PREFACE

Telomere is the nucleoprotein complex located at the endmost chromosomal terminus and one of the essential cis-acting structural elements of chromosomes. Telomeres are composed of DNA, proteins, and RNA. In this book, the authors present current research in the study of the biological functions, sequencing and aging of telomeres. Topics discussed include the biology of telomeres in multipotential stromal cells; telomere maintenance in the blight fungus ustilago maydis; telomere length in lymphoid malignancies; unraveling the genetic basis of the alternative lengthening of telomeres and the adjustment of telomeres by cell-matrix interaction.

Chapter 1 - Multipotential stromal cells, also known as mesenchymal stem cells (MSCs), reside within the bone marrow (BM) and other skeletal and connective tissues. They serve as a reserve of progenitors for continuous cell replacement during normal tissue homeostasis. Furthermore, MSCs are responsible for production of replacement cells lost as a result of acute or chronic injury to bone, cartilage, tendon, meniscus and other connective tissues and are involved in physiological bone remodeling. Loss of MSC function is apparent in the older individual, potentially contributing to the development of osteoporosis and osteoarthritis; dyskeratosis and Werner's syndromes are also linked to premature aging of skeletal and connective tissues. Telomere attrition has been proposed as a possible mechanism for the loss of MSC function, and may represent a therapeutic target in these diseases. MSCs can be culture-expanded with relative ease and are already used therapeutically to repair cartilage and bone defects following injury. Also, because of their well-described immunoregulatory capacity, MSC therapy is used to treat patients with graft-versus-host disease. It is therefore very important to monitor telomere dynamics during MSC manufacture, for the purpose of controlling their therapeutic potency and potential for spontaneous transformation. The authors will outline the current understanding of MSC ageing *in vitro* and observed telomere loss during culture expansion, primarily focusing on MSCs derived from adult tissues (BM, adipose and dental pulp), but also discussing perinatal tissues such as umbilical cord. Various telomere length measurement methods will be described and compared, in terms of their utility and practicality. Safety concerns relating to genetic manipulation of MSCs to extend their proliferation span, and the subsequent quantity of manufactured MSCs will be discussed. Finally, the authors evaluate *in vivo* MSC ageing compared to that of hematopoietic stem cells highlighting both similarities and differences, which in the authors' view stems from the current lack of robust methods for native MSC isolation directly from skeletal tissues. This combined knowledge should undoubtedly advance the authors'

understanding of MSC function during ageing and is likely to contribute to the development of novel therapies for age-related skeletal diseases.

Chapter 2 - Telomeres, the ribonucleoprotein complexes located at the chromosomal termini, are committed to cap and protect the endmost chromosomal sequences from being recognized as double strand breaks by the DNA damage repair machinery. Telomeres are synthesized by the functions of the telomerase, a ribonucleoproteic enzyme-complex with reverse transcriptase properties that maintains telomere length homeostasis in eukaryotic cells. In *Ustilago maydis*, a basidiomycetous phytopathogen that infects many plant species of the *Zea* genus, the telomere is composed af a TTAGGG repeated motif, which is commonly found in vertebrates and other fungal species. Both the telomere organization and the characterization of the telomerase catalytic subunit have been characterized in this fungus. Adjacent to the telomere are mosaics of sequences, which are largely species-specific and commonly termed telomere-associated sequences (TAS), The TAS are not fully understood and require further characteriation. *UTASa*, a class of TAS in *U. maydis,* harbors an open reading frame with homology to RecQ enzymes. Those sequences resemble a long non-coding RNA (lncRNA) and exhibit a variable expression pattern during the cell cycle in synchronous cell cultures. The authors propose that this type of RecQ-like sequence may be part of a surveillance mechanism that operates in the absence of telomerase.

Chapter 3 - Human telomeres are nucleoprotein complexes located at the end of chromosomes, composed of tandem repeats of the non-codificant DNA sequence TTAGGG. A core of six telomere binding proteins, the shelterin complex, serves to protect telomeric ends and to prevent recognition of chromosome ends as damaged DNA. Both critically short telomeres and direct disruption of the shelterin structure can initiate telomere dysfunction. The enzyme telomerase is a ribonucleoprotein that compensates the telomere reduction by adding new repeats to chromosome ends. Different studies have demonstrated that reduced telomere length (TL) contribute directly to genomic instability, usually found in several types of cancer. Short telomeres and high telomerase activity have been associated with tumorigenesis in patients with hematological neoplasm. Lymphoid malignancies constitute a heterogeneous group of lymphoproliferative disorders that represent distinct disease entities, according to their clinical, morphological, immunophenotypic and genetic features. In this study, the authors analyzed the TL in 262 patients with different B-cell malignancies and its association with genetic characteristics and telomere-associated proteins expression. TL was highly heterogeneous and significantly reduced in B-cell disorders compared to normal controls. Patients with diffuse large B-cell lymphoma secondary to follicular lymphoma (DLBCL-S) had the shortest telomeres, followed by follicular lymphoma (FL). Mantle cell lymphoma (MCL) and *de novo* DLBCL showed similar mean TL, whereas monoclonal gammopathy of undetermined significance (MGUS) and chronic lymphocytic leukemia (CLL) cases displayed the longest TL. Mean TL for multiple myeloma (MM) were intermediate among those entities. Significant shorter TL in MM and CLL patients with chromosomal abnormalities with respect to cases with normal karyotypes were observed ($p<0.03$). In FL, a significant difference between *BCL2* positive and negative cases was found ($p<0.05$). The analysis of telomere-associated proteins expression was performed in MGUS and MM. A significant increase of *TRF1* levels and a lower expression of *TRF2* and *TANK1* in MGUS with respect to MM patients were found ($p=0.006$). The analysis of TL according to *hTERT* expression showed that patients with the highest mRNA values had the shortest TL. These findings are of interest considering the new therapeutic approaches with diverse

telomere-targeting inhibitors and suggest that a number of patients with B-cell malignancies may be particularly sensitive to telomere-damage drugs.

Chapter 4 - Telomeres are specialized nucleoproteic complexes localized at the physical ends of linear eukaryotic chromosomes that maintain their stability and integrity. In mammalian cells, telomeres consist of tandem arrays of the repetitive sequence (TTAGGG)n, oriented 5′ to 3′ towards the end of the chromosomes and associated proteins, and a large non-coding RNA (named TERRA) which forms an integral component of telomeric heterochromatin. Telomeres provide a protective "cap" for chromosomal DNA against illegitimate recombination, exonucleolytic attack and degradation, and oxidative damage. The main role of telomeres is to preserve the integrity of the chromosomes, protecting them from degradation, recombination or fusion, by preventing the ends of linear chromosomes from being recognized as DNA double-strand breaks (DSB) by the DNA repair machinery, i.e., they distinguish natural DNA ends from DNA ends resulting from breakage events. Besides the abovementioned function, telomeres regulate the replicative life span of somatic cells, contribute to maintenance of chromosome topology in the cell nucleus, play a fundamental role in the proper alignment of chromosomes for recombination during the first meiotic prophase, assure accurate chromosome segregation during mitosis, and silence genes flanking the telomere repeat sequence, a phenomenon called the "telomere position effect". The maintenance of telomere function is crucial for genomic stability and cell viability. Cells respond to dysfunctional telomeres by undergoing senescence, cell death, or genomic instability. Moreover, telomere dysfunction has been identified as a primary mechanism involved in the chromosomal instability observed in cancer cells. Cellular response to dysfunctional telomeres is governed by proteins that also control the DNA damage response. In addition, several aspects of telomere form and function are modulated by the enzymes helicases. The present review offers an overview of the many biological functions of telomeres in mammalian cells.

Chapter 5 - Telomeres are present at the ends of all eukaryotic chromosomes. Human telomeres play an important role in critical processes underlying genome stability, cancer, and aging. Recent studies suggest that telomeres may form higher order structures to provide critical protection of chromosomes. Telomerase or its telomere DNA substrate was first recognized as a unique and exciting anticancer target. A large number of approaches have been developed for targeting human telomeres and telomerase for cancer therapy. For a long time, telomeres have been considered transcriptionally silent. A recent finding demonstrated that telomere DNA is transcribed into telomeric repeat-containing RNA (referred to as TERRA) in mammalian cells. The existence of TERRA aRNA may reveal a new level of regulation and protection of chromosome ends that could facilitate valuable insight into fundamental biological processes such as cancer and aging. Revealing the structure and function of telomere RNA will be essential for understanding telomere biology and telomere-related diseases. In this chapter,the author provides an overview of the advances in understanding of the structures and functions of human telomeres. Next, he focuses on the current efforts on developing various approaches to targeting human telomeres and telomerase for the treatment of cancer. Last, studies on the newly discovered TERRA RNA are discussed in detail. Future perspectives in the field are outlined.

Chapter 6 - Telomere shortening and maintenance plays a crucial role in cancer and aging. About 10% of all cancers use a recombination-based telomerase-independent pathway named ALT which is not fully understood yet. However, in recent years advances have been

made that shed light on both the exact mechanisms that ALT employs and the genes underlying it. A small but interesting body of evidence has been published that points to ALT as a potentially dysregulated form of a physiologically occurring process that takes place during development. Several proteins have been found to be essential for ALT, but none appears to provide a complete explanation yet of when and how ALT is activated in cells undergoing malignant transformation, and why it only accounts for a small proportion of cases. Once the genes and proteins governing the ALT switch are found, proposed treatments that could open up new avenues for intervention in cancer and in the aging process will become possible. In this chapter, the current understanding of ALT activation in humans cells is reviewed with a particular focus on regulatory molecular targets.

Chapter 7 - Aging is classically defined as a state of irreversible growth arrest, accompanied by telomere shortening, cumulative DNA damage and mitochondrial dysfunction. Recently, the authors have reported that the simple change of the extracellular matrix from senescent cells to young cells can induce the senescent phenotype of the aged cells to the young cell like phenotype. In this change of the phenotype, the authorscould observe that the age-related telomere shortening and dysfunction could be restored but without any evidence of telomerase activation. In mammals, several proteins, involved in the nonhomologous end joining (NHEJ) DNA repair pathway, are reported to be telomere-bound and to modulate telomere length maintenance in addition to telomerase. Among them, Ku70 was found to be essential, since Ku70-deficient cells failed to restore telomere length after induction of the cellular phenotype change by alteration of cell to matrix interaction. For the functional modulation of Ku70, its acetylation status was shown to be important, regulated by SIRT1. Therefore, it can be summarized that the interaction between Ku70 and SIRT1 is essential for the restoration of telomere length despite the absence of telomerase activation. These new findings can be applied to adjustment of the aging process.