Telomeres/Telomerase

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Telomerase Discovery: The Excitement of Putting Together Pieces of the Puzzle (Nobel Lecture)**

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Autobiography

I was born in San Diego, California, in 1961. My brother Mark was born in January of the previous year. My father Kenneth Greider was a physicist who had recently graduated with a PhD from University of California at Berkeley. My mother Jean Foley Greider also had received her PhD from UC Berkeley in botany. My father worked high-energy nuclear physics and my mother was a mycologist and a geneticist. After both parents completed postdoctoral fellowships in San Diego in 1962, my father took a faculty position in the Physics Department at Yale and so the family moved to New Haven, Connecticut. My mother took a post-doc position at Yale in the laboratory of Norman Giles, where she worked on *Aspergillus* as well as other fungal species. A few years later in 1965, my father took a faculty position in the Physics Department at UC Davis and so the family moved back to California. My mother first took a teaching position at Sacramento community college and then later at American River College in nearby Sacramento.

Davis

Mark and I grew up in Davis where we could walk to school. My parents built a house in a development in West Davis shortly after we moved to Davis. The street was conveniently located about four blocks walk from the West Davis Elementary School (Grades K–4) and half a block from the new West Davis Intermediate School (Grades 5 and 6). Mark and I would walk to school together as kids, and later bike to high school, year-round. It gave us a sense of independence to come and go. The idea of parents driving their kids to school was one I had never heard of until moving to the east coast and becoming a parent myself. This early responsibility was something that shaped my sense of independence. For me school was something that was a kid's responsibility. Parents were not really involved.

In December of 1967 my mother died when I was in first grade and Mark was in second. In retrospect this event played a major role in my learning to do things on my own. Mark and I continued to get ourselves to school and to go on with our lives as best we could. School was not easy for me. I was put in remedial spelling classes because I could not sound words out. I remember a special teacher coming into the classroom every week to take me out for special spelling lessons. I was very embarrassed to be singled out and removed from class. As a kid, I thought of myself as "stupid" because I needed remedial help. It was not until much later that I figured out that I was dyslexic and that my trouble with spelling and sounding out words did not mean I was stupid, but early impressions stuck with me and colored my world for a time.

Heidelberg

In 1971 my father was invited take his sabbatical at the Max Planck Institute for Nuclear Physics in Heidelberg, Germany. We moved to Germany for the year and Mark and I went to the "Englisches Institut", a private school. Despite its name, it was a typical German Gymnasium and all of the instruction was in German. So for the first six months or so, we learned German by immersion. Mark and I took the city bus to school each day so we quickly learned to navigate the public transportation system, as well as navigate our way around a new school and new language and new culture. In Davis we had been used to getting to school on our own, so we welcomed this independence and developed an appreciation for how things were done in a very different culture.

I remember my grades were particularly poor in this school and especially so in the English class. The English teacher would give a dictation and we were supposed to write down what she said in English. It seemed too simple and pointless to me, but when I got my graded notebooks back, the scores were usually D's or F's because every other word was misspelled. Looking back over those notebooks later, I saw the pattern of backwards words and letters and gross misspellings that led me to suspect I was dyslexic. The other

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confusing thing about school for me was the "religion" class. You had to declare if you were Catholic or Protestant (as if those were the only choices) and then each group had their own class. Back home, my father was music director for the Unitarian Church, but as kids, we rarely went to church. It was too hard to translate what Unitarian meant to the Germans, so my father asked the school to excuse me from this religion class, and instead have a free period to do homework. This is how I met my friend Jiska, who was one of the few Jewish kids in the school and who was also excused from religion class. In my friendship with Jiska, both of us different from the rest, I began to develop an appreciation for people who were not like the others and who stood a bit outside the mainstream. This understanding of and affinity for people outside the mainstream served me well later in life. In high school and college I never felt the need to be part of a popular group, but rather sought out friends for their personal qualities. This appreciation may have also shaped many choices later in life; for example, working on the unusual organism *Tetrahymena*.

I spent a lot of time on my own in Heidelberg, playing down by the stream near our house or hiking the hill to the top of Boxberg. I took the bus into town on my own and learned to dress and speak like a German. There was a large American army base in town and I did not want to be mistaken as an army kid. I liked being more unusual: an American kid who understands German culture. By the middle of the year, I had learned German and became fluent in speaking and reading, but like all other written tasks, the writing and grammar eluded me. Mark and I had some German–American friends a few stories up in our apartment complex and we made up games like tapping out a code on the radiators and sending notes on string outside the kitchen windows to communicate. These games irritated the other apartment residents and resulted in the building manager coming to talk to my father. We were typical kids of that fashion, breaking some rules, where we could, but not going too far.

Davis—Part II

When we returned from Germany I went into 6th grade, which was a transition year, the last in Intermediate school before junior high school. I spent much of it readjusting to being back and making new friends. Unlike many scientists I know, I was not a kid who knew from early on that I wanted to be a scientist. I think one important thing I learned in my early years was to focus intently on the task at hand, such as learning German when we were in Heidelberg, to the exclusion of other things going on around me. This survival skill served me very well in later years. Focusing on certain goals and ignoring obstacles came naturally to me.

In junior high school I learned that I could be good at school. I remember liking the freedom to choose classes and the pleasure of learning and doing well. My perseverance and love of reading had somehow allowed me to overcome many disadvantages of dyslexia, and I read a lot of books for pleasure. I found I had to memorize words to spell them, as sounding them out did not work for me. This coping mechanism proved also to have an up-side; memorization in biology and history was easy for me. My father encouraged us to do well in school and to do it for ourselves. He said that we should want to do well because it would "open doors" for us. He emphasized that being able to choose what you want to do in life is so important, and doing well early on will allow more possibilities in the future. I also discovered the pleasure of the outside reward of getting all A's in classes, it made me feel good and I got positive feedback from people outside the family.

In high school I focused on doing well in my classes and finding a supportive group of friends. In junior high I had been attracted to outsiders, perhaps from my experience in Germany. But the outsider group I found myself with in junior high was not as interested in school as I was. I took the opportunity of the change in schools from Emerson Junior High to Davis Senior High as an opportunity to find a new group of friends. I met Lori Lopez and Resi Zapfel at an American Field Service (AFS) Club meeting in the first weeks of high school, and they quickly became friends. Resi was an exchange student from Austria and Lori was the AFS club president. Lori's family and Resi's host family the Robertsons became like a second family to me. I liked the foreign students in the ASF group, and the American kids who were a part of this group were not interested in the mainstream popularity. I affiliated myself with the AFS student group throughout high school and was even president of the club my senior year. I did not focus particularly on science in high school, or join any science-related groups, although I continued to do well in all of my classes; I considered it a challenge to get all A's. I never considered myself one of the smart kids, they seemed confident and driven. I just enjoyed learning and especially spending time with friends.

After my junior year at Davis High School, I knew I needed to think about where to go to college. I had done well in biology in school and was particularly captivated by my 12th grade biology class, where we learned a lot of physiology from a very motivated science teacher who had a PhD. I loved learning new material and being challenged, so I decided to major in biology in college. Many of my fellow high school graduates intended to go to nearby schools, either UC Davis or UC Berkeley. I did not want to go to either. I wanted to do something different from the norm, get out, and have new experiences. My friend Alyssa Ingalls, whom I had known since 6th grade, was taking a trip to visit several University of California schools with her family Liz and Bob Young. I was happy to be invited along on this school tour. We visited UC Santa Cruz, UC Los Angeles, and UC Santa Barbara.

I had a contact at UCSB, Beatrice Sweeney, who was a professor there and who had known and worked with my mother at Yale. My father put me in touch with Beazy, as she was known, and Alyssa and her family and I got a tour of the campus from her. Beazy was a cell biologist by profession but a naturalist in her heart. She took us for a walk on the beach near her house and told us fascinating stories about the biology of all the marine animals and plants that we walked by. I was captivated by her and by the beautiful UCSB

campus. I decided I wanted to study Marine Ecology at UCSB.

Santa Barbara

Beazy was on the faculty of the College of Creative Studies, a small college that is part of UCSB. The College of Creative Studies was founded by Marvin Mudrick, a professor in the English Department, to foster independent learning and interaction between disciplines. The requirements to get into CCS were significantly higher than to get into UCSB. My grades were very good, but my SAT scores were not. I never spent time practicing to take standardized tests and the dyslexia made them hard for me. I was very happy when CCS accepted me. So off I went in the fall to Santa Barbara.

The most important thing about UCSB and CCS was that Beazy encouraged me to begin working in a lab in my freshman year. I was scared that I needed more time to adjust to college, but she said to start as soon as possible. I did a project first with Adrian Wenner studying sand crab populations in Santa Barbara. Though I thought I wanted to be a marine ecologist, this experience did not captivate my attention. The science was mostly statistics, which I did not understand or relate to. Beazy kept in close contact with me and saw I needed a different experience. So I then worked with a post-doc in Beazy's lab studying the movements of chloroplasts during dark–light circadian cycles in *Pyrocystis,* a dinoflagellate. I enjoyed the work in the lab. I liked coming in to do my own experiments and was challenged when Beazy said I had to come up with a way to plot and describe my experiment on my own, with no set form. The simultaneous pain and joy of trying to create something that made sense to describe my observations was exhilarating.

I enjoyed watching cells and describing the circadian rhythms, but after a while I felt the work was too descriptive. So next Beazy took me to work in Les Wilson's lab on microtubule dynamics. I am not sure if it was the topic or the personalities in the lab, though it was likely both, that captivated me. I worked first with Kevin Sullivan and later with David Asai studying microtubule-associated proteins. The work in the lab was focused on understanding molecules and how they interact and behave. We would do experiments to examine how fast microtubules would assemble from the tubulin building blocks, then make a change to the tubulin preparation and see how that affected the results. Being able to manipulate molecules and understand the mechanics of how things worked fit my way of thinking. In addition, talking with both Kevin and David and the others in the lab was fun. People knew each other well, were playful, and would tease each other a lot. There were inside jokes and an easy way of laughing about experiments as well as everyday life that was infectious. I worked with Kevin for my sophomore year studying the assembly kinetics of chick brain microtubules under different conditions. The experience that Beazy and the CCS program provided me, to try out several different laboratory experiences, was instrumental in my finding how much I loved mechanistic thinking and biochemical experiments. By comparing several different labs, it became clear to me when I was having fun and when I was not. I saw that laboratory work was about people and interactions as well as about science. It could be playful and was appealing as a potential path I could enjoy.

Göttingen

My junior year in college I spent as a student at the University of Göttingen in Germany. Ever since my early experience in Heidelberg and visits to see Resi Zapfel (now Schmall) in Austria, I wanted to experience what it was like to live as a student in a foreign country. I took the opportunity to go to Germany for a year on the University of California's Education Abroad Program (EAP). Before I left, Kevin Sullivan and Les Wilson encouraged me to continue lab work in Germany. They contacted Klaus Weber who had a lab at the Max Planck Institute for Biophysical Chemistry, and he agreed I could work there. I would split my time between classes at the University, such as Biochemistry and Genetics, and time at the Max Planck working on intermediate filaments in the Weber lab. In addition to lab work, I also became close friends with a number of Americans in Göttingen who were also on exchange programs.

At the beginning of the second semester I was looking for biology courses in the course catalogue and found one on chromosomes that looked interesting. When I showed up in the assigned room at the right time it turned out it was the regular lab meeting for Professor Ulrich Grossbach. Professor Grossbach had listed his lab meetings as a course so the graduate students could get credit. I was very embarrassed to walk into a private lab meeting, but the researchers in the group were all very nice and they asked me to stay. Michel Robert–Nicoud, a research associate in the group, took me under his wing and asked if I wanted to help in a study of polytene chromosomes of *Chironomus*, a diptera distantly related to *Drosophila*. I enjoyed learning how to do the preparations. It was satisfying to prepare the salivary glands just right and see the giant polytene chromosome under the microscope. I finished the work I had begun in the Weber lab and moved to work with Michel in the Grossbach lab.

Michel collaborated with Tom and Donna Jovin, who were also at the Max Planck Institute for Biophysical Chemistry, on an unusual left-handed helical form of DNA called Z-DNA. Tom and Donna had studied the biophysics of sequences that could form this unusual DNA structure. To understand if Z-DNA is found in natural chromosomes and where it might be located, they developed antibodies to Z-DNA.

They were collaborating with Michel Robert–Nicoud to locate the Z-DNA by staining the giant *Chironomus* polytene with their Z-DNA antibody. There were controversies about whether Z-DNA might be located in bands or interband regions of the chromosome. There was also discussion about whether the regions that stained with the antibody normally had Z-DNA or if the binding of the antibody itself induced Z-DNA where it might not normally be. There was a lot of excitement in the lab about this project and Donna Jovin was preparing to submit a paper on these findings. It was thrilling

to know that my work staining chromosomes was of use for *real* experiments and not just as make-work, and might be part of a publication. This experience with *Chironomus* polytene chromosomes gave me an appreciation for the beauty of chromosomes. It may be that I gained an affection for chromosomes that I brought with me several years later when I first met Liz Blackburn.

Santa Barbara—Part II

When I returned to Santa Barbara for my senior year I wanted to go back to work in the Wilson lab. Kevin Sullivan was writing his thesis and planning to move to a post-doctoral position. Kevin suggested I work with David Asai who was a research associate in the Wilson lab. Kevin was very excited about his future studying the genes for tubulin, because he said genes and DNA were the exciting work going on. Talking to Kevin and David helped me decide that I wanted to go to graduate school. I enjoyed the camaraderie in the lab and liked the challenge to think creatively. I worked hard my senior year, and CCS made it possible for me graduate in four years by their flexibility about transferring credit from my course work in Germany.

For graduate school entrance I took the GRE exams and, as with the SAT's, did not do well. I applied for admission to eight different graduate programs, but did not make it through the numerical cut off for grades + GRE's. I got many rejection letters. However, two schools did decide to interview me. I may have seemed like an interesting case to those people who actually read the applications, rather than pre-screening with a numerical cut-off. I had a 3.9 GPA and A+ grades in Organic Chemistry, Physical Chemistry and Pharmacology, a lot of lab experience, but poor GRE scores. California Institute of Technology interviewed me, and each of the ten professors with whom I talked asked me why my GRE's were low. I talked science with all of them and also explained the dyslexia and poor scores on standardized tests. After the interview I was accepted to Cal Tech. UC Berkeley also accepted me and asked me to come for an interview. It was during that interview that I met Elizabeth (Liz) Blackburn. I felt her enthusiasm for chromosomes and telomeres was infectious. I wanted to talk to her more after the allotted interview time so I made plans to come back again the next week to talk in more depth about her telomere work. After that interview, I decided I wanted to go to Berkeley and work with Liz.

Both of my advisors at UCSB, Bea Sweeney and David Asai, encouraged me to go to Cal Tech instead. David had done his PhD there and felt it was a special place, so he wanted me go there too; Beazy did not want me to go to Berkeley "just because my parents had gone there". Somehow my interest in potentially working with Liz was great enough to for me to go against the recommendations of two mentors. So I signed up as a PhD student in the Department of Molecular Biology at Berkeley.

UC Berkeley

When I got to Berkeley I had missed the week of orientation for new students, because I decided to attend the wedding of my friends Monica and Chris Morakis whom I had met in Göttingen. My first few weeks at Berkeley felt overwhelming. Although I had done biochemistry, I had not taken any molecular biology courses and had never worked with DNA. My classmates were an impressive bunch with a strong background in molecular biology and it seemed they were all clearly smarter and better prepared than I was. It was thrilling to be part of such an impressive group of interesting people, and soon we all became very close friends.

Although I had come to Berkeley to work with Liz Blackburn, all first year students had to do three laboratory "rotation projects" for 2–3 months each before decisions were made about which lab to join. My first rotation was with Richard Calendar studying phage P2 and P4 interactions. I was very fortunate that that year two of us first-year students were both assigned to Rich's lab at the same time. My fellow "roton", Jeff Reynolds, was very smart, very friendly and it seemed he knew everything about DNA. So I could lean on Jeff and his knowledge to get me started at Berkeley. From those first days, Jeff became, and still is, one of my best friends.

My second rotation project was in Liz Blackburn's lab. There was a certain amount of anxiety among the first-year students as we could not choose our rotation labs; assignments were made by the Department chair, Nick Cozarelli. I was very happy to get assigned to Liz's lab because of my strong interest in working with her. For the rotation I worked on a project to clone telomeres from trypanosomes and the related species *Leishmania*. By the time I arrived in the lab Liz and Jack Szostak had already shown that telomeres from *Tetrahymena* would function as telomeres in yeast. This was incredible because *Tetrahymena* and yeast are in different kingdoms phylogenetically. They had shown that when *Tetrahymena* telomeres were ligated to both ends of a plasmid, they allowed that plasmid to be grown as a linear chromosome in yeast. By removing one *Tetrahymena* telomere they were able to clone a functional yeast telomere. I was using this same technique to try to capture telomere fragments from *Leishmania*. I enjoyed the laboratory environment and by talking to people I got a sense of what projects I found most interesting; I was intrigued by the question of how telomeres get elongated.

In the second quarter, I also took a graduate course on chromosomes taught by Liz in which students were assigned papers that they then presented to the entire class. I was assigned the Szostak and Blackburn 1982 *Cell* paper that identified yeast telomeres. I was petrified, having never presented a paper in front of a large group before. I studied the paper inside and out. I was scared, but I was energized and got a thrill out of presenting that paper. I found it satisfying to convey the excitement I had about telomeres to my fellow students.

Janice Shampay, a student in Liz's lab had recently published an important follow-up paper to the *Cell* paper. They showed that *Tetrahymena* telomeres had yeast sequen-

ces added to them as the linear plasmid was maintained in yeast. The excitement grew with the idea that these telomere sequences must be somehow added to chromosome ends. A previous rotation student and friend of mine, Jim Bliska, had done his first rotation in Liz's lab. He had been testing ways to find an activity that might elongate telomeres. From what I knew about telomeres, I thought this project was exciting because it directly approached the heart of the biggest question: How are telomeres elongated?

I had to wait until after my third rotation before I could ask Liz about working with her, according to the graduate program rules. Toward the end of the third rotation, I made an appointment to talk to Liz. As I went into her office I was both scared and excited. I asked her first if I could work in her lab, and second, whether I could work on the telomere elongation project. I was thrilled when she said "yes" to both. I think the conversation lasted all of a minute, but it was a very momentous minute for both of us.

The Blackburn Lab

I joined Liz's lab in May of 1984 and I set out to see if I could find biochemical evidence for telomere elongation in *Tetrahymena*. Liz had first sequenced telomeres in *Tetrahymena* and she reasoned that this single-celled ciliate would be a good source for a telomere elongation activity. Each cell has over 40000 telomeres and perhaps more importantly, there is a stage of its life cycle where new telomeres are added onto fragmented chromosomes. I made extracts from *Tetrahymena* cells and examined whether artificial telomeres could be elongated by enzymes present in the extracts. These experiments are described in detail in the accompanying lecture.

After about nine months of trying variations on experiments, we found our first strong evidence for telomere elongation. A 18-nucleotide telomere "seed" was elongated with a repeated sequence that was six bases long—precisely the length of the TTGGGG telomere repeat in *Tetrahymena*. Now we had a biochemical assay that we could use to determine if this was a new telomere elongation mechanism. We set out to critically examine whether the 6-base pattern we were seeing was indeed due to a new activity or perhaps instead was a well known polymerase fooling us. Liz and I worked very well together. We would talk most every day and each of us would assert what we thought should be done next. Often we agreed but sometimes we did not and we would try to convince the other of our reasoning. I remember for one experiment we talked for a long time and neither of us would give up our stance. It was an impasse. The next day when I came in to the lab and we talked, we had both shifted sides, I decided to do her proposed experiment first. We both laughed that we had each convinced each other.

I learned many important lessons that first year after the initial telomerase discovery. Mostly, I learned the importance of questioning your own assumptions. We did not set out to prove we had a new enzyme, rather we imagined all the ways our own thinking could be deceiving us and allowing us to interpret our results in a way that favored our bias. I learned that getting the correct answer is more important than getting an answer you might hope for. I learned to step aside from myself and view my data through the eyes of a skeptic. We worked for a year before we convinced ourselves that the telomere terminal transferase was indeed a unique activity. The initial discovery was in December of 1984 and the paper was published in *Cell* in December 1985.

Stanley Hall Cold Room—UC Berkeley

We first called the activity we identified "telomere terminal transferase" because it transferred telomere sequences onto termini, but later shortened it to "Telomerase". My friend and fellow student, Claire Wyman and I would joke around in the lab a lot. Claire pointed out telomere terminal transferase was too long and suggested various humorous names as alternatives. Names were further discussed later that night over a few beers and telomerase was one Claire had proposed initially as a joke. She thought it was funny, but Liz and I both liked it.

The next most exciting question was: where does the information for the TTGGGG repeat addition come from? I wondered if there might be an RNA component that specifies the TTGGGG sequences added. I set out to do an experiment to pre-treat the *Tetrahymena* extract with either DNase or RNase or nothing and see if that affected the activity. I remember that day Tom Cech was visiting Berkeley for a seminar. Tom had a longstanding interest in both telomeres and in RNA biology. Liz and I met with Tom in the morning and I told him about my idea of testing to see if the activity was RNase-sensitive. He agreed that was an interesting experiment. Throughout the day, as he was being walked around the department from appointment to appointment, Tom would stop by the lab and see how the experiment was going. I was flattered that he was so interested.

The RNase experiments indicated that activity was eliminated when RNA was degraded, implying there was an RNA component. Liz and I felt that the best way to really show that an RNA was involved was to find the actual RNA. So I went into the cold room to try to purify the enzyme. I read as many books on biochemical purifications as I could, and set out to purify telomerase. As a complete amateur, I spent an inordinate amount of time in the cold room setting up and running columns to purify telomerase. My friends would come to find me to go to get coffee at Café Roma, and I would have on my puffy down jacket covered over with an extra-large white lab coat. They joked that I looked like the Pillsbury Doughboy.

The friends in Stanley hall were a very close group. We would walk to get a latte at least once every day. We would talk science, tell jokes, tease each other, and complain to each other about experiments that did not work. There were a lot of practical jokes that we played on each other. I was having trouble with experiments one afternoon and complained to Jeff that I was "bored". So late that night Jeff filled my umbrella with home made confetti with the word *bored* on each piece. The next day I was leaving genetics class, it was raining so I opened my umbrella and thousands of pieces of paper fell out. I knew I had to retaliate. The next day I got into

the lab very early. I went to Jeff's lab bench; he had 40 bottles of different chemical reagents for his experiments lined up on the shelf above his bench. They were all glass bottles filled with clear liquid, I removed the labels that were taped on for every one of them (I marked each with a number underneath and kept a paper key). When Jeff came in to work in the morning, he started his experiment for the day, reached up for his TE buffer and found 40 identical unlabeled bottles. He was shocked at first, then, being clever, he saw the small numbers on the bottom and realized what I had done. He came into our lab and said "OK so where is the key?". I pretended to not know what he was talking about, but was glad when he admitted I had gotten him back. These kinds of jokes were common in Stanley Hall. Often they involved dry ice inside plastic tubes, which would burst and sounds like a bomb when placed in a metal garbage can.

We found every excuse imaginable to have parties at one of our graduate student houses. One party invitation flyer copied a departmental memo that said "Emergency water outage—Party time" we decided this was a good reason for a time for a party at Jeff's house. Some of our parties involved making up skits for the "follies" where we would roast our professors and fellow students. We all worked very hard and we played hard too. The creativity was not just at the lab bench, but spilled over to our daily life together at work; being creative in all aspects of our lives in the lab and out was wonderful. Spending time with people who understood me and what I was doing and who loved to laugh and play was extremely rewarding.

After four years in graduate school, my thesis committee members encouraged me to finish up and look for a postdoctoral position. I remember Jasper Rine specifically telling me it would be good to finish the thesis in four years, because I had enough material and it looked good to finish quickly, so why not try? Mike Botchan who was on my committee, strongly encouraged me to apply for postdoctoral fellowship positions at Cold Spring Harbor laboratory, where he had been for a number of years before coming to Berkeley. So I sent letters inquiring about positions to four people at CSH, Bruce Stillman, Yasha Gluzman, Doug Hanahan, and Mike Wigler and was asked to go there for an interview.

Cold Spring Harbor Laboratory

I think there may have been only eight or ten people in the audience for my interview talk at CSH. I gave a talk on telomerase activity in the James library. All four lab heads with whom I had applied to work were there, as well as Jim Watson whom I had never met before. It was a cold and rainy day, and afterwards Jim Watson wanted to take me to lunch. I was both excited and terrified at lunch and did not know what to say, but he was clearly interested in telomerase. Several days after the interview when I was back in Berkeley, I got a call from Bruce Stillman, he said that he would be happy to have me as a postdoc in the lab if I wanted to come, but that there was also an opportunity to have an independent position as a Cold Spring Harbor Fellow and work on whatever I wanted. I had not heard of or applied to an independent fellowship position so I was a bit surprised. I later found that Mike Botchan from Berkeley had quietly nominated me for this without my knowing. When Bruce called, I first said that I would just work with him as a postdoc; but then I thought it over for a week and realized there were so many interesting questions I still wanted to ask about telomerase that I would love to keep working on it. So I called Bruce back and told him that I would like to accept the independent Fellow position. So I filed my thesis in November of 1987 and continued to work on trying to identify and sequence the RNA that co-purified with telomerase for a few months. January 1, 1988 I started as a Cold Spring Harbor Fellow.

My main goal in my new lab at CSHL was to clone the gene for the telomerase NA. I had already obtained several partial sequences through direct RNA sequencing using specific RNases. I made short oligonucleotides to the regions of RNA sequence and used them to probe genomic libraries from *Tetrahymena*. After searching through many libraries, I found one clone where the sequence matched the partial RNA sequence *and* also contained CAACCCCAA, the complement of the TTGGGG telomere sequence. I was excited and told my friends in the building about the sequence. I was very surprised to hear later at lunch in Blackford Hall that many other people knew of the result. A few hours later Bruce Stillman stopped me on the street to say he heard I got a great result. News traveled fast at CSH and people really cared about what other people were doing. It was fun to again be with people who cared about each other and who kept up with what science people around them were doing, it was a very exciting time.

Having a clone with a telomere repeat was tantalizing, but how could I show that it was required for telomerase activity? I devised a series of experiments using antisense oligonucleotides and RNase H to show that this RNA was indeed required for telomerase activity. I wrote a draft of a paper and sent it to Liz since I had initiated the sequencing efforts while working in her lab. I presented my work in Bruce Stillman's lab meeting and he encouraged me to propose a model for how I thought the enzyme might work in the paper. This model, drawn crudely on a Macintosh SE, has stood the test of time. It turned out what I conceived of as a possible mechanism is indeed the way telomere repeats are made by copying the RNA template. I sent Liz the clone encoding the RNA component before our paper was published, and she and her student Gou-Liang Yu were able to express a telomerase RNA with a change in the template sequence in *Tetrahymena* and show that change was incorporated into the telomere repeats. This was definitive evidence for the templating model proposed.

Having success in cloning telomerase soon after arriving at CSH was a great start. I soon had my first graduate student Lea Harrington and was rapidly promoted through the different scientific staff positions to the position of "Rolling 5". We continued to pursue our curiosity about the function of telomerase and role of telomerase in cells that are discussed in more detail in the Nobel lecture. In 1993 I married Nathaniel Comfort, whom I met when he was the science writer in the Public Affairs office at CSHL. In 1996 our son Charles

Comfort was born in Huntington, New York. Nathan completed his PhD in history of science at the University at Stony Brook in 1997 and was offered a position on the faculty at George Washington University. I was concurrently offered a position in Tom Kelly's department of Molecular Biology and Genetics at Johns Hopkins University. So when Charles was one year old, we moved to Baltimore to start new lives.

Johns Hopkins University School of Medicine

I was very fortunate to come to Johns Hopkins to a very interactive and cohesive department. Although the institution as a whole is much bigger that CSH, the department of Molecular Biology and Genetics felt as small and homey as CSH. I was fortunate to have outstanding graduate students and postdocs come to work with me. I was able to branch out into both yeast genetics and mouse genetics and follow my interests in what happens to cells when they don't have telomerase. I enjoy having smart people around to talk to who are excited by the work on telomeres. The different directions the lab has gone has been driven not just by my own interests but by the interests of the students in the lab. Finding something new that nobody knew before is exhilarating, and discussing ideas with students and postdocs and helping them to pursue their most interesting questions leads to new insights.

Two years after I moved to Johns Hopkins, my daughter Gwendolyn was born. Having two kids and a full time job in the lab is a challenge, but having Charles and Gwendolyn is the best thing that has ever happened to me. My lab knows that I am a mom first, and the flexibility that academic science provides makes having a career and a family possible. I can go home when needed, or to a school play in the middle of the day, then come back and finish my work-day; or work from home on the computer. The main thing is to find the time to get things done, it is not the hours at work but the overall productivity that counts. Having flexibility takes a huge amount of pressure off.

In 2002 Tom Kelly, the Department Director (the Hopkins name for Chairman) told me he was leaving Hopkins to take a position at Memorial Sloan–Kettering Cancer Center in New York. I kept the note that my assistant left on my desk that day that said "Tom Kelly wanted to talk to me"—and marked it as a Black Day; I actually cried when he told me he was leaving. Tom was an ideal director for the department, he cared about everybody and worked hard to help create the collegial environment that attracted so many top scientists. After a two-year search process, I was appointed as the Daniel Nathans Professor and Department Director for Molecular Biology and Genetics. I am extremely honored to hold the Daniel Nathans Chair, as it was Dan who created the department and established the interactive environment that Tom Kelly helped build. Dan Nathans, who died in 1999, personified thoughtfulness, caring, and above all integrity; traits that we all strive to show the way that Dan did.

I know that I cannot fill the shoes of either Tom Kelly or Dan Nathans, but I try to bring my own style of leadership to the department. Being director is made easy by the terrific faculty in the department; the science is outstanding and everybody talks and cares about the other faculty. The flat structure of the department that was established early on makes it clear that everyone has a voice. Decisions are made by discussion and consensus and not in a top-down fashion. I have been able to learn about leadership in a hands-on fashion and the faculty have all helped me tremendously in that.

Mentors, Friends, and Lessons

One of the lessons I have learned in the different stages of my career is that science is not done alone. It is through talking with others and sharing that progress is made. Work done today, of course, builds on the past work of many others, but in addition, experiments are often suggested by friends and colleagues either directly or indirectly. The ideas generated are not always the result of one person's thoughts but of the interaction between people; new ideas quickly become part of collective consciousness. This is how science moves forward and we generate new knowledge.

I am grateful to the many scientists who have influenced and helped me in my journey from Davis to Baltimore: Bea Sweeney, Michel Robert, Kevin Sullivan, David Asai, Les Wilson, Elizabeth Blackburn, Jasper Rine, Mike Botchan, Bruce Stillman, Rich Roberts, Dan Nathans, Tom Kelly. These colleagues and many others have helped me move from one stage to the next and taught me many essential lessons along the way. I would not have been able to do the science that I have done without the students, postdocs, and wonderful technicians who brought their energy and great ideas to the lab. Finally, the close friends I have made in Davis, Berkeley, CSHL, and Baltimore are my constant support group. I value them above all else.

Nobel Lecture

The story of telomerase discovery is a story of the thrill of putting pieces of a puzzle together to find something new. This story represents a paradigm for curiosity-driven research and, like many other stories of fundamental discovery, shows that important clinical insights can come from unlikely places. In this Lecture I describe the process of scientific discovery—at times frustrating, at times misleading and perplexing, but yet also at times wonderfully exciting. The willingness to keep an open mind, to enter uncharted waters and try something new, along with patience and determination, came together to tell us something new about biology. Fundamentally this story shows how curiosity and an interest in solving interesting problems can lead to a lifetime of exciting discoveries.

Identifying the Puzzle: Telomere Sequences Defined

Telomeres posed a puzzle for biologists for a many years: it was clear that a chromosome end differed from a

chromosome break—but how? In the 1930s, Hermann Müller and Barbara McClintock showed that natural chromosome ends have special properties that afforded their protection; they coined the word *telomere* to describe these natural chromosome ends, deriving from the Greek *telo* = end and *mere* = part.[1–3] The puzzle of how these ends functioned remained unsolved for many years, until their molecular structure was characterized.

In 1978, Blackburn and Gall identified the first telomere sequence using the ciliate *Tetrahymena*, which contains 40000 telomeres. They found that the chromosome end was made up of tandem, consecutive repeats of the simple sequence CCCCAA.[4] Their discovery of this simple repeated sequence turned out to be the key to understanding telomere function.

The identification of tandem repeats in the telomeres of *Tetrahymena* was followed by the identification of similar repeats in the telomeres of other organisms, including *Oxytricha, Physarum*, yeast, and trypanosomes.[5–8] This conservation of telomere sequence across a wide variety of organisms suggested that telomere function might also be similar in these species. This conservation also suggested that telomeres and their function—of maintaining chromosome ends, and hence protecting them from destruction—arose early in evolution, such that all species with linear chromosomes evolved with the same ancestral mechanism of maintenance, albeit with some variation.

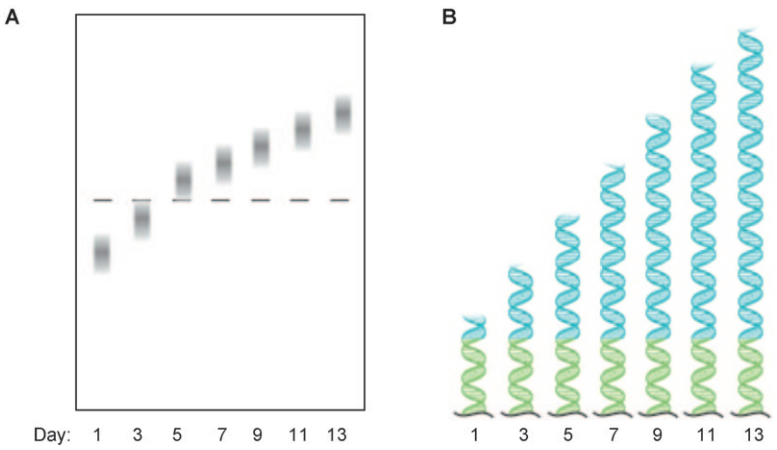

Figure 1. Telomere elongation in Trypanosomes and *Tetrahymena*: Keeping cells in continuous log phase growth results in progressive elongation of the telomeres. A) Diagram of a gel showing the heterogeneous length telomeres from *Tetrahymena*. Each lane represents increased numbers of cell divisions. B) Diagram of the interpretation of telomere elongation seen in gel in part (A).

Curious Facts about Telomeres: Some Pieces of the Puzzle

Liz Blackburn and others were interested to know how the simple repeats functioned as telomeres to confer protection upon a chromosome end. Soon after the telomere sequence was first identified, several curious facts about telomeres were uncovered. First, telomeres were heterogeneous in length: in a population of cells, different chromosome ends possessed telomeres comprising different numbers of repeats.[4] How was this established? When a population of telomeres was digested using restriction enzymes it generated so-called "fuzzy bands" when the digest was separated using agarose gel electrophoresis (Figure 1). Why was this? The variable-length telomeres had generated heterogeneous fragment lengths, which all migrated to slightly different positions on the agarose gel, yielding blurred (or "fuzzy") bands, rather than the sharper, more distinct bands that are typical of restriction fragments which are all of a similar size.

A second curious fact was that the telomeres in trypanosomes grew longer as the cells were grown in culture.[5] The same surprising result was found when similar experiments were performed with *Tetrahymena*: when kept in continuous log phase growth, the telomere fragments grew progressively (Figure 1).[9] This elongation was unexpected. In fact, it was the opposite of what had been predicted on the basis of published models for telomere replication. In 1974, James Watson suggested that a linear chromosome should get shorter as cells divided, based on the mechanism by which DNA polymerase replicates DNA during cell division.[10] A similar idea was also proposed by Alexi Olovinkov, who suggested that chromosome ends might shorten after rounds of DNA replication.[11,12] Why, then, were the telomeres getting longer? And why were they fuzzy?

Collecting More Pieces of the Puzzle: Telomere Sequence Addition

When Liz Blackburn and Jack Szostak met for the first time at a conference, they were both interested in DNA ends. They knew about the curious structure of telomeres and their elongation, and they saw a way to use these puzzle pieces to perform a long-shot experiment: they decided to test whether *Tetrahymena* telomeres could function as telomeres in yeast. The experiment was a long-shot because these two species are very distantly related, having diverged from a common ancestor millions of years ago. Despite being a long-shot it was an experiment they could do, so they forged ahead.

Blackburn and Szostak took a circular yeast plasmid and cut it once with a restriction enzyme to make it linear. Jack knew from his earlier experiments in yeast that this linear DNA would be rapidly degraded if put into yeast cells. However, they wondered whether the addition of *Tetrahymena* telomeres to this linear DNA would cause the ends to be protected—as they are in *Tetrahymena*—so preventing the normal rapid degradation of the DNA when transformed into (that is, added to) yeast cells. Liz purified the *Tetrahymena* telomeres and Jack ligated them onto the linearized yeast plasmid (Figure 2). When this construct was transformed into yeast, it was stable: it replicated and was maintained as a linear chromosome fragment.

This maintenance of the linear plasmid was a stunning result: it indicated that the *Tetrahymena* telomeres functioned and protected DNA ends in yeast, a very distantly related

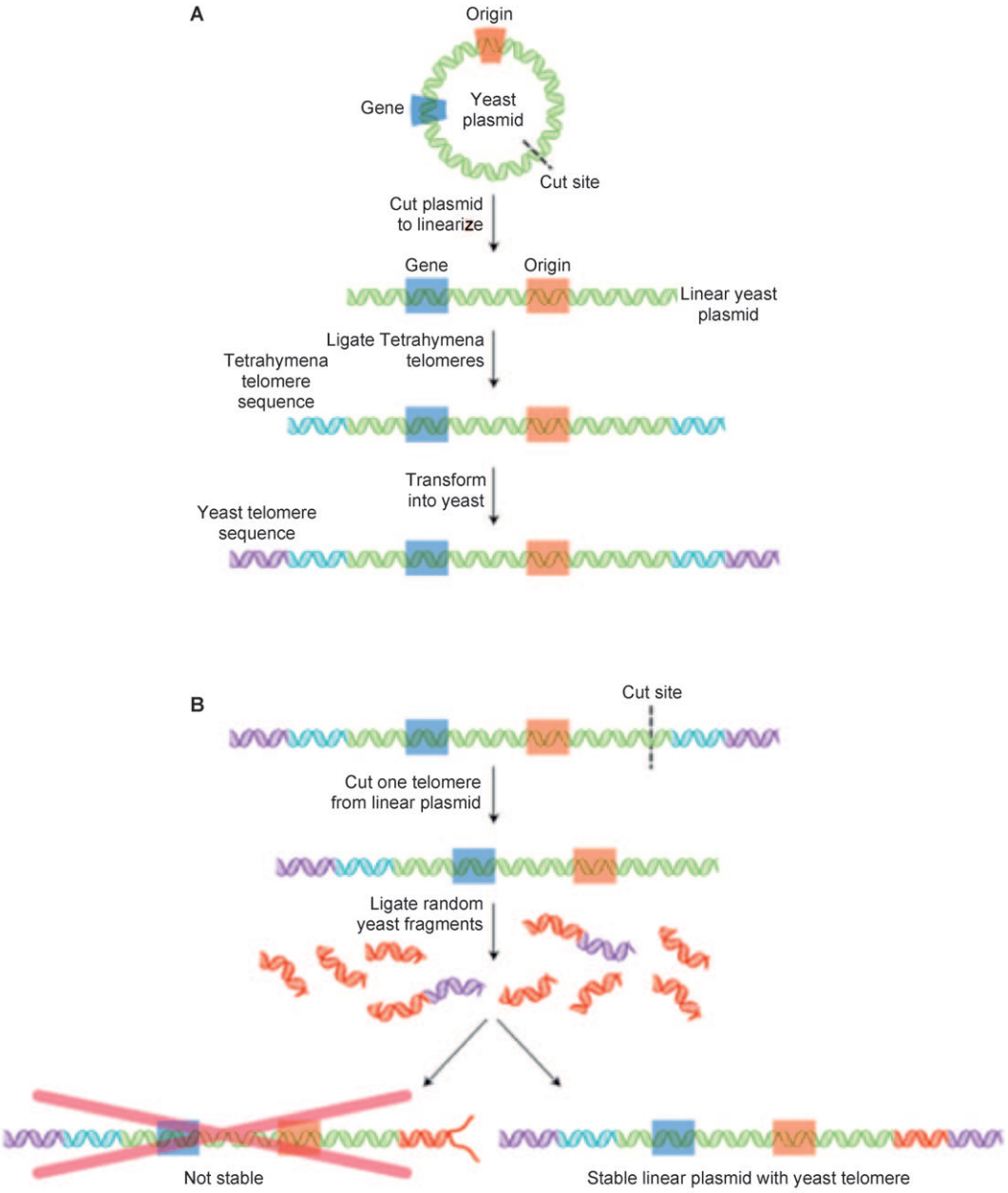

Figure 2. Evidence for telomere elongation: A) *Tetrahymena* telomeres function in yeast and are elongated. A circular yeast plasmid was linearized at a unique cut site and *Tetrahymena* telomeres were added. This linear DNA was transformed into yeast and the linear plasmid was stable. During propagation in yeast, the terminal DNA was elongated by addition of yeast telomeres sequences. B) Cloning of a yeast telomere. The linear plasmid shown in part (A) was cut to remove one end and random pieces of yeast genomic DNA were ligated onto the end. Those products that had internal yeast DNA added were not stable; they would be degraded by nucleases. However, when a piece of DNA that contained a telomere was ligated, this plasmid was stable and was propagated as a linear plasmid. Yeast telomere fragments were first identified using this strategy.

organism.[8] Emboldened by this success, Liz and Jack took one step further forward when they realized they had a method to identify the naturally occurring yeast telomere sequence: instead of attaching *Tetrahymena* telomeres to the linear plasmid in order to protect and maintain it, find the parts of the yeast genome that protect the plasmid in the same way, and which therefore contain the yeast telomere. To do this, they removed one end of the linear plasmid to which *Tetrahymena* telomeres had been added, and ligated random genomic fragments of yeast DNA to this free end. Those random fragments that harbored a telomere allowed stable maintenance of the linear plasmid.

In doing these experiments, Liz and Jack noticed another curious fact: the *Tetrahymena* telomeres on the plasmid maintained in yeast were longer than they had started out. They were curious to know why. So, together with Janice Shampay, they set out to sequence the cloned yeast telomeres and the *Tetrahymena* telomere end. When they sequenced the

yeast telomere they found it to have the same kind of tandemly-repeated sequences as had been found in all other organisms so far. More strikingly, determining the sequence of the *Tetrahymena* telomeres from the linear plasmid showed why they were longer: the initial *Tetrahymena* telomeres had been extended by terminal addition of yeast-specific sequences (Figure 2).[13]

This last puzzle piece was a very important one. Models of telomere maintenance and elongation had been proposed, which involved telomere–telomere recombination (Figure 3A). Even though Jack's expertise lay in understanding these recombination models, both he and Liz recognized that the addition of yeast-specific sequences onto the *Tetrahymena* ends could not really be explained by those models. Instead, the data suggested to Liz and Jack the existence of an active elongation mechanism in yeast, whereby DNA was generated de novo rather than being the result of a recombination event (Figure 3B). They wrote in their paper, "*we propose that terminal transferase-like enzymes are responsible for extending the 3′ G + T rich strand of yeast telomeres*". By continually following the clues (and keeping an open mind) they had come to the conclusion that no known enzyme could do the sequence addition, and so proposed instead that there must be an unknown enzyme that adds telomere sequences.

Looking for Telomere Elongation: Defining the Edges of the Puzzle

When I joined Liz's lab in May of 1984 I set out to look for this unknown enzyme. We decided to use biochemistry to see if we could identify an enzyme that might elongate telomeres. There was no established protocol for finding an unknown enzyme, so we had fun and made one up. In fact, more precisely, we continually made up new protocols. It was like biochemical improvisation: we started with one concept of an assay that might allow detection of addition onto telomeres, but kept modifying the assay after each set of experiments. We changed the reaction conditions, the substrates, and even method of detection. After nine months we found something (!) and so we had another piece of the puzzle. But how did our nine-month search for this puzzle piece unfold?

First we needed an assay—a way to detect if telomere elongation was happening. The first assay we tried explored whether a piece of DNA that included a telomere would incorporate DNA precursors more readily than a piece of DNA containing non-telomeric sequences. The idea was that if there *was* an enzyme that actively elongated telomeres, we might be able to detect it through its activity in association with telomere DNA. For this assay, we developed a substrate

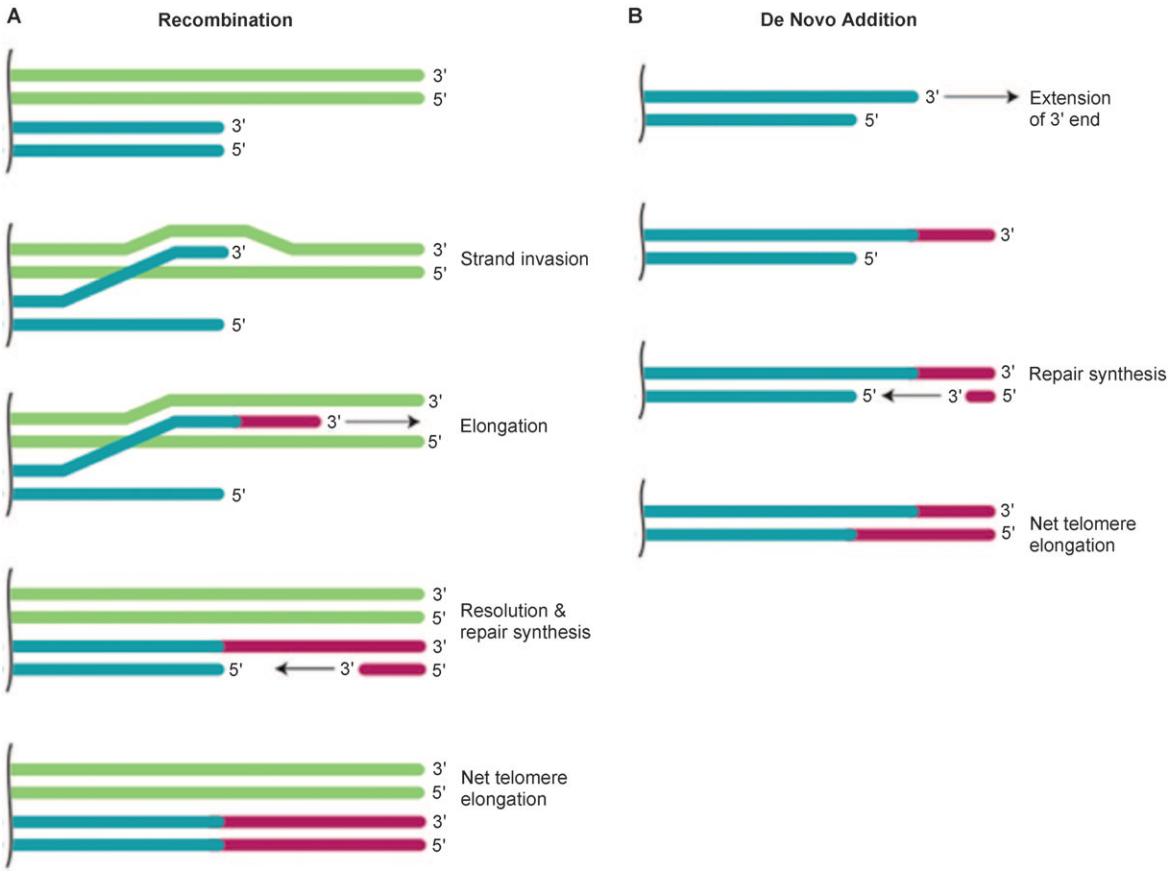

Figure 3. Two models for telomere elongation: A) Recombination can elongate telomeres. Since telomeric repeats are homologous on all chromosome ends a short telomere may copy off of a long telomere in a gene-conversion type of recombination. The 3′ end of the short telomere base pairs with the longer telomere and polymerase extends the end of their strand. The other strand can be copied by conventional polymerase activity. This results in the net elongation of the short telomere, while the long telomere remains long. B) Alternative model for telomere elongation proposes that de novo elongation lengthens telomeres. The discovery of telomerase indicated that this mechanism is the predominant mechanisms for telomere elongation in most species.

that was meant to mimic a telomere in the cell: a linear DNA fragment that contained a telomeric sequence at one end but not at the other (Figure 4). I incubated this linear fragment of DNA with the nucleotides dA, dC, dG, and dT—the building blocks of DNA—which had been labeled with the radioactive isotope ^{32}P. We reasoned that an elongation enzyme might preferentially elongate the end of the DNA fragment that contained the telomeres; if it did, we expected to see more of the ^{32}P label incorporated into the telomeric end than the end lacking a telomere.

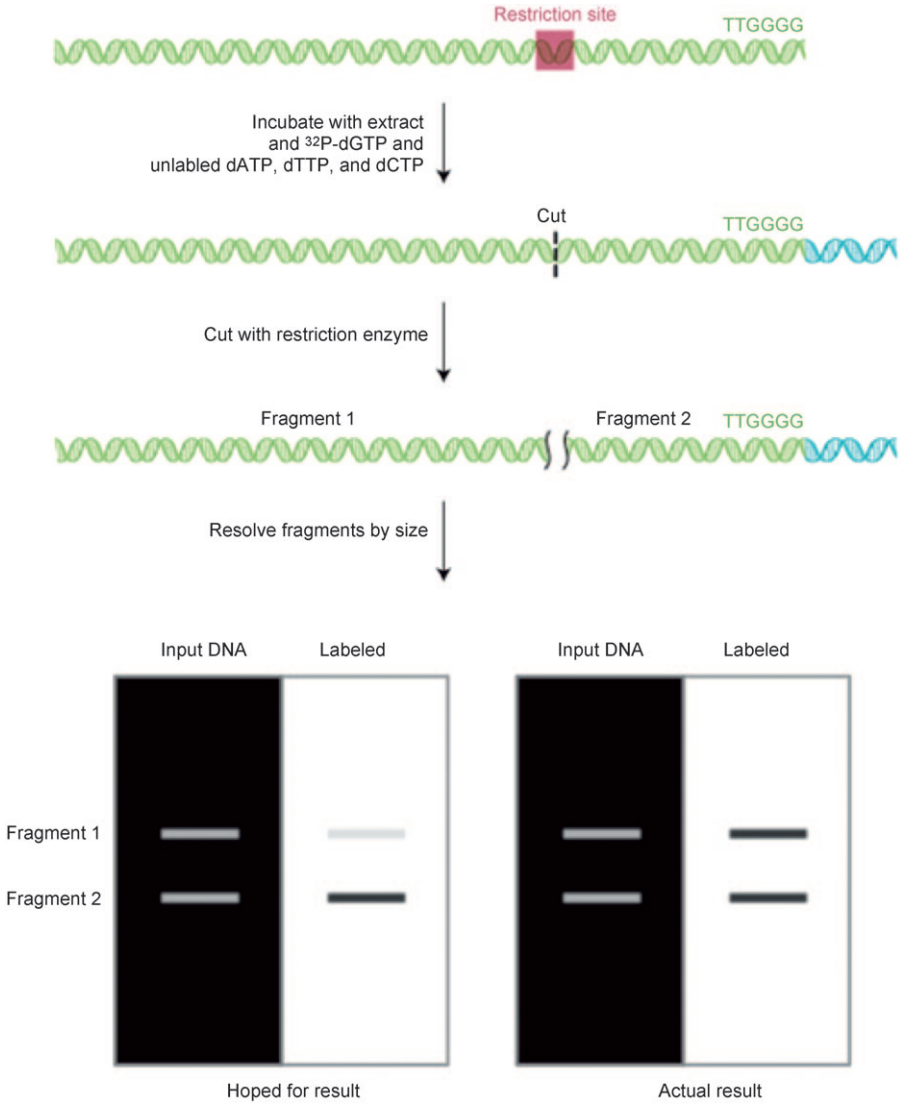

Figure 4. Initial assay for telomere-specific end labeling. A restriction fragment was purified from a plasmid. The fragment had telomeric DNA on the right end and non-telomeric DNA on the left end. There was a restriction enzyme cut site that would allow two different sized pieces (Fragment 1 and Fragment 2) to be generated. After incubation with ^{32}P dGTP and dCTP and unlabeled dATP and dTTP the fragment was purified and cut with the restriction enzyme. The products were resolved on an agarose gel and then the gel was exposed to X-ray film. The hoped for result was that Fragment 1 with the telomeric end would be preferentially labeled (left bottom panel). The actual results showed that both ends were equally labeled (right bottom panel), prompting a revision of the assay.

I incubated the linear DNA substrate in an extract made from *Tetrahymena* nuclei. The extract was prepared in a manner that we hoped would allow all of the enzymes normally present in the nuclei to be active. We also added radiolabeled dCTP and dGTP and unlabeled dATP and dCTP to serve as DNA precursors. After incubation for an hour, I purified the linear fragment from the extract to examine what had happened to the ends. Following its purification, I cut the DNA fragment to generate two unequal sized fragments to distinguish between the telomeric and non-telomeric ends. (The smaller of the two pieces contained the telomere end.) We could then separate and identify the two different-sized fragments on an agarose gel.

After separating the two pieces by size, we exposed the gel to X-ray film, because the fragments that had incorporated the ^{32}P-labeled precursor would generate a dark band on the film and we could thus identify them. We then looked to see if there was more radioactive label incorporated in the fragment that had the telomere end than the non-telomere end (Figure 4). Unfortunately, no matter how we prepared the extract, both fragments always incorporated similar amounts of label. We knew we needed a different way to approach this problem.

A Puzzle-Solving Strategy: Getting the Assay Right

The most productive way to solve a puzzle is to attack it with the right strategy. Since we did not know precisely what we were looking for, we tested a number of different approaches to see which plan might be successful. After each experiment, we thought of new changes to make to the next experiment. Two of the many changes we tried proved important: using sequencing gels to resolve the DNA fragments after incubation, and using synthetic oligonucleotides as telomere substrates instead of large restriction fragments. How did these two changes affect the outcome of the experiments?

After our initial attempts that I've described above, we sat and puzzled about the fact that both the telomere end and the non-telomere end showed incorporation of the radioactive

label. We realized that exonucleases, which were expected to be present in the extract, were likely generating some single-stranded DNA within the linear DNA fragment; repair polymerases would then fill out the single strand region, causing radioactive label to be incorporated into both ends of the fragment.

We thought hard about a way to get around this problem. First, we changed the approach: rather than looking for increased label incorporation, we decided to look for changes in the size of the fragment. If there was an enzyme that extended telomeres not only should the telomeres become labeled with the radioactive precursors, but *also* the size of the fragment should increase as the telomere is extended. (The repair polymerases in the extracts, which could cause both telomere and non-telomere ends to be labeled would not be capable of generating DNA that was *longer* than the fragments added at the start of the assay.)

To see a change in size of a fragment we needed to have a short fragment: a small change in size of a large fragment would be too hard to detect, but a small change in size of a very small fragment would be noticeable. We repeated the experiment as described above but, when cutting the fragment, made the cut very close to the telomere end to generate a telomere fragment just 34 base pairs long. The non-telomere and telomere fragments were then separated on a gel that was usually used for DNA sequence analysis and which could distinguish between fragments that differed in length by just a single base pair.

I worked from May through December trying different variations of this experiment, staring hard at the sequencing gels but never quite convincing myself there was much of a change in fragment size. So, in December of 1984, we decided to make another change to the assay: we changed the substrate that we were adding to the reaction. Instead of a long linear DNA fragment, I tested a synthetic 18-residue oligonucleotide $(TTGGGG)_4$ as the substrate. Eric Henderson, a post-doctoral fellow in the lab, was studying the unusual structure of DNA oligonucleotides made of these G-rich telomere sequences, and offered some of his synthetic oligonucleotide, which I decided to use instead of the DNA restriction fragment to see if it might be elongated.

So, I set out to examine the elongation of the telomeric oligonucleotide. I made cell extracts from *Tetrahymena* and incubated them with the oligonucleotide and radiolabeled nucleotide precursors (Figure 5A). It took over a week to set up the experiment, do the reactions, and then run the sequencing gel. To maximize the signal generated by the radioactive label, I exposed the gel to X-ray film for three days. When I went to the lab to develop the X-ray film, I was thrilled to see a repeating pattern of elongation products that extended up the gel (Figure 5B). The oligonucleotide substrate was being elongated to give products that varied in size by six bases, giving the repeating pattern seen on the gel. This was the first visualization of telomerase activity.

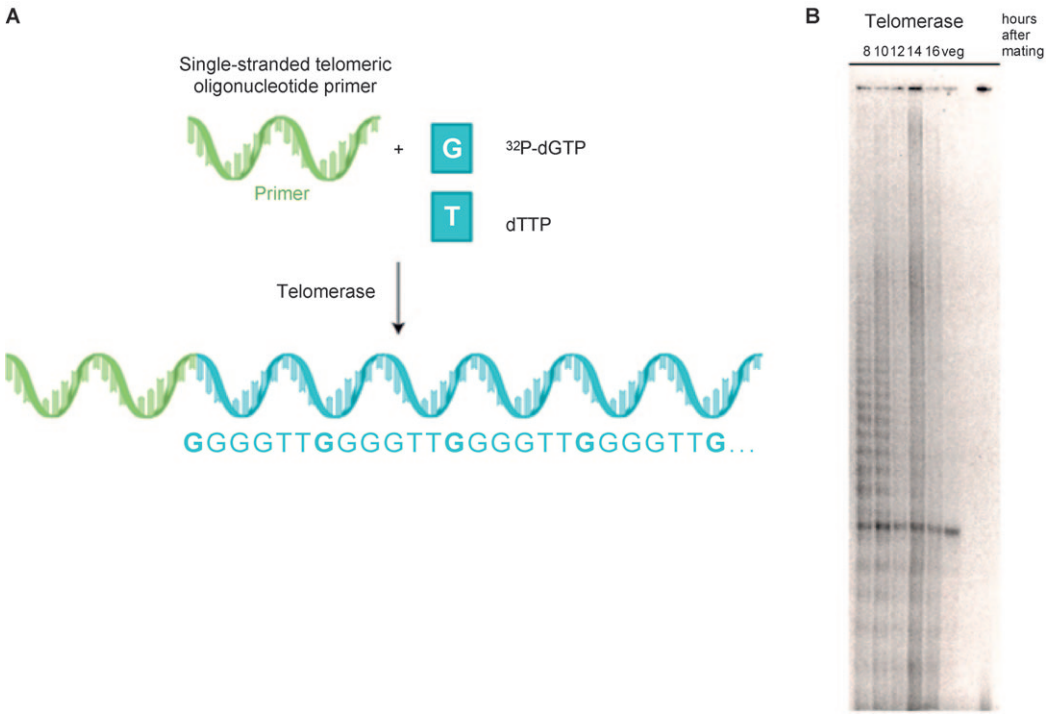

Figure 5. Telomerase activity assay: A) Diagram of primer elongation assay. A single-stranded DNA oligonucleotide with the sequence (TTGGGGTTGGGGTTGGGG) was added with ^{32}P dGTP and unlabeled dTTP and incubated in *Tetrahymena* cell extracts that we hoped would have telomerase activity. The primer was extended by addition of a size base repeating pattern B) Telomerase elongation of telomeric primer. Extracts from a time course of *Tetrahymena* development were made at different numbers of hours of development (indicated at top by hours). Extracts were also made from vegetatively growing cells (marked Veg). The banding pattern represents the six-base repeat of GGGGTT that is added to the primer. This autoradiogram was the first experiment in which telomerase activity was seen on December 25, 1984.

Testing Ourselves: Do the Pieces Really Fit, or Are We Forcing Them?

I talked to Liz the next day and showed her the gel. We were both talking at the same time, trying to understand the meaning of the repeating pattern. We knew that this could be the result of the enzymatic activity we were looking for, but we also wanted to be sure the pattern we were seeing was indeed generated by a novel enzyme. Were we truly seeing something new, or was our own wishful thinking coloring our interpretation of the result?

To be sure we were not forcing the interpretation of the elongation pattern, we set out to test various alternative explanations that might generate a repeating pattern like the one we were seeing. For example, we thought the TTGGGG primer might be annealing to double-stranded genomic DNA that might be present as a contaminant, such that a conventional polymerase could generate the TTGGGG repeat addition when replicating the DNA (Figure 6A). Alternatively, the primer might be self-annealing (that is, pairs of the primer might be sticking to one another), generating a double-stranded substrate for a conventional polymerase to copy (Figure 6B).

To address these and other concerns, we devised an ever-evolving set of control experiments to determine if the repeat addition was the result of the activity of a previously identified enzyme. For one control, we treated the samples with aphidicolin, which inhibits conventional DNA polymerases. More importantly, we used a CCCCAA primer, which would be expected to be elongated if simple copying of telomere repeats by DNA polymerase was occurring (rather than nucleotides being added de novo). The fact that the CCCCAA primer was not elongated ruled out the trivial explanation that the repeating pattern was coming from the copying of endogenous DNA.

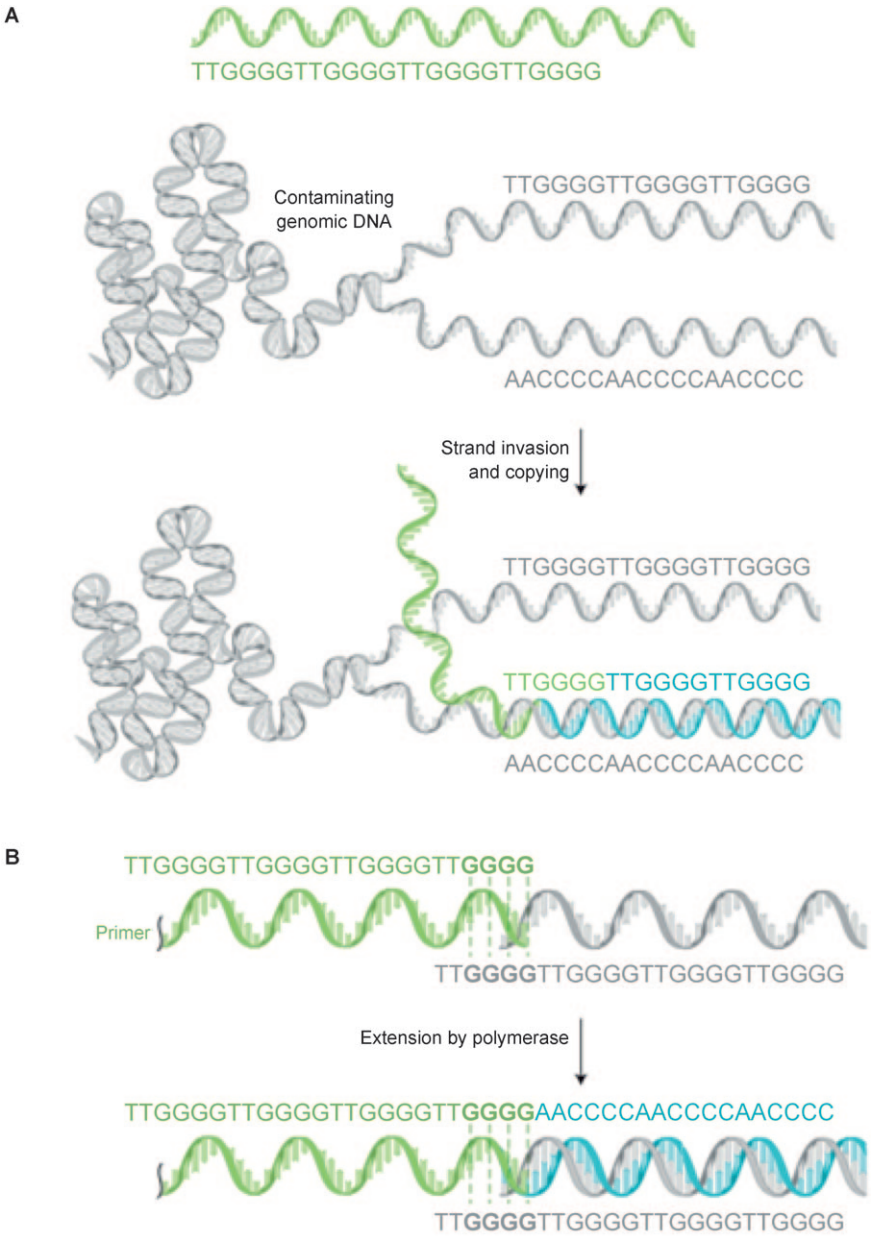

Figure 6. Possible telomere elongation artifacts: A) If the primer telomeric oligonucleotide were to anneal to contaminating DNA in the extract, any conventional DNA polymerase could copy the telomeric sequence and might generate the six-base repeating pattern. B) An alternative artifact might come from two telomeric primers self-annealing thought G–G non-Watson–Crick base pairing. Extension of these "primer dimers" might also generate a six-base repeat pattern.

These were exciting times. Once I could repeatedly see the primer elongation activity, it was fun to test various ideas about how it might be generated. I would come in to the lab every day, eager to test the next set of experiments and find something new. The final experiment that convinced both Liz and I that we had something new was when we did the converse of the experiment that Liz and Jack Szostak had done, which had been published in *Cell* in 1982. They had put *Tetrahymena* telomeres into yeast cells and shown that a yeast telomeric sequence was added to the ends. By contrast, we made a synthetic yeast sequence telomere oligonucleotide primer and put it in *Tetrahymena* extracts—and found that the *Tetrahymena* telomere repeats were added to the yeast telomere.

How could we tell that *Tetrahymena* and not yeast telomere repeats were being added? The yeast sequence primer had three G's at the end, while the *Tetrahymena* telomere sequence had four. The banding pattern of sequences added onto the yeast primer was one base longer than would have been the case if the yeast sequence had been

repeated: this extra base was the extra G required to complete the GGGG found in the *Tetrahymena* sequence. This result was quite stunning. The shift in banding pattern convinced us a new enzyme was in action: we could not imagine how conventional polymerases would elongate a yeast sequence with *Tetrahymena* repeats. We wrote up the paper, which was published in *Cell* in December of 1985.[14]

The Next Part of the Puzzle: Sequence Information

The next big question was where the information for the addition of TTGGGG repeats was coming from. Liz and I talked through several different models. The one I wanted most to test was that there might be an RNA component that specifies the sequences added to the chromosome end. I set out to do an experiment to pre-treat the extract with either DNase (which would digest DNA) or RNase (which would digest RNA) or nothing (as a control) to see if this pre-treatment affected the activity of telomerase. On the day of this experiment, Tom Cech, who had a long-standing interest in both telomeres and RNA, was visiting Berkeley to give a seminar. Liz and I met with Tom in the morning and described my idea of seeing whether the activity would be sensitive to treatment with RNase. He agreed that was an interesting experiment.

Throughout the day, as Tom was being escorted around the department from appointment to appointment, he would stop by the lab and see how the experiment was going. We found that pre-treatment of the *Tetrahymena* extract with RNase did indeed block the elongation activity. Establishing that RNA was needed for elongation was a key clue: it allowed us to think about possible mechanisms by which the enzyme would specify the TTGGGG repeats that were added.

Following the Clues: Is There a Template?

The inactivation of telomerase by RNase treatment suggested a clear hypothesis: the TTGGGG repeats are made by copying from an RNA template, which is a component of the functioning telomerase. The most powerful way to test the validity of this hypothesis was to find the actual RNA template. To isolate a component of telomerase, we needed first to purify the enzyme from all of the other proteins present in the crude extract. I established a multistep purification protocol for telomerase, using conventional

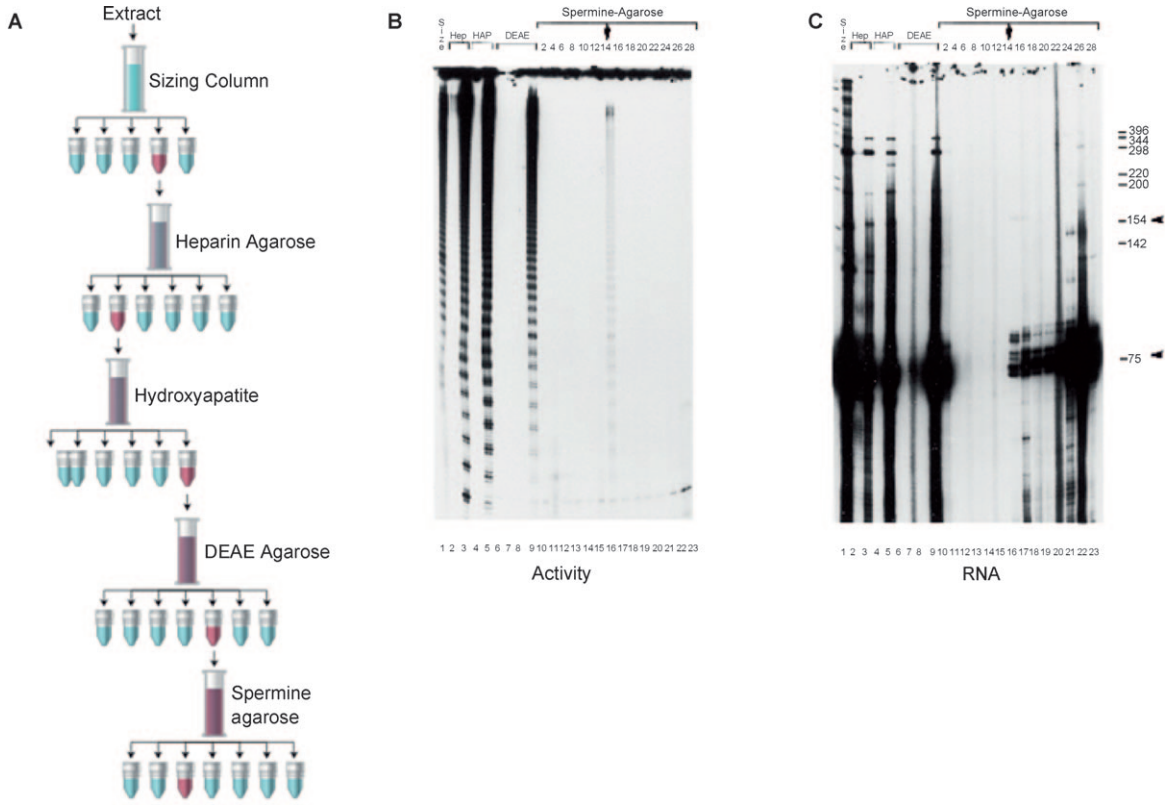

Figure 7. Purification of telomerase and identification of the co-purifying RNA: A) Extracts were fractionated first on a sizing column, the fractions were all assayed for activity and those that had activity were again fractionated on a heparin agarose column. Further purification continued in the same manner, after each column the active fractions were loaded on a subsequent column including hydroxyapatite, DEAE agarose, and finally spermine agarose B) Gel showing the active fractions for each of the columns diagrammed in (A). C) To identify the co-purifiying RNAs, all RNAs from each of the active fractions were end-labeled with ^{32}P and resolved by gel electrophoresis. The faint RNA band in lanes 6 and 7 migrating near the 154-base marker (indicated on the far right) was later found to be the telomerase RNA. B) and C) reprinted from Ref. [15] with permission from Elsevier.

column chromatography (Figure 7A). I would separate the extract into fractions and test each fraction for activity (to identify the fraction containing telomerase). I would then take the active fractions and subject them to another, different separation step. I used size exclusion, ion exchange, dye binding, and heparin binding columns to successively purify telomerase (Figure 7B). I then examined the active fraction to look for an RNA that was always present when telomerase was active. I purified the RNAs from active fractions, and labeled them with ^{32}P (Figure 7C); we then narrowed down the likely candidates by determining which RNAs reproducibly co-purified with telomerase.

We then faced our next challenge: we needed to have the sequence of the RNA component to determine if a template mechanism was, indeed, working. We tried a number of different methods to obtain the RNA sequence, including the very newly developed method termed PCR (polymerase chain reaction), which we had heard about but which had not yet even been published.

After trying to obtain a sequence for a number of months, I decided to take a more direct approach: I used direct RNA sequencing techniques to determine a partial sequence from those RNAs that emerged as good candidates. To do this, the RNAs of interest were cut out of a high-resolution gel and sequenced. This sequencing revealed that the very small RNAs that co-purified with telomerase activity were, in fact, tRNA contaminants that were present in the active fractions due to the high abundance of tRNA in the cell. For example, I sequenced one RNA that ran near a marker of 175 bases and found it was related to 7SL RNA, an RNA that had recently been associated with the signal recognition particle involved in ribosome function. Finding known RNAs was somewhat reassuring as it told us that the sequencing technique was working. However, it was somewhat of a disappointment as we were hoping to identify a new RNA that might provide the template for telomerase.

One RNA that I had my eye on after staring at many different purification experiments ran near the 154-base marker. No single experiment had pinpointed this RNA as the best candidate; my interest was really a hunch since I had seen this RNA repeatedly in many experiments. I decided to test this hunch, however this "154-base RNA" as I referred to it proved harder to sequence. I was able to obtain only partial sequences from different regions of the RNA, but none of them contained a telomere repeat as expected for a template—and the full length RNA proved impossible to sequence.

All of the accumulated evidence indicated there must be a template in telomerase, but we did not have the final key piece of the puzzle. Since telomerase was an interesting enzyme and our experiments clearly suggested that an RNA was involved, we decided to write a paper about what we knew about telomerase, despite not having the actual RNA in hand. We wrote the paper on the biochemical characterization of telomerase and the fact that there was likely an RNA component; it was published in *Cell* in 1987.[15] Looking back it is now clear that many of the experiments in this paper that helped to characterize telomerase served as the basis for identifying telomerase in other organisms. After the identification of *Tetrahymena* telomerase, telomerase enzymes from other organisms such as *Euplotes*, *Oxytricha*, and human were characterized by other groups.[16–19]

A Change in Venue: Seeing the Puzzle From a Different Perspective

I finished my PhD at Berkeley in November 1987 and took a position as an independent fellow at Cold Spring Harbor Laboratory in January 1988. At Cold Spring Harbor I was still focused on identifying the RNA, but my exploration took a different approach. Rather than continuing with more RNA sequencing, I decided to see if I could clone the RNA gene directly from the partial sequence information that I had already obtained.

I designed several different oligonucleotide probes that were complementary to the regions of partial RNA sequence I had obtained, and made size-selected genomic libraries that were enriched for sequences that hybridized to these oligonucleotides. After a number of attempts, I obtained one clone that possessed both the correct partial sequence and the sequence CAACCCCAA. Seeing this sequence on the sequencing gel was exciting, as it mirrored what would be expected if a template mechanism for TTGGGG addition was indeed used by telomerase. This clone was clearly a central puzzle piece. I went on to verify that this RNA was expressed in *Tetrahymena* and was around 160 nucleotides in length. All

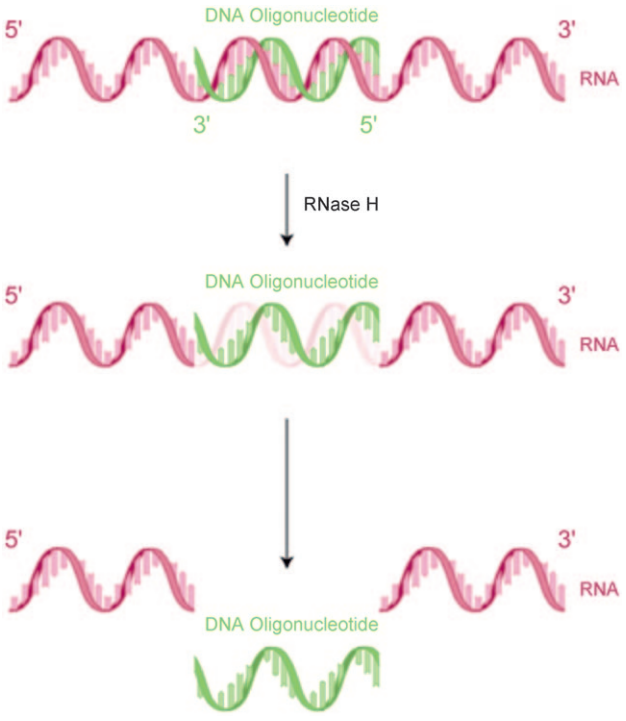

Figure 8. RNase H cleaves the RNA of a DNA–RNA duplex: Single-stranded regions of RNA will hybridize to single-stranded complementary oligonucleotides. When such hybrids are incubated with RNase H, the RNA part of the duplex will be degraded in only the regions that is complementary. In this example the RNA is cleaved in the middle leaving the two RNA halves intact.

the signs were that my earlier hunch about "154-base RNA" being a key player in telomerase activity had been right.

Is This the Right Puzzle Piece?

It was exhilarating to have in hand the sequence of an RNA that might encode the RNA component of telomerase. But I needed some evidence that this RNA was indeed the template. Again, and just as when we were trying to first identify telomerase, there was no precedent for the experiments I was trying to do. It was fun to be creative and dream up ways to test whether this RNA was the right candidate. I talked to my friends at Cold Spring Harbor, listened to their advice and read about other enzymes and about how functional RNAs were identified.

My next step in characterizing telomerase activity was to test the function of my candidate RNA template. To do this, I decided to use the oligonucleotides I had used in the cloning experiments, which were complementary to telomerase. I thought that if I could inactivate the enzyme by specifically cleaving the candidate RNA, I would have strong evidence for the involvement of this specific RNA in telomerase activity. I think it was Adrian Krainer, a colleague at CSH, who suggested using RNase H in this experiment. RNase H is a specific RNase that will cleave the RNA of a DNA–RNA duplex. The thought was that, if the oligonucleotide hybridized specifically to regions of complementary RNA in telomerase, and subsequent RNase H cleavage inactivated the telomerase, we would have evidence for the involvement of the RNA to which the oligonucleotide had hybridized in telomerase-mediated telomere elongation (Figure 8).

As is often the case in science, unexpected results provided the most important puzzle pieces. The RNase H experiment relied on the oligonucleotide having access to the RNA to be able to hybridize with it, even when the RNA is bound by telomerase proteins. As this was not always the case, some of the oligonucleotides had no effect. However, two oligonucleotides did give completely unexpected results. Incubation of telomerase with *Oligo 3* inactivated telomerase even before RNase H was added, while, amazingly, incubation of telomerase with *Oligo 8* resulted in elongation of *Oligo 8* itself (Figure 9). I had to sit and think about what this meant.

At first it was frustrating: if telomerase was already being inactivated by *Oligo 3* and adding RNase H had no further effect, how could I do the experiment? This frustration soon faded when, having talked about this result with my friends and puzzling more, I realized there was a much more interesting explanation for these results. I had fortuitously

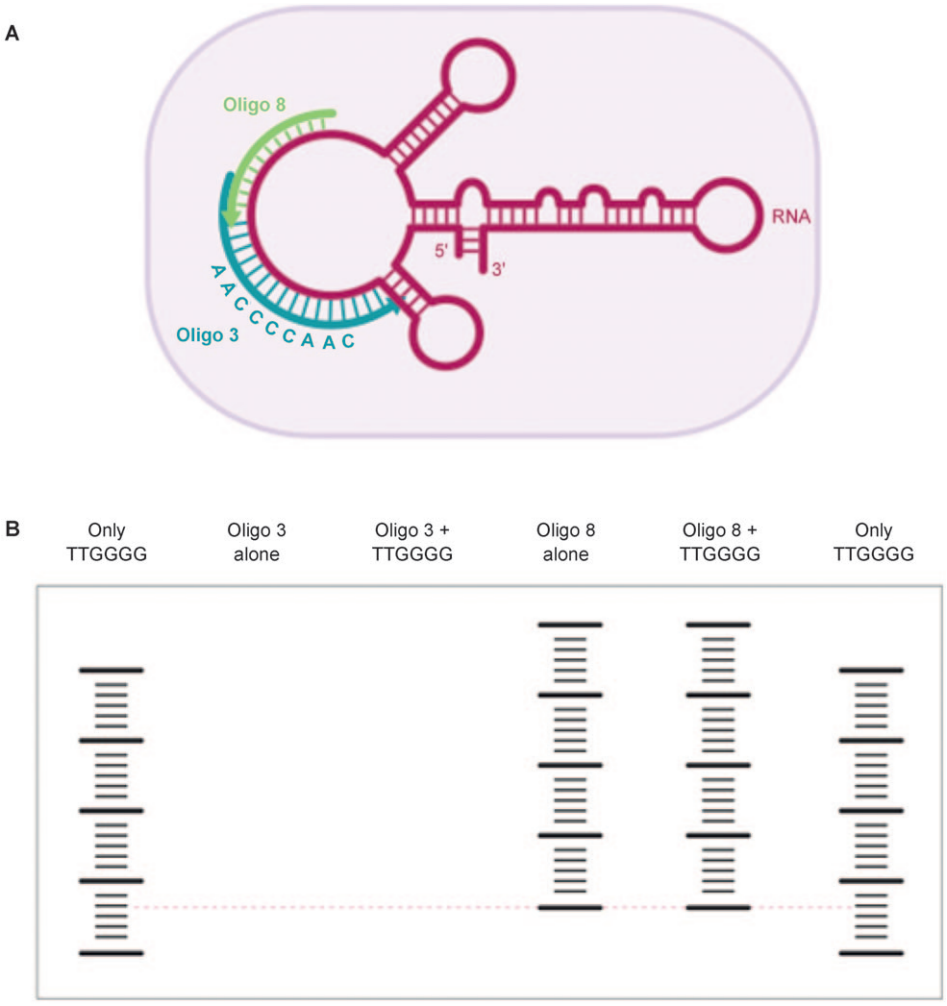

Figure 9. Elongation of oligonucleotides complementary to the telomerase RNA. Top: the secondary structure of *Tetrahymena* telomerase. *Oligo 8* hybridizes adjacent to the template region with its 3′ end positioned one base before the template. *Oligo 3* also hybridized adjacent to the template but also extends across the template region. Bottom: Diagram of gel showing effects of *Oligo 3* and *Oligo 8* on telomerase activity. Lane 1, telomerase will elongate $(TTGGGG)_3$ primer when it was added alone. Lane 2, *Oligo 3* alone was not elongated even though the 3′ end contains TTGGGG sequence. Lane 3, *Oligo 3* and TTGGGG are added together, and there is no activity because *Oligo 3* binds to and blocks the active site of telomerase. This inhibition is the basis for the first telomerase inhibitor to be used in clinical trials against cancer (GRN163).[22] Lane 4, *Oligo 8* was added alone and it was extended by telomerase generating a distinct banding pattern. This is because *Oligo 8* itself was being extended. Lane 6, When both *Oligo 8* and TTGGGG primer were added, the *Oligo 8* banding pattern was seen because it can outcompete the TTGGGG primer through its ability to hybridize to the telomerase RNA. Reprinted from Ref. [20].

found additional evidence of a role for the 159-nucleotide RNA in the telomerase reaction. *Oligo 3* was unique in that it hybridized to my 159-nt RNA in a region adjacent to the template region and also across it (Figure 9A). When anchored to the RNA by hybridization, this oligonucleotide would block binding of the TTGGGG oligonucleotide substrate, and thus block telomerase activity (Figure 9B). Subsequent work showed that RNase H would cleave the 159-nt RNA when incubated with *Oligo 3*, showing that *Oligo 3* does indeed hybridize to the 159-nt RNA as part of telomerase.

The other unusual puzzle piece was *Oligo 8*. This oligonucleotide shared sequence similarity with *Oligo 3* but its 3′ end stopped just before the template region. This oligonucleotide also hybridized to the 159-nt RNA and the 3′ end was positioned in exactly the right place for it to be elongated by telomerase (Figure 9A). This implied that the CAACCCCA sequence in the putative RNA did indeed serve as a template for TTGGGG repeat addition. Putting all of these pieces of the puzzle together, I felt there was good evidence to support the cloned RNA being the RNA component of telomerase.

Models Can Show the Solution to the Puzzle

In writing the paper on the identification of the telomerase RNA,[20] I was encouraged by Bruce Stillman to include a diagrammatic model for how I thought the enzyme might work. I was so caught up in the data that drawing a model was not foremost in my mind. However, I found that drawing out how I interpreted the results made everything even clearer. We knew from our early experiments that there must be a protein component to telomerase in addition to the RNA. I did not know how many protein components there might be but decided to draw just one for simplicity. The CAACCCCAA sequence in the RNA represented one and a half copies of the complementary strand of the TTGGGG repeat sequence. So the model I drew had the sequence GTTGGG base paired with the CAACCC, which left CAA free in the template sequence to be copied (Figure 10). With this in mind, I proposed that telomerase has an elongation phase during which the CAA is copied, followed by a translocation to reposition the growing sequence for another round of elongation. I wrote up the paper and, together with Liz Blackburn, in whose lab I had begun the sequencing, published a paper in *Nature* describing the RNA.[20]

Solutions to Puzzles Show the Way to more Interesting Questions

Drawing out the telomere elongation model helped to clarify my thinking about telomerase. Thinking about the

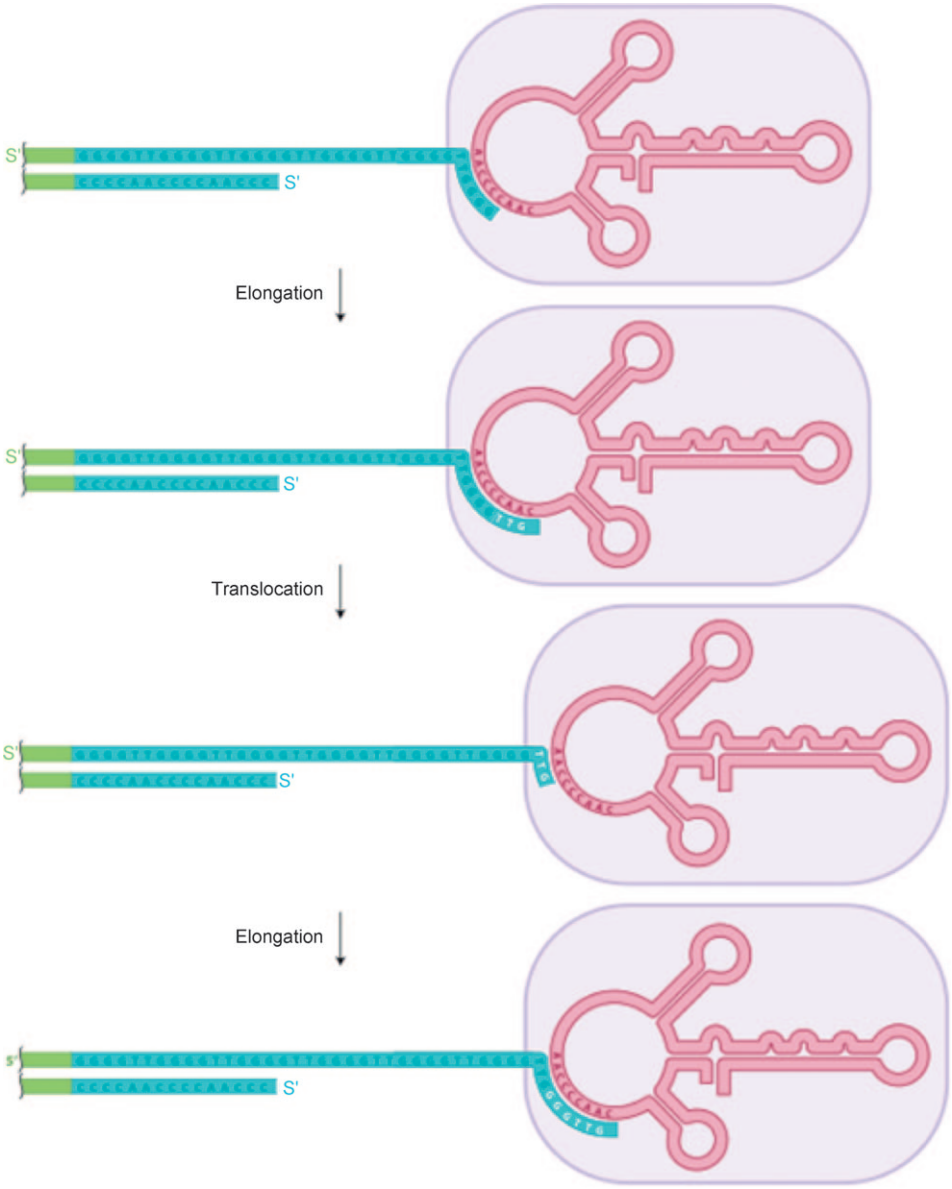

Figure 10. Model for telomerase elongation of telomeres: The sequence AACCCCAAC in the telomerase RNA serves as a template for extension of the telomere TTGGGG strand. The terminal TTGGGG on the telomere can base pair with the AACCCC in the RNA. This leaves three bases that can serve as a template for elongation. The activity of telomerase adds TTG and the end of the template is reached. Translocation can then reposition the 3′ end such that base pairing between the terminal TTG and the telomerase RNA is maintained.

model also immediately raised several new questions that I was curious to address. For example, does the proposed translocation step actually occur? That is, does telomerase hold on to the substrate it is elongating for a while, or does one enzyme only add one repeat, with a second repeat added during a second round of binding by a separate enzyme molecule? This question, which I had not thought of before I drew the model, was suddenly a burning one for me. I went on to tackle this next puzzle in a later paper.[21] Many other questions arose as we continued our work on telomerase. The many different paths the research took and our later focus on telomerase in cancer and human disease is described in the Nobel lecture presentation online at the Nobel Foundation website.

Putting together puzzle pieces is challenging, fun, and extremely gratifying, especially when they lead to new understanding in biology. This process of making a hypothesis and following leads is not a linear one: there are many twists and turns in the path. But the key is to keep the excitement and to follow the leads that are the most rewarding. I learned this during the first six years of working on telomerase, and it is the approach that I continue to follow. Many new questions often arise after one part of a puzzle is solved; the rewarding thing about curiosity-driven science is being able to pick from these new questions those that seem the most interesting to me. The pleasure of figuring out the puzzle and finding out things not known before is a great reward. Sharing that experience with friends and colleagues makes the reward even greater.

I would like to thank all of the talented scientists who have worked with me over the years for their energy and ideas that have made solving the puzzles fun, and opened up new puzzles. I would also like to thank Jonathan Crowe and Mary Armanios for editing help with this manuscript and David Robertson for helpful advice on the accompanying autobiography. I would like to thank Jennifer Fairman for the figures in this manuscript and Bang Wong for the figures used in the Nobel lecture Powerpoint presentation.

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