ANTIOXIDANTS & REDOX SIGNALING
Volume 13, Number 4, 2010

DOI: 10.1089/ars.2009.2994

FORUM REVIEW ARTICLE

Well-Known Signaling Proteins Exert New Functions in the Nucleus and Mitochondria

Nicole Büchner,[1,*] Joachim Altschmied,[1,*] Sascha Jakob,[1] Gabriele Saretzki,[2] and Judith Haendeler[1]

Abstract

One distinguishing feature of eukaryotic cells is their compartmentalization into organelles, which all have a unique structural and functional identity. Some proteins are exclusively localized in a single organelle, whereas others are found in more than one. A few proteins, whose function was thought to be completely understood, were only recently found to be present in the mitochondria. Although these proteins come from diverse functional classes, their common new denominator is the regulation of respiratory chain activity. Therefore, this review focuses on new functions of the Signal Transducer and Activator of Transcription 3, originally described as a transcription factor, the most prominent Src kinase family members, Src, Fyn, and Yes, which were so far known as plasma membrane-associated molecular effectors of a variety of extracellular stimuli, the tyrosine phosphatase Shp-2 previously characterized as a modulator of cytosolic signal transduction involved in cell growth, development, inflammation, and chemotaxis, and Telomerase Reverse Transcriptase, the key enzyme preventing telomere erosion in the nucleus. Their unexpected localization in other organelles and regulation of mitochondrial and/or nuclear functions by them adds a new layer of regulatory complexity. This extends the flexibility to cope with changing environmental demands using a limited number of genes and proteins. *Antioxid. Redox Signal.* 13, 551–558.

Introduction

One distinguishing feature of eukaryotic cells in comparison to prokaryotes is their compartmentalization into organelles, which are obvious already at the microscopic level. Each compartment or organelle contains a characteristic set of proteins providing it with a unique structural and functional identity. Therefore, proteins, which—with the exception of a few respiratory chain components in the mitochondria—are translated in the cytoplasm, have to be targeted to their place of final destination. Eukaryotic cells have evolved highly specialized mechanisms to perform this task. Most commonly, specific topogenic sequences within proteins are used to target them to a distinct subcellular localization, such as the nucleus, mitochondria, peroxisomes, and the endoplasmic reticulum, from where they are transported through the Golgi apparatus to become secreted or membrane proteins. All these targeting sequences are characterized by conserved amino acids and are recognized by highly specialized transport complexes that are required to carry their cargo to the respective organelle. Specific sequences of amino acids can easily be recognized by appropriate analysis software and therefore a large number of programs are available to predict the subcellular localization of a protein based on its primary structure (Table 1). However, not all proteins contain such conserved targeting sequences despite a highly specific subcellular distribution (7, 8, 11, 20, 21).

Besides proteins that are exclusively localized in a single organelle, others exist, which are present in more than one compartment. One cellular strategy to achieve distribution to several or different locations is to produce different polypeptides possessing or lacking one or the other targeting sequence, either from separate genes or from a single gene by means of alternative transcription or translation initiation, differential splicing, or post-translational modification. However, several proteins possess two targeting signals leading to distribution between several organelles. In these cases, the desired and/or required localization can be achieved by different relative affinities to the transport machineries, accessibility of the targeting signals, incomplete translocation or redistribution via retrograde transport, leakage out of the organelle, or active export (for review, see Refs. 9 and 23).

[1]Molecular Aging Research, LIUF (Leibniz-Institute for Molecular Preventive Medicine), University of Duesseldorf, Duesseldorf, Germany.
[2]Crucible Laboratory, Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, United Kingdom.
*These authors contributed equally to the work.

Table 1. Publicly Available Programs for the Prediction of Protein Localization or Specific Targeting Sequences

Program	*Prediction*	*Homepage*	*Reference*
WoLF PSORT	Subcellular localization	http://wolfpsort.org	(21)
TargetP	Subcellular localization	www.cbs.dtu.dk/services/TargetP/	(11)
MultiLoc TargetLoc	Subcellular localization	www-bs.informatik.uni-tuebingen.de/Services/MultiLoc/	(20)
Mitoprot	Mitochondrial targeting sequence and cleavage site	http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html	(7)
SignalP	Signal peptide	www.cbs.dtu.dk/services/SignalP/	(11)
PredictNLS	Nuclear localization sequence	http://cubic.bioc.columbia.edu/services/predictNLS/	(8)

As the distribution of single translation products to more than one destination within the cell is less well understood than targeting to a single compartment, this review will be far from comprehensive, but rather a compilation of a few interesting proteins, for which a role in the nucleus and in the mitochondria has been shown just recently. The aim is not to describe the regulation of targeting of these proteins, but rather their function in different organelles. The major emphasis will be on proteins newly discovered in the mitochondria, which play a role in regulating the electron transfer chain. Mitochondria contain the most reducing compartment, have the highest rate of electron transfer, and are highly sensitive to oxidation. They are the most redox-active compartment of mammalian cells, accounting for more than 90% of electron transfer to O_2 as the terminal electron acceptor. Therefore, proteins that have well-described functions in other cellular compartments and were recently shown to be involved in the regulation of respiratory chain regulation are in the focus of this review. Specifically, we will discuss functions of four proteins in compartments where they had not been suspected before: a) the Signal Transducer and Activator of Transcription 3 (STAT3), originally described as a transcription factor; b) the most prominent Src kinase family members, Src, Fyn, and Yes, which were so far known as plasma membrane-associated molecular effectors of a variety of extracellular stimuli; c) the tyrosine phosphatase Shp-2 previously characterized as a modulator of cytosolic signal transduction involved in cell growth, development, inflammation, and chemotaxis; and d) Telomerase Reverse Transcriptase (TERT), the key enzyme preventing telomere erosion in the nucleus.

Signal Transducer and Activator of Transcription 3

Signal transducers and activators of transcription (STATs) were originally described as key components of a direct signal transduction pathway from the cell surface to the nucleus in response to cytokines and growth factors. For a long time, the tyrosine phosphorylation of STATs by ligand activated receptors was thought to be an obligatory requirement for dimerization in an active conformation, nuclear import, and transcriptional activation (10, 25). More recently it has been shown that nonphosphorylated STATs shuttle between the cytoplasm and the nucleus at all times in a constitutive manner and that also these nonphosphorylated STATs can be transcriptionally active, either as homodimers or in a complex with other transcription factors. However, these nonphosphorylated STATs regulate a different set of target genes than their phosphorylated counterparts (36, 42) (Fig. 1).

Lately, new functions for STAT3 outside the nucleus became evident. STAT3 was shown to be present in the mi-

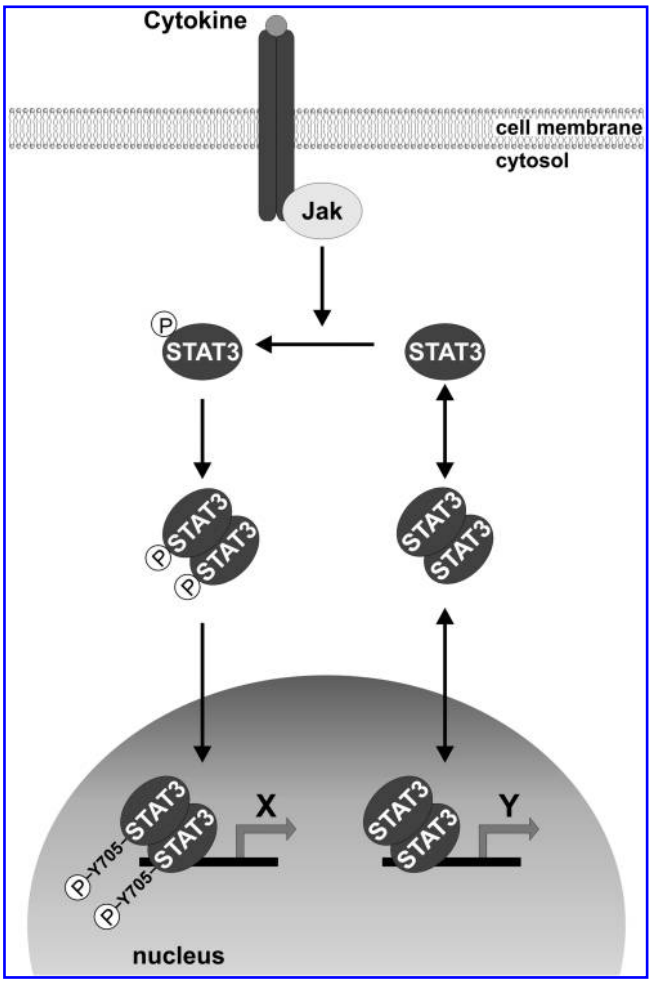

FIG. 1. Nuclear functions of STAT3. STAT3 is tyrosine phosphorylated by Janus kinase (Jak) activation in response to cytokine binding to the corresponding receptor. Phosphorylation of tyrosine 705 leads to nuclear translocation of the STAT3 protein. Dimerized, phosphorylated STAT3 causes transcriptional activation of a specific set of target genes (X). Nonphosphorylated STAT3 can shuttle between the cytoplasm and nucleus. Dimers of unphosphorylated STAT3 activate a different set of target genes (Y) than phosphorylated dimers.

tochondria of cultured cells and primary tissue, although it does not contain a mitochondrial targeting sequence. Immunoprecipitations demonstrated an association with complex I and possibly with complex II of the electron transport chain. On the functional level, an influence of STAT3 on the respiratory chain was demonstrated in STAT3-deficient pro-B cells, where the activities of complexes I and II were reduced by 40% and 85%, respectively, although the mitochondrial content in the STAT3 -/- cells was unaltered. These findings were confirmed in hearts of mice with cardiomyocyte specific ablation of STAT3. Reconstitution of STAT3-deficient cells with different STAT3 mutants specifically targeted to the mitochondria revealed that mitochondrial STAT3 is sufficient to modulate respiratory chain activity and that phosphorylation on serine 727 and a monomeric conformation play a crucial role in this process. In addition, the effects of STAT3 on the respiratory chain were unrelated to its actions as a transcription factor (39). A second report described a function of mitochondrial STAT3 in cellular transformation by the nontyrosine kinase oncogene Ras (14). Ras mediated transformation *in vitro* and tumor growth in mice were impaired in STAT3-deficient cells. Mutational analysis demonstrated that the N-terminal DNA binding domain, the Src homology 2 (SH2) domain, phosphorylation on tyrosine 705, and nuclear localization of STAT3 are dispensable for supporting malignant transformation by Ras. In contrast, tyrosine phosphorylation and presence in the nucleus are required for transformation by the tyrosine kinase oncogene v-Src. This newly discovered function of STAT3 was ascribed to its mitochondrial localization accompanied by augmentation of respiratory chain activity, particularly that of complex II and V, and a dependence on phosphorylation of serine 727. In summary, these reports lead to the conclusion that mitochondrial STAT3 can modulate the activity of the electron transport chain and that the structural requirements are completely different than the ones for transcriptional activation in the nucleus (Fig. 2).

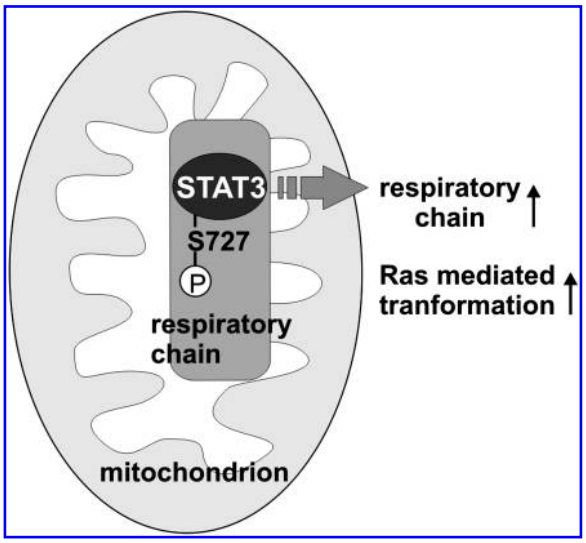

FIG. 2. Mitochondrial functions of STAT3. Phosphorylation on serine 727 is required for STAT3 translocation to the mitochondria. Here, monomeric STAT3 binds to complexes of the respiratory chain and thereby enhances their activities. This newly discovered function is unrelated to its actions as a transcription factor. Mitochondrial STAT3 is also required for malignant transformation induced by the proto-oncogene Ras.

Src, Fyn, and Yes Kinases

The Src family of nonreceptor protein tyrosine kinases consists of at least 9 members, some of which, like Src, Yes, and Fyn, are ubiquitously expressed, whereas others show more limited expression patterns (28). In this review we will focus on the most prominent kinases, Src, Fyn, and Yes, because they can compensate for each other. These three kinases are important for the regulation of cell proliferation by modulating cell metabolism, division, survival, and migration. Their function as plasma membrane-associated molecular effectors of a variety of extracellular stimuli is well known. However, recent studies demonstrated that at least Src fulfills also important functions in the nucleus and mitochondria. Changes in the chromatin structure indicative of active or inactive transcription are observed during cell cycle, tumorigenesis, and senescence. Increased euchromatic hypocondensation and heterochromatic hypercondensation are detected upon growth factor stimulation. These processes depend on nuclear tyrosine phosphorylation by Src, Fyn, and/or Yes, since they are not observed in Src, Fyn, Yes-triple deficient mouse embryonic fibroblasts (MEFs) (38). Recently, our group revealed a different cellular function for nuclear Src and Yes in endothelial cells by demonstrating that they contribute to the hydrogen peroxide-induced nuclear export of telomerase reverse transcriptase (TERT) (22), which will be discussed in more detail later in this review.

A mitochondrial localization of Src has been demonstrated by several groups (2, 26, 30). In the experiments described in these publications, several complexes of the respiratory chain have been identified as substrates for Src. First, the cytochrome c oxidase, the terminal complex of the electron transport chain was shown to be activated by Src (26). Recently, it has been discovered that Src has also effects on other complexes of the respiratory chain. Arachiche *et al*. (2) reported an increase of Src activity in response to ATP in rat brain mitochondria. ATP addition induced an autophosphorylation of Src at its catalytic site, which leads to its activation. This activated Src increased the activity of the complexes I, III, and IV, and decreased that of complex V (2). Taken together, these data indicate that respiratory chain activity is partially dependent on tyrosine phosphorylation by Src.

Protein Tyrosine Phosphatase Shp-2

The ubiquitously expressed protein tyrosine phosphatase Shp-2 contains two N-terminal SH2 domains and a C-terminal protein tyrosine phosphatase domain. Shp-2 plays an important role in cytosolic signal transduction. It modulates different pathways involved in cell growth, cell development, tissue inflammation, and cellular chemotaxis. These cytosolic functions of Shp-2 are well known and reviewed elsewhere (5). However, over the last years also nuclear and mitochondrial functions of Shp-2 have been identified.

In 2002 Chughtai *et al.* (6) reported a nuclear localization of Shp-2 associated with the signal transducer and activator of transcription 5 (STAT5). After stimulation of mammary cells

with prolactin, Shp-2 forms a complex with STAT5, which translocates into the nucleus. Formation of this complex is dependent on tyrosine phosphorylation of STAT5 in response to prolactin. Shp-2 binds to tyrosine phosphorylated STAT5 for which it requires the distal SH2 domain and an intact catalytic center. The nuclear Shp-2/STAT5 complex binds to DNA and regulates transcription of milk protein genes (6), demonstrating a transcriptional regulation by nuclear Shp-2. Given the fact that Shp-2 does not dephosphorylate STAT5 and that binding of Shp-2 to STAT5 is required for keeping STAT5 in its tyrosine phosphorylated and thereby active state, nuclear Shp-2 acts as an enhancer of transcription by binding to STAT5 and not as a phosphatase. Thus, these findings revealed a new phosphatase independent function of Shp-2. In contrast, an inhibition of the transcriptional activity of STAT1 by dephosphorylation of tyrosine and serine residues of STAT1 in the nucleus by Shp-2 has been shown (41), demonstrating that nuclear Shp-2 can also function as a phosphatase. In line with these findings are data from our group. We demonstrated that hydrogen peroxide-induced nuclear export of TERT is dependent on the tyrosine kinases Src and Yes. We identified nuclear Shp-2 as the counterplayer for this export, demonstrating again a phosphatase activity of Shp-2 in the nucleus (22). The maintenance of TERT in the nucleus by Shp-2 under conditions of oxidative stress will be discussed in more detail later in this review.

Recently, a tyrosine phosphatase activity was detected in the mitochondria of rat brains. The responsible phosphatase was identified as Shp-2. It was mainly located inside the mitochondria associated with cristae and the intercristal space (31). Arachiche *et al.* also showed a mitochondrial localization of Shp-2 (2). As mentioned above, the same group also detected the tyrosine kinase Src in the mitochondria and suggested that Src is partially involved in the regulation of respiratory chain activity. For cytosolic Src and Shp-2, it has long been demonstrated that the two proteins regulate each other in their activity. Therefore, it is tempting to speculate that mitochondrial Shp-2 acts as a phosphatase and inhibits Src activity and is thus also involved in the regulation of respiratory chain activity.

Telomerase Reverse Transcriptase

Telomeres, the physical ends of chromosomes, are necessary for their stability and integrity. They are shortened during each cell division. This shortening is counteracted by the enzyme telomerase. Telomerase is a large ribonucleoprotein complex and consists of the reverse transcriptase subunit telomerase reverse transcriptase (TERT), which contains the catalytic activity of the enzyme, and the associated RNA component TERC, which serves as the template for synthesis of the telomeric sequence (15, 27). Several telomerase-associated proteins are involved in the assembly and activity of the holoenzyme (Fig. 3).

TERT is differentially regulated on transcriptional and post-translational levels but also via its localization. One major post-translational event is the phosphorylation of TERT by kinases such as Src, Akt, PKC, and ERK1/2. The kinase Akt has a dominant role in the activation of TERT (3). Upon phosphorylation of serine 823 in TERT by Akt, telomerase activity is increased. Also the binding of HSP90 to TERT is essential for telomerase activity via stabilization of a TERT/Akt/Hsp90 complex (19) (Fig. 4). The localization of TERT is highly regulated. Our group demonstrated that TERT is exported from the nucleus after treatment with tumor necrosis factor alpha in combination with cycloheximide as well as with hydrogen peroxide in a CRM1/Ran-GTPase-dependent manner. This export is mediated by phosphorylation of TERT on tyrosine 707 by Src kinases in several cell types, including endothelial cells (19). During induction of replicative senescence in the latter cell type, an increase in active Src kinase phosphorylated on tyrosine 416 is observed, which induces nuclear export of TERT (18). Taking into account that cytosolic Shp-2 and Src kinases can regulate and antagonize each other under certain conditions, we hypothesized that a nuclear Shp-2 also exists in endothelial cells which may counteract the Src kinase-dependent nuclear export of TERT. Indeed, ablation of endogenous Shp-2 resulted in increased tyrosine phosphorylation of nuclear TERT and a reduction of telomerase activity in the nucleus. Moreover, overexpression of Shp-2 inhibited hydrogen peroxide-induced tyrosine phosphorylation and export of TERT from the nucleus. It has to be noted that this process requires the catalytic activity of Shp-2, since the catalytically inactive mutant Shp-2(C459S) cannot prevent nuclear export of TERT (22), suggesting that either nuclear Src or TERT or both are dephosphorylated by Shp-2 (Fig. 5).

Interestingly, nuclear TERT was shown to be not only involved in telomere elongation but also in the regulation of

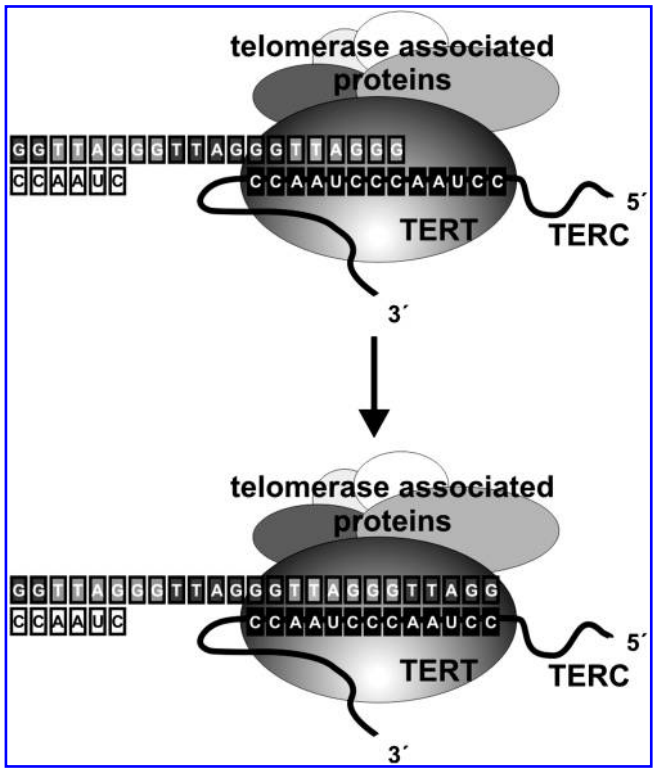

FIG. 3. Nuclear function of TERT. Telomerase is composed of the catalytic subunit telomerase reverse transcriptase (TERT) and the telomerase RNA component (TERC). TERC binds to the telomeric repeats ($TTAGGG_n$) and thus serves as a template for the reverse transcriptase. Several telomerase binding proteins are involved in the assembly and activity of the holoenzyme.

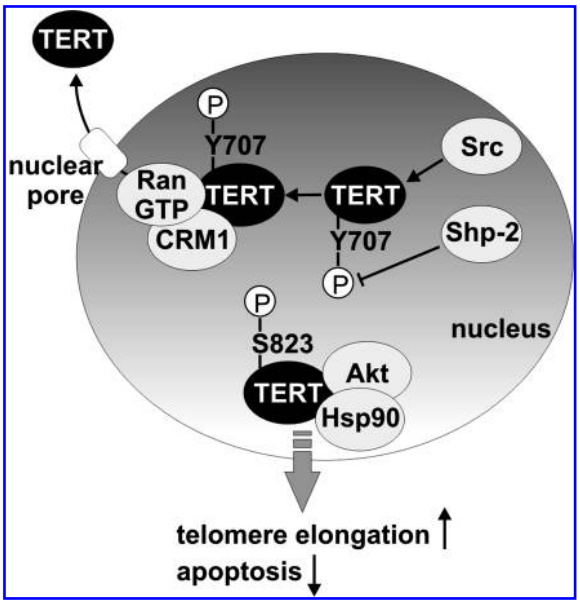

FIG. 4. In the nucleus TERT forms a complex with Akt and Hsp90, which keeps TERT phosphorylated on serine 823 and therefore in its active state. Active nuclear TERT prevents telomere erosion and can inhibit apoptosis. Under conditions of oxidative stress, Src kinases induce phosphorylation of nuclear TERT on tyrosine 707 resulting in nuclear TERT export via the nuclear pore in a CRM1/RanGTP dependent manner. Protein tyrosine phosphatase Shp-2 inhibits phosphorylation and TERT export.

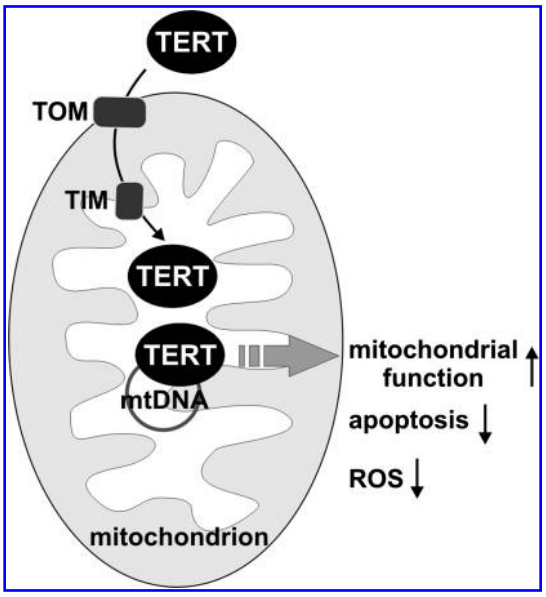

FIG. 6. TERT is imported into the mitochondria via the translocases of outer and inner membrane (TOM, TIM). Here, TERT is located in the matrix and can bind mitochondrial DNA (mtDNA). Mitochondrial TERT improves mitochondrial function, decreases apoptosis, and lowers ROS levels in the cell.

apoptosis (13, 17, 19). The anti-apoptotic capacity of TERT occurred within few hours after transfection, which indicates a function independent of direct telomere elongation. Further studies supported telomere-independent functions of TERT. In cell culture models, the suppression of TERT or TERC in cancer and stem cells has been shown to reduce proliferation and render the cells more vulnerable to apoptosis in a largely telomere length independent fashion (12, 34, 43). Similar results were obtained by ectopic expression of TERT. Stewart *et al.* demonstrated that TERT enhances tumorigenesis independent of its telomeric function although the mechanism for this effect is not entirely clear (37). Importantly, Sarin *et al.* showed that conditional transgenic induction of TERT can activate epidermal stem cells independent of its catalytic function (35). This demonstrated for the first time that TERT has an important telomere-independent function in stem and progenitor cells. In line with the emerging nontelomeric functions, Santos *et al.* showed that telomerase activity and TERT protein can be detected in mitochondria (32, 33). Although this came as a surprise to the scientific community, it is supported by the finding that TERT has a N-terminal mitochondrial targeting sequence. In addition, we showed that TERT is imported into mitochondria by the translocases of outer and inner membrane (16) (Fig. 6). However, the functions that TERT fulfills in mitochondria are still controversial. Santos *et al.* associated the mitochondrial localization of TERT with an increased apoptosis induction and interpreted this as a potential selective mechanism for the elimination of damaged stem cells (33). Recently our laboratories have contradicted these findings by demonstrating a beneficial role of TERT within mitochondria (1, 16). Independent of each other we found an improved mitochondrial function, decreased apoptosis, and reduced mitochondrial reactive oxygen species measured as a decrease in Mitosox fluorescence in cells expressing TERT (Fig. 6). Furthermore, we demonstrated that TERT directly or indirectly binds to mitochondrial DNA. Moreover, we showed that mouse lung fibroblasts from TERT knockout animals are more sensitive to ultraviolet B (UVB)-induced decrease in proliferation and respiration than their

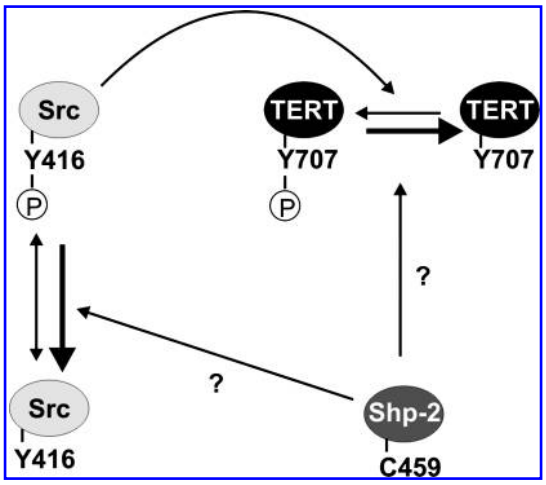

FIG. 5. Regulation of nuclear TERT tyrosine phosphorylation by Src and Shp-2. Active Src, phosphorylated on tyrosine 416, phosphorylates TERT on tyrosine 707, leading to nuclear export of TERT. This is counteracted by catalytically active Shp-2 (C459), which either dephosphorylates TERT directly or indirectly through inactivation of Src by dephosphorylation.

wild-type counterparts. UVB radiation causes cell death and DNA damage. It induces the formation of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts (44). Together with our finding that TERT associates with mitochondrial DNA, one could speculate that TERT protects mitochondrial DNA against the deleterious effects of UVB. However, there is accumulating evidence that other mechanisms, such as free radical formation, play important roles in the cellular responses caused by UVB radiation (24). This would offer an additional explanation for the protective function of mitochondrial TERT, which reduces reactive oxygen species in this organelle. We also demonstrated that TERT overexpression enhances respiratory chain activity and found that the respiration rate is decreased in heart, but not in liver from TERT knockout animals (16).

In accordance with TERT expressing cells having lower reactive oxidative species levels, it has recently been demonstrated that cells and tissues from mice deficient for the RNA component of telomerase (TERC) have an imbalance in their redox systems, resulting in higher levels of oxidative stress. Perez–Rivero and colleagues (29) found increased MnSOD level in MEFs and tissues from first generation TERC knockout mice, which do not display telomere shortening, but a decrease in catalase accompanied by a higher oxidative stress and oxidative damage. Elevated reactive oxygen species were shown by increased dichlorofluorescein diacetate and dihydroethidine fluorescence and oxidative damage of proteins was assessed by quantitation of 4-hydroxynonenal protein adducts. Most importantly, re-introduction of TERC restored the redox balance (29). This *in vivo* demonstration of a direct relationship between telomerase deficiency and oxidative stress is supported by data from our laboratories. We showed a reduced oxygen uptake in heart tissue from TERT knockout mice and a decreased UVB resistance in lung fibroblasts derived from these animals (16). These data are complemented by our *in vitro* findings demonstrating a decrease of reactive oxygen species and improvement of mitochondrial function in TERT overexpressing fibroblasts (1). In accordance with this, higher catalase protein levels were found in TERT overexpressing fibroblasts while there was no change in the levels of MnSOD (Saretzki, unpublished data). Moreover, the influence of TERT on heart function has been further investigated in a voluntary running mouse model (40). We showed that physical exercise can stimulate telomerase in the heart and has beneficial anti-aging effects measured by a decrease in senescence-associated markers such as p16, p53, and Chk2. In TERT-deficient mice, however, the effect of exercise was absent, pointing to an important role of telomerase in this process. This leads to the conclusion that the running-induced upregulation of telomerase reduces oxidative stress and thereby may slow down senescence. Given the facts that serum levels of insulin-like growth factor 1 (IGF-1) are increased with voluntary running and that IGF-1 has been shown to activate Akt in cardiomyocytes, we wanted to determine whether increased IGF-1 levels serve as a mediator of increased telomerase activity. Therefore, mice were treated with IGF-1 and as expected, the IGF-1 treatment resulted in an activation of Akt in the heart and a substantial increase of telomerase (40). In addition, an increased proliferation rate in cardiomyocytes was observed after voluntary running. One possible explanation might be a change in pro-proliferative transcriptional programs due to increased TERT levels, because changes in the cellular transcriptome have been observed upon overexpression of TERT (4). However, it is undeniable that mitochondrial function is required for cardiomyocyte proliferation, suggesting that increased TERT levels, which result in enhanced respiratory chain activity, are one of the reasons for cardiomyocyte proliferation.

In conclusion, it is tempting to speculate that nuclear and mitochondrial TERT act in concert to improve cardiomyocyte and thereby heart function.

Conclusion

In this review we have summarized recent evidence for several proteins extending their functions to cellular compartments beyond the ones, which have been textbook knowledge for a long time. These proteins can have similar roles in different organelles or can perform completely different, so far unexpected tasks depending on their subcellular localization. These new functions are not restricted to a specific class of proteins, as they have been described for transcription factors, protein kinases and phosphatases, and the only eukaryotic reverse transcriptase, TERT. Interestingly, all these proteins are involved in regulatory processes, which help cells to adapt to changing environmental situations. Thus, one may speculate that such additional functions in other cellular compartments, especially in the mitochondria, are not restricted to the few examples described here, but could be a more general phenomenon, which might have been overlooked in the past. Changing the subcellular distribution of a particular protein and thereby sometimes making use of other functional properties, adds a new layer of complexity in addition to the well described regulatory processes on the transcriptional, translational, or post-translational levels. Thereby cells, organs and whole organisms would extend their flexibility to cope with changing environmental demands using a limited number of genes and proteins.

Acknowledgments

We apologize for the failure to cite many of the important and relevant papers in this field due to space limitations. This study was in part supported by the Deutsche Forschungsgemeinschaft to J.H. (SFB 728 B5 and HA-2868/2-3).

References

1. Ahmed S, Passos JF, Birket MJ, Beckmann T, Brings S, Peters H, Birch–Machin MA, von Zglinicki T, and Saretzki G. Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. *J Cell Sci* 121: 1046–1053, 2008.
2. Arachiche A, Augereau O, Decossas M, Pertuiset C, Gontier E, Letellier T, and Dachary–Prigent J. Localization of PTP-1B, SHP-2, and Src exclusively in rat brain mitochondria and functional consequences. *J Biol Chem* 283: 24406–24411, 2008.
3. Breitschopf K, Zeiher AM, and Dimmeler S. Pro-atherogenic factors induce telomerase inactivation in endothelial cells through an Akt-dependent mechanism. *FEBS Lett* 493: 21–25, 2001.
4. Choi J, Southworth LK, Sarin KY, Venteicher AS, Ma W, Chang W, Cheung P, Jun S, Artandi MK, Shah N, Kim SK, and Artandi SE. TERT promotes epithelial proliferation

through transcriptional control of a Myc- and Wnt-related developmental program. *PLoS Genet* 4: e10, 2008.
5. Chong ZZ and Maiese K. The Src homology 2 domain tyrosine phosphatases SHP-1 and SHP-2: Diversified control of cell growth, inflammation, and injury. *Histol Histopathol* 22: 1251–1267, 2007.
6. Chughtai N, Schimchowitsch S, Lebrun JJ, and Ali S. Prolactin induces SHP-2 association with Stat5, nuclear translocation, and binding to the beta-casein gene promoter in mammary cells. *J Biol Chem* 277: 31107–31114, 2002.
7. Claros MG and Vincens P. Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur J Biochem* 241: 779–786, 1996.
8. Cokol M, Nair R, and Rost B. Finding nuclear localization signals. *EMBO Rep* 1: 411–415, 2000.
9. Danpure CJ. How can the products of a single gene be localized to more than one intracellular compartment? *Trends Cell Biol* 5: 230–238, 1995.
10. Darnell JE, Jr., Kerr IM, and Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264: 1415–1421, 1994.
11. Emanuelsson O, Brunak S, von Heijne G, and Nielsen H. Locating proteins in the cell using TargetP, SignalP and related tools. *Nat Protoc* 2: 953–971, 2007.
12. Folini M, Brambilla C, Villa R, Gandellini P, Vignati S, Paduano F, Daidone MG, and Zaffaroni N. Antisense oligonucleotide-mediated inhibition of hTERT, but not hTERC, induces rapid cell growth decline and apoptosis in the absence of telomere shortening in human prostate cancer cells. *Eur J Cancer* 41: 624–634, 2005.
13. Gorbunova V, Seluanov A, and Pereira–Smith OM. Expression of human telomerase (hTERT) does not prevent stress-induced senescence in normal human fibroblasts but protects the cells from stress-induced apoptosis and necrosis. *J Biol Chem* 277: 38540–38549, 2002.
14. Gough DJ, Corlett A, Schlessinger K, Wegrzyn J, Larner AC, and Levy DE. Mitochondrial STAT3 supports Ras-dependent oncogenic transformation. *Science* 324: 1713–1716, 2009.
15. Greider CW and Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. *Nature* 337: 331–337, 1989.
16. Haendeler J, Dröse S, Büchner N, Jakob S, Altschmied J, Goy C, Spyridopoulos I, Zeiher AM, Brandt U, and Dimmeler S. Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. *Arterioscler Thromb Vasc Biol* 29: 929–935, 2009.
17. Haendeler J, Hoffmann J, Brandes RP, Zeiher AM, and Dimmeler S. Hydrogen peroxide triggers nuclear export of telomerase reverse transcriptase via Src kinase family-dependent phosphorylation of tyrosine 707. *Mol Cell Biol* 23: 4598–4610, 2003.
18. Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM, and Dimmeler S. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. *Circ Res* 94: 768–775, 2004.
19. Haendeler J, Hoffmann J, Rahman S, Zeiher AM, and Dimmeler S. Regulation of telomerase activity and anti-apoptotic function by protein–protein interaction and phosphorylation. *FEBS Lett* 536: 180–186, 2003.
20. Höglund A, Donnes P, Blum T, Adolph HW, Kohlbacher O. MultiLoc: Prediction of protein subcellular localization using N-terminal targeting sequences, sequence motifs and amino acid composition. *Bioinformatics* 22: 1158–1165, 2006.
21. Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams–Collier CJ, and Nakai K. WoLF PSORT: Protein localization predictor. *Nucleic Acids Res* 35: W585–587, 2007.
22. Jakob S, Schroeder P, Lukosz M, Büchner N, Spyridopoulos I, Altschmied J, and Haendeler J. Nuclear protein tyrosine phosphatase Shp-2 is one important negative regulator of nuclear export of telomerase reverse transcriptase. *J Biol Chem* 283: 33155–33161, 2008.
23. Karniely S and Pines O. Single translation–dual destination: mechanisms of dual protein targeting in eukaryotes. *EMBO Rep* 6: 420–425, 2005.
24. Masaki H, Atsumi T, and Sakurai H. Detection of hydrogen peroxide and hydroxyl radicals in murine skin fibroblasts under UVB irradiation. *Biochem Biophys Res Commun* 206: 474–479, 1995.
25. Mertens C and Darnell JE, Jr. SnapShot: JAK-STAT signaling. *Cell* 131: 612, 2007.
26. Miyazaki T, Neff L, Tanaka S, Horne WC, and Baron R. Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. *J Cell Biol* 160: 709–718, 2003.
27. Nakamura TM and Cech TR. Reversing time: Origin of telomerase. *Cell* 92: 587–590, 1998.
28. Parsons SJ and Parsons JT. Src family kinases, key regulators of signal transduction. *Oncogene* 23: 7906–7909, 2004.
29. Perez–Rivero G, Ruiz–Torres MP, Diez–Marques ML, Canela A, Lopez–Novoa JM, Rodriguez–Puyol M, Blasco MA, and Rodriguez–Puyol D. Telomerase deficiency promotes oxidative stress by reducing catalase activity. *Free Radic Biol Med* 45: 1243–1251, 2008.
30. Salvi M, Brunati AM, Bordin L, La Rocca N, Clari G, and Toninello A. Characterization and location of Src-dependent tyrosine phosphorylation in rat brain mitochondria. *Biochim Biophys Acta* 1589: 181–195, 2002.
31. Salvi M, Stringaro A, Brunati AM, Agostinelli E, Arancia G, Clari G, and Toninello A. Tyrosine phosphatase activity in mitochondria: Presence of Shp-2 phosphatase in mitochondria. *Cell Mol Life Sci* 61: 2393–2404, 2004.
32. Santos JH, Meyer JN, Skorvaga M, Annab LA, and Van Houten B. Mitochondrial hTERT exacerbates free-radical-mediated mtDNA damage. *Aging Cell* 3: 399–411, 2004.
33. Santos JH, Meyer JN, and Van Houten B. Mitochondrial localization of telomerase as a determinant for hydrogen peroxide-induced mitochondrial DNA damage and apoptosis. *Hum Mol Genet* 15: 1757–1768, 2006.
34. Saretzki G, Ludwig A, von Zglinicki T, and Runnebaum IB. Ribozyme-mediated telomerase inhibition induces immediate cell loss but not telomere shortening in ovarian cancer cells. *Cancer Gene Ther* 8: 827–834, 2001.
35. Sarin KY, Cheung P, Gilison D, Lee E, Tennen RI, Wang E, Artandi MK, Oro AE, and Artandi SE. Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature* 436: 1048–1052, 2005.
36. Sehgal PB. Paradigm shifts in the cell biology of STAT signaling. *Semin Cell Dev Biol* 19: 329–340, 2008.
37. Stewart SA, Hahn WC, O'Connor BF, Banner EN, Lundberg AS, Modha P, Mizuno H, Brooks MW, Fleming M, Zimonjic DB, Popescu NC, and Weinberg RA. Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. *Proc Natl Acad Sci USA* 99: 12606–12611, 2002.
38. Takahashi A, Obata Y, Fukumoto Y, Nakayama Y, Kasahara K, Kuga T, Higashiyama Y, Saito T, Yokoyama KK, and Yamaguchi N. Nuclear localization of Src-family tyrosine kinases is required for growth factor-induced euchromatinization. *Exp Cell Res* 315: 1117–1141, 2009.

39. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, Moh A, Moghaddas S, Chen Q, Bobbili S, Cichy J, Dulak J, Baker DP, Wolfman A, Stuehr D, Hassan MO, Fu XY, Avadhani N, Drake JI, Fawcett P, Lesnefsky EJ, and Larner AC. Function of mitochondrial Stat3 in cellular respiration. *Science* 323: 793–797, 2009.
40. Werner C, Hanhoun M, Widmann T, Kazakov A, Semenov A, Poss J, Bauersachs J, Thum T, Pfreundschuh M, Muller P, Haendeler J, Bohm M, and Laufs U. Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. *J Am Coll Cardiol* 52: 470–482, 2008.
41. Wu TR, Hong YK, Wang XD, Ling MY, Dragoi AM, Chung AS, Campbell AG, Han ZY, Feng GS, and Chin YE. SHP-2 is a dual-specificity phosphatase involved in Stat1 dephosphorylation at both tyrosine and serine residues in nuclei. *J Biol Chem* 277: 47572–47580, 2002.
42. Yang J and Stark GR. Roles of unphosphorylated STATs in signaling. *Cell Res* 18: 443–451, 2008.
43. Yatabe N, Kyo S, Kondo S, Kanaya T, Wang Z, Maida Y, Takakura M, Nakamura M, Tanaka M, and Inoue M. 2-5A antisense therapy directed against human telomerase RNA inhibits telomerase activity and induces apoptosis without telomere impairment in cervical cancer cells. *Cancer Gene Ther* 9: 624–630, 2002.
44. You YH, Lee DH, Yoon JH, Nakajima S, Yasui A, and Pfeifer GP. Cyclobutane pyrimidine dimers are responsible for the vast majority of mutations induced by UVB irradiation in mammalian cells. *J Biol Chem* 276: 44688–44694, 2001.

Address correspondence to:
Judith Haendeler, Ph.D.
Molecular Aging Research
Institute for Molecular Preventive Medicine
University of Duesseldorf gGmbH
Auf'm Hennekamp 50
40225 Duesseldorf
Germany

E-mail: j.haendeler@web.de

Date of first submission to ARS Central, November 12, 2009; date of acceptance, December 2, 2009.

Abbreviations Used

Chk2 = checkpoint kinase 2
CRM1 = chromosome region maintenance 1
ERK = extracellular regulated kinase
Hsp90 = heat shock protein 90
IGF-1 = insulin-like growth factor 1
MEF = mouse embryonic fibroblast
MnSOD = manganese superoxide dismutase
PKC = protein kinase C
SH2 = Src homology 2
STAT = signal transducer and activator of transcription
TERT = telomerase reverse transcriptase
UV = ultraviolet

This article has been cited by:

1. Charlie Mantel, Steven V Messina-Graham, Hal E Broxmeyer. 2011. Superoxide flashes, reactive oxygen species, and the mitochondrial permeability transition pore: potential implications for hematopoietic stem cell function. *Current Opinion in Hematology* **18**:4, 208-213. [CrossRef]
2. Thomas Kietzmann . 2010. Intracellular Redox Compartments: Mechanisms and Significances. *Antioxidants & Redox Signaling* **13**:4, 395-398. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]