Abstract

Chromosomes end are capped by protective structures that maintain the integrity of the genetic material and play key roles in aging and cancer. These structures, termed telomeres, are protein-DNA complexes, their length being critical to their function. The filamentous fungus, Aspergillus nidulans, possesses very short and tightly regulated telomeres, but nothing is currently known about the mechanisms of telomere length regulation. A telomere-binding protein, POT1, has been identified in A. nidulans, and it has been hypothesized to bind to the 3’ overhang of the telomeres and function in length regulation and protection. However, no studies have been conducted to determine its importance to telomere maintenance in A. nidulans until now. By altering a previous method, I have developed a new approach to measuring the C-rich strand length of telomeres. Results show that the POT1 mutant displays heterogeneous telomere length at both the G-rich and C-rich strands, which is evidence when compared to the tightly regulated telomeres of the wild-type.

Introduction

Curiously is the force behind continuous discovery and an apparent constant in human nature. It is a quality that we all have possessed and something that has steered me to become a student of science and the scientific method. Scientific research is the result of natural causes, an outlet for this curiosity. In the development of our understanding of the world, there is nothing more intriguing than the unknown, a reality suggested in Mein Weckbote by Albert Einstein, one of the greatest minds to ever live. 

The most beautiful thing we can experience is the mysterious. It is the source of all true art and all great knowledge. Whosoever this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead: his eyes are closed. (Einstein, 1934).

In science, we constantly question mysterious phenomena. As we pursue this question, the many theoretical answers we gain a better understanding of the world around us, helping improve our quality of life. Our development as a people. With each theoretical answer comes endless novel questions, one of which I hope to answer in the following thesis.

Abstract

Telomerase and Aging

Telomeres are the reason why this mechanism of replication does not present an irrevocable problem for future generations of the cell. They are the protective caps found at both ends of eukaryotic chromosomes and are necessary for the stability of genetic information. Telomeres are short, specific, non-coding DNA-protein structures composed of specific repeating sequences of T, A, G, and C nucleotides—the same nucleotides that make up our DNA.

Telomeric DNA sequence and structure is similar across all species, even in the most widely divergent species. An example of this would be the telomeric repeat of 5’-TTAGGG-3’ (which is found in all vertebrates (Myers, Rafitt, & Moyzis, 1989), and numerous other species including slime molds (Muller, 1939), and some species of fungus, such as Aspergillus nidulans (Bhattacharya & Blackburn, 1997, Kasumoto, Suzuki, & Kashiwagi, 2003) and A. nidulans (Olovnik, 1973). However, this phenomenon is not the same across all eukaryotes, for example, Tetrahymena thermophila has the telomeric repeat of 5’T-TGGGG-3’ (Blackburn & Gal, 1978).

In addition to this, telomere sequence repetition, the average length of telomeres differs across organisms as well. For example, human telomere lengths range from 1000-2000 bp. or 250-1000 telomeric repeats (Moyzis et al., 1988), whereas telomeres of Aspergillus nidulans are about 110 bp (Bhattacharya & Blackburn, 1997; Vahedi Thesis, 2005). This variation is seen within a species and a potential indicator of how many times a cell could divide. 

Telomeres are a buffering zone, allowing chromosomes to be replicated completely, without the loss of important terminal bases at the 5’ end of each strand, leaving coding DNA intact (Olovnikov, 1973). This function, however, is only one of the many functions of the telomere, and I am intrigued by the observation that telomere length negatively correlates with age (Jiang et al., 2008; Song et al., 2008). Telomere length and lifespan in humans, but also to telomere length and aging of an organism are linked to changes in genomic DNA replication. Older cells showed significantly shorter telomeres than younger cells, suggesting for the first time that cellular aging could be linked to changes in genomic DNA replication (Harley et al., 1990). Even after these convincing results achieved by Hayflick et al. (1961), more positive evidence was needed in order to confirm that telomere length represented the cell’s biological clock. This necessary evidence was found in a study done by Boehnig (1998). In their research, they transfected human fibroblasts with TERT. This protein is a key component of the telomerase enzyme, which catalyzes the lengthening of telomeres and is normally absent or present at very low levels in human somatic cells. These TERT+ cells that had elevated telomerase levels showed elongated telomeres and were maintained in culture for an average of about 90 population doublings. In contrast, fibroblasts that contained telomeres and senesced after about 55 population doublings (Boehnig et al., 1998). The fact that the cell span was extended through longer lengths of time with no signs that telomeres were directly tied to cellular aging.

Analysis

The data presented show a linkage between telomere length and cellular function, suggesting that telomere length may be an indicator of the health of the cell. This is supported by the finding that shorter telomeres correlate with cellular senescence, as determined by the p53 tumor suppressor gene. The p53 gene is activated when DNA damage occurs, and the cell cycle is arrested, preventing further cell division and allowing repair of the damaged DNA. If the DNA damage is too severe, the cell may undergo apoptosis, or programmed cell death, to prevent the propagation of genetic mutations. The p53 gene is also involved in regulating telomere length, as it can bind to telomeric DNA and induce telomerase activity. This mechanism ensures that telomeres are maintained at a constant length, which is necessary for proper chromosome function. The p53 gene is therefore a key regulator of telomere length and cellular senescence, and its activation is a critical checkpoint in the cell cycle that prevents the propagation of genetic mutations and maintains genomic stability.

Conclusion

Thus, I conclude that telomere length is a predictive indicator of cellular aging and may be an important factor in determining overall health and lifespan. Further research is needed to fully understand the molecular mechanisms underlying telomere length regulation and its relationship with cellular senescence. The development of new strategies for maintaining telomere length may provide novel therapeutic targets for the prevention and treatment of age-related diseases. This research is critical for advancing our understanding of cellular aging and developing potential interventions to extend healthy lifespan.
that individuals with shorter telomeres had a lower survival rate that was caused by an assortment of infectious diseases and heart disease. Additional studies have also been done linking accelerated aging to diabetes (Sampson et al., 2006), heart failure (Van der Harst et al., 2007), osteoporosis (Virmani et al., 2007), and an increased cancer risk (Wu et al., 2003). From these observations it has been suggested that telomere length may serve as a biomarker determining general health, lifespan, and the pace of biological aging (Babichayk et al., 2011).

Telomeres, Cancer, and Smoking

As explained previously, telomeres in human somatic cells shorten with each DNA replication event. This is because the enzyme that lengthens telomeres, telomerase, is normally absent or present at very low levels in these cells. Once a cell's telomeres reach a critical length, a DNA damage response is activated before the genetic material can be compromised, and the cell enters a senescent state (Harris & Levine, 2005). One of the speculations as to why organisms age has to do with the fact that cells enter this senescent state where they continue to live and function, but no longer go through cell division (Jeyalaya & Siddiq, 2009). Thus, aging would occur as a result of fewer new daughter cells being produced.

However, a problem arises when telomeres reach a critically short length, and this DNA damage response does not get activated. In these cases, senescence does not occur and the cell continues to divide but with severe telomere shortening, marked genetic instability, and massive cell death; this event is commonly called crisis. Most cells going through crisis die due to the occurrence of chromosomal abnormalities and the near complete loss of telomeres (Harris & Blackbum, 1996). However, as a result of possible mutations and chromosomal alterations that occur during crisis, some mutated cells survive that possess the ability to maintain their telomere length, either through the activation of telomerase or through an alternative pathway. This is known as crisis-induced telomere stabilization of genetically compromised cells in the crisis state contributes to the formation of cancers (Chin et al., 1999). These mutated cancerous cells divide rapidly, maintain very short telomeres, and are immortal, leading to the formation of malignant tumors. This claim is supported in a study by Griffith et al. (1997), they found that almost all malignant tumor cells showed upregulated telomerase activity. Other research has also shown that cancer cells line possess telomeres than regular somatic cells (Shammas, 2011). Therefore, we should naturally want to maintain the length of our telomeres as long as possible, as we are at risk for many different life-threatening diseases once they get too short.

In order to maintain telomere length, one must avoid factors that can contribute to their accelerated shortening. There are many different components that have been seen to speed up the shortening process, including obesity (Furukawa et al., 2004), pollution (Hoxha et al., 2009), and stress (Furukawa et al., 2004), as well as increased oxidative stress in the body (Furukawa et al., 2004). It has been speculated through research that these factors cause telomere shortening due to a heightened exposure to agents that cause telomere damage. With respect to pollution, the intake of genotoxic agents causes damage to telomeres, increasing their shortening process (Hoxha et al., 2009; Pavaniello et al., 2010). Obesity, stress, and diets high in fats and proteins have been seen to cause high oxidative stress in the body (Furukawa et al., 2004; Epp et al., 2004; Pavaniello et al., 2010). This increased amount of free radical species causes direct damage to telomeres, increasing the pace of their shortening. Furthermore, it has been seen that exercise has positive effects on telomeres.

Research done by Werner et al. (2009) on the leukocytes of athletes showed they had increased telomerase activity and longer telomeres in comparison to non-athletes. The telomere connection between exercise and telomere length is thought to be mediated through the activation of telomerase or through an alternative way of telomere stabilization (McEachern & Blackbum, 1996; Stewart & Weinberg, 2006). This has led to the speculation that exercise may serve as a biomarker determining general health, lifespan, and the pace of biological aging (Babichayk et al., 2011).

Telomerase is a ribonucleoprotein because it is an enzyme that possesses both a protein component and a RNA component. The protein component is known as the Telomerase Reverse Transcriptase (TERT) and the RNA component is known as the Telomerase RNA (TER). In order for telomerase to elongate telomeres, it first must localize to the 3' end of the chromosome. After binding, telomeric repeats are added by TERT complementary to the template present in the TER component. For example, if the template region of TER contains the sequence 5'-CCUCA3-3', it will add a sequence repeat that is complementary to the template region of TERT. Once a telomeric repeat has been added to the 3' end of the G-rich strand, telomerase realizes its TER template to the end of the sequence in order to synthesize an additional telomeric repeat, continuing elongation of the telomere (Figure 2).

Shelterin: The Master Regulator

Telomere functions by extending telomeres, but this extension cannot go on infinitely. This activity is understood with the discovery of telomere and binding proteins, which have been shown to function in the telomere length regulation system of different organisms. At the vertebrate telomere, a six-protein complex termed shelterin is found that functions to protect the end of the telomere (de Lange, 2005). The three main shelterin protein subunits, which directly associate with repeats at the end of the telomere, are TRF1, TRF2, and POT1. These proteins are interconnected by three additional proteins, TR2, TP1, and Rap1 (de Lange, 2005). Research has shown that these components localize specifically to telomeres, are abundant at the ends of the cell cycle, and only function at the telomeres (Palm & de Lange, 2008). With each protein having a unique role towards the intersection system, this six-protein complex, as a whole, has been observed to control access of telomerase to the telomeres and allow cells to distinguish telomeres from DNA damage sites (Figure 3) (Palm & de Lange, 2008).

T-loop

T-loop is in addition to the protective roles of the shelterin complex, there is also the formation of a t-loop structure (Griffith et al., 1999). In eukaryotes, the telomere is not blunt ended, but instead has a 3' single-stranded G-rich overhang which is required for telomeric extension by telomerase (Makarov et al., 1997; Froelich-Ammon et al., 1998). The current prediction on formation of the T-loop is that it includes processing, or resection, of the telomeric G-rich strand by an unknown nuclease (Makarov et al., 1997). T-loops form when the single-stranded 3' overhang of the telomere invades the duplex region of the telomere, displacing the G-rich strand, forming a D loop (displacement loop) (Figure 4) (Griffith et al., 1999). It is not yet known to form in vertebrates (Griffith et al., 1999), but also in ciliates (de Lange, 1996). It is possible in hind the chromosomal end from DNA damage responses.
binding occurred through the presence of OB-folds, three in the α strand and two in the β strand, which bound to, and protected the G-rich 3' overhang during stranded DNA damage, as opposed to ATM, which occurs during issues with replication forks, including single-stranded DNA damage, as opposed to ATM, which occurs during issues with replication forks.

This theory was proven correct in future CHIP studies, which proved that RNA was loaded onto telomeres during telomere degradation and chromosome end fusions (Baumann & Cech, 2001). In further support of its function, it was also observed that this protein, now referred to as POT1, bound the G-rich overhang but not the C-rich strand or double-stranded region of the telomeres in vitro (Tijoluo et al., 2005). As further studies were performed, scientists realized that this protein had not only telomere end protection, but also in telomere length regulation, something that was consistent with ciliate TEBP and budding yeast Cdc13 proteins (Munro et al., 2004).

The next discovery was that of a human protein that was hypothesized to have similar functions to the fusion yeast POT1 due to a conserved sequence. However, when knockdown experiments were done with human POT1, mainly telomere elongation occurred (Ye et al., 2004). This result was puzzling to researchers who expected telomere degradation and chromosome end fusions. However, in this experiment, it was concluded that the function of POT1 in humans must have closer ties to telomere length regulation, rather than protection of the G-rich end, because POT1 with POT1 discovered another interesting quality. In humans, it was found that the telomeres in the C-rich strand of the human POT1 protein, which bound to, and protected the G-rich 3' overhang during stranded DNA damage, as opposed to ATM, which occurs during issues with replication forks, including single-stranded DNA damage, as opposed to ATM, which occurs during issues with replication forks.

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prior observations, I hypothesize that a significant increase in telomere length will be seen in the A. nidulans POT1 mutant when compared to the short and tightly regulated telomeres of the wild-type strain. I predict that this increase in telomere length will be seen at both the G-rich and C-rich strands.

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