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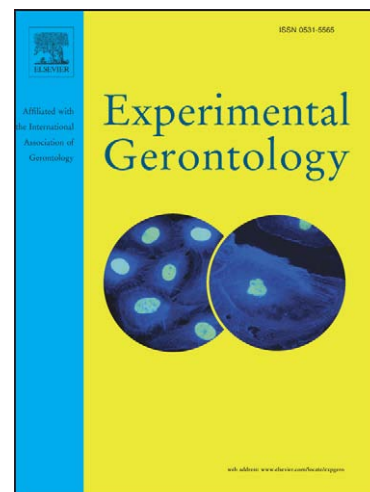
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Telomerase, mitochondria and oxidative stress

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Abstract

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3 Telomerase plays an important role in cellular proliferation capacity and survival under
4 conditions of stress. A large part of this protective function is due to telomere capping and
5 maintenance. Thus it contributes to cellular immortality in stem cells and cancer. Recently,
6 evidence has accumulated that telomerase can contribute to cell survival and stress
7 resistance in a largely telomere-independent manner. Telomerase has been shown to shuttle
8 dynamically between different cellular locations. Under increased oxidative stress telomerase
9 is excluded from the nucleus and can be found within the mitochondria. This phenotype
10 correlates with decreased oxidative stress within telomerase expressing cells and improved
11 mitochondrial function by currently largely unknown mechanisms. Our data suggest that
12 mitochondrial protection could be an important non-canonical function for telomerase in cell
13 survival and ageing. This review summarises briefly our knowledge about extra-telomeric
14 functions of telomerase and discusses the potential significance of its mitochondrial
15 localisation.
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Telomerase in senescence and immortality

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Telomerase is a unique reverse transcriptase with the main function of elongating and maintaining telomeres. The ribonucleoprotein consists of two main components - the RNA component (TR) containing the antisense template for telomere synthesis and the catalytic protein, hTERT. *In vivo* the enzyme is associated with multiple regulatory proteins which might be involved in various intracellular pathways. There exist many different levels of regulation of the enzyme, such as epigenetic modification, transcriptional activation and repression by multiple transcription factors and hormones and alternative splicing of hTERT mRNA where only one product generates a biochemically functioning enzyme. In addition, telomerase undergoes post-translational modifications and can be found in multiple sub-cellular locations.

Telomerase is expressed at varying levels in human cells and tissues. There is a high level of expression during early embryonic development, but the expression is greatly down-regulated in most adult tissues. In dividing human somatic tissues that express no or only low levels of telomerase, telomere shortening is an essential contributor to cellular senescence. A critical length of telomere repeats is required to ensure proper telomere function and avoid the activation of DNA damage pathways that result in replicative senescence or cell death.

Telomeres shorten due to the end-replication problem and to oxidative stress mediated telomere DNA damage. Stably capped telomeres contribute greatly to genome stability and a healthy lifespan (Geserick and Blasco, 2006). Telomerase can counteract replicative senescence by maintaining telomeres and genomic stability. Telomerase plays also a role in ageing and longevity of organisms via its telomere stabilising function as demonstrated in a cancer-protected mouse model (Tomás-Loba et al., 2008).

Telomerase activity and telomere maintenance are important prerequisites for cellular immortality mainly due to its telomere stabilising and proliferation promoting function.

However, there have been described also telomere independent functions of telomerase in tumourigenesis (Stewart et al., 2002).

A high expression level is found within the germ line, embryonic stem cells and cancer cells. Mutations in either the RNA component or the catalytic part are rare, but frequently result via their telomere-dependent function in premature ageing syndromes and human diseases such

1 as dyskeratosis congenita (Blasco, 2007). Lymphocytes, endothelial cells and adult stem cells
2 express varying amounts of telomerase and the expression is tightly regulated. Since most
3 cells express at least moderate levels of the RNA component, the expression of the catalytic
4 subunit hTERT is sufficient to generate high levels of telomerase activity in normal cells such
5 as fibroblasts (Bodnar et al, 1998). Over-expression of hTERT is sufficient to counteract
6 telomere shortening and to confer a greatly extended lifespan and often immortalise cells
7 which would otherwise senesce due to continuous telomere shortening. In cancer cells
8 telomerase is constitutively active and contributes substantially to an unlimited proliferation
9 potential and tumour development. Thus, telomerase activity and telomere maintenance are
10 associated with the immortality of cancer, germ-line and embryonic stem cells. Telomerase
11 plays an important role in stem cell proliferation, function and self-renewal. Embryonic stem
12 (ES) cells express high levels of telomerase that decreases during differentiation due to
13 epigenetic modification of telomerase promoter activity. At the same time telomeres shorten
14 while DNA damage and oxidative stress increase in the differentiated progeny from hES cells
15 (Saretzki et al., 2008). Over-expression of TERT in mouse embryonic stem cells conferred
16 increased stress resistance, improved antioxidant defence and differentiation capacity to the
17 cells whereas in human embryonic stem cells TERT over-expression contributes to
18 pluripotency and suppresses the *in vitro* differentiation capacity while it increases survival
19 under mild stress (Armstrong et al., 2005, Yang et al., 2008). Down-regulation of TERT in
20 human embryonic stem cells was incompatible with stem cell characteristics and pluripotency
21 and these cells went into differentiation immediately, emphasising the importance of
22 telomerase for pluripotency and stress resistance in hES cells (Yang et al., 2008). It has been
23 shown very recently that activation of telomerase is a necessary step for the generation of
24 induced pluripotent cells (iPS) and that iPS cannot be derived from TR knockout mice without
25 re-introduction of telomerase (Marion et al., 2009). The role of telomerase for the function of
26 adult stem cells is less clear and can be rather diverse depending on the respective tissue
27 analysed. Whereas hematopoietic stem cells express high levels of telomerase,
28 mesenchymal stem cells, for example, do not express any telomerase at all. Although it is
29 known that telomerase influences telomere length and regenerative potential and replicative
30 lifespan of many tissue stem cells and telomere length in tissue stem cells shortens with age
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(Allsopp et al., 2003, Blasco, 2007, Flores et al., 2008) not much is known about telomere independent functions of telomerase in stem cells. The only report to date is that of Sarin and co-workers who demonstrated a role for hTERT without hTR in cell cycle activation of hair follicle stem cells leading to enhanced hair growth in mice (Sarin et al., 2005). Increased expression of genes involved in cell cycle progression such as cyclinD1 in certain but not all stem cell compartments could be a possible mechanism for this telomere and telomerase activity independent function of hTERT.

Mitochondria, oxidative stress and telomeres

Mitochondria are important organelles for cellular respiration and energy supply through ATP generation. Mitochondria are also the major cellular source for generation of reactive oxygen species (ROS) and at the same time the main targets of ROS-induced oxidative damage. Mitochondrial dysfunction and oxidative stress have been implicated in cellular senescence and the ageing process. The “free radical or oxidative stress theory of ageing” developed by D. Harman (Harman 1956, 1972) postulates that ageing results from the accumulation of molecular damage caused by reactive oxygen species (ROS) generated during normal metabolism and that this ultimately determines the lifespan of an organism. This theory has been further developed and refined by Miquel and colleagues as the mitochondrial theory of ageing (Miquel et al., 1980), who suggested that the accumulation of somatic mutations in the mtDNA induced by oxidative stress is the major contributor to ageing and age-related degenerative diseases

There is substantial evidence indicating that oxidative stress can induce or accelerate the development of cellular senescence. The respiratory function and ATP production of mitochondria declines with age while oxidative damage and mutations in mitochondrial DNA increase during human ageing (Krishnan et al., 2007, Sastre et al., 2003,). Oxidative stress and cellular senescence are increasingly recognised as important underlying mechanisms contributing to intrinsic ageing, frailty and loss of vitality. Mitochondrial DNA (mtDNA) damage has long been suggested as a sensitive indicator of intracellular oxidative stress and many correlative studies have demonstrated an increased ROS generation as well as reduced mitochondrial function and increased oxidative damage to mtDNA with age (Van

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2 Remmen and Richardson, 2001). Experiments in model organisms indicate that mitochondrial
3 function and reactive oxygen species in particular play a major role in determining lifespan
4 (Sedensky MM, Morgan PG.2006). However, more recent studies using genetically modified
5 animal models showed inconsistent results and therefore, the exact relationship between
6 oxidative metabolism and ROS as determinants of ageing and longevity are far from clear and
7 the subject of a controverse debate (Lapointe and Hekimi 2008, Jang and van Remmen,
8 2009, Trifunovic et al., 2004).

9
10 *In vitro* oxidative stress can be modulated in experimental systems. Using increased oxidative
11 stress on human fibroblasts von Zglinicki and colleagues demonstrated that telomere
12 shortening accelerates under these conditions whereas a lower oxidative stress decreased
13 telomere shortening and increased the lifespan of cells in culture (von Zglinicki et al., 1995,
14 Saretzki et al., 2003, Serra et al., 2003). We also demonstrated that mitochondrial dysfunction
15 via induction of telomere damage is an important component in the occurrence of senescence
16 within dividing cellular populations (Passos et al., 2007).

17
18 Telomeres emerged recently as important indicators of oxidative stress that accumulates *in*
19 *vivo* in the human body and have been suggested as an underlying event in many diseases.
20 Shortened telomeres in blood cells during ageing can be caused by oxidative stress (von
21 Zglinicki et al., 2000) or decreased telomerase activity (Plunkett et al., 2007). Telomeres are
22 very sensitive indicators of cumulative oxidative stress, whereas more acute measurements
23 of oxidative stress could be below the detection limit for short lived oxygen radicals.

24
25 Accordingly, telomeres have been identified as potential biomarkers for age-associated
26 disease risk, progression, and mortality in many studies. For instance, telomere length in
27 blood cells is a good indicator for the likelihood of vascular dementia after stroke (Martin-Ruiz
28 et al., 2006, von Zglinicki et al., 2000). Telomere shortening and increased oxidative stress
29 have been described even for processes such as high psychological stress in caring mothers
30 (Epel et al., 2004). At the same time, stress and senescence can influence telomerase levels
31 in lymphocytes (Plunkett et al., 2007, Porton et al., 2008). Changes in telomerase activity in
32 cell types such as lymphocytes, endothelial cells and tissue stem cells could influence
33 processes relevant for healthy ageing. Importantly, even lifestyle factors known to promote
34 cancer and cardiovascular disease could affect telomerase function. It has been shown
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1 recently in a longitudinal study that improvements in nutrition and lifestyle were associated
2 with increases in telomerase activity correlating with decreases in low-density lipoprotein
3 cholesterol and psychological distress (Ornish et al., 2008). Thus, comprehensive lifestyle
4 changes could significantly increase telomerase activity, stabilise telomeres and decrease
5 oxidative stress within tissues and organs.
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10 **Telomere independent functions of telomerase**

11 Beyond the clear role of telomerase in maintaining telomere length, accumulating evidence
12 suggests that telomerase has additional physiological functions. Telomere-independent
13 functions of telomerase have been related to improved DNA repair, increased apoptosis
14 resistance or changes in chromatin structure and gene expression. This data suggests that
15 telomerase can promote cell survival and stress resistance independent from its effect on
16 telomere length maintenance although stress resistance can also depend on telomere length
17 (Rubio et al., 2004). In addition, more subtle changes in telomere capping could be involved.
18 It is often difficult to discriminate experimentally between telomere dependent and
19 independent functions, as both will lead to overlapping outcomes.
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32 Specifically, it has been reported that telomerase expression confers increased resistance to
33 agents that induce cellular stress resulting in apoptosis and/or senescence in various cell
34 types such as neurons and cancer cells. At the same time telomerase failed to protect
35 telomeres under increased oxidative stress in over-expressing model systems and was
36 therefore not able to prevent premature senescence (Ahmed et al., 2008, de Magalhães et
37 al., 2002, Gorbunova et al., 2002,). However, in our hands, at least under conditions of mild
38 chronic oxidative stress this effect is reversible as soon as telomerase re-enters the nucleus
39 (Ahmed et al., 2008) and Gorbunova and colleagues demonstrated that hTERT
40 overexpression protects the cells from stress induced apoptosis and necrosis (Gorbunova et
41 al., 2002).
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52 Data is contradictory as to whether the protective function of telomerase is (Cao et al., 2002,
53 Santos et al., 2006, Zhang et al., 2003,) or is not (del Bufalo et al., 2005, Lee et al., 2008,
54 Kang et al., 2004) dependent on telomerase catalytic activity (i.e., elongation of telomeres). It
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1 is even possible that particular non-canonical properties of telomerase require catalytic
2 activity, whereas others do not.

3
4 Identification of mechanisms and a clear separation between telomeric and non-telomeric
5 functions of telomerase are of major importance to better understand the significance of
6 telomerase for sensitivity against oxidative stress as well as senescence of telomerase
7 positive cells such as endothelial cells, lymphocytes, stem cells and tumour cells.

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10 In most experimental systems it is, however, difficult to clearly distinguish between telomere-
11 related and telomere unrelated effects of telomerase on survival of cells because they can
12 both co-exist and contribute to the same effect. Knock-out mice for different components of
13 telomerase are good experimental models to address the role of TERT, TR and telomerase
14 separately. Sarin and colleagues showed an effect of TERT over-expression in an mTR
15 knockout background on the proliferation of hair follicle stem cells (Sarin et al., 2005). This is
16 in line with data from Kang and co-workers who found a beneficial effect of TERT over-
17 expression on certain mitochondrial functions such as mitochondrial membrane potential and
18 calcium storage capacity in mouse neurons upon treatment with N-methyl-D-aspartic acid
19 (NMDA), without any measurable RNA component or telomerase activity (Kang et al. 2004).

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22 Using mTERT and mTR knockout models and transgenic mouse systems, the same lab
23 found recently that the withdrawal of TERT has substantial implications on the survival and
24 stress resistance of cells, whereas that of the RNA component TR has not (Lee et al., 2008).

25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 **Telomerase and gene expression**

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43 Telomerase actively promotes cell growth and changes global patterns of gene expression
44 that seem unrelated to telomere maintenance. Micro-array studies in various experimental
45 systems revealed rapidly occurring changes in growth promoting genes (EGFR, FGF), cell
46 cycle regulation (cyclins D1, G2), metabolism and cell signalling after manipulating
47 telomerase levels (Choi et al., 2008, Smith et al., 2003, Yang et al., 2008). Switching TERT
48 expression on or off in an inducible system changes Wnt and Myc- signalling pathways in
49 mouse epithelial cells (Choi et al, 2008). These rapid changes were not associated with
50 changes in telomere length. In cancer cells the knock-down of telomerase resulted in
51 expression changes of genes implicated in metastasis, angiogenesis and cell differentiation
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(Li et al., 2005, Bagheri et al., 2006). This data presents evidence for a functional interference of hTERT with the cell cycle and cyclins. However, whether telomerase physically interacts with transcription factors and signalling pathways or via rather indirect mechanisms such as telomere stabilisation or chromatin modification is not clear at present. Telomerase has been shown to be involved in the regulation of chromatin status and DNA damage responses that are independent of its telomere-interacting function (Masutomi et al., 2005).

Mitochondrial dysfunction in yeast and human cells is known to induce significant changes in nuclear gene expression, a process termed retrograde signalling. Examples for such genes are glutamine synthase, pyruvate kinase 4 (PK4), mono amine oxidase A (MAOA), adenylate cyclase 3 (ADCY3), sulfatase1 (Sulf1), desmoglein2 (DSG2), BAMBI, growth hormone receptor (GHR) and IGFBP3. Mitochondrial dysfunction and retrograde signalling has been found to be part of the changes that occur during replicative senescence (Passos et al., 2007). Surprisingly, we found that over-expression of hTERT in human fibroblasts suppressed the gene expression patterns characteristic for retrograde response. Remarkably, hTERT over-expression did not only reverse the changes seen in senescent parental fibroblasts (senescence being generated by exhaustive replication under either normoxia or 40% hyperoxia) back to the level of young fibroblasts, but for several genes a direct opposite regulation compared to senescent cells occurred that partially was even maintained when the telomerase positive cells were cultivated until a senescence-like growth arrest under hyperoxia (Ahmed et al., 2008, see also Fig. 1). Therefore, telomerase expression seems to rejuvenate cells and to improve mitochondrial function. Again, this happened without a measurable effect on telomere length, although it cannot be excluded that a better telomere capping contributes to the improved cellular and mitochondrial function in these cells.

Therefore, a better understanding of the mitochondrial function of telomerase is imperative.

Telomerase, stress resistance and apoptosis

The first to analyse telomere-independent functions of telomerase was the neurophysiologist Mark Mattson. Telomerase is highly expressed in embryonic brain and seems to play an important role in neuronal differentiation and survival. When TERT levels are decreased in cultured embryonic brain neurons they undergo apoptosis demonstrating a requirement for

1 TERT in survival of these embryonic neurons. In contrast, over-expression of TERT in PC12
2 pheochromocytoma cancer cells suppresses apoptosis induced by trophic factor withdrawal
3 (Fu et al., 2000). The study showed as well for the first time that TERT exerts its anti-
4 apoptotic action at an early stage of the cell death process prior to mitochondrial dysfunction
5 and caspase activation. TERT over-expression counteracted the loss of mitochondrial
6 membrane potential and the increase of ROS in these cells which occurs during withdrawal of
7 trophic factors. Therefore, it was suggested that TERT may serve as a neuron survival-
8 promoting factor in the developing brain, and down-regulation of TERT in the adult brain may
9 contribute to increased neuronal vulnerability in various age-related neurodegenerative
10 diseases. These actions of telomerase at a pre-mitochondrial step prior to cytochrome-c
11 release and caspase activation were confirmed by the same lab in another cell model where
12 apoptosis was induced with etoposide and staurosporine (Zhang et al., 2003).

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14 Another paper from the same lab showed additional evidence of an anti-apoptotic role of
15 telomerase by counteracting mitochondrial dysfunction, oxidative stress and caspase
16 activation. It has been shown that the beta-amyloid peptide induces free radical oxidative
17 stress in Alzheimer's disease brain neurons both *in vitro* and *in vivo* giving rise to the
18 oxidative stress hypothesis of Alzheimers' pathogenesis (Butterfield, 2002). The beta-amyloid
19 peptide (ABP) accumulates within mitochondria where it disturbs respiration and increases
20 oxidative stress (Manczak et al., 2006). ABP also changes intracellular calcium homeostasis
21 in cells and both processes can result in apoptosis induction. Telomerase suppression in
22 embryonic neurons promoted apoptosis upon ABP treatment whereas over-expression of
23 TERT in PC12 cells counteracted ABP induced apoptosis and cytotoxicity (Zhu et al., 2000).
24 Therefore it is tempting to speculate that telomerase might decrease the oxidative stress
25 caused by ABP in the mitochondria and thereby preserve the function of the organelle and
26 prevent apoptosis induction.

27
28 These results are in line with those reported by Kang et al. (2004) that showed an increased
29 resistance against NMDA-induced toxicity and hypoxic-ischemic injuries in mice transgenic for
30 TERT. Transgenic neurons did not show any measurable TR levels or telomerase activity, but
31 reduced the accumulation of cytosolic free calcium by sequestering it into mitochondria.
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1 These data show that TERT can alter mitochondrial properties and change sensitivity to
2 apoptosis induced by neuro-toxic stress signals, however, no mechanism was suggested.

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4 A possible mechanism for the involvement of hTERT in apoptosis via interacting with
5 mitochondrial components has been presented by 2 groups recently. Massard and colleagues
6 demonstrated that depleting hTERT facilitated apoptosis induction by cisplatin, etoposide
7 and ROS in various cancer cell lines in a p53 independent manner as well as activation of the
8 pro-apoptotic protein Bax via a conformational change (Massard et al., 2006).

9
10 Del Bufalo and co-workers found that over-expression of Bcl2, an important anti-apoptotic
11 protein, increased hTERT levels and telomerase activity and that over-expression and
12 decrease of hTERT correlated with decrease or increase in apoptosis induction and cell
13 survival, respectively. They also showed that this activity was not dependent on hTERT
14 catalytic activity or p53 status of the cells. This study confirms the finding of others about an
15 interference of hTERT at an early, pre-mitochondrial step of apoptosis.

16
17 Since high levels of telomerase are characteristic for the majority of human cancers the
18 inhibition of telomerase activity by targeting either hTR or hTERT has been suggested as a
19 new strategy for anti-cancer treatments.

20
21 If telomerase is inhibited in tumour cells there is either a delayed response that depends on
22 progressive telomere erosion (Herbert et al., 1999) or a rapid effect on cell viability without
23 any measurable telomere shortening (Kondo et al., 1998a, Saretzki et al., 2001). The
24 mechanism for the latter is not clear at present.

25
26 Over-expression of TERT in various cellular systems confers higher resistance to either
27 physiological signals or different DNA damaging and apoptosis inducing agents, including
28 chemotherapeutic drugs (Ahmed et al., 2008, Ludwig et al., 2001, Sharma et al., 2003, Zhang
29 et al., 2003,) while knock-down of telomerase sensitizes cells to induced and spontaneous
30 apoptosis (Fu et al. 2000, Ludwig et al., 2001, Kondo et al., 1998b).

31 32 **Extra-nuclear localisation of telomerase and oxidative stress**

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34 Although extra-nuclear localisation has been described by various groups, a direct
35 association between specific functions and certain subcellular compartments is at present
36 lacking. The hTERT protein harbours a nuclear and nucleolar localisation signal as well as a
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1 nuclear export signal. In lymphocytes hTERT protein is transported into the nucleus upon
2 activation by antigen exposure or treatment with TNF-alpha (Akiyama et al., 2004, Liu et al.,
3 2001). Telomerase can be found in the nucleolus and can be associated with the signalling
4 protein 14-3-3 that is involved in the sub-cellular shuttling of several proteins (Seimiya et al.,
5 2000, Wong et al., 2002, Zhang et al., 2003). Endogenous TERT can be found in the nucleus
6 and cytoplasm of mouse hippocampal neurons and human cells and tissues (Fu et al., 2000,
7 Haendeler et al., 2009, Yan et al., 2004,). This data demonstrates that sub-cellular shuttling is
8 not an artefact of forced hTERT expression but occurs naturally within cells and is
9 dynamically regulated. This shuttling process depends on various factors such as cell cycle
10 phase, DNA damage and oxidative stress.

11 It has recently been shown by three independent groups that telomerase is excluded from the
12 nucleus upon stress (Ahmed et al., 2008, Haendeler et al., 2003, 2009, Santos et al., 2004,
13 2006). In addition to the determination of telomerase activity in different subcellular fractions
14 various models and methods have been used: over-expression of a GFP/hTERT fusion
15 protein in cancer cells or hTERT over-expression in normal and tumour cells and immuno-
16 cytochemistry of hTERT in telomerase over-expressing fibroblasts ((Haendeler et al., 2003,
17 2009, Santos et al., 2004, Ahmed et al., 2008). Fibroblasts over-expressing hTERT display
18 lower levels of intracellular ROS and therefore lower oxidative stress under basal condition
19 and under increased chronic oxidative stress compared to parental fibroblasts under the
20 same conditions (Ahmed et al., 2008).

21 Importantly, Haendeler and colleagues showed that nuclear export of endogenous telomerase
22 occurs in endothelial cells approaching senescence due to increased oxidative stress,
23 whereas treatment with antioxidants was able to reverse this process (Haendeler et al.,
24 2004). Nuclear exclusion of telomerase could play an important role in senescence and loss
25 of function of these and other telomerase positive cells. Inhibition of hTERT expression using
26 siRNA in endothelial cells and shRNA in HEK293 increased oxidative stress (Ahmed et al.,
27 2008, Haendeler et al., 2009). This data suggests an intimate relationship between
28 telomerase and oxidative stress in these cells. Dysfunction of endothelial cells is involved in
29 the development of cardio-vascular diseases such as arteriosclerosis. On the other hand,
30 treatment with agents such as aspirin, estrogen and statins could potentially delay changes in
31

telomerase, oxidative stress and endothelial cell senescence (Jacob and Haendeler, 2007).

Oxidative stress such as H₂O₂ activates nuclear export of TERT by a Src kinase dependent phosphorylation of TERT on tyrosine 707 and might involve other factors such as CRM1 and the cytosolic 14-3-3 proteins (Haendeler et al., 2003, Seimiya et al., 2001). The localisation of hTERT within different cellular sub-compartments seems to be dynamically regulated and dependent on various signals such as antigens, growth factors or oxidative stress. Since telomerase can exert its telomere-interacting function only in the nucleus one can suggest that extra-nuclear localisations of hTERT and telomerase could have additional, non-telomeric functions.

Mitochondrial localisation of telomerase

A growing number of proteins have been shown to shuttle between the nucleus and mitochondria, including p53, HMG A1, VHL, amyloid beta, prohibitins, Lon protease, ARF etc. Recently it has been shown that telomerase can shuttle from the nucleus to the mitochondria upon oxidative challenge and drug treatment (Santos et al., 2004, 2006, Ahmed et al., 2008, Haendeler et al., 2009). Santos and co-worker have described a specific mitochondrial import sequence at the N-terminus of hTERT (Santos et al., 2004). Thus, transport of hTERT to the mitochondria seems to be an induced, directed and naturally occurring process. Contradicting data has been obtained regarding the impact of hTERT on mitochondrial DNA integrity. Santos and colleagues reported that mitochondrially localised hTERT increases mitochondrial DNA damage and apoptosis frequency after H₂O₂ treatment and suggested a potential role of iron metabolism. The interpretation of the authors for the observed effect was that it could function as a selection mechanism in order to drive stem cells with high oxidative damage into apoptosis, thereby ensuring a high quality of the stem cell pool.

In contrast, our and J. Haendeler's groups have both demonstrated a protective effect of mitochondrially localised telomerase in independent model systems and under various experimental settings. Our lab showed that hTERT over-expression in human fibroblasts protected mtDNA from DNA damage upon acute (H₂O₂ treatment) and chronic (hyperoxia) oxidative stress (Ahmed et al., 2008). In accordance, the frequency of apoptosis after

1 treatment with H₂O₂ and etoposide decreased in hTERT over-expressing cells in comparison
2 to parental or vector-transfected fibroblasts (Ahmed et al., 2008).

3
4 Translocation of telomerase into the mitochondria upon oxidative stress occurs in a time and
5 dosage dependent manner (Ahmed et al., 2008, Fig. 2). This process can occur within less
6 than 3 hours under H₂O₂ treatment which essentially excludes *de novo* synthesis as the
7 source of the mitochondrial hTERT protein pool as it has been reported for mitochondrial p53
8 (Marchenko et al., 2007). We found that oxidative stress causes 80–90% of all telomerase to
9 enter the mitochondria, with the remaining telomerase in the nucleus being unable to maintain
10 telomere length under conditions of chronic hyperoxia (40% oxygen) (Ahmed et al., 2008).

11
12 We had shown previously that cultivation of parental fibroblasts under these conditions
13 resulted in a dramatic acceleration of telomere shortening (von Zglinicki et al., 1995). Shifting
14 the hTERT over-expressing cells back from hyperoxia to normoxia reversed the nuclear
15 exclusion of telomerase correlating with re-elongated telomeres and regaining proliferation.

16
17 The gradual nuclear exclusion and shuttling of telomerase to mitochondria could present an
18 important physiological mechanism in human cells that are positive for telomerase such as
19 lymphocytes, endothelial cells and stem cells. It could mean that telomerase that protects
20 telomeres under normal growth conditions shifts its protective function towards mitochondria
21 under conditions of increased oxidative stress. We have preliminary data showing that a sub-
22 cellular shuttling of telomerase from the nucleus to mitochondria also occurs in cancer cells
23 under increased stress very similar to fibroblasts with a constitutively over-expressed hTERT
24 transgene (unpublished results). Nuclear exclusion might be a novel regulatory mechanisms
25 of telomerase catalytic activity in addition to regulation of expression, in particular at the level
26 of transcription. There is data suggesting that the mitochondrial function might co-exist with
27 the telomeric function: 20-30% of telomerase has been found outside the nucleus and
28 partially within mitochondria already under normal basal conditions in various cell types
29 (Ahmed et al., 2008, Haendeler et al., 2009).

30
31 To what extent telomerase localises to other cytoplasmic sites is not entirely clear at present.
32 Haendeler and colleagues have analysed the distribution of telomerase activity in three
33 cellular sub-fractions of HEK 293 cells, showing around 60% of telomerase activity in the
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1 nucleus, 20% within mitochondria and 20% in the remaining cytoplasm (Haendeler et al.,
2 2009)..

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4 Most importantly, we found decreased cellular ROS levels and an improved mitochondrial
5 function characterised by an increased mitochondrial membrane potential (see also Kang et
6 al., 2004), lower mitochondrial mass and mitochondrial DNA copy number and lower levels of
7 mtDNA damage in stressed hTERT over-expressing cells. A high membrane potential
8 together with low expression levels of the (mRNA encoding for the) uncoupling protein UCP2
9 underlined the fact that mitochondria in these cells are tightly coupled and generate ATP with
10 a lower degree of oxidative stress and generation of ROS.

11
12 Haendeler and colleagues published recently that telomerase is transported into the
13 mitochondrial matrix by TOM and TIM, binds to mitochondrial DNA coding for complex I
14 genes and increases complex I respiratory efficiency (Haendeler et al., 2009). In addition, the
15 authors demonstrated that heart mitochondria from TERT knockout mice had a less efficient
16 respiration in comparison to wild type mice. The authors confirm our data supporting a
17 beneficial, protective effect of mitochondrial hTERT by using UV and ethidium bromide
18 induced DNA damage. At the same time they demonstrated a positive correlation between
19 mitochondrially localised hTERT and apoptosis resistance as well as an increase in
20 mitochondrial ROS generation after hTERT ablation (Haendeler et al.2009).

21
22 However, it is not clear by what mechanism hTERT protects mitochondrial DNA. Possible
23 mechanisms include lowering of mitochondrial ROS generation by improved coupling or more
24 effective respiration, direct binding to and protection of mtDNA, improved DNA repair or an
25 accelerated degradation of mitochondria harbouring damaged DNA.

26
27 It is not clear at present whether telomerase activity is indeed needed for the protective effect
28 or whether it is the presence of hTERT alone that causes the effects. Haendeler and
29 colleagues demonstrated a better protection of mitochondria by wild-type hTERT compared to
30 a catalytically inactive mutant (Haendeler et al., 2009). Contradictory results exist regarding
31 the dependency of various protective and pro-survival function of telomerase on its catalytic
32 activity. It is possible that some functions do depend on the enzyme being catalytically active
33 whereas others do not.

1 Haendeler and co-workers have shown that hTERT is transported into the mitochondrial
2 matrix (Haendeler et al. 2009). The fact that we and others found telomerase activity within
3 the mitochondria demonstrates indirectly that hTR, the RNA component of telomerase that is
4 necessary for the enzymatic activity of telomerase, localises to mitochondria as well. We have
5 preliminary results showing that hTR is localised to mitochondria under oxidative stress
6 (unpublished). This is intriguing because RNA import into mammalian mitochondria is a rare
7 event. However, co-import of protein and RNA components into mammalian mitochondria has
8 been demonstrated for two RNases (RNaseP and MSP), both fulfilling vital function during
9 mitochondrial replication and mitochondrial tRNA processing. In addition the import of a small
10 amount of 5S RNA into mammalian mitochondria has been demonstrated *in vivo* and *in vitro*
11 (Entelis et al., 2002).
12

13 Finding a mitochondria-protective function of telomerase from two labs (Ahmed et al., 2008,
14 Haendeler et al., 2009) directly contradicts the observations of Santos et al. (2004, 2006) who
15 showed an adverse effect of mitochondrial localisation of telomerase on the integrity of
16 mitochondrial DNA leading to apoptosis. However, a protective function of telomerase in
17 mitochondria corresponds well with earlier findings of protective anti-apoptotic and pro-
18 survival functions of telomerase in various cellular systems.
19

20 The demonstration that telomerase can improve mitochondrial functions and therefore
21 contribute to a decreased oxidative stress suggests an entirely new function of telomerase in
22 protecting mitochondria and, consequently, cells under stress. A summary of known
23 localisations and respective functions of telomerase is depicted in figure 3.
24

25 Although the exact biological significance of shifting telomerase from the nucleus to the
26 mitochondria upon oxidative stress is not entirely clear we speculate that under increased
27 stress mitochondrial protection could be of more immediate importance for cell survival than
28 telomere capping. This process is reversed once the high stress situation is over.
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30 In addition there seems to be a certain amount of telomerase that is localised in mitochondria
31 under normal physiological conditions. Since ROS and mitochondrial damage have been
32 implicated as important components of the ageing process our data suggests that
33 mitochondrial telomerase adds an additional level of complexity to the ageing process.
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Conclusion

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2 Oxidative stress and mitochondrial dysfunction are suggested as possible causes for
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4 replicative senescence and ageing. Telomeres are protective structures at the ends of
5
6 chromosomes that may be involved in many pathological conditions and age-related diseases
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8 both causal and as potential biomarkers. Telomerase is a reverse transcriptase that primarily
9
10 maintains telomeres. Although telomerase activity is greatly reduced after embryonic
11
12 development several adult cell types, including stem cells retain it. Recently it has been
13
14 demonstrated that telomerase activity can be modified by oxidative stress *in vivo* and
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16 therefore contribute to telomere shortening in cells. Consequently, oxidative stress can
17
18 shorten telomeres by two independent mechanisms- by increasing telomeric DNA damage
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20 and decreasing telomerase levels. In addition, oxidative stress has been shown to induce a
21
22 nuclear exclusion of telomerase and a shuttling of the enzyme to mitochondria. In contrast to
23
24 previous data of an adverse effect of mitochondrial telomerase, two groups demonstrated
25
26 recently that mitochondrial telomerase correlates with an improvement of mitochondrial
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28 function and a decrease of cellular oxidative stress. These data correlate well with a
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30 previously shown pro-survival and anti-apoptotic functions of telomerase. Although our
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32 understanding of the underlying mechanisms is limited and the biological significance of this
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34 process requires further investigation, mitochondrial telomerase adds to the already
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36 established non-canonical functions of telomerase that correlate to an extra-nuclear
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38 localisation. Further analysis of the association of telomerase with mitochondria *in vivo*, in
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40 particular in stem cells and other telomerase positive cells, should enhance our understanding
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42 about the role of the enzyme for oxidative stress and the ageing process.
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Figure legends:

1
2 **Figure 1:** Selection of genes involved in retrograde response under 5 different conditions
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4 obtained by using Affymetrix expression arrays: H: MRC-5 under hyperoxia, S: senescent
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6 MRC-5, Y; young MRC-5, T: hTERT over-expressing MRC-5 under normoxia, TH: hTERT
7
8 over-expressing MRC-5 under hyperoxia. Upper field: genes for proteins that are involved in
9
10 mitochondrial function and metabolic regulation. Lower field: proteins involved in Ca^{2+}
11
12 signalling. Expression colour code of fold changes are indicated at the bottom. For technical
13
14 details see Passos et al., 2007 and Ahmed et al., 2008.
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18 **Figure 2:** Confocal images of nuclear exclusion of hTERT, using Rockland anti-hTERT
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20 antibody (red). Upper row: MRC-5 parental fibroblasts as negative control, hTERT-over-
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22 expressing MRC-5 under normoxic conditions as positive control: hTERT shows nuclear
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24 localisation. Treatment with H_2O_2 for 3 hours leads to a dosage-dependent nuclear exclusion
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26 of hTERT. Middle row: gradual exclusion of hTERT in a time-dependent manner under
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28 hyperoxia (40% ambient oxygen) for the indicated times. Lower row: colocalisation of hTERT
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30 (red) and mitotracker green after 10 weeks under hyperoxia. For more details see Ahmed et
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32 al., 2008.
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36 **Figure 3:** Schematic representation of known telomerase localisations and respective
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38 functions
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