The maintenance of telomeres, the ends of linear chromosomes, appears to play a crucial role in senescence, aging, and cancer. The stabilization of telomere length is believed to be strongly associated with unlimited proliferation and cellular immortalization. The enzyme telomerase stabilizes telomere length by de-novo adding of telomeric repeats that had been lost by incomplete DNA replication, nucleolytic degradation, and oxidative stress. Since the telomerase is active in the majority of human tumours, thus allowing indefinite growth, it has become an attractive diagnostic and prognostic marker as well as a promising target for therapeutical intervention. Furthermore, telomerase has been talked about as a potential rejuvenation enzyme, counteracting the effects of aging. Telomere length, on the other hand, could serve as a tool for prognosis and predictive parameter in the search for the most promising approach in cancer treatment.

The role of telomeres and telomerase in cancer and aging

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Deciphering the end

- Scientists discovered that in eukaryotic cells the ends of linear chromosomes behave differently to chromosomal breaks as early as the late 1930s. They postulated that these ends form specialized structures which prevent end-to-end fusions and nucleolytic degradation, thus ensuring genomic stability\(^{(1,2)}\). These structures were called telomeres, with reference to the greek words telos, which means end, and meros, which means part.

It took almost another 40 years before the telomeric structure could be deciphered. In 1978, Elizabeth Blackburn and Joseph Gall described the Tetrahymena telomere as consisting of an extremely short and simple sequence of nucleotides – TTGGG – arranged in multiple tandem repeats\(^{(3)}\). Subsequently, this telomeric structure with similar T- and G-rich telomeric repeats was found to be highly conserved in all eukaryotic organisms\(^{(4)}\). However, the number of these telomeric repeats is remarkably heterogeneous in various organisms, as well as in different cell types of the same organism\(^{(5)}\).

Today, we know that telomeres are not merely linear DNA elements, but highly organized nucleoprotein complexes consisting of telomeric DNA and various telomere-associated proteins. At its 3’ end, the telomeric DNA contains a single-stranded overhang which loops back and invades internal telomeric repeats. It thereby forms a well-defined three-dimensional structure, referred to as the t-loop\(^{(6)}\). Formation and maintenance of the t-loop requires the presence of telomere-associated proteins. The most prominent of these proteins are the telomere repeat binding factors TRF\(^1\) and TRF\(^2\). While the involvement of the telomeric proteins in t-loop formation and maintenance is well established, their role in the regulation of telomere length and telomerase activity is still poorly understood. However, considerable progress has been made over the last few of years. Surprisingly, data from such diverse model systems as fission yeast, budding yeast, and even plants also help to increase the information on protection and length regulation of telomeres in mammalian cells.

Telomeres are not stable. Their structure is compromised by a phenomenon referred to as telomere erosion. It is the consequence of the so-called DNA end-replication problem first described by James Watson\(^{(8)}\), who discovered that, due to the nature of DNA polymerase and the mechanism of DNA replication, linear chromosomes cannot be copied to their outermost ends. Alexy Olovnikov\(^{(9)}\) made the same observation. However, he went one step further and proposed that the end-replication

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problem results in the loss of telomeric DNA at each round of cell division. He also suggested that this telomere erosion is the reason why somatic cells cultured \textit{in vitro} have only limited proliferation capacity. This was already observed in the early 1960s: Leonard Hayflick recognized that cultures of primary fibroblasts undergo a finite number of cell divisions before entering a state of irreversible cell cycle arrest, which he defined as replicative senescence or cellular aging\cite{hayflick1960}. When the respective control mechanisms triggering replicative senescence are impaired, cells with dysfunctional telomeres continue to proliferate and enter a period of slow growth – the so-called crisis – characterized by a high degree of genomic instability and, eventually, cell death. If cells find a way to stabilize their telomeres, they overcome the crisis and become immortal and, in some cases, even tumourigenic.

**Telomerase: composition and function**

- In most cell types, telomere maintenance is achieved by the reactivation of the enzyme telomerase. The telomerase was first discovered in 1984 by Elizabeth Blackburn and Carol Greider, who identified this enzyme in \textit{Tetrahymena}\cite{blackburn1984}. Subsequently, it was also found in yeast\cite{gray1984}, mouse\cite{weinrich1987}, and man\cite{reddy1988}. Today, we know that the telomerase is synthesized by almost every organism at least in one period of its lifetime. The exact composition of the enzyme may differ from species to species, but it always contains a RNA component telomerase RNA (TR), which acts as an anchor and template for the telomeric DNA, and a catalytic subunit, the telomerase reverse transcriptase (TERT). TERT catalyses the addition of telomeric repeats to the 3’ ends of the chromosome.

- The human telomerase also contains multiple accessory proteins which bind either to TR or TERT, thus forming a large ribonucleoprotein complex with a molecular weight of more than 1,000 kDa\cite{reddy1988}.

**Are telomeres the genetic clock for aging?**

- Although it was long accepted that telomere length regulates replicative senescence, the role of telomere shortening in this process is now rather controversial. Some researchers found that reintroduction of telomerase inhibits and even reverses replicative senescence, thereby increasing the lifespan of a cell\cite{lewis1995, gross1995, mitchell1995}. Their data suggest that these effects are solely due to the extension or stabilization of the telomeres, and cannot be attributed to exogenous telomerase expression \textit{per se}\cite{lewis1995}. Moreover, inhibition of the telomerase results in progressive telomere shortening, eventually leading to replicative senescence\cite{mitchell1995}. These results imply that telomere shortening is a major mechanism of replicative senescence. However, conflicting data exists and begs the question whether telomere length can be considered as the ultimate biological clock. And if yes, does this refer to the mean telomere length\cite{lewis1995} or to the length of the shortest telomere\cite{mitchell1995}? While telomere length correlates very well with replicative senescence in some cases, in other cases such a correlation does not exist at all and the telomeres in senescing cells shorten at the same rate and are of the same length as telomeres in cycling cells. It is therefore suggested that not telomere length \textit{per se}, but rather proper telomere function is an essential for continuous proliferation. This proper telomere function is only guaranteed when the telomeric structure is maintained. This depends not only on a certain telomere length, but also on the existence and integrity of the telomeric proteins. If they are depleted or mutated, replicative senescence can be induced even if telomeres have not yet reached their critical lengths.

Besides replicative senescence, we must consider a phenomenon re-

\begin{figure}[h]
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\includegraphics[width=\textwidth]{cell_cycle.png}
\caption{The cell cycle. (Reprinted from reference 39 by kind permission of Elsevier Press, Amsterdam, The Netherlands)}
\end{figure}
ferred to as stress-induced senescence. Both processes are characterized by irreversible growth arrest and involve similar or even the same morphological and biochemical changes. Stress-induced senescence is triggered by a variety of exogenous and endogenous acute or chronic stress signals and can occur in young cells which have not yet exhausted their in vitro replicative potential. It was therefore assumed that induction of this type of senescence is not based on telomere shortening. However, there is now growing evidence that stress-induced senescence is accompanied by abrupt telomere erosion\(^{[24,25]}\). This effect may be mediated by homologous recombination involving a circular telomeric structure comparable to the t-loop\(^{[26,27]}\).

So far, senescence has only been found in cell culture and it is a subject of much debate as to whether it also occurs in vivo. Thus it may not be causatively linked to the aging of organisms. In some tissues, however, proliferative capacity is reduced during aging and in at least some cell types, a correlation has been found between telomere length and age. There are some indications that changes occurring at the cellular level with the increasing age of an organism, may play a role in human aging. Some questions still remain unanswered: are these changes a consequence rather than a cause of aging? Are they more liable to be involved in age-related disorders than in aging in general\(^{[28,29]}\)?

Diseases characterized by premature aging such as Dyskeratosis congenital and Werner syndrome, are helpful models for the research on human aging. There is substantial evidence that genetic aberrations – which result in increased rates of telomere erosion and impaired DNA repair functions – cause premature aging by synergistic effects. Surprisingly, it became evident that both processes are closely linked, and that proteins involved in DNA repair are also able to interact with telomeres and telomere-binding proteins, thus contributing to telomere stability\(^{[30]}\). As in transgenic mouse models, human cell lines derived from patients with defective DNA repair pathways also exhibit alterations in telomere maintenance. On the other hand, mice with dysfunctional telomeres also show a decreased DNA repair in response to radiation. Consistent with these findings, inhibition of Poly ADP-ribose polymerases – a protein family involved in DNA repair but also present at telomeres – leads to progressive telomere shortening. However, the telomere repeat binding factor TRF2 plays an interesting and controversial role. On the one hand, TRF2 is upregulated upon UV irradiation, and thus may induce DNA repair. On the other hand, over-expression of TRF2 in basal keratinocytes of mice leads to UV hypersensitivity and skin abnormalities similar to those observed in the human Xeroderma pigmentosum syndrome: these are probably due to impaired DNA repair. These observations suggest that TRF2 might act as DNA damage sensor. Physiological upregulation of TRF2 might induce DNA repair, while stable, unphysiological over-expression of the protein might trigger senescence or apoptosis.

**Telomeres: the guardians of genomic integrity**

- The major function of telomeres is to stabilize chromosomes and to ensure genomic integrity. Telomeric dysfunction leads to end-to-end fusions, resulting in highly unstable dicentric chromosomes. In a continuous »fusion-bridge-breakage cycle«, these unstable chromosomes give rise to chromosomal aberrations. For such end-to-end fusions to occur, the telomeres must come into close vicinity of each other. To define this process and the mechanisms underlying it, studies on the three-dimensional distribution of telomeres within the nucleus have been made. Considerable progress has also been made in this area over the last couple of years, thanks mainly to the steady improvement of optical and computational equipment and the development of new experimental tools.

These studies revealed that, within the nucleus, the chromosomes are arranged in so-called territories, which are characterized by certain morphological, functional, and dynamical features. Telomeres are believed to contribute to the maintenance of these chromosomal territories. Scientists were long of the opinion that the telomeres have a static position within the nucleus. Indeed, this was confirmed for most of the telomeres that display merely Brownian movements, and that are constrained in their mobility. Other telomeres, however, can move rapidly over long distances, particularly during the G1 phase of the cell cycle. Since telomeric motion also includes movement of the chromosomes in question, alterations in transcription and transformation might be induced. It therefore seems reasonable to assume that the movements of the telomeres play a role in the formation of chromosomal rearrangements and oncogenesis.

Interestingly enough, striking differences can be observed between the localization and organization of telomeres in normal and tumour cells. Discoveries made in the last few years show that telomeres are localized in non-overlapping territories in the inner third of the nucleus in aderently growing normal human cells\(^{[30]}\). In at least some telomerase-positive tumour cells, this distribution is altered due to the formation of telomere aggregates (TAs). These are characteristic for all tumour cells with deregulated c-Myc expression, and can be reversibly induced in normal cells by transient induction of c-Myc\(^{[30]}\). TA formation is associated with changes in chromosome territories and correlates with the appearance of chromosome aberrations. It is tempting to speculate that TAs are responsible for this illegitimate chromosome distribution during mitosis. Their formation may represent a novel telomere length-independent mechanism to induce genomic instability in telomerase-positive cells. These telomere associations resemble the »chromosomal bouquet«, a
Telomeres and telomerase: two new players in cancer therapy

- Telomerase activity is observed in about 85% of all human tumours. Telomere stabilization is essential for their long-term proliferation. This makes telomerase and telomeres attractive targets for cancer diagnosis, prognosis, and therapy. Investigations in this area include the development of chemotherapeutic agents mediating inhibition of telomerase and disadvantage of telomerase inhibitors, as well as telomerase-based immunotherapy.

- It was once thought that, in non-pathological situations, telomerase is functional only during embryonal development and in the germ-line of the adult organism, and that normal somatic cells are telomerase-negative. However, it is now generally accepted that cells of highly proliferative and periodically or continuously renewing tissues, such as the haematopoietic system and the epidermis, also show telomerase activity. New anticancer therapies based on telomerase inhibition must therefore take the potentially negative side effects in these tissues into consideration.

- Telomerase inhibitors are generally considered as relatively safe drugs due to the fact that normal cells have longer telomeres than cancer cells. The impact of telomere erosion induced by telomerase inhibition may therefore be negligible. The disadvantage of telomerase inhibitors is that they may not become immediately effective, since cancer cells have to continue with proliferation until their telomeres are short enough. Furthermore, they may be unable to completely eliminate tumour cells, particularly in cases where they induce replicative senescence rather than cell death. Induction of replicative senescence leaves tumour cells alive and metabolically active. These senescent tumour cells could produce tumour promoting factors, thus inducing the re-growth of the tumour when cancer therapy is stopped. Indeed, the rate of reoccurrence is high in certain tumours. However, promising results have been obtained by combining telomerase inhibition and conventional cytotoxic chemotherapeutic drugs and radiation.

The telomerase inhibitors currently receiving most attention are the G-quadruplex-binding agents. These interact with and stabilize four-stranded guanine-quadruplex structures that can be formed by folding of the single-stranded overhang at the 3' end of the telomere. The G-quadruplex is incompatible with telomerase extension, and may also trigger DNA damage responses. Of these, BRACO-19 shows the best results. In telomerase-positive cells, BRACO-19 seems to act via telomerase inhibition and prevents the stabilization of telomeres, thus inducing replicative senescence and apoptosis. In a telomerase-negative background – as found in approximately 15% of all human tumours in which telomere stabilization is achieved by the telomerase-independent ALT mechanism – BRACO-19 induces cell cycle arrest. This might be through activation of DNA damage response pathways that are independent of telomere erosion.

These investigations are encouraging. Nevertheless, they are to be taken with a pinch of salt since they have been performed predominantly in vitro – mainly due to the lack of appropriate in-vivo models. The main reason for this is the difficulty in transporting the drugs to cancer cells in vivo. Although the potential toxicity for humans has not yet been com-
pletely characterized, the first telomerase inhibitors are about to advance into the clinical trial stage.

Other anticancer strategies involve telomerase-based immunotherapy and TERT promoter-driven expression of therapeutically active genes, both aimed at the selective killing of tumour cells. Apoptosis inducing genes and genes coding for enzymes which convert harmless prodrugs into cytotoxic agents are to be found among these. Again, the potential toxic effects on normal telomerase-positive cells and tissues should be taken into consideration in these approaches. However, initial data from the first clinical studies did not show any severe treatment-related side effects\(^{(10)}\).

**Telomerase: the key to immortality?**

- Another issue to be addressed critically is the use of telomerase to immortalize cells as sources for medical applications e.g. TERT-immortalized cells to treat deep-burnt lesions or non-healing ulcers. Although these cells were long believed to be genotypically and phenotypically equal to their normal counterparts, recent experimental data suggests otherwise. There is evidence that ectopic expression of telomerase selects for an abnormal karyotype. Telomerase seems able to immortalize only certain cells of the cell population, namely those which are predestined to overcome replicative senescence by additional genetic alterations. Due to these genetic changes, the telomerase-immortalized cells may have a higher probability of tumorigenic transformation. If they are used for transplantation, they could increase patient’s risk of developing cancer. A further aspect to be considered is the functional consequence of ectopic telomerase expression itself. Over the last few years, it has become evident that, besides its role in telomere maintenance, telomerase also has functions related to cell growth and survival, DNA repair and tumorigenesis when its expression is constitutive and not regulated by normal physiological control mechanisms\(^{(10)}\). Potential negative side effects must therefore be carefully evaluated before using telomerase-immortalized cells for clinical applications.

**Telomerase regulation**

- During the last years, the investigation of telomerase regulation became more intensive. This is mainly due to the fact that scientists want to increase the knowledge about telomerase and to develop appropriate tools to manipulate it. The regulatory mechanisms modulating telomerase activity are diverse and complex, involving epigenetic regulation such as histone acetylation and methylation, transcription, alternative splicing, cellular localization, phosphorylation, telomerase complex assembly and interaction with telomere-binding proteins\(^{(10)}\).

Various studies indicate that expression of TERT is the rate-limiting step for telomerase activity, and transcription has been proposed as its major control mechanism. Numerous binding sites for transcription factors have been identified and several transcription factors have been implicated in the regulation of telomerase expression. These transcription factors are activated and/or inhibited by different signalling cascades, which in some cases operate in a cell type-specific manner. While considerable progress has been made in recent years in shedding light on the regulation of telomerase, many questions remain. Above all, the mechanism of tumour-specific activation of telomerase has not yet been established. Since telomerase inhibition has become attractive in cancer treatment, further investigations are required in this field to identify new therapeutic targets. In this context, it is also important to comprehend the molecular mechanisms underlying physiological down-regulation of telomerase – as seen, for example, during epidermal differentiation – as an alternative to chemically engineered telomerase inhibitors. Studies in this area also involve the generation of transgenic in vivo models to analyse telomerase expression under normal physiological and tumorigenic conditions. Special interest is paid to the elucidation of signal transduction pathways correlating oncogenes and telomerase expression. In addition, numerous proteins have been found to associate with telomerase. However, with a few exceptions, their functional role in telomerase activity remains unclear. For this reason, great efforts are now being made to develop new methods to analyse active telomerase complexes.

**References**