

Telomeres and Tetrahymena: an interview with Elizabeth Blackburn

Elizabeth Blackburn knows that loose ends contribute to aging and many of its associated diseases. She, together with Carol Greider and Jack Szostak, received the 2009 Nobel Prize in Physiology or Medicine for their work on the synthesis and function of telomeres, the unusual DNA sequences at the ends of chromosomes. Telomere changes are now recognized in human diseases ranging from cancer and cardiovascular disease to depression. Here, she discusses her approach to mentorship, how scientists might inform public policy, and new directions in telomere research.

Telomeres are made of repeating DNA sequences at the end of chromosomes that enhance their stability and protect their ends during replication. Changes in telomere length, usually a shortening of the sequences, are associated with disease. As we age, telomeres become shorter. Elizabeth Blackburn and Carol Greider discovered the enzyme, telomerase, that makes and can replenish the telomeric sequences at the ends of DNA. These initial discoveries used simple, single-celled organisms and lots of radiolabeling. As a postdoc in Joe Gall's laboratory, Elizabeth Blackburn kept her eyes fixed on the pear-shaped protozoan *Tetrahymena*, and in her hands, this single-celled ciliate soon laid the foundation for telomerase biology and a better understanding of chromosome structure, aging and disease.

What lead you to focus on such a simple organism as *Tetrahymena* to understand chromosome organization?

I used *Tetrahymena* because it is a system that is good for doing biochemistry that requires large amounts of biological material, and because it contained a very large number of short chromosomes that are linear.

My postdoctoral adviser, Joe Gall, had a very good sense that important biological processes are conserved throughout evolution. So, you choose a model organism that you can most readily get your hands on and

that allows you to work efficiently. This philosophy was especially important at this time, back in the seventies, when there were not many DNA technologies available. Joe found that *Tetrahymena* have high copy numbers – literally tens of thousands – of tiny, short linear chromosomes. My PhD training in Fred Sanger's lab was in the world of DNA sequencing, which was still just beginning. The so-called 'Sanger method' for DNA sequencing was still being invented, so I was using techniques of radiolabeling and piecing DNA sequences together. You had to sequence DNA from the very ends. I realized that, for big molecules, there was very little access to most of the sequence. Remember, this happened in precloning days, which is now hard to imagine! You had to have short abundant molecules because of the problem with background noise. I decided on *Tetrahymena* because it had abundant short linear molecules and I thought I could use the available sequencing techniques to see what was really at the chromosome ends.

Joe Gall was a great mentor and model. Having a good mentor yourself gives you a fantastic jump-start on trying to become a mentor. Once you've experienced working with a good mentor, you realize just how valuable good mentorship can be.

The current literature reflects a sort of alternation between the use of ciliates and budding yeast as models to understand telomeres and their function. Is one model better than the other?

People were using budding yeast as a common model when the initial work was done, but at first it was not good for understanding telomerase. David Prescott showed



Elizabeth Blackburn © 2007 Micheline Pelletier.

in the 1970s that some ciliates had DNA in the form of short linear molecules that were amenable to direct DNA sequencing by end-labeling techniques. The right molecules in yeast were not known until we used a special trick by adding *Tetrahymena* telomeres to the end of a linear yeast episome, which allowed for molecular cloning of yeast telomeres. This yeast work came later. It was useful and yeast are small inexpensive organisms that you can grow easily and do lots of genetics on fast. There were many powerful genetic tools for the yeast system, but the *Tetrahymena* system had the biochemical advantages. So, the telomere and telomerase work began in the ciliates.

How might telomerase be used as a therapeutic target in people?

I think that it is helpful to think of telomeres in two different contexts. In cancer cells,

changes in telomerase promote certain cancer characteristics and make some cells immortal. Almost immediately, people realized that cancer cells had telomerase. In 1989, Greg Morin reported the first human telomerase isolated from HeLa cells, using the same methods that we had used in *Tetrahymena*. Others did much broader surveys later in the 1990s and showed that lots of telomerase was present in cancer cells relative to less telomerase in normal cells. The simple question was, 'if the cancer cells are made immortal by telomerase, could you turn the telomerase off?' This idea could be tested by inhibition of telomerase. There are now some early Phase I experiments looking at inhibitors of telomerase. I think that the search for inhibitors of telomerase is one that can be addressed best by pharmaceutical companies.

What intrigued me, was really trying to understand what telomerase was doing in cancer. Our experiments in *Tetrahymena* proved that telomerase is a ribonucleoprotein reverse transcriptase. If we mutated the template of a cloned telomerase RNA sequence, we found that the mutant repeats were inserted into the telomeres. As soon as you add these mutant repeats to the telomeres in *Tetrahymena* cells, the cells look very sick. So in the mid-1990s, I was talking with cancer researchers at UCSF (University California, San Francisco) and said, 'I've got this interesting finding that suggests that we could add mutant sequences to telomeres to kill cancer cells right away,' but they were initially much more interested in telomerase inhibition. We did some early screens but never published them. We were not, ourselves, experts in developing small molecules.

My idea was to use the telomerase to make cancer cells really sick. It seems effective to use the naturally occurring high concentration of something that is already in cancer cells to turn against the cancer cell from within. We are now at a very, very early preclinical stage with this idea. We are able to induce a very robust apoptotic response. We are testing the idea in mouse models, using delivery methods with clever liposome-derived nanoparticles, with our

UCSF colleagues. We think this is a good approach but at the moment it involves a gene insertion, which may or may not be a good thing to do. It is just a little telomerase RNA gene that is inserted, with 451 nucleotides in the coding sequence. It is not a big gene and we have collaborators who use clever targeting methodologies, such as immunoliposomes that target overexpressed receptors on cancer cells. All of this is progressing very slowly, but teaching us quickly that all cancer cells really hate having mutations added to their telomeres.

There is another exciting context in which to think of telomerase. Experts in the field had a totally good rationale of why telomerase activity would be increased in

Telomerase may affect important cancer stem cell-like properties in cells. This is exactly the kind of thing you really want to target therapeutically, and the results are quite unexpected

cancer cells. Cancer cells, of course, are very abnormal in lots of ways and most of their abnormalities contribute to their immortality. We did a very simple experiment to determine the influence of telomerase level in cancer cells. In my lab, Shang Li knocked down the telomerase RNA with standard RNA interference. We expected to have to sit around and wait for a long time before the telomeres ran down and the cells stopped dividing, but to our great surprise, the cells responded very quickly. They didn't immediately stop dividing but they immediately slowed. We looked at their gene expression profiles by microarray and found a changed profile that appears quite quickly after telomerase knockdown.

Since we were changing telomerase RNA by knocking it down, it suggests that there is something special about reducing telomerase expression which is different from inhibiting the enzyme and leaving the ribonucleoprotein concentration high. The experiment that we did was in melanoma and when we just turned the telomerase activity down a bit, the cells continued to grow, just a little more slowly. Then we knocked down the level of the telomerase RNA component so that the total telomerase ribonucleoprotein concentration was lower. When we did that, the melanoma cells became more differentiated and less malignant. It is a fast-acting effect. The cells do not have time to run their telomeres down and then go into crisis. This hints that

telomerases are doing other interesting things. They have a night job as well as a day job.

It is still not clear what is going on, but the cell program is changing. So, ideas for polymerization-independent functions of telomerase are starting to emerge. Steven Artandi at Stanford has evidence that the telomerase protein affects Wnt signaling. That would not be expected if the role of the telomerase in cancer depended on telomere length alone. Telomerase may affect important cancer stem cell-like properties in cells. This is exactly the kind of thing you really want to target therapeutically, and the results are quite unexpected.

If it was possible to manipulate telomerase, either its activity or level in cancer, how does the evidence that telomerase has a fundamental role in the cell program and ubiquitous signaling pathways affect its therapeutic potential?

That I don't know, because a cancer cell is a whole different beast from a normal cell. A cancer cell doesn't have proper checkpoints, but instead has all sorts of anti-apoptotic pathways activated. Its signaling is really different from a normal cell. We don't want to knock telomerase down in normal cells because maintaining telomere lengths is probably a healthy thing. So, if you're going to inhibit telomerase or knock it down in cancer cells, you want to be pretty careful about specificity. Based on in vitro data from our lab and others, I'm convinced that any cancer therapy that involved telomerase as a target would be a combination therapy. Telomerase would not be the only target because it may be dangerous to knock telomerase all the way down in normal cells.

In normal cells, telomerase creates a whole different picture because these cells have highly regulated telomerases and telomere lengths. Normal cells show changes in telomeres as they age. In 2001, Inderjeet Dokal reported initial genetic findings that mutations in the RNA component of telomerase cause a severe type of premature aging. It's very clear that these mutations were in the telomerase RNA and now many more have been identified. We looked at the in vitro telomerase activity of the mutants that occur in human disease. We took some of the mutations that Dokal described and showed that they, in effect, knock telomerase telomere synthesis activ-

ity out – either completely, or down to about 1%. People with these mutations have one good gene and one bad gene. In these situations, people get terrible bone marrow failure and other symptoms like fibrosis. It is clear that defective telomerase RNA results in defective telomerase activity, which in turn is associated with diseases involving bone marrow failure and even pulmonary fibrosis. So, common conditions are caused by telomere dysfunction. Curiously, telomeres seem to be limiting for humans and there was no a priori reason to expect this result.

It seems that changes to telomeres may influence many areas of human disease.

From about the year 2000 onwards, an impressive compendium has accumulated that is derived from all sorts of cohort studies around the world, showing that telomere shortness is associated with just about all the major diseases of aging. Right now it is all association, but very interesting. Scientifically, one has to be very careful not to say anything about causality except for the rare genetic diseases. But, over and over again, telomere changes are associated with everything from cardiovascular disease, death from cardiovascular disease, risks of cardiovascular disease, diabetes, diabetes risks such as insulin resistance, vascular dementia, to osteoarthritis. The list goes on and on and the correlation is always in the same direction: shorter telomere length is associated with more disease. The association is absolutely solid now because it has been found in so many cohorts that it cannot be a statistical accident. What does this mean? That's a really exciting question and I don't know yet. I am skeptical that the answer is going to be very simple.

Are there models to support the correlation between short telomeres and disease?

In mouse models, only when telomerase is completely ablated is there a phenotype, which is generally delayed. Mice normally die naturally at 2 years of age and still have long telomeres. But humans live 80 years, which is a very different life expectancy from mice. I'm very intrigued by what telomeres do in humans. To address it, we collaborate with people who do clinical studies.

We have found that pessimism is one of the psychometric measures that one can

use to analyze people. We have even used cognitive dietary restraint, which is also striking. It's basically a fancy name for people who are under stress and have eating disorders; they are constantly trying to diet but they don't actually lose weight and they beat themselves up about it. It's a common problem in our very food-exposed world. There are various questionnaires and scales that are validated to measure this. Sure enough, when we look at telomere length in people, there is an inverse relationship between telomere length and pessimism, and between telomere length and eating disorders. This doesn't demonstrate causality, but it indicates a strong association that is quite striking.

Does stress cause telomere shortening?

Most of these big cohort studies weren't looking at stress directly, but it is a really interesting relationship. We became involved in the stress part through a colleague of mine at UCSF, Elissa Epel, who was working initially with Nancy Adler. They both look at physiological effects but are really interested in the world of psychology. Elissa emailed me in 2000 and said could she come and talk to me about her ideas to look at chronic stress. She wondered, 'Has anybody ever looked at telomere maintenance in people with chronic stress?' and I said, 'No, but we should look'. She gathered her cohorts very, very carefully. Richard Cawthorn in Utah assayed the telomere lengths and we analyzed the telomerase activity. We did all of our samples blinded. Elissa called up very excited and said, 'Wow! There is really a correlation'. The more severe the stress that a patient reported, the more the telomerase activity was damped down. This opened the door for us to look at so many other things. So, it seems that the best model for human telomeres is humans, and that's our model system now.

We talk a lot with our clinical colleagues because there are really two different cultures between scientists and clinicians. You have to learn to talk their language and they have to learn enough about telomeres and telomerase to see what makes sense for their patients. One of our colleagues once had a beautiful study and he sent us samples to test. My postdoc looked at them and said, 'Oh, where are the cells!?' and he said, 'Oh! I thought you wanted serum'. We realized that we hadn't talked carefully enough about what we were looking at, that telom-

erase is a cellular ribonucleoprotein so you need to have actual cells. It is a mutual education. There was so much that I did not know about clinical design, so I always work with collaborators. They know what makes a good clinical study and whether a study should take into account certain things – for example, who is on medications – to be able to draw important conclusions. They know the pitfalls that I'm now learning. It's very interesting because we really have to talk and have lots of face-to-face meetings to make sure that we're getting each other's language.

You have a reputation of being a good mentor. What qualities do you think are necessary to be an effective mentor?

I try to remember what it is like for someone at the early stages of their career. Sometimes I draw on my own personal experience and sometimes it's what I've learned from other people. I do talk a lot with younger colleagues. It's important to just remember what it was like, although it's really easy to forget. I remember positive things about my postdoc mentor, Joe Gall. When Joe said positive reinforcing things to me, I remember how much it meant and how very insecure I was. I was insecure enough that these things were important for me to hear. For him to say 'you are a good scientist' told me things that I didn't allow myself to think.

Scientists often forget something that most of the world realizes, that you actually have to say positive things to people too. I try to remember the experience I had, but I have to be reminded sometimes because in science we are trained to be critical. We just go straight, very efficiently, to the faults and often we forget that it's quite difficult to be on the receiving end of criticism, even when the intention is good. So, I try to put myself in other people's shoes. Often when people are cocky on the outside they're not on the inside. Rather, they are really very vulnerable and I try to stay aware of that all the time.

Does being a good mentor have any direct influence on your career?

If people do come and ask me to be a mentor, it is much the same as when people come and talk to me about other things. The time commitment is minor and doesn't take time away negatively from something precious. The positive thing is that men-

toring allows me to have really good students and postdocs. People are now smart enough to take someone's mentoring reputation into account when choosing a lab. I was told that Joe Gall was a terrific mentor when I went to Yale. That was a really helpful thing for me to know. When you have really terrific people in your lab, it helps the science so much.

I have never really thought of myself as a great mentor. But, I think that being a mentor means caring about people so that people want to come and do good science. And the people who come are fantastic. This, of course, makes the science happen.

I don't know how much of the lab dynamic is cause and effect. If I were a horrible mentor, if I were known to be really mean or something like that, then would I still attract really good scientists that were different kinds of people? We all know people who are very poor mentors but who do really good science. The question is, 'is there a cost for their poor mentorship style?' Maybe their science could have been even better.

After a frustrating term as an invited member of President Bush's Council on Bioethics, do you have any advice about how scientists might inform public policy in a useful way?

Yes, I really do. I went on the Bioethics Council wondering what I could bring to it. I know how science works, I know good science from bad science and I know the field. I'm not directly a stem cell scientist, which I knew would be a big question for the Bioethics Council, but I do know cell biology and I'm in the general field, so I could speak about it. I also felt that I could think about evidence, since that is what I am trained to do. That's what I think scientists really can bring to public policy, we are very good at listening to arguments and we can tell when an argument is weak. We are trained to think critically and I think that's very useful for public policy.

Scientists have a culture, I discovered, of being rather honest. We tend to just say things, whereas I think in many other walks

of life there is much more circumspection. Our culture is direct. If we think it's the case, we say it clearly and this approach is valued. So, it seems to me that scientists can offer public policy a different way of approaching questions that is clear headed, focused on evidence and that clearly discusses the issues. I keep saying that we've got to get the science right, because that is what we do as scientists. I used to joke that, in that respect, it is just like our lab group meeting. So, I think the best thing a scientist can do in public policy is to try to get the scientific facts right.

So the critical directness that is characteristic of scientists should be saved for public policy and not for mentoring?

Right! That's a really good comparison. With mentorship, to be most effective, we have to actually do what we're not trained to do. The evidence is that mentoring is more successful if you do it in certain ways. That's a kind of training too. It's just not the subject matter that we're as used to.

If you were to start out all over again as a new scientist, is there a different field where you would choose to work?

If I had to start again now I would choose neuroscience, since it has advanced so much recently. I see how important this area is from the chronic stress tests where we can see how the brain feeds in to all of the rest of human physiology. That's what I would love to have as my field.

I'd like to understand how the brain commands the body because in the field of telomeres we see the role that it plays. It is also a very interdisciplinary field, bringing so many different physiological consequences together. Once the view of medical science was that the impact of the brain on physiological disease was complicating. Separate cultures and ideologies formed and I think there was a sort of dismissal of brain effects as just psychosomatic. Wait a minute, that's the whole point! There are real effects of the brain on disease and the science for understanding this was not adequate for a long time.

But I wouldn't have liked to enter this area when I started in the 1970s. At that time, molecular biology was all the rage and that was where things were exciting and fast. I don't think that neuroscience had the capabilities then that it has now.

Have you looked at telomeres in the brain or neurons?

No, I'm more interested in how the brain sends all sorts of messages to the rest of the body, such as vagal innervation. All we used to know about the brain is that it churns the stomach and beats the heart. But there is even a vagal innervation that goes right to stem cells in the spleen. It is physically right there. I also learned recently about pretty direct effects of the brain on the immune system, and these are just a tiny example of what the brain must do. I would love to know how it works. The brain integrates the whole organism much more than we ever thought, and this is an area of emerging science. The molecular biology has gone very far and now we have to re-integrate all of this information about the human organism. I think the human is the best one to study for this.

That will make it a difficult area to model.

Yes, that's what the great molecular biologist, Sydney Brenner said: 'The model for human organisms is humans'. I think there are some very powerful mouse models and animal models that have been very powerful for highly conserved things. There's no question that they are very important.

We greatly appreciate Elizabeth Blackburn's retelling of her exciting story and her thoughts about mentorship, politics and the future of science. Kristin H. Kain, Associate Reviews Editor for DMM, interviewed Elizabeth Blackburn. This piece was edited and condensed with approval from the interviewee.

DMM congratulates Elizabeth Blackburn, Carol Greider and Jack Szostak for being awarded this year's Nobel Prize in recognition of their exciting telomere research.