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GUEST EDITORIAL

2009 Nobel Prize in Physiology or Medicine: telomeres and telomerase

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Elizabeth H Blackburn, Carol W Greider and Jack W Szostak were acknowledged with this year's Nobel Prize in Physiology or Medicine for their discoveries on how chromosomes are protected by telomeres and the enzyme telomerase.

In the first half of the twentieth century, classic studies by Hermann Müller (Nobel Prize 1945) working with the fruit fly (*Drosophila melanogaster*) and by Barbara McClintock (Nobel Prize 1983) studying maize (*Zea Mays*) proposed the existence of a special structure at the chromosome ends (Müller, 1938; McClintock, 1939). This structure would have the essential role of protecting chromosome ends from fusing to each other, thus ensuring the correct segregation of the genetic material into daughter cells during every cell division cycle. Such structures, which Müller called telomeres (from the Greek *telos* 'end' and *meros* 'part'), conferred identity to the ends of chromosomes such that the cell could distinguish between native chromosome ends and the DNA fragments resulting from double-strand breaks, which must be fused back together to safeguard the integrity of the genetic material (McClintock, 1941). This protective function of telomeres is known as 'telomere capping'. The molecular mechanisms underlying telomere capping involve specialized protein complexes bound to telomeres (Blackburn, 2000, 2001, 2005; Blackburn *et al*., 2006) as well as telomere-associated noncoding RNAs known as TERRA (reviewed by Schoeftner and Blasco, 2009a,b; Luke and Lingner, 2009). If telomere capping is disrupted, telomere fusions generate dicentric chromosomes that are susceptible to breakage during mitosis, ultimately leading to aneuploidy and disease states including premature aging pathologies and cancer (Hackett *et al*., 2001; Hackett and Greider, 2002).

In 1961, Leonard Hayflick discovered that human cells could undergo only a limited number of cell divisions when cultured *in vitro* (Hayflick and Moorhead, 1961), a phenomenon known as replicative senescence or the Hayflick limit (reviewed by Hayflick, 1998). In fact, this is another feature of telomeres; their length determines the number of cell divisions that a cell can undertake. Alexei Olovnikov linked the Hayflick limit to the replication of telomeric DNA (Olovnikov, 1971, 1973). Replication of the 5′-3′ strand requires RNA primers that are removed afterward, leaving gaps. These gaps are filled-in using the adjacent Okazaki fragments as primers. The very terminal gap at the 5′ end of telomeres cannot be filled because of the lack of such a primer. Olovnikov proposed that the DNA replication machinery could not copy chromosomal ends completely and, therefore, cells could not compensate for the chromosomal shortening produced associated with cell division, suggesting that progressive telomere shortening may be a key factor to limit the number of cell divisions. James D Watson (Nobel Prize 1962) also recognized that the unidirectional nature of DNA replication was a problem for the complete copy of chromosomal ends (Watson, 1972). This was called the 'end-replication problem'. In this manner, during each cycle of cell division, a small fragment of telomeric DNA is lost from the end. After several rounds of division, telomeres eventually reach a critically short length, which activates the pathways for senescence and cell death (Hermann *et al*., 2001; Samper *et al*., 2001).

Uncovering the solution to the end-replication problem took several years of intense research. In the 1970s, several models speculated that chromosome ends contained inverted repeats, which would generate hairpins and intermediate replicative structures, able to bypass the end-replication problem (Cavalier-Smith, 1974; Bateman, 1975; Dancis and Holmquist, 1979). In 1978, Blackburn and Gall showed that the telomeres of the ciliated-protozoan *Tetrahymena thermophila* comprised a hexanucleotide tandomly repeated sequence (CCCCTT), which was variable in length (Blackburn and Gall, 1978). *Tetrahymena* had a number of advantages for the study of telomeres, among them that it contained linear (and circular) extrachromosomal rDNA molecules with palindromic termini, which were replicated in the cell nucleus. In addition, these terminal sequences were well characterized (Blackburn and Gall, 1978). For these reasons, Blackburn and her collaborator Szostak decided to further use it for their studies. They showed that the terminal-repeated sequence of *Tetrahymena* could protect a linear plasmid from degradation in yeast. Thus, these repeated sequences conferred telomere function. In addition, they showed that hairpin structures alone were not sufficient to provide telomere function (Szostak and Blackburn, 1982). Importantly, their discoveries also indicated that the structural properties of telomere function were evolutionarily conserved from yeast to *Tetrahymena* (Fungi to Animalia kingdoms, respectively).

Blackburn had shown that *Tetrahymena* telomeres varied in length (Blackburn and Gall, 1978) and that they could be lengthened when cells were grown long term in logarithmic phase. Moreover, this lengthening of the telomeres was always attributable to an increase in the number of hexanucleotides repeats. Blackburn and co-workers reasoned that the conventional replication system was not likely responsible for the extra addition of telomere repeats and that recombination was not required for telomere lengthening (Dunn *et al*., 1984). On the basis of these considerations, they proposed that telomere replication should involve a terminal

transferase-like activity able to add telomeric repeats onto chromosome ends *de novo* (Shampay *et al*., 1984). In 1985, Carol W Greider, a PhD student working in Blackburn's laboratory, first showed the existence of a transferase-like activity in *Tetrahymena* cell-free extracts, which accurately added telomere repeats *de novo* onto chromosome ends (Greider and Blackburn, 1985). Two years later, Greider and Blackburn (1987) purified the enzyme and showed that it was a ribonucleoprotein complex, being both components (RNA and protein) required for activity. Thus, they had discovered a new type of DNA polymerase, which they called telomerase. Cloning of the *Tetrahymena* telomerase RNA component in 1989 further suggested that it contained the template for the addition of repeats onto telomeres (Greider and Blackburn, 1989). Thus, telomerase was a new type of reverse transcriptase, specialized in telomere replication. Although most of these discoveries were made in the very unusual organism *Tetrahymena*, we now know that the enzyme telomerase is the main mechanism used to elongate telomeres throughout the eukaryotes. This mechanism is widely conserved among higher eukaryotes, with the exception of *D. melanogaster*, whose chromosome ends consist of small retrotransposons (Pardue and DeBaryshe, 2003; Chan and Blackburn, 2004). Mutation of telomerase components in yeast (Lundblad and Szostak, 1989) first showed that telomerase was responsible for telomere length maintenance in this organism. Later on, generation of the first knockout mice for the telomerase RNA component (Blasco *et al*., 1997) showed a conserved role for telomerase as the main activity responsible for maintaining telomere length in mammals, and further uncovered its importance in tissue renewal and organismal life span. In the absence of telomerase, mice suffered an accelerated rate of telomere shortening associated with mouse aging, which led to premature development of age-associated pathologies and a reduced longevity (Lee *et al*., 1998; Herrera *et al*., 1999). Indeed, telomerase-deficient mice could only be bred for a limited number of generations, showing the essential function of telomerase for the maintenance of the species. Interestingly, in the absence of telomerase, both yeast and cultured mammalian cells can maintain telomeres through activation of alternative mechanisms based on recombination, also known as ALT in mammalian cells (Bryan and Reddel, 1997); however, ALT is not sufficient to grant organismal fitness and species propagation in mammals.

Very similar to *Tetrahymena* telomeres (formed by tandem repeats of the TTTGGG sequence), vertebrate telomeres consist of TTAGGG repeats, which end in a 3′ overhang of the G-rich strand. In turn, telomeric repeats are bound by a six-protein complex known as shelterin, whose components have functions in chromosome protection and control of telomere length (De Lange, 2005). Proper telomere function requires a minimal telomere length, the integrity of the shelterin complex, and a higher-order DNA structure called the T-loop, which is proposed to protect the 3′ overhang from degradation and DNA repair activities (Griffith *et al*., 1999; Blackburn, 2001; de Lange, 2005; Blasco, 2005). In addition, early studies with yeast showed that telomeres are able to create a repressive chromatin environment able to silence nearby genes, a phenomenon known as 'telomere position effect' (Gottschling *et al*., 1990; Palladino *et al*., 1993; Cooper *et al*., 1997), suggesting analogies with other silenced chromatin domains in the genome. Telomere position effect was later shown to operate in higher eukaryotes (Baur *et al*., 2001). More recently, mammalian telomeric and subtelomeric regions were found to be enriched in repressive histone modifications and heterochromatin protein 1 binding, whereas subtelomeric DNA was found to be densely methylated (reviewed by Blasco, 2007). These heterochromatic marks are proposed to negatively control telomere length and telomere recombination (reviewed by Blasco, 2007; Schoeftner and Blasco, 2009). In particular, decreased heterochromatin protein 1 binding to telomeres, as well as a decrease in the density of trimethylation of histone 3 at lysine 9 and trimethylation of lysine 20 of histone 4 in cells deficient for the Suv39 H1 and H2 histone methyltransferases, leads to substantial telomere elongation (Garcia-Cao *et al*., 2004). Furthermore, loss of subtelomeric DNA methylation also results in telomere length increase (Gonzalo *et al*., 2006). In both studies, the loss of repressive marks correlated with increased recombination rates, suggesting that heterochromatic marks prevent promiscuous telomere-recombination events and control telomere length. In addition to being enriched in heterochromatic marks, telomeres are bound by telomere-associated noncoding RNAs called TelRNAs or TERRAs (Azzalin *et al*., 2007; Azzalin and Lingner, 2008; Schoeftner and Blasco, 2008, 2009a, b), which have been proposed to exert a negative control over telomere length based on their ability to behave as potent telomerase inhibitors *in vitro* (Schoeftner and Blasco, 2008).

Telomerase activity is undetectable in somatic tissues, except in stem cell compartments (reviewed by Flores *et al*., 2006), but the level of activity is not sufficient to prevent telomere shortening associated with cell division, tissue renewal and age as first shown by Harley *et al*. (1990). Because telomeres are necessary for genome integrity, their shortening was also proposed to cause cell senescence or apoptosis in the so-called 'telomere hypothesis' (Harley *et al*., 1990). Confirmation for this hypothesis in *in vitro* cultured cells came when reintroduction of the TERT telomerase catalytic component into telomerase-negative primary human cells was sufficient to bypass replicative senescence (or the Hayflick limit) and to confer immortal growth (Bodnar *et al*., 1998). In addition, the generation of the telomerase knockout mice showed *in vivo* that short telomeres could cause multiple organismal defects associated with decreased adult stem cell functionality (Blasco *et al*., 1997; Lee *et al*., 1998; Herrera *et al*., 1999; Flores *et al*., 2005; Hao *et al*., 2005). These organismal defects were more pronounced with each following generation, known as 'genetic anticipation'. Interestingly, some patients characterized by poor telomere maintenance due to mutations in telomerase components

(for example, dyskeratosis congenita, aplastic anemia, pulmonary fibrosis) also show disease anticipation (Mitchell *et al*., 1999; Collins and Mitchell, 2002; Mason *et al*., 2005), highlighting the similar phenotypes associated with telomere dysfunction in mice and humans.

All these findings gave rise to the interesting idea that increasing telomerase activity in somatic cells could have a positive effect, enlarging the life span of the organism. The generation of three mouse models with increased telomerase activity showed a greater susceptibility than wild-type to tumor formation, with a higher rate of mortality (Gonzalez-Suarez *et al*., 2001; Artandi *et al*., 2002; Canela *et al*., 2004), in agreement with telomere shortening representing a barrier for the uncontrolled growth of cancer cells. Interestingly, these mice also showed improved tissue regeneration and those that did not develop tumors displayed an increased life span (González-Suárez *et al*., 2005). Particularly, overexpression of the telomerase catalytic component TERT increased the capacity of epidermal stem cells to regenerate skin and hair (Flores *et al*., 2005; Sarin *et al*., 2005). However, the function of telomerase in organismal aging remained elusive until recently, in part due to the cancer-promoting activity of telomerase. To circumvent this problem, Blasco and co-workers constitutively expressed TERT in mice engineered to be cancer-resistant by means of enhanced expression of the tumor suppressors p53, p16 and p19ARF. In this context, TERT overexpression improved the fitness of tissues and produced a systemic delay in aging accompanied by extension of the median life span (Tomás-Loba *et al*., 2009). These results showed that constitutive expression of *Tert* provides anti-aging activity in the context of the organism.

Short telomeres are a barrier for cell division; therefore, it is not surprising that most cancer cells activate telomerase during tumorigenesis to maintain a minimum telomere length, even if telomeres are not lengthened (Counter *et al*., 1994; Kim *et al*., 1994, Harley, 2008). Indeed, some human tumors show amplification of both telomerase genes, *TERC* and *TERT*, suggesting that telomerase supports tumor growth, allowing cells with oncogenic mutations to divide (Kolquist *et al*., 1998; McKay *et al*., 2008; Rafnar *et al*., 2009; Shete *et al*., 2009; Stacey *et al*., 2009). Conversely, the lack of telomerase activity in most somatic cells works as a tumor suppressor mechanism. Furthermore, telomerase inhibition stops cancer cell growth (Li *et al*., 2005) and telomerase knockout mice with short telomeres are resistant to carcinogenesis (González-Suárez *et al*., 2000) even in backgrounds with higher susceptibility to tumor emergence, such as mice defective for INK4a/Arf, p21, ATM, Ku, DNAPK or PMS2 (Greenberg *et al*., 1999; Wong *et al*., 2003; Espejel *et al*., 2004; Choudhury *et al*., 2007; Siegl-Cachedenier *et al*., 2007). Nonetheless, primary cells from telomerase-deficient mice can also form tumors, maintaining their telomere length by recombinational mechanisms (Blasco, 2007; Hande *et al*., 1999; Herrera *et al*., 2000; Morrish and Greider, 2009). Interestingly, increasing evidence supports the link between stem cell biology and cancer, as some cancers emerge from transformation of normal stem cells (Bell and Van Zant, 2004; Blasco, 2005; Flores *et al*., 2005).

Recently, a further link between telomere biology and cancer was made by showing that subtelomeric regions are normally hypomethylated in human cancer cells and that this epigenetic defect influences both telomere length and telomere recombination (Vera *et al*., 2008). These findings indicate that epigenetic status of telomeres is altered during tumorigenesis, thus influencing telomere maintenance mechanisms in cancer cells. In addition to hypomethylation of subtelomeric DNA, many human cancers show decreased levels of telomere-associated TERRA (Schoeftner and Blasco, 2008), something that has been proposed to favor telomere elongation based on the observations that TERRA are potent telomerase inhibitors *in vitro* (Schoeftner and Blasco, 2008).

In summary, the early discoveries on telomere structure and function by Blackburn, Greider and Szostak unveiled a route to understanding the molecular bases underlying aging, stem cell biology, cancer and other human diseases. These topics remain of much interest and under intense investigation. Since those days, Elizabeth H Blackburn (professor at the University of California, San Francisco) and Carol W Greider (professor in the Johns Hopkins University of School of Medicine in Baltimore) continue studying the pathways that control telomere maintenance, function and its impact in human health and disease. Jack W Szostak (professor at Massachusetts General Hospital in Boston) switched focus to the fascinating question of the origin of life. He investigates threose nucleic acid molecules, progenitors of RNA and 'steady-state' replication, as well as the primitive formation of cell compartments, cell growth and cell division.

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