Telomerase: The Elixir of Life? An Interview with Professor Kathleen Collins

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Telomeres are short, repeating units of DNA located on the ends of chromosomes to protect genetic information during DNA replication. Telomere loss has been frequently associated with aging, suggesting that longer telomeres correlate with longer life span. Although not quite literally an "elixir of life," components of the telomere complex have the exciting possibility to lead the path in creating new therapies for diseases, such as those involved in tissue failure. The enzyme responsible for generating telomeres, telomerase, is necessary in actively dividing cells like skin and blood cells, but also poses a danger when left uncontrolled in cancer cells. BSJ had the privilege to speak with Professor Kathleen Collins about her work, ideas, and remarks about the current state of telomere research.

BSJ: Our topic this semester is on death and dying. We interviewed one of the astrophysicists here, Alexei Filippenko, on death of stars and core collapse. We thought your research in telomeres would be a nice parallel since we started our journey in astrophysics and now are working our way down to the inner workings of a cell.

Primarily, to know more about you, can you tell us a bit about your background and how you first got involved in telomere research?

Collins: What science exactly you wind up studying is often not something you predict because if you knew what research was going to be interesting, then you wouldn't have to do that research. You would already know the answer. The way I wound up studying telomeres is slightly random. I had been in graduate school working on myosin and actin, so I was very interested in cell architecture and how cells build different kinds of surfaces. A leading edge of a cell looks very different than its contracting back edge, and I was working on polarized intestine epithelia, which have to transport nutrients from the gut epithelial cells. The surface area that faces their gut is highly contoured and the surface area that faces where the nutrients are going to pass is not. So I was studying how that surface area gets highly contoured. And it turned out that there were molecular motors, these myosins, that were taking actin filaments and remodeling this whole area. From there I got interested in how molecular motors work, because in order to move, these actin filaments had to exert force. They had to translocate along their polymer. The idea is that you can translocate along a substrate and these enzymes do it incredibly efficiently, much more than any car anyone has ever built. And furthermore, these enzymes know which direction to go. Although there is a whole family of myosin proteins, there's just one that knows to do

this surface remodeling. So how do different motors get specialized for their cellular task? How do motors work and how do motors get specialized? I would go to conferences and there were people who had worked for decades on actin and myosin, muscle contraction and I would offer some new hypothesis. And they'd say, "Yeah, I heard that twenty years ago!" So I was thinking, I want to study motors, but I want to study something that no one can get up and say, "Oh I heard that twenty years ago!"

So I went to a conference on molecular machines, and in addition to all the actin, myosin, kinesin, dynamin talks, there was a talk about RNA polymerase and how it moved along DNA. And I thought, "Great! Instead of cytoplasm, I'll study a polymerase because there are far fewer people who have thought about this and it must be different." For example, how you move on nucleic acid is very different than how you move on a protein. So I thought about what to do as a post-doc and I interviewed in a myosin lab and a kinesin lab along the lines of what I was doing. But, a friend of mine had showed me a paper about this new polymerase—telomerase— and there were maybe five papers on it. But it seemed to be a very simple polymerase in the sense that it carried its own template and it bound one sequence-specific single-stranded DNA primer and it added simple sequences of DNA. I thought, that's much simpler than RNA polymerase binding a double stranded DNA making an RNA. I'll study how polymerases move by studying telomerase. And there were two people working on telomerase, Elizabeth (Liz) Blackburn and Carol Greider. So I visited Liz at UCSF and Carol at Cold Spring Harbor, and I just realized, "Boy, this is cool. I am going to be able to do this because there is no biochemist working on this at all. I am going to purify this enzyme and then I am going to do this single-molecule assay where I'll add this template and I'll watch it step. And it will translocate each time it makes a repeat and I'll watch." And so I wrote my post-doc proposal on this: what is the mechanis ple came to me (I was at the Whitehead Institute at MIT), and asked, 'What is telomerase?' And it makes sense, there were just a handful of papers on it, but they read the proposal and they liked the proposal, and so I got a post-doc fellowship and I went to Cold Spring Harbor. That was the incredibly naïve choice, one of many, because we didn't know anything about telomerase. Right now, we are still reconstituting that enzyme. The very first single molecule assay was done just about five years ago by a former Berkeley graduate student, Michael Stone who had decided to do a post-doc in a single molecule lab at UC Santa Cruz. He decided, 'I'm going to study telomerase and I am going to do single-molecule method.' And you know, that's twenty years after from when I was working on myosin and actin. So this idea that I am going to use this simple system to study translocation events and molecular motors got me into telomerase. However, we have had to solve so many other questions before we can even think about this translocation mechanism. A current student in my lab, Alex Wu, is doing that right now. He is at the point where he is doing a collaboration with Ahmet Yildiz. He is going to watch this translocation event and he may accomplish it. So that's the long-winded story of a really simple answer, which is, not at all for the reasons that we actually wound up studying telomerase.

"What's surprising about telomerase is that it is evolving very rapidly."

BSJ: Telomerase is an RNP (ribonucleoprotein) complex. Is there a hypothesis or advantage for using RNA as template rather than a DNA template?

Collins: That's a great question. So I could have thought to ask myself that twenty years ago, but I didn't. It turns out that now we know the answer to that question. We spent quite a while trying to get telomerase to work as a protein with a little RNA and it would never work. It would never take a little template, whereas other reverse transcriptases, like viral reverse transcriptases take any RNA template base-paired to a DNA primer. They don't need a big RNA component. The just need any ol' RNA. But telomerase wouldn't do that. A student, Michael Miller, performed an experiment with the following question: is it the template that is the problem or are there factors that the telomerase RNA provides, other than a template? So we took the template region as an RNA oligonucleotide, and we took the rest of the telomerase RNA as the RNA body. The template alone wouldn't work, but as two physically separate molecules, he put back that rest of RNA. Now telomerase could copy an external exchangeable RNA template. So the template doesn't need to be internal within the telomerase RNA. Telomerase needs the non-template motifs as a cofactor for its basic activity. We begin to understand that this constitution experiment lets us ask separately what is needed for template recognition and what is needed from the non-template motifs of the RNA. Protein-RNA interactions help fold the active site of telomerase, so the RNA is providing allosteric modulation of the protein. It's bringing together different protein domains. One positions the template and one binds the primer and it's doing other things we haven't figured out yet.

But the reason telomerase has to be a ribonucleoprotein (RNP) with an RNA template, is that it needs the non-template RNA portions for its activity. And this may be a part of the bigger question of evolution. There was an RNA world: RNA can fold and RNA can have many functions due its ability to fold. The way that RNA folds is very different than how proteins fold . So the RNA world may have given rise to the early RNP world where catalytic RNAs were helped by proteins. Protein active sites came to dominate. But, now we're in a world where in evolution there is an explosions of non-coding RNA function. These new non-coding RNAs, they're not catalytic, but they still can fold and have protein interactions. And, that combination of RNA and protein folding gives much greater structural and functional repertoire than a protein alone. So telomerase is just expanding from the protein world into this new RNP renaissance where these non-coding RNAs can give the protein new functionality.

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BSJ: That perfectly transitions to our next question. In a review article you authored, you discussed the evolutionary conservation of various telomerase RNAs, TERs. Could you explain the evolutionary advantage of these conserved motifs?

Collins: So evolutionary conservation is a way to look at important regions of a molecule because if they can't mutate, they are selected for their function. What's surprising about telomerase is that it is evolving very rapidly. If an enzyme is essential for chromosome replication, it could not tolerate much change. But in fact, over evolution, you can't even recognize telomerase RNAs of yeasts versus vertebrates versus plants. And that's abnormal for an enzyme that is highly conserved in DNA replication. But RNA has an interesting property that comes from its folding; if there is a region of secondary structure, like a base-paired stem, it doesn't necessarily matter what the sequence is. All that matters is that it base pairs. So the sequence can diverge and the structure can stay the same. And you can't predict structure from sequence. But the other difference for RNA is because small sequences of RNA can fold independently. RNA tolerates insertions and deletions very well compared to a protein, where a much longer amount of protein is required to make a tertiary structure fold. Another reason it's difficult to find telomerase RNAs is because they may be 150 nucleotides in one species and 1500 in others. Yet amidst all of this, there are a few motifs that are conserved. One is called a pseudoknot, which is a base triple pairing (three strands coming together). The other is an RNA stem-loop. The stem-loop is the binding site that binds to the reverse transcriptase protein and somehow allosterically gives it an active confirmation. We are pretty sure why the stem-loop is conserved, but we have absolutely no idea what the pseudoknot motif does despite studying it for a very long time.

BSJ: Telomerase is commonly associated with aging, and although there are other factors that influence lifespan, what is the potential application for prolonging life by controlling telomerase?

Collins: That's a good question. When I was working on telomerase because of this link of telomere shortening and aging, people started saying, "Oh! It's the fountain of youth! If we could activate telomerase, we would cure aging." And I would always said, "Oh, come one! That's ridiculous!" So, I was very skeptical. This idea came about because in the lab we were trying to understand the composition of the enzyme and we discovered that human telomerase had a protein in it that had been previously cloned as the locus of a disease in humans. Through that, we were able to show—to great skepticism in the community—that mutations in a particular protein called Dyskerin gave rise to disease by decreasing the amount of telomerase. So if you reduce the amount of telomerase you have, you die of bone marrow failure. If you decrease your amount of telomerase by half, you die of bone marrow failure in your 30s and 40s. And if you reduce it by ¼ its normal amount, you die in your teens. What this forced me and many other people to think about is if you have a little reduction, you run out of renewal, but only in certain highly proliferative tissues. Blood cells, for example, are turning over constantly—you need to renew them all the time in addition to the cells of the intestinal and epithelial tracts. If you look at people who inherit telomerase deficiency, they have many epithelial problems. So then, now you can go and look in the population and ask these questions. There are new population studies that try to correlate telomere length with either longevity, which is life span, or something we like to talk about more, health span, which is how healthy your tissues are.
There is a correlation, in fact, between telomere length and cellular renewal capacity and lifespan. Telomerase is one of many things that would determine telomere length. We can't do this experiment in humans (we can't activate telomerase

overall). And I don't think aging per se is a complex thing, but the people who die of inability to fight off infections, of immune deficiency, maybe those people would have lived longer with telomerase. Obviously if you get hit by a bus you're not going to live longer if your telomeres were longer. So not all aging and all diseases of aging are going to have anything to do with telomere length.

But some of them and in particular things that compromise our health span, in the setting of chronic infection, like Hepatitis or HIV or environmental exposure to toxin that force high rates of cellular renewal in a certain tissue, will help. But there is a downside. Just recently, there was a publication that people who have two times the normal amount of telomerase in certain tissues inherit a higher risk of developing melanoma. So it is going to be a fine balance between anti-aging and anti-cancer, a tissue-by-tissue, person-by-person choice, and I think we are going to need to check telomere length before we consider treating a patient with a telomerase activator.

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BSJ: Is there a happy medium between too much or too little telomerase activity? Is there a known "on-and-off" switch?

Collins: No, not really. So what's interesting is that this telomere phenotype is unlike any other genetic basis of disease. If you have inherited sickle-cell anemia, every cell in your body has a mutant in this gene, and that gene product is functional or dysfunctional; you genetically test for the disease. Telomere length is not that way. You can have long telomeres, medium telomeres, short telomeres—everybody is equal until the telomeres become critically short. Cancer doesn't need telomerase until it wants to go metastatic, right? It can grow and grow and grow and then it hits telomeres that are too short and if it doesn't have a telomere, it stops. Benign cancers don't have to have telomerase. And likewise, you can fight off all the infection you want if your telomeres are short, or long, or intermediate. So no, there's no way to say what's a perfect telomere length, because a whole range of telomere lengths are just fine. It's the risk of having very short telomeres that's the problem, because it's the very short telomeres that will cause the cell to die, cause genome instability, or convert to cancer. How to interpret a telomere length test is a very interesting thing because longer lengths are not better; too little is bad, but everything else is okay.

BSJ: Elizabeth Blackburn gave a talk that we attended and through her data, she brought up the idea that women's telomere lengths are substantially longer, and that telomere length correlated perfectly with age . It was outstanding to see that striking correlation.

Collins: I think that is a useful indicator of the amount of stress, potentially, or the load of replication stress on a person. And I think what Liz was trying to say was you can use the rate of loss of telomere length as a warning sign, just like we use cholesterol as a warning sign. Now, you have an intermediate range of cholesterol; one number doesn't necessarily tell you how to do therapy. If your numbers are increasing you might want to modify your lifestyle – if you're old and your numbers are high you might want to modify your lifestyle. So if you're young and your telomeres are short, Liz would say you want to modify your lifestyle because you need those telomeres for the rest of your life. Or if your rate of loss of telomeres is very high, again that might be a thing to concern yourself with because if you continue that rate, your telomeres are going to wind up too short. So as a monitor of lifestyle, I believe that would be the best theory for that.

BSJ: The broader implications of telomere research might relate to your earlier statement about "the fountain of youth," but that is a goal for the long-term future. What do you think is the next step for the telomere field in the short-term?

Collins: I think there are implications that are relevant to clinical therapy right now. So, for example, bone marrow failure patients, aplastic anemia, or any disease remotely associated with tissue failure, the choice of therapy is important, because we know that not all therapies are successful. Many people get the same drug, and ten people recover and ten people will not recover. And the great leap forward is going to be predicting. It would be great if we could tell in advance which people are going to respond positively to the chemotherapy and whose lives are going to be made worse by taking that chemotherapy than if they hadn't taken it to begin with. You could both use treatments more selectively, but you could also greatly improve quality of life by treating the people who are going to benefit from it and not giving the side effects to people who aren't.

So for example, if I had any anemia, I would get my telomeres tested, because the standard therapy is to give someone a hormone that will stimulate blood stem cells to make more blood. But if you exhausted telomere length in your blood stem cells, that hormone is pointless. So why wait for that to fail? The standard way to do a bone marrow transplant is to oblate your entire existing bone marrow, and then put in the new marrow, but if your existing bone marrow is not going to proliferate anyways, why radiate somebody and cause more harm that needs to be repaired, right? And also if you're going to do a bone marrow transplant, studies have shown very clearly that the proliferative demand on the transplanted cells is very high. We lose telomeres in our immune system very rapidly, from ages about 1 to 4, and then it levels off, which is good, because we couldn't continue to lose telomere lengths at the rate we lose them at that age.

But if you transplant bone marrow, telomeres are lost at a very high rate, because it's replicating a lot. So you transplant bone marrow for someone who is healthy, but maybe their telomeres are long enough that they are going to be fine with the short rate of telomere attrition to support their immune system. But maybe the marrow transplanted doesn't have long telomeres, so I would type all bone marrow transplant donors for telomere length in their system before taking cells to transplant. And I would only take a transplant if I knew those cell were going to have the capacity to renew. I think there are immediate therapies for these things that have medical precedence where short telomeres are going to determine the outcome of the therapy. And I think in broader cases, there may be ways to evaluate the toxicity of therapies based on that. In the choice of future therapy, we should not rely on just using telomerase, but using whole realms of information, to be able to pick, and individualize therapy. I'm not saying to sequence everyone's genome and design a life for them based on that, but if you have a certain cancer, and we want to treat it, I think there needs to be a way—including telomeres and other genetic tests—to make that choice of treatment.

BSJ: With the telomere field growing so rapidly, and knowing that you've been following it since the beginning, how do you think the telomere has changed since you first began research?

Collins: Wow, it's a good question. I think early on this aging idea took over, and it was just too early. There was a phase when every question I got at a seminar would be about aging. And people realized that we didn't know enough yet to ask that. So that kind of died down. Then there was an, 'Oh my gosh we can cure cancer' phase, and a lot of studies researched different kinds of cancer: how many telomeres there were. People realized it was still too early to do things about that, because we still didn't know enough basic mechanisms about how telomerase works. You are not going to cure cancer if you don't actually know what step to treat. So that sort of died down. And right now there is a lot more of the field doing very fundamental biology about what a telomere looks like, and how it is dynamic over the cell cycle. Not just what a telomere looks like in an average cell, but what telomeres look like at different points. How does it cause— at a molecular level— cell death or cancer? How is telomerase brought to a telomere, how does the cell know how much telomerase to make? I think it's also really important for any field to bring in new ways of thinking and new expertise. For example, we are now seeing people who are interested in high-resolution imaging and new model systems, which will be helpful.

BSJ: Our last question is about HeLa cells. In regard to HeLa cells, which are considered immortal, what was the basic mechanism of the telomerase in the cells that renders them immortal?

Collins: We like to say proliferatively immortal when we are talking about telomerase, because you can destroy HeLa cells with a little bit of bleach, a little bit cold, or a little bit heat. So it's not really like Tuck Everlasting, but it's true that they have the capacity to be immortal. And what gives them that is a de-regulation of the limit on telomerase production. HeLa cells are a little different, also, in that they have more telomere ends than normal, because cancer cells are often amplified in their chromosome content. So, probably in order to support those amplified chromosomes, the cell had to dramatically up-regulate telomerase, more than it would in normal cell. Of course, this would not occur in any normal cell development and it therefore had to mutate to do that. So we don't know what causes that. And, we all use HeLa cells as a canonical model system. In fact, Dirk Hockemeyer, a new MCB faculty recruit is studying human embryonic stem cells. He is going to look at telomerase regulation in those cells and that will be a very interesting model, because he can ask: how are expression levels of the components controlled? We have a collaboration to help him ask: what controls telomerase getting to a telomere in human embryonic stem cells? I think only in comparing those cells to HeLa cells will we know what makes HeLa, "HeLa", because right now "HeLa" is all we know. If it were not for HeLa cells, there would be no human telomerase work.

BSJ: I think that is a great place to end. Thank you so much for your time!