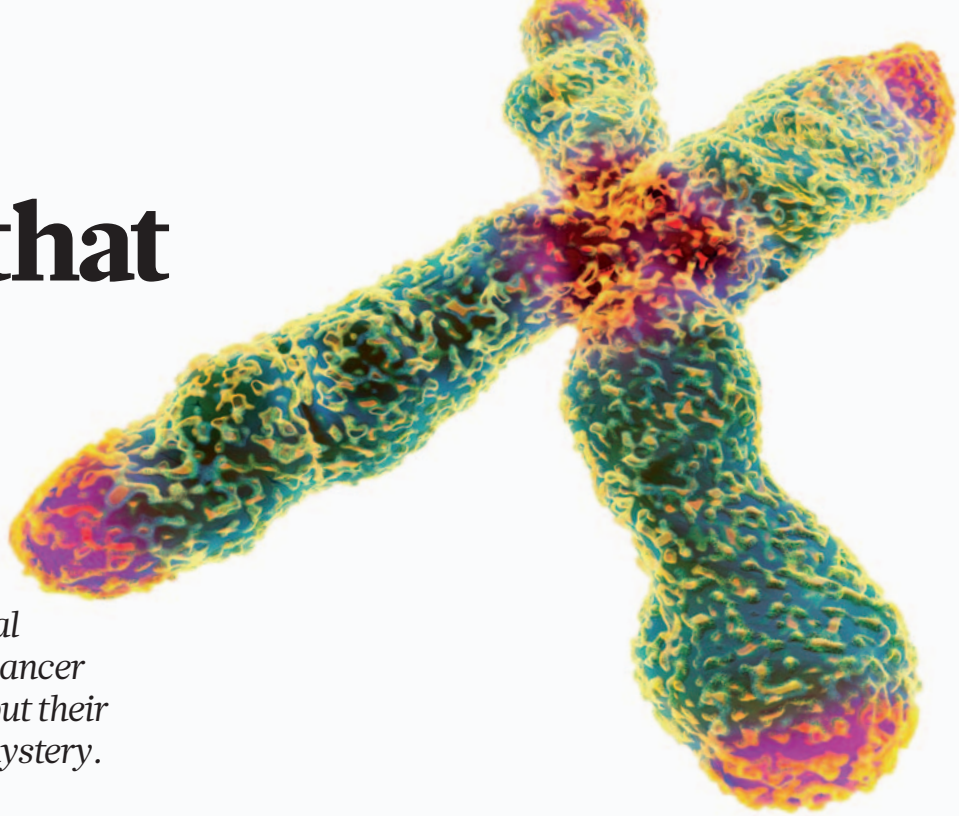
TELOMERES

# All's well that ends well



*Elizabeth Blackburn gave the first lecture at the 2011 Lindau meeting, describing her Nobel prizewinning work on telomeres. These chromosomal caps are known to play a role in cancer and are implicated in ageing — but their full biological utility remains a mystery.*

**BY MICHAEL EISENSTEIN**

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TTAGGG. TTAGGG. TTAGGG. And so on. And so on, hundreds and hundreds of times. It seems hard to believe that such mind-numbing repetition could conceal anything of interest.

Yet these nucleotide sequences, which cap the ends of every mammalian chromosome, appear to act as both an important bulwark against tumour growth and a buffer that delays onset of a number of the degenerative processes that take hold as we age. The Nobel Committee recognized the importance of these chromosomal end-pieces — called telomeres — when they awarded the 2009 Nobel Prize in Physiology or Medicine to Elizabeth Blackburn, Carol Greider and Jack Szostak for their trailblazing efforts to map details of telomere function and the mechanism by which these structures are generated and maintained.

When Blackburn began investigating these phenomena in single-cell organisms in the late 1970s with her mentor Joseph Gall, "it was much more of an esoteric, yeast and protozoan biology field," says Jerry Shay, a cell biologist specializing in telomere research at the University of Texas Southwestern Medical Center in Dallas. "Now it's really becoming a major area of investigation, and there are lots of new and exciting work that's going to be coming out."

The story that has emerged is not a simple one. For example, truncated telomeres can be seen as a harbinger of both good and bad: slowing cancer cell division but also impeding natural tissue repair processes. In both humans and mice, short telomeres are implicated in disease progression and ageing, yet scientists are still struggling to understand how measures and manipulation of these enigmatic end-caps might benefit human health.

***"If I had short telomeres, I would probably be a more compliant patient."***

## PLAYING A DOUBLE GAME

Owing to a quirk in DNA replication, chromosomes become slightly shorter each time they are copied. Excessively short telomeres could disrupt essential DNA sequences, and introduce the risk that cellular repair mechanisms might mistake frayed chromosome tips for broken DNA strands and graft these loose ends together. In the late 1930s, Barbara McClintock — a Nobel prizewinner in 1983 — showed that unprotected chromosome ends are prone to cycles of fusion and breakage, resulting in severe disruption and rearrangement of chromosomal structure. The main function of telomeres is to prevent such problems: these repetitive caps of DNA erode over many rounds of cell division while insulating essential sequences against injury.

Telomeres are made and maintained by the enzyme telomerase. Telomerase is intermittently active within populations of adult stem cells, which contribute to the maintenance and repair of various tissues throughout the body. Most other adult cells do not produce detectable telomerase, so their telomeres become shorter with every division. When telomeres reach a critical length, they trigger crisis management mechanisms such as p53, a tumour-suppressor protein that monitors DNA integrity and can induce cells to either self-destruct or enter a dormant state known as senescence (see 'A tale of two ends').

Because chromosomal damage can promote uncontrolled cell growth, one might expect that rampant telomere erosion could introduce the same risks. Fortunately, p53 halts this process by preventing cell division. Greider and others have shown that senescence induced by telomere shortening might offer protection against tumour formation. "It's clear that, in general, short telomeres can limit cell division and stop the growth of tumours in several mouse models," says Greider, a molecular biologist at Johns Hopkins Medical School in Baltimore, Maryland. "The question is to what degree that translates to different human settings."

If genetic mutation disrupts p53 function, as occurs in a variety of cancers, a key safeguard is lost and short telomeres become a major liability. Cancer biologist Ronald DePinho studied shortened telomeres in the absence of p53 activity in mouse models. "We saw an increase in cancer," says DePinho, president of the MD Anderson Cancer Center in Houston, Texas. What's more, although cancer-prone mice with normal length telomeres usually develop different cancers than are typically observed in older humans, DePinho's short-telomere mice developed tumours in the breast, colon and skin. "These mice changed their tumour spectrum towards a humanized spectrum of epithelial cancers," a finding that he says highlights the importance of telomere length in human cancer.

Tumour progression is limited without telomerase. "The almost universal way in which cells continue to divide and become an advanced, robust malignancy is by somehow upregulating telomerase, so they can maintain the telomeres that are being lost with every division," says Shay. Indeed, Shay and long-time collaborator Woodring Wright, also at Southwestern Medical Center, have found that telomerase might be active in 90% or more of human tumours. Researchers once thought that this restored activity required full reactivation of the dormant telomerase gene in the tumour cell. More recent findings suggest that tumour growth is instead initiated in stem cells that already produce telomerase, necessitating only a small bump in

expression rather than a dramatic upswing. "The vast majority of tumour cells have very short telomeres," says Wright. "They maintain length, and are not actively elongating."

Given that cancer cells tend to exhibit steadier overall telomerase activity than healthy stem cells, this enzyme could offer a useful target for a variety of cancer treatments. Menlo Park, California-based biotech company Geron is in the midst of several phase II trials to test the efficacy of imetelstat, a telomerase inhibitor, against lung cancer, breast cancer and myeloma. According to Stephen Kelsey, Geron's chief medical officer, this molecule is most effective against the rapidly dividing, immature tumour cells that some cancer researchers have tentatively labelled 'cancer stem cells'. He suggests that drugs like imetelstat might work best following surgery or chemotherapy, when surviving tumour cells are proliferating aggressively in an attempt to rebuild.

Inhibiting telomerase is expected to halt tumour growth either by forcing the cells to enter senescence or through excessive genomic damage, although this has not been demonstrated in humans. Geron hopes to have preliminary efficacy data by late 2012; initial safety studies at clinically useful dosages have found no evidence that the drug has lasting adverse effects on healthy telomerase-expressing cells. "Generally, tumour cells have shorter telomeres, which would provide a therapeutic window where they get selectively killed off," says Calvin Harley, former chief scientific officer of Geron and now CEO of Telome Health, a diagnostics company also based in Menlo Park.

CH FLEMMING/NOBEL LAUREATE MEETINGS

**Laureate Elizabeth Blackburn, a pioneer of telomere research, chats with a young researcher in Lindau.**

## THE LIMITS OF REGENERATION

Aside from the cancer connection, some of the greatest excitement in this field focuses on how telomere shortening contributes to human age-related disease. "I wouldn't say that cancer has moved to the back seat, but rather that the connection to human age-related disease has moved to the forefront in the last few years," says Mary Armanios, an oncologist at Johns Hopkins.

An extremely rare disease known as dyskeratosis congenita (DC) was the first condition to be directly linked to mutations in genes involved in telomere maintenance. Patients with DC lose the capacity to produce new blood cells owing to a collapse of their precursor stem cell population, a state known as bone marrow failure. More recently, telomerase gene mutations were also linked to risk of idiopathic pulmonary fibrosis (IPF), a more common, fatal disease characterized by extensive scarring in the lung.

Both DC and IPF appear to represent a failure by the body to repair aged or damaged tissues. "We know that telomerase is maintaining telomeres to enable self-renewal in stem cells, and when that goes awry it leads to disease," explains Steven Artandi, a haematologist at Stanford University in California. "In many cases these patients appear normal at birth; then, as they enter late childhood or early adulthood, depending on the mutation, they start to experience failure of different tissue compartments."

People who inherit these telomerase mutations might also inherit prematurely shortened telomeres, leading to earlier and earlier onset of IPF. Studies by Armanios and colleagues have demonstrated that this intergenerational shortening is associated with increasingly severe pathology[1]. "Older generations develop IPF, but as the disease gets worse with each generation, the younger generations develop a bone marrow failure disorder called aplastic anaemia," she says.

Telomere shortening appears at least partially culpable in a growing list of diseases. Early attempts to characterize the physiological effects of telomere shortening hit a wall when researchers found that mice lacking telomerase appeared to suffer no ill effects. Closer inspection revealed that standard inbred strains of laboratory mice are born with telomeres several times longer than in humans, limiting the effects of age-associated shortening. Scientists have overcome this limitation via serial breeding of telomerase-deficient animals and use of newly derived mouse strains with human-like telomere lengths. With these short-telomere strains, it is now possible to model in mice many diseases previously intractable to *in-vivo* investigation. For example, standard laboratory mice "don't develop liver cirrhosis even after you ablate the liver many times", says DePinho. "Once we generated mice with shorter telomeres, these mice developed florid liver cirrhosis." This result, DePinho explains, showed that shortened telomeres are "integral to the pathogenesis of this disease state". Similarly, Artandi and Stanford colleague Helen Blau showed that developing mouse models of Duchenne muscular dystrophy requires mice with short telomeres[2]. "If you take telomerase away, short telomeres impair stem cell function and you suddenly reveal the full effect of the Duchenne mutation on muscle physiology in mice — and it looks very much like it does in humans," says Artandi.

***"Then we flipped the switch back on, and the animals had improved cognition, they became fertile and so forth."***

## COUNTING THE DAYS

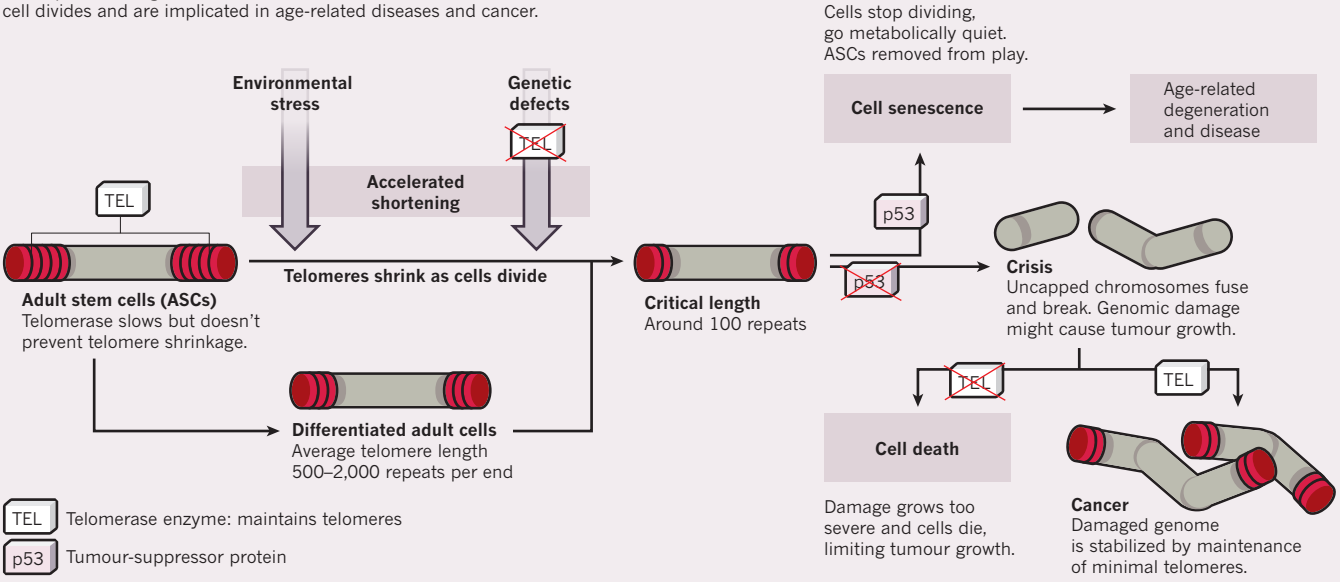
Many of the above pathologies seem to recapitulate the tissue degeneration we experience as we grow old, and the idea that telomere shortening helps drive the ageing process has been a central focus of the field.

Studies in mice, at least, have suggested that telomere maintenance can have an impact on both quantity and quality of life. "Work in mouse models clearly indicates that having shorter telomeres than normal is a cause of ageing," says Maria Blasco, a cell biologist at the Spanish National Cancer Research Centre (NCIO) in Madrid. Using genetically manipulated animals, Blasco and colleagues have shown that increasing telomerase at the same time as increasing expression of the p53 pathway could "extend the lifespan of mice by 40%" (ref. 3). More recently, DePinho's team assessed the impact of reactivating telomerase in mice whose telomeres had reached a critical length[4]. "The mice were not in good shape — equivalent to somebody in their 80s or 90s," he says. "Then we flipped the switch back on, and the animals had improved cognition, they became fertile and so forth; this told us that there's a point of return for tissues, even those in a severe state of degeneration."

Indeed, the correlation of ever-shortening telomeres with the passing of the years lends

## A TALE OF TWO ENDS

Chromosomes are capped by telomeres, a series of TTAGGG repeats, which protect the genetic material. Telomeres shrink each time the host cell divides and are implicated in age-related diseases and cancer.




itself to a tidy narrative in which telomere length somehow records biological age, like rings in a tree stump. However, scientists working with telomeres caution that this is a gross oversimplification. "There is clear evidence against the concept that telomeres are the sole determining factor in ageing," says Greider. "There may be five, six or seven different pathways that play into what we consider as ageing, and telomeres play one role in that process."

All the same, the tantalizing association between short telomeres and various diseases has persuaded several leading researchers to launch companies that measure telomeric length in blood cells for prognostic and diagnostic purposes. Repeat Diagnostics, based in Vancouver, Canada, was founded in 2005 by oncologist Peter Lansdorp to help clinicians identify patients with genetic mutations affecting telomere function. Two others companies are reaching out directly to consumers and healthcare providers: Blackburn and Harley teamed up to launch Telome Health in March 2011; Blasco and colleagues founded Life Length in Madrid in December 2010. Indeed, Life Length claims to be able to measure 'biological age' and recommends annual measurements of telomere length.

The customer focus of these latter companies has been the subject of debate. "People want to know their telomere length because there are more and more papers being published that show that the lowest percentiles of telomere length have a higher risk for disease," says Blasco. Harley points out that even if the association between telomere length and specific conditions is ambiguous, people might still be able to make lifestyle decisions based on these measurements. "It allows you to monitor your health more closely and work with your doctor to get more specific information about where it is that you're most likely at greatest risk," says Harley. "If I had short telomeres, I would probably be a more compliant patient."

Other scientists are concerned that too little is known about the impact of short telomeres for such a measurement to be meaningful. "It could certainly be useful — I just think it's premature," says Greider.

### MEASURE FOR MEASURE

Clarifying the relationship between telomere length and disease will require more prospective studies of large cohorts of people. "Unfortunately, there's a lot of data out there that I think is very questionable," says Lansdorp. "The studies that have been done are primarily epidemiological." As a consequence, he says, "a lot of these observations may be correlative and indirect".

Methodology is also a point of contention. Telome Health, in common with most epidemiological studies performed to date, employs a DNA amplification-based technique that quickly determines average telomere length within cells, but which might be prone to technical artefacts. Blasco, Lansdorp and others use more time-consuming and costly techniques that use fluorescent probes to examine individual telomeres withina cell, uncovering individual outliers that might be masked by averaging. "Each cell has 92 telomeres, and each telomere has its own unique length," says Armanios. "The shortest telomere is the one that determines the phenotype." These measurements are typically taken from white blood cells, which are easy to obtain and closely reflect the telomere length of the stem cell reservoir from which they emerge; however, it remains unclear whether these measurements reflect telomere length throughout the body.

Telomere length can be affected by environmental factors, further complicating the interpretation of length measurements taken at any one time. Studies led by Blackburn and Elissa Epel at the University of California, San Francisco, have uncovered evidence that telomeres undergo accelerated shortening in direct response to lifestyle and emotional stress, such as from post-traumatic stress disorder or major clinical depression[5]. "When this first came out I was sceptical, but I'm starting to believe a little bit of this stuff now," says Shay. "I'm still on the fence about how much it really means, but I believe it shows that that the shortest telomeres can be modified by environmental stressors."

Shay, who is also a scientific advisor for Life Length, hopes that the data collected by the direct-to-consumer companies will shed light on the influence of lifestyle and other mysteries by providing population-wide data from long-term, longitudinal telomere profiling studies that might otherwise be too expensive to support via public funding.

From DePinho's perspective, efforts to figure out the role of telomeres in cancer and ageing are a race against the clock. "By 2025, there will be 1.2 billion individuals over the age of 60," he says. "We need to understand the circuitry of ageing, and we need to understand how to control it so that we can increase the years of healthy living, diminish age-associated diseases and maintain productivity in our aged population." ■

**Michael Eisenstein** *is a freelance journalist based in Philadelphia, Pennsylvania.*

1. Parry, E.M. *et al. Blood* **117**, 5607–5611 (2011).
2. Sacco, A. *et al. Cell* **143**, 1059–1071 (2010).
3. Tomás-Loba, A. *Cell* **135**, 609–622 (2008).
4. Jaskelioff, M. *et al. Nature* **469**, 102–106 (2011).
5. Wolkowitz, O.M. *et al. PLoS One* **6**, e17837 (2011).