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Review

Cellular functions of the dual-targeted catalytic subunit of telomerase, telomerase reverse transcriptase — Potential role in senescence and aging

Niloofar Ale-Agha [b,1], Nadine Dyballa-Rukes [b,1], Sascha Jakob [b], Joachim Altschmied [b], Judith Haendeler [a,b,*]

[a] Central Institute of Clinical Chemistry and Laboratory Medicine, University of Duesseldorf, Germany
[b] IUF — Leibniz Research Institute for Environmental Medicine at the University of Duesseldorf gGmbH, 40225 Duesseldorf, Germany

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ABSTRACT

Over the last 40 years it has become clear that telomeres, the end of the chromosomes, and the enzyme telomerase reverse transcriptase (TERT), which is required to counteract their shortening, play a pivotal role in senescence and aging. However, over the last years several studies demonstrated that TERT belongs to the group of dual-targeted proteins. It contains a bipartite nuclear localization signal as well as a mitochondrial targeting sequence and, under physiological conditions, is found in both organelles in several cell types including terminally differentiated, post-mitotic cells. The canonical function of TERT is to prevent telomere erosion and thereby the development of replicative senescence and genetic instability. Besides telomere extension, TERT exhibits other non-telomeric activities such as cell cycle regulation, modulation of cellular signaling and gene expression, augmentation of proliferative lifespan as well as DNA damage responses. Mitochondrial TERT is able to reduce reactive oxygen species, mitochondrial DNA damage and apoptosis. Because of the localization of TERT in the nucleus and in the mitochondria, it must have different functions in the two organelles as mitochondrial DNA does not contain telomeric structures. However, the organelle-specific functions are not completely understood. Strikingly, the regulation by phosphorylation of TERT seems to reveal multiple parallels. This review will summarize the current knowledge about the cellular functions and post-translational regulation of the dual-targeted protein TERT.

1. Introduction: telomeres and telomerase

Already in the first half of the last century Hermann Muller and Barbara McClintock described the instability of broken chromosomes in species as distantly related as *Drosophila* and maize (McClintock, 1941; Muller, 1938). However, in their studies they never found structural changes involving the end of the chromosomes, which led Muller to the conclusion that this terminal region (in his words "terminal gene") must have a special function in sealing the chromosome. To describe the uniqueness of these regions and to set them apart from the remainder of the chromosomes, he termed them telomeres combining the Greek words for end (telo) and part (meros).

Almost 40 years later Joseph Gall and Elizabeth Blackburn described the presence of a tandem hexanucleotide repeat sequence CCCCAA at the end of extrachromosomal rDNA molecules in *Tetrahymena* (Blackburn and Gall, 1978). After it was shown by Elizabeth Blackburn and Jack Szostak that these were functionally equivalent to the ends of yeast chromosomes, they concluded that such repeated end sequences correspond to functional telomeres (Szostak and Blackburn, 1982). Nowadays, it is common knowledge that telomeres consist of tandem repeats of the hexanucleotide sequence TTAGGG (in mammals), adopt a higher order structure and are capped with a large number of single- and double-strand DNA binding proteins (Blasco, 2005; de Lange, 2005; Pinto et al., 2011).

A connection between telomeres, senescence and aging was derived from several independent lines of evidence. In 1965 Leonard Hayflick demonstrated that isolated fibroblasts show only a limited proliferative potential in culture and concluded that this finite lifetime of cells *in vitro* may be an expression of aging or senescence at the cellular level (Hayflick, 1965). A molecular basis and a link to telomeres were proposed by Alexey Olovnikov after he and James Watson had independently recognized the problem that during DNA replication, a small

Abbreviations: TERT, telomerase reverse transcriptase; TERC, non-coding telomerase RNA component; ROS, reactive oxygen species; ETC, electron transport chain; mtDNA, mitochondrial DNA; NLS, nuclear localization signal; NES, nuclear export signal; MTS, mitochondrial targeting sequence.

* Corresponding author at: Central Institute of Clinical Chemistry and Laboratory Medicine, University of Duesseldorf, and, IUF — Leibniz Research Institute for Environmental Medicine, Auf'm Hennekamp 50, 40225 Duesseldorf, Germany. Tel.: +49 211 3389 291; fax: +49 211 3389 331.
E-mail addresses: Niloofar.Ale-Agha@uni-duesseldorf.de (N. Ale-Agha), Nadine.Dyballa@uni-duesseldorf.de (N. Dyballa-Rukes), Sascha.Jakob@uni-duesseldorf.de (S. Jakob), Joachim.Altschmied@uni-duesseldorf.de (J. Altschmied), juhae001@uni-duesseldorf.de (J. Haendeler).

[1] Both authors contributed equally to the work.

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part of DNA from the 3′-ends of linear chromosomes is unreplicated. Olovnikov explained Hayflick's theory of limited somatic cell division suggesting that this now called end-replication problem leads to loss of telomeric sequences, which he called "telogenes", during successive mitoses finally resulting in cellular senescence and elimination of such cells (Olovnikov, 1973). However, up to now it is not clear whether telomere length could be used as a biomarker for aging because the findings in humans are contradictory as reviewed by Mather et al. (2011).

Again Elizabeth Blackburn, together with her graduate student Carol Greider, discovered that telomere shortening is counteracted by the activity of a cellular ribonucleoprotein complex called telomerase, which they identified in *Tetrahymena* (Greider and Blackburn, 1985; Greider and Blackburn, 1987). The presence of a similar activity in human cells was demonstrated shortly thereafter establishing that telomerase-mediated telomere maintenance is conserved throughout eukaryotes (Morin, 1989). The telomerase holoenzyme requires the catalytic proteinaceous subunit telomerase reverse transcriptase (TERT) and, besides multiple telomerase associated proteins, the non-coding telomerase RNA component (TERC), which serves as the template for the elongation of the telomere repeat sequences by TERT (Blasco, 2005).

If telomerase is absent, telomeres shorten during each round of cell division until they reach a critically short-length threshold (Harley et al., 1990). This causes a DNA damage response and induces cell cycle arrest triggering senescence (Herbig et al., 2004). Along the same lines, the introduction of TERT into cells prolongs both their lifespan and their telomeres to lengths typical of young cells and reduces signs of senescence (Bodnar et al., 1998; Counter et al., 1998). These findings also suggest that permanent, high-level expression of TERT can lead to uncontrolled proliferation, which is reflected by the fact that many tumors exhibit reactivation of telomerase (Blasco, 2005). Therefore, telomerase activity must be tightly regulated, which on one hand is achieved by transcriptional regulation of the TERT gene, and on the other hand by controlling TERT activity on the post-translational level (Aisner et al., 2002; Cong et al., 2002; Ducrest et al., 2002).

It has been proposed for a long time that telomerase activity is absent from most human somatic cells and only present in tumor cells and adult stem cells of highly regenerative tissues, such as the immune system, skin and intestine (Forsyth et al., 2002). However, there is accumulating evidence that substantial telomerase activity is present in differentiated somatic cells, e.g. endothelial cells, smooth muscle cells and hepatocytes (Haendeler et al., 2004; Leri et al., 2000; Minamino and Kourembanas, 2001; Vasa et al., 2000; Werner et al., 2009, 2011; Yamaguchi et al., 1998).

2. Non-telomeric functions of TERT

Besides its pivotal role in ensuring telomere maintenance in the nucleus, a large number of studies provided evidence for non-telomeric functions of TERT. Amongst other things, TERT was shown to be involved in the regulation of gene expression (Geserick et al., 2006; Park et al., 2009; Smith et al., 2003). In addition, nuclear TERT protects against apoptosis independent of changes in telomere length (Haendeler et al., 2003a; Werner et al., 2011). Furthermore, it is essential for the beneficial effects of physical exercise (Werner et al., 2008, 2009).

Surprisingly at that time, a number of years ago TERT was also detected in mitochondria independently by several groups (Ahmed et al., 2008; Haendeler et al., 2009; Santos et al., 2004, 2006). From the textbooks mitochondria are known to produce reduction equivalents in the citric acid cycle and ATP via oxidative phosphorylation. They are also a main source of reactive oxygen species (ROS) produced by complexes I, II and III of the electron transport chain (ETC.). While complexes I and II produce ROS only into the matrix, complex III generates ROS on both sides of the inner mitochondrial membrane (Murphy, 2009; Turrens, 2003). In addition, mitochondria play a crucial role in intrinsic apoptosis pathways and have thus been described as the central executioner of apoptosis (Estaquier et al., 2012). Newer proteomic studies have revealed the presence of several hundred to more than 1000 proteins in these organelles, depending on the cell type and physiological situation (Balaban, 2010; Pagliarini et al., 2008) suggesting that mitochondria serve much broader functions than originally anticipated. When the functions of mitochondrial TERT were assessed, the first descriptions reported that it renders cells more susceptible to oxidative stress-induced mitochondrial DNA (mtDNA) damage, which can lead to apoptotic cell death (Santos et al., 2004, 2006). In contrast to these findings, we and others described a protective function for mitochondrial TERT by increasing the membrane potential, reducing reactive oxygen production, protecting mtDNA against damage and inhibiting apoptosis induction (Ahmed et al., 2008; Haendeler et al., 2009). Interestingly, TERT has also an impact on the ETC., as overexpression of TERT enhances overall respiratory chain activity in HEK 293 cells, with the most pronounced effect on complex I activity. This was corroborated *in vivo* by demonstrating significantly reduced respiratory chain activity in hearts of TERT-deficient mice in comparison to their wildtype littermates. Interestingly the respiration was unaltered in liver mitochondria of TERT-deficient mice as compared to their wildtype littermates. Thus, one could speculate that in regenerative organs, which are rich in mitochondria like the liver, mitochondrial TERT is not as important for respiration as in post-mitotic tissues, which depend on mitochondria like the heart. Further studies e.g. in liver-injured animals are needed to support this hypothesis. It was shown in cell culture that the effect of TERT on respiration requires the reverse transcriptase activity of the enzyme (Haendeler et al., 2009). Later on the group around Santos also showed that the absence of TERT has a negative impact on mitochondria supporting a positive role for this protein on mitochondrial functions. In addition, it was demonstrated that mitochondrial TERT works as a TERC-independent reverse transcriptase (Sharma et al., 2012). Furthermore, Indran et al. demonstrated in cancer cells that TERT overexpression reduces the basal cellular ROS levels, which was accompanied by a lower level of cytochrome C release to the cytosol and inhibition of endogenous ROS production in response to oxidative stress (Indran et al., 2011). In line with these findings was the study from Singhapol et al. demonstrating that mitochondrially localized TERT decreases mitochondrial ROS and thereby prevents nuclear DNA damage (Singhapol et al., 2013).

The findings reviewed in this chapter support an anti-apoptotic function of nuclear as well as of mitochondrial TERT. These at first glance contradictory results could be explained by the different roles of TERT in the two compartments, namely regulation of gene expression in the nucleus and protection against increased ROS formation in the mitochondria.

3. TERT transport to nucleus and mitochondria

The finding that TERT is present in two different cellular compartments raises the question of how TERT is distributed between these organelles. The passage of larger proteins across the nuclear pore complexes, which allow the passive diffusion of ions, metabolites and macromolecules up to a size of approximately 40 kDa, requires selective transport mechanisms. Therefore, proteins above this exclusion limit possess nuclear localization signals (NLS) of different kinds, which are recognized by specific import machineries (Marfori et al., 2011). Additionally, the traffic of proteins between the nuclear compartment and the cytosol can be controlled by nuclear export signals (NES) (Pemberton and Paschal, 2005). The nuclear-cytoplasmic shuttling of proteins can also be affected by phosphorylation, one of the most common post-translational modifications in the regulation and fine-tuning of many biological processes (Nardozzi et al., 2010). For TERT it has recently been shown that its nuclear import requires a so-called bipartite NLS and phosphorylation on a serine residue in this NLS by protein kinase B/Akt (Chung et al., 2012). This is in accordance with older studies demonstrating Akt phosphorylation of a synthetic peptide

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