of the extract with RNase abolished the activity. A complex series of controls eventually showed that this loss of activity was not an artifact: the enzyme contained an essential RNA component[27]. However, finding the specific RNA was trickier. Carol set out to purify telomerase and sequence the RNAs that copurified with the enzyme activity. Several years of cold-room work led to highly purified fractions, but the RNA remained elusive. After moving to an independent position at the Cold Spring Harbor Laboratory, Carol was able to use partial sequence information from copurifying RNAs to clone the RNA component of telomerase. The appearance on a sequencing gel of the sequence CAACCCCAA in one clone immediately heralded success. Carol then set out to test whether this RNA was required for enzyme activity. She accumulated evidence that not only did the RNA copurify with telomerase, but oligonucleotides that blocked the CAACCCCAA proposed template region also blocked enzyme activity[28]. The presence of nine nucleotides in the template region then implied a mechanism for how telomerase might synthesize tandem TTGGGG repeats. The model, presented in 1989, is still the accepted mechanism for telomerase action[28].

Carol sent the cloned RNA gene back to Liz at Berkeley. Guo-Liang Yu and John Bradley, graduate students in Liz's lab, made mutations in the template. Guo-Liang overexpressed the mutant telomerase RNA in *Tetrahymena* cells by microinjecting the genes into *Tetrahymena* using a new vector system he had devised. He found that the telomeres in these cells now incorporated the altered telomere repeat sequence. This showed definitively that telomerase uses the CAACCCCAA template sequence to specify the addition of telomeric repeats and therefore established its reverse transcriptase mode of action *in vivo* as well as *in vitro*[29].

Evidence for telomerase in yeast

In parallel with the biochemical effort to identify the components of telomerase in *Tetrahymena*, Vicki Lundblad in Jack's lab initiated a genetic screen in yeast for mutants defective in telomere maintenance[30]. The most interesting mutant had the phenotype predicted for one defective in telomere maintenance—a continuous shortening of the telomeric DNA. Because the telomeres in this strain became shorter and shorter over time, the gene was named *EST1* for 'ever shorter telomeres.' Most gratifyingly, this strain also showed a delayed senescence phenotype, in which many generations of normal growth were followed by an increase in chromosome loss and a significant loss of growth potential, as predicted from the gradual loss of telomeres. It was not known at the time whether *EST1* was related to

telomerase or had some other role in telomere maintenance.

In characterizing the *Tetrahymena* telomerase RNA mutants, Liz found that one mutant had a phenotype that paralleled the *EST1* mutant of yeast[29]. This template mutation somehow blocked all DNA addition onto telomeric ends *in vivo*. Instead, the telomeres shortened progressively, and the cells grew for about 20–25 more cell divisions, and then they senesced (stopped dividing)[29]. This result helped support the idea that the *EST1* mutant in yeast might indeed be a telomerase mutant, as the phenotype was so similar to that of this *Tetrahymena* telomerase mutant. Together, these experiments established that a functional telomerase is necessary for the indefinite replicative capacity of yeast and *Tetrahymena*.

Biochemical identification of the yeast telomerase activity came from the work of postdoctoral fellows Marita Cohn and John Prescott in Liz's lab[31,32]. An initially puzzling result was that this *in vitro* activity did not require *EST1* (refs. 31,33). However, subsequent biochemical evidence showed that the Est1 protein is indeed a component of the yeast telomerase complex, although it is not the catalytic component[34]. The *EST2* gene, which was later identified by Vicki Lundblad[35], turned out to encode the catalytic subunit. *EST2* was shown to be homologous to a ciliate telomerase component purified and identified by Joachim Lingner and Tom Cech, leading the three of them to show that *EST2* encodes the catalytic protein component of telomerase—the telomerase reverse transcriptase (TERT)[36].

Telomeres in cancer and cell renewal

The discovery of telomerase in *Tetrahymena* and yeast was pure curiosity-driven research, with no obvious medical impact. That soon changed. Although the sequence of the human telomere took longer to identify, there was early evidence that it had a similar structure[37–39]. In 1988, the sequence of the human telomere was identified as simple repeats of TTAGGG, very similar to the *Tetrahymena* repeats[38,39]. This and the identification of human telomerase[40] sparked research in two areas: cellular senescence and cancer. Alexei Olovnikov had predicted that the end-replication problem would result in telomere shortening that might be the cause of the limited cell division potential of primary fibroblasts in culture—cellular senescence[41]. Carol, in collaboration with Calvin Harley, then found that telomere shortening does indeed occur in human cells in culture[42]. This is due to a lack of telomerase expression and a consequent inability to maintain normal telomeres. If telomerase is expressed in these cells, telomeres do not shorten and the cells do

not show senescence[43]. Thus, short telomeres can limit the ability of cells to divide, indicating that telomerase inhibition might limit the growth of cancer cells[42].

This idea of a role for telomeres in cancer was pursued independently by two independent groups. Nick Hastie and Titia de Lange both showed that telomeres were shorter in cancer cells than in normal tissue[44,45]. This shortening is likely to be due to the large number of cell divisions that cancer cells undergo without sufficient telomerase. For cancer cells to overcome the limit to cell division imposed by very short telomeres, telomerase must be present to allow telomere maintenance. The subsequent demonstration that telomerase is active in many human tumor cells but is not detectable in most normal tissues further strengthened the idea that telomerase inhibition might be an effective way to kill cancer cells[46].

These initial findings stimulated the cancer community to study telomerase activity in a variety of tumor types, which led to an explosion of publications on telomerase (**Fig. 3**). Subsequent work using cultured human cells and a telomerase knockout mouse model confirmed that inhibition of telomerase expression can limit cancer cell division and tumor production[47]. However, loss of telomere function in some situations may lead to chromosome rearrangements that can fuel tumor progression[48]. The details of the pathways that determine whether telomerase inhibition will be effective in fighting particular types of cancer are still being worked out. This is a very complex and rich area of current research.

How much telomerase is enough?

The continuing biochemical studies of telomerase throughout the 1990s led to an entirely new discovery: short telomeres have a role in genetic disease. The structure of the human telomerase RNA component[49] contains a motif that is present in an unrelated class of small RNAs, the snoRNAs[50]. This motif binds to a protein termed dyskerin that functions in ribosomal RNA modification. Alterations in dyskerin lead to altered rRNA[51], but they also result in a reduction in human telomerase RNA concentration and, most importantly, short telomeres[52,53]. Dyskerin mutations underlie the X-linked human genetic disease dyskeratosis congenita, which is characterized by abnormal skin and by bone marrow failure. This initial association of dyskerin with telomerase paved the way for the demonstration that the autosomal dominant form of dyskeratosis congenita is caused by mutations in human telomerase RNA[54].

Remarkably, mutations in both the RNA and the protein components of telomerase result in progressive telomere shortening over many generations in humans, which limits tissue renewal capacity[55–57]. This limited tissue renewal manifests clinically as dyskeratosis congenita, aplastic anemia or other syndromes depending on the tissue that is most affected in the individual. The striking thing about the autosomal dominant inheritance pattern is that it implies that one functional copy of telomerase is not sufficient for telomere maintenance. Indeed Carol's lab demonstrated this directly in mice by showing that having half the normal amount of telomerase results in progressive telomere shortening and

loss of tissue renewal capacity[58]. This implies that the remarkable ability of telomerase to recognize and efficiently elongate telomeres is strictly limited in the cell. Experiments aimed at understanding the mechanisms that limit telomere elongation and at understanding why telomerase activity is so tightly regulated in mammals promise more exciting discoveries in telomere research in the coming years.

The power of curiosity-driven research

The stories of the functional analysis of the telomere, the discovery of telomerase and the initially unseen implications of both serve to illustrate several points. First, there is great value in talking to people working in different fields. As the specialization of science grows— perhaps inevitably, as a result of the increase in knowledge—it becomes more likely that scientists will remain cocooned within their own research specialty. But big problems such as cancer, infectious disease and aging will never be solved purely by studying one aspect of one biological system; such targeted work is necessary but not sufficient. We can see this clearly in, for example, recent advances in understanding the biology of aging, in which the integration of studies in species from yeast through humans has been critical for the progress that has been made. Beyond this, the emerging discipline of systems biology is helping to integrate entirely new and unfamiliar techniques, such as the mathematical analysis of network architecture, into our methodological armamentarium.

A second important lesson is the value of high-risk, high-payoff experiments. When we were first discussing the idea of putting *Tetrahymena* telomeres into yeast, the experiment seemed unlikely to work. After all, these organisms are in distantly related phyla and their cell biology is remarkably diverse. However, a large body of experimental work showed that, in all eukaryotes that had been studied, the DNA ends located at chromosomal termini were unusual. Given that there had to be some mechanism for telomere replication and stabilization, it seemed possible that this mechanism might be conserved across eukaryotes. Likewise, initiating experiments aimed at finding telomerase in *Tetrahymena* seemed very risky at the time. The success of these experiments also illustrates the value of work on 'nonstandard' organisms—we will miss a lot if we focus exclusively on a few well worked-out model systems. We can learn so much from the diversity of life. Biology sometimes reveals its general principles through that which appears to be arcane and even bizarre. But in evolution, function is frequently fundamentally conserved at the mechanistic and molecular levels, and what changes is the extent and setting in which
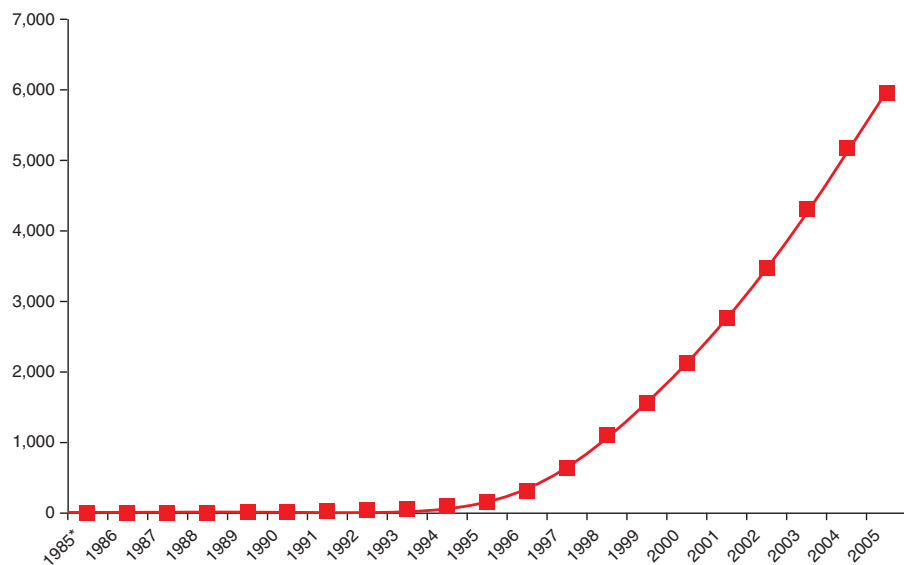
Figure 3 Cumulative citations for telomerase in Medline. The number of citations for "telomerase" is shown from every year since its discovery. While there were many important fundamental papers on telomerase published between 1985 and 1996, it was after this time that the medical relevance of telomerase became established in the scientific community. The number of cancer publications on telomerase then skyrocketed. *The first paper, in 1985, did not use the name "telomerase"; this term was not used until 1987.

the various molecular processes are played out. Thus, what may at first look like an exception in biology often turns out to be the manifestation of a molecular process that is much more fundamental. Such was the case with the ciliated protozoa and their telomeres and telomerase.

Even more broadly, the quiet beginnings of telomerase research emphasize the importance of basic, curiosity-driven research. At the time that it is conducted, such research has no apparent practical applications. Our understanding of the way the world works is fragmentary and incomplete, which means that progress does not occur in a simple, direct and linear manner. It is important to connect the unconnected, to make leaps and to take risks, and to have fun talking and playing with ideas that might at first seem outlandish. Fundamental advances are often stimulated by unexplained observations made in the course of applied work, and the resulting growth in understanding directs our attention to new avenues of research. This cycle of interaction between fundamental and applied research is autocatalytic and contributes greatly to the explosive increase in scientific knowledge. If the cycle is broken, through a failure to recognize the importance of basic science, the continuation of progress in more applied domains of science, medicine and engineering will surely be limited.

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