Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mutation Research 577 (2005) 252–259
Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Review

Deficient DNA repair in the human progeroid disorder, Werner syndrome

Vilhelm A. Bohr*

Laboratory of Molecular Gerontology, National Institute on Aging, NIH, 5600 Nathan Shock Dr., Baltimore, MD 21224, USA

Received 11 February 2005; received in revised form 4 March 2005; accepted 4 March 2005
Available online 23 May 2005

Abstract

The study of how DNA repair mechanisms change with aging is central to our understanding of the aging process. Here, I review the molecular functions of a key aging protein, Werner protein (WRN), which is deficient in the premature aging disorder, Werner syndrome (WS). This protein plays a significant role in DNA repair, particularly in base excision repair and in recombination. WRN may be a key regulatory factor in these processes and may also play a role in coordinating them. WRN belongs to the RecQ helicase family of proteins, often referred to as the guardians of the genome. These proteins appear to integrate with the more classic DNA repair pathways and proteins.
Published by Elsevier B.V.

Keywords: Werner syndrome; RecQ helicases; DNA repair

Contents

* Fax: +1 410 558 8157.
E-mail address: vbohr@nih.gov.

0027-5107/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.mrfmmm.2005.03.021

Prologue

It is a pleasure to contribute to this special issue in honor of Philip Hanawalt. I have known Phil for about 25 years. I came from Copenhagen, Denmark in 1982 to join his laboratory at Stanford and I spent 4 years there during a very intense time when many advances were made. Phil had been a postdoc in Måløes laboratory in Copenhagen 1958–1960, and thus had a good appreciation of Denmark and its culture and even some tidbits of its language. On festive occasions Phil would sing some Danish songs and I would often be the only one there who could truly appreciate the sophisticated details of his pronunciation. Phil often tells good stories from the Måløe lab in 1950s. This was one of the birthplaces of molecular biology with many of Måløe's students going on to become leading scientists. While there was a stimulating atmosphere in this lab, it was also full of smoke from Måløe's cigars. It is fun to hear Phil describe this situation. One of Phil's great attributes is his sense of humor, and this gift comes to use in many a situation. The years in Phil's laboratory were important for my career development. The discoveries we made in those years did have significant impact on the field and opened opportunities that I have later pursued within the study of DNA repair mechanisms. Also, my interest in aging began through discussions with Phil and others in his lab. Several years later, when I was offered the opportunity to head a department at the National Institute on Aging, I saw this as a great opportunity to explore the interphase of aging and DNA repair, a line of work that I have followed since.

1. Introduction

It is not a new idea that a deficiency in DNA repair may be an important factor in the aging phenotype. Various studies have documented a decline in DNA repair capacity in analyses of aging. Deficiencies in DNA repair may lead to age associated diseases such as cancer and neurodegeneration rather than changes in longevity, and this is the general hypothesis with which we are working. The study of DNA repair has broadened enormously in recent years and so has the mechanistic insight and the tools for analysis. The study on aging has also broadened considerably and the model systems used have expanded to allow for more mechanistic studies. Thus, the convergence of DNA repair and aging is a revived area of study and provides new and challenging opportunities.

In this review I will briefly discuss some of the studies on human premature aging, one important approach to the study of aging. As a model system, we have been interested in Werner syndrome (WS), because it has a particularly close resemblance to the normal aging phenotype. The Werner protein (WRN) belongs to the family of RecQ helicases, guardians of the genome, and this is also befitting for a tribute to Phil Hanawalt, because he has contributed significantly to the understanding of the function of RecQ from *Escherichia coli*, the original member of the family.

2. Multiple roles of the Werner protein in DNA repair

For many years, we have been used to thinking about the different DNA repair pathways as being distinct processes defined to a large degree by the nature of the lesion that is being repaired. But in recent years it has become more and more evident that the different pathways interplay in important ways. As will be argued in the following, Werner syndrome protein (WRN) is an example of a protein that functions in at least two pathways of DNA repair.

2.1. RecQ helicases and the Werner protein

RecQ helicases are a family of conserved enzymes that play roles in maintaining genomic integrity and suppressing deleterious recombination events. Mutations in *E. coli* RecQ and in the *S. cerevisiae* RecQ homolog, Sgs1, result in an increased frequency of illegitimate recombination [1]. In humans, defects in three RecQ family members, WRN, Blooms syndrome (BLM), and RECQL4, are associated with rare autosomal-recessive disorders characterized by genomic instability and increased cancer susceptibility. Mutations in WRN, BLM, and RECQL4 give rise to the disorders Werner syndrome (WS), Bloom syndrome (BS) and Rothmund–Thomson syndrome (RTS), respectively, whose clinical features have been extensively reviewed elsewhere [2]. Briefly, BS patients are predisposed to many types of cancer with a mean age of onset of 24. WS patients are especially predisposed to sarcomas, premature aging and aging-associated dis-

eases. RTS patients have a characteristic rash, poikiloderma, and are predisposed to osteosarcomas and some features of premature aging. The molecular basis of genomic instability in patients with these syndromes is not well understood. Gene expression profiles in WS individuals are similar to those of normal older individuals [3], indicating that WS may be a useful model for normal aging.

E. coli RecQ is the prototypical member of the RecQ protein family, which is conserved from bacteria to humans. Helicases separate complementary strands of nucleic acids in a reaction coupled to NTP hydrolysis. RecQ helicases have a common helicase domain with seven conserved motifs which bind and hydrolyze ATP. A recent report on the X-ray crystal structure for the *E. coli* RecQ catalytic core indicates that the RQC domain contains DNA and protein binding motifs [4]. RecQ helicases prefer substrates that resemble DNA metabolic intermediates, including forked and flap structures (intermediates in DNA replication and repair), bubble structures (intermediates in DNA repair and transcription), D-loop and Holliday junction structures (intermediates in DNA recombination) and G-quadruplex DNA and D-loop structures (associated with telomere DNA). Thus, the in vitro biochemistry suggests that WRN, BLM and other RecQ helicases may play roles in DNA repair, recombination, replication, telomere processing and transcription (reviewed in [5,6]). The mammalian genome contains a variety of sequences that may generate specific substrates for RecQ helicases. These sequences can form DNA triple helices and G-quadruplex structures that have the potential to block unwinding and other DNA transactions.

Consistent with their ability to act on multiple intermediates in DNA processing, RecQ helicases interact with many proteins involved in DNA metabolism [5–7]. Some are significant functional interactions. For example, the Ku heterodimer strongly stimulates WRN exonuclease [8–10] and the telomere binding protein, TRF2, stimulates WRN helicase activity [11]. Other proteins, such as p53 and BLM, inhibit WRN exonuclease activity [12,13], while nucleolin inhibits its helicase activity (Indig et al., unpublished). Based on the biochemistry described above and on the known protein partners of WRN, it is likely that the main functions of WRN are in DNA repair and in telomere maintenance. Some of the important functional protein interactions between WRN and other proteins are shown in Fig. 1.

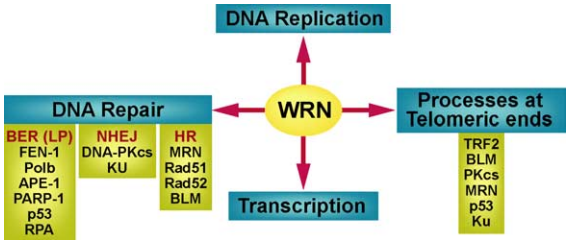

Fig. 1. Major protein partners for WRN protein. Protein interactions representing documented functional interactions are shown. These interactions suggest that WRN is involved in the specific processes listed and particularly in DNA repair.

Functional interaction implies that the proteins affect each other's catalytic activity. In some cases, there are reciprocal interactions.

2.2. Role of WRN in DNA double strand break repair (DSBR)

Two important pathways of DSBR are homologous recombination (HR) and non homologous end-joining (NHEJ). RecQ helicases may function in HR to promote proper intermediate resolution and suppress strand cross-over events. HR is important for repairing chromosomal double strand breaks (DSB), which result when the replication fork encounters a single strand break (SSB) or gap. The substrate specificity of the RecQ helicases and their protein interactions are consistent with roles in HR. BS cells and yeast *sgs1* mutants display a high frequency of HR-mediated sister-chromatid exchanges, which likely result from DSBs during DNA replication [14]. WS and RTS cells display an increased frequency of chromosomal rearrangements, including translocations and deletions [5,14], which may result partly from DSBs. WRN may participate both at early and late stages of HR. WRN colocalizes and interacts with Rad52, and in vitro shows modest stimulation of Rad52 mediated ssDNA annealing [15]. Recent in vivo data suggest that WRN participates in late stages of HR resolution [16,17]. This is supported by the observation that WRN interacts functionally with Nbs1 [9] as Nbs1 is thought to function downstream of Rad51 [18].

Essential components of the NHEJ pathway of DSBR [19] have been found to interact with WRN. A search for protein interactions with the WRN C-terminal region identified the Ku heterodimer as

the most prominent binder [8]. Ku stimulates the WRN 3′–5′ exonuclease and increases its processivity [8,20,21]. Ku is part of the DNA–PK complex, and associates with DNA and the DNA–PK catalytic subunit to form an active kinase [19]. DNA–PK-dependent phosphorylation of WRN has been observed in vitro and in vivo, and regulates the WRN helicase and exonuclease activities [22,23]. Aberrant products of NHEJ reactions have been observed in WS cells [24]. Precise roles for WRN, and potentially other RecQ helicases, in NHEJ, remain to be determined.

2.3. Role of WRN in single strand break repair/base excision repair (SSBR/BER)

Roles for RecQ helicases in repair of single strand breaks (SSB) have also been proposed. WS cells display sensitivity to 4-nitroquinoline 1-oxide (4 NQO) and ionizing radiation [5], which generate various types of DNA damage, including oxidative damage. Recently it has been shown that WRN knockdown cells are hypersensitive to the methylating agents methyllexitropsin and temozolomide compared to isogenic controls [25]. In addition, WRN has been shown to physically interact with several proteins involved in SSBR/BER including: DNA polymerase δ (pol δ) [26], DNA polymerase β (pol β) [27], proliferating cell nuclear antigen (PCNA) [28], replication protein A (RPA) [29], flap endonuclease 1 (FEN-1) [30], and poly(ADP-ribose) polymerase 1 (PARP-1) [31]. WRN has been shown to stimulate FEN-1 flap cleavage [30] and nucleotide incorporation by pol δ [32]. WRN helicase activity stimulates pol β strand displacement DNA synthesis [27] and cooperates with pol β on 3′ mismatches (Harrigan et al., unpublished). RPA stimulates WRN helicase activity [29] and the poly(ADP-ribosyl)ation state of PARP-1 regulates WRN helicase and exonuclease activities [33]. PARP-1 binds strongly to strand breaks and acts in the DNA damage surveillance network, partly by ribosylating a variety of nuclear proteins in response to DNA damage. WS cells are deficient in poly(ADP-ribosyl)ation in response to H_2O_2 and methyl methane sulfonate [31], indicating that the strand breaks may not be properly processed in WS cells. These findings suggest that WRN, together with its protein partners, functions in SSBR/BER.

The BER pathway is a hand-off process [34] where the DNA substrate is passed through a number of proteins and complexes. We have recently examined the potential role of WRN in this process. AP-endonuclease 1 (APEndo) inhibited the helicase activity of WRN [35], and this inhibition was relieved in the presence of pol β [35]. Thus, it is possible that WRN participates in the BER process, affecting other proteins in this pathway and its functions are regulated such that the helicase activity can be involved in the pol β dependent strand displacement synthesis, but otherwise is inhibited from performing promiscuous, undesirable functions.

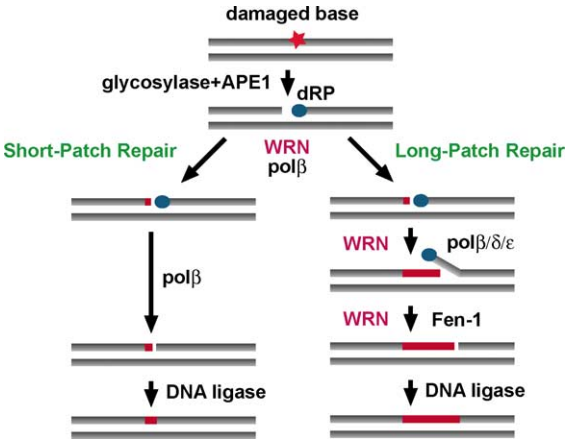

Fig. 2. WRN protein participates at different steps in the base excision repair process. See text for discussion.

Fig. 2 shows the general steps in BER as subdivided into the long patch and short patch subpathways. The figure also shows some of the auxiliary proteins involved. Protein partners of WRN are indicated as are the steps in the process where WRN protein may be operating. This model is based on in vitro studies, but recently, we have also obtained cellular data demonstrating that WS cells accumulate the oxidized DNA base lesion 8-oxoG [36], thus supporting the in vitro work with more in vivo observations, and further corroborating that WS cells are defective in BER.

3. Roles for the Werner protein at telomeres

3.1. Role of RecQ helicase activity in telomere maintenance

Telomeres function to protect and cap the chromosome ends and prevent them from being recognized as

DNA DSBs. Indeed, the induction of telomere dysfunction, by interfering with telomere maintenance proteins, activates a DNA damage response pathway [37]. The exact nature of the telomere cap is not understood, but it is known to consist of telomeric DNA and associated proteins. Human telomeres consist of 5–15 kb of TTAGGG tandem repeats and end in a 3′ single strand G-rich tail that is at least 100 nucleotides long. Maintenance of this precise sequence is critical for proper function [38] and loss of telomeres has been linked to the aging process [39]. Telomere repeat binding factors 1 and 2 (TRF1 and TRF2) bind specifically to human telomeric sequence, and regulate telomere length and access [40] and these proteins interact with other proteins that function in DNA DSBR [40]. Human Pot1 binds specifically to human single strand telomeric DNA and regulates telomere length. Loss of Pot1 in yeast results in rapid loss of telomeric DNA [41]. How these proteins cooperate with telomeric DNA to protect telomeres and to regulate accessibility of telomeric 3′ tail is an active area of investigation. We previously identified a physical and functional interaction between TRF2 and the RecQ helicases WRN and BLM [11]. We and others have evidence that WRN and BLM likely function in recombination repair pathways that lengthen telomeric ends. It is becoming increasingly apparent that specialized DNA helicases function in telomere maintenance. In yeast the helicases Rrm3 and Pif1 influence DNA replication at the telomeres [42], and other helicases have been found to regulate telomere length in mice [43]. Other, recent evidence supports the idea that WRN has an important function at the telomere end. While WRN knockout mice have been reported to have no phenotype, a mouse with telomerase knockout and WRN knockout has distinct premature aging features, much resembling those seen in a WS patient [44]. This finding suggests that WRN has an important function at the telomere end. Further, Crabbe et al., recently found defective telomere lagging strand synthesis in cells lacking WRN helicase activity [45].

3.2. DNA damage and repair of telomeres

Increasing evidence indicates that DNA damage may contribute directly to telomere dysfunction. A number of studies have reported increased telomere erosion in response to mild chronic oxidative stress induced by various means including hyperoxia and mitochondrial dysfunction [46]. In these studies, treatment with antioxidants prevented the telomere attrition. Hydrogen peroxide treatment increased extrachromosomal fragments in cultured fibroblasts, and at a higher frequency in ATM-deficient cells [47]. The mechanism of telomere attrition is unknown, but studies in human fibroblasts [46] and with telomeric DNA in vitro, indicate that telomeric DNA may be highly susceptible to oxidative and alkylation lesions [46]. A preferential accumulation of single stranded regions was found in telomeres of primary fibroblast compared to bulk DNA in response to oxidative and alkylation damage [46]. In addition, the telomeric sequence is highly susceptible to UVA irradiation-induced 8-oxo-guanine adducts, which induces telomere erosion in primary fibroblasts [48]. The roles of repair pathways at telomeric ends, including BER and recombination repair, in maintaining telomere function are virtually unknown. Recently, we observed that TRF2 binds specifically to a number of proteins involved in BER including pol β and FEN-1 (our unpublished results). TRF1 and TRF2 also interact with proteins that function in DNA DSB repair [40]. It has been proposed that defects in telomere repair may result in accelerated senescence and aging [47]. Consistent with this idea, it was observed that WS cells were deficient in repair of the telomere repeat sequence [49].

4. Role of the Werner protein in coordinating DNA repair pathways

There appears to be good evidence for involvement of Werner protein in two major DNA repair pathways, BER and DNA DSB repair (DSBR). WRN might coordinate within the individual pathways and it may coordinate between them. For example, in the course of end-joining there is a step of helicase activity followed by resection of single stranded regions by exonuclease. To avoid undesired functions at steps in this pathway, one of the two catalytic activities of WRN could be temporarily down regulated. This regulation could be affected by posttranslational modification of the protein. For example, phosphorylation effected by different kinases can greatly change WRN catalytic activities. WRN is phosphorylated in vivo and in vitro, and extensive phosphorylation can lead to complete inhi-

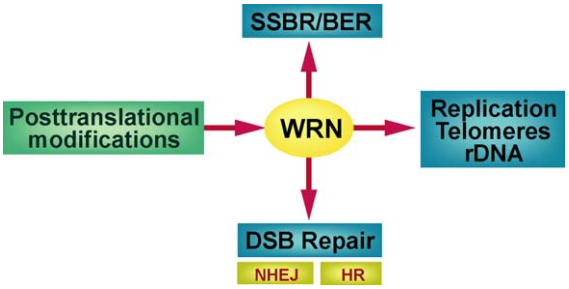

Fig. 3. WRN protein as a coordinator in DNA repair. WRN participates in BER and recombination and its functions are greatly affected by its state of post translational modification, such as phosphorylation, acetylation and oxidation. It prefers substrates such as replication forks, G-quartet structures and triple helical structures, and these are found throughout the genome, but with a preponderance for telomeric DNA and ribosomal regions. In its function, WRN likely cooperates with other RecQ helicases such as Blooms protein.

bition of its helicase and exonuclease functions [22]. These aspects are depicted in Fig. 3. WRN may coordinate the BER and double strand break repair (DSBR) pathways and this coordination may be regulated at least in part by the extent of WRN posttranslational modification. WRN operates in association with the DNA repair machinery, and particularly at stalled replication forks or at other special DNA structures, such as Holliday junction recombination intermediates and G4 quartet structures. Here, WRN may cooperate with BLM or other RecQ helicases. An interaction between WRN and BLM has been demonstrated [13], the first observation of functional interaction between human RecQ helicases, and there may well be others.

An interesting feature about WRN is that possesses three different enzymatic activities, the helicase, exonuclease and ATPase functions. It is a special challenge to speculate on metabolic pathways where these different catalytic activities are needed and thus where WRN might operate. In the process of non homologous end joining (NHEJ) these three activities are required, and this has helped fuel the idea that WRN operates here. Since the helicase function would be needed before that of the exonuclease in the course of this process, it is tempting to speculate that the relative activities of the different WRN activities might be regulated throughout a pathway such as NHEJ by relative changes in the phosphorylation of the protein allowing one and not the other catalytic activity to function at a given time. It is also likely that the different WRN catalytic activities are needed in telomere maintenance [50], and this in another process where relative post translational modifications of WRN could play a regulatory role.

RecQ helicases are called “guardians of the genome” or “genome caretakers”, but they are also participants in the more classic schemes of DNA repair pathways. The helicases may preferentially operate at specific DNA structures such as arrested replication forks, hairpins, G-quartets, or triple helix structures in DNA. They represent an integrated component of the DNA repair machinery by working in conjunction with the repair proteins of the BER and recombination pathways.

Acknowledgements

I wish to acknowledge my many colleagues in this laboratory and collaborators who have all greatly contributed to the ongoing studies on WRN and other premature aging proteins.

References

[1] K. Hanada, T. Ukita, Y. Kohno, K. Saito, J. Kato, H. Ikeda, RecQ DNA helicase is a suppressor of illegitimate recombination in Escherichia coli, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 3860–3865.
[2] I.D. Hickson, RecQ helicases: caretakers of the genome, Nat. Rev. Cancer 3 (2003) 169–178.
[3] K.J. Kyng, A. May, S. Kolvraa, V.A. Bohr, Gene expression profiling in Werner syndrome closely resembles that of normal aging, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 12259–12264.
[4] R.J. Bennett, J.L. Keck, Structure and function of RecQ DNA helicases, Crit Rev. Biochem. Mol. Biol. 39 (2004) 79–97.
[5] P.L. Opresko, W.H. Cheng, C. von Kobbe, J.A. Harrigan, V.A. Bohr, Werner syndrome and the function of the Werner protein; what they can teach us about the molecular aging process, Carcinogenesis 24 (2003) 791–802.
[6] P.L. Opresko, W.H. Cheng, V.A. Bohr, Junction of RecQ helicase biochemistry and human disease, J. Biol. Chem. 279 (2004) 18099–18102.
[7] J.A. Harrigan, V.A. Bohr, Human diseases deficient in RecQ helicases, Biochimie 85 (2003) 1185–1193.
[8] M.P. Cooper, A. Machwe, D.K. Orren, R.M. Brosh, D. Ramsden, V.A. Bohr, Ku complex interacts with and stimulates the Werner protein, Genes Dev. 14 (2000) 907–912.
[9] W.H. Cheng, K.C. von, P.L. Opresko, L.M. Arthur, K. Komatsu, M.M. Seidman, J.P. Carney, V.A. Bohr, Linkage between

Werner syndrome protein and the Mre11 complex via Nbs1, J. Biol. Chem. 279 (2004) 21169–21176.
[10] W.H. Cheng, V.A. Bohr, Diverse dealings of the Werner helicase/nuclease, Sci. Aging Knowledge. Environ. 2003 (2003) E22.
[11] P.L. Opresko, C. von Kobbe, J.P. Laine, J. Harrigan, I.D. Hickson, V.A. Bohr, Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases, J. Biol. Chem. 277 (2002) 41110–41119.
[12] R.M. Brosh Jr., P. Karmakar, J.A. Sommers, Q. Yang, X.W. Wang, E.A. Spillare, C.C. Harris, V.A. Bohr, p53 Modulates the exonuclease activity of Werner syndrome protein, J. Biol. Chem. 276 (2001) 35093–35102.
[13] C. von Kobbe, P. Karmakar, L. Dawut, P. Opresko, X. Zeng, R.M. Brosh Jr., I.D. Hickson, V.A. Bohr, Colocalization, physical, and functional interaction between Werner and Bloom syndrome proteins, J. Biol. Chem. 277 (2002) 22035–22044.
[14] C.Z. Bachrati, I.D. Hickson, RecQ helicases: suppressors of tumorigenesis and premature aging, Biochem. J. 374 (2003) 577–606.
[15] K. Baynton, M. Otterlei, M. Bjoras, K.C. von, V.A. Bohr, E. Seeberg, WRN interacts physically and functionally with the recombination mediator protein RAD52, J. Biol. Chem. 278 (2003) 36476–36486.
[16] Y. Saintigny, K. Makienko, C. Swanson, M.J. Emond, R.J. Monnat Jr., Homologous recombination resolution defect in Werner syndrome, Mol. Cell Biol. 22 (2002) 6971–6978.
[17] R.J. Monnat Jr., Y. Saintigny, Werner syndrome protein—unwinding function to explain disease, Sci. Aging Knowledge. Environ. 2004 (2004) re3.
[18] H. Tauchi, J. Kobayashi, K. Morishima, G. van, T. Shiraishi, N.S. Verkaik, D. vanHeems, E. Ito, A. Nakamura, E. Sonoda, M. Takata, S. Takeda, S. Matsuura, K. Komatsu, Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells, Nature 420 (2002) 93–98.
[19] J.A. Downs, S.P. Jackson, A means to a DNA end: the many roles of Ku, Nat. Rev. Mol. Cell Biol. 5 (2004) 367–378.
[20] P. Karmakar, C.M. Snowden, D.A. Ramsden, V.A. Bohr, Ku heterodimer binds to both ends of the Werner protein and functional interaction occurs at the Werner N-terminus, Nucleic Acids Res. 30 (2002) 3583–3591.
[21] B. Li, L. Comai, Requirements for the nucleolytic processing of DNA ends by the Werner syndrome protein-Ku70/80 complex, J. Biol. Chem. 276 (2001) 9896–9902.
[22] P. Karmakar, J. Piotrowski, R.M. Brosh Jr., J.A. Sommers, S.P. Miller, W.H. Cheng, C.M. Snowden, D.A. Ramsden, V.A. Bohr, Werner protein is a target of DNA-dependent protein kinase in vivo and in vitro, and its catalytic activities are regulated by phosphorylation, J. Biol. Chem. 277 (2002) 18291–18302.
[23] S.M. Yannone, S. Roy, D.W. Chan, M.B. Murphy, S. Huang, J. Campisi, D.J. Chen, Werner syndrome protein is regulated and phosphorylated by DNA-dependent protein kinase, J. Biol. Chem. 276 (2001) 38242–38248.
[24] L. Chen, S. Huang, L. Lee, A. Davalos, R.H. Schiestl, J. Campisi, J. Oshima, WRN, the protein deficient in Werner syndrome, plays a critical structural role in optimizing DNA repair, Aging Cell 2 (2003) 191–199.
[25] A. Blank, M.S. Bobola, B. Gold, S. Varadarajan, D. Kolstoe, E.H. Meade, P.S. Rabinovitch, L.A. Loeb, J.R. Silber, The Werner syndrome protein confers resistance to the DNA lesions N3-methyladenine and O(6)-methylguanine: implications for WRN function, DNA Repair (Amst) 3 (2004) 629–638.
[26] A.M. Szekely, Y.H. Chen, C. Zhang, J. Oshima, S.M. Weissman, Werner protein recruits DNA polymerase delta to the nucleolus, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11365–11370.
[27] J.A. Harrigan, P.L. Opresko, K.C. von, P.S. Kedar, R. Prasad, S.H. Wilson, V.A. Bohr, The Werner syndrome protein stimulates DNA polymerase beta strand displacement synthesis via its helicase activity, J. Biol. Chem. 278 (2003) 22686–22695.
[28] M. Lebel, E.A. Spillare, C.C. Harris, P. Leder, The Werner syndrome gene product Co-purifies with the DNA replication complex and interacts with PCNA and topoisomerase I, J. Biol. Chem. 274 (1999) 37795–37799 [in process citation].
[29] R.M. Brosh Jr., D.K. Orren, J.O. Nehlin, P.H. Ravn, M.K. Kenny, A. Machwe, V.A. Bohr, Functional and physical interaction between WRN helicase and human replication protein A, J. Biol. Chem. 274 (1999) 18341–18350 [in process citation].
[30] R.M. Brosh Jr., C. von Kobbe, J.A. Sommers, P. Karmakar, P.L. Opresko, J. Piotrowski, I. Dianova, G.L. Dianov, V.A. Bohr, Werner syndrome protein interacts with human flap endonuclease 1 and stimulates its cleavage activity, EMBO J. 20 (2001) 5791–5801.
[31] K.C. von, J.A. Harrigan, A. May, P.L. Opresko, L. Dawut, W.H. Cheng, V.A. Bohr, Central role for the Werner syndrome protein/poly(ADP-ribose) polymerase 1 complex in the poly(ADP-ribosyl)ation pathway after DNA damage, Mol. Cell Biol. 23 (2003) 8601–8613.
[32] A.S. Kamath-Loeb, E. Johansson, P.M. Burgers, L.A. Loeb, Functional interaction between the Werner Syndrome protein and DNA polymerase delta, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 4603–4608.
[33] K.C. von, J.A. Harrigan, V. Schreiber, P. Stiegler, J. Piotrowski, L. Dawut, V.A. Bohr, Poly(ADP-ribose) polymerase 1 regulates both the exonuclease and helicase activities of the Werner syndrome protein, Nucleic Acids Res. 32 (2004) 4003–4014.
[34] S.H. Wilson, T.A. Kunkel, Passing the baton in base excision repair, Nat. Struct. Biol. 7 (2000) 176–178.
[35] B. Ahn, J.A. Harrigan, F.E. Indig, D.M. Wilson III, V.A. Bohr, Regulation of WRN helicase activity in human base excision repair, J. Biol. Chem. 279 (2004) 53465–53474.
[36] K.C. von, A. May, C. Grandori, V.A. Bohr, Werner syndrome cells escape hydrogen peroxide-induced cell proliferation arrest, FASEB J. 18 (2004) 1970–1972.
[37] J. Karlseder, D. Broccoli, Y. Dai, S. Hardy, T. De Lange, p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2, Science 283 (1999) 1321–1325.
[38] J.P. Hanish, J.L. Yanowitz, L.T. de, Stringent sequence requirements for the formation of human telomeres, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 8861–8865.
[39] P.M. Lansdorp, Repair of telomeric DNA prior to replicative senescence, Mech. Ageing Dev. 118 (2000) 23–34.
[40] L.T. de, T-loops and the origin of telomeres, Nat. Rev. Mol. Cell Biol. 5 (2004) 323–329.

[41] P. Baumann, E. Podell, T.R. Cech, Human Pot1 (protection of telomeres) protein: cytolocalization, gene structure, and alternative splicing, Mol. Cell Biol. 22 (2002) 8079–8087.
[42] J.B. Bessler, J.Z. Torredagger, V.A. Zakian, The Pif1p subfamily of helicases: region-specific DNA helicases? Trends Cell Biol. 11 (2001) 60–65.
[43] H. Ding, M. Schertzer, X. Wu, M. Gertsenstein, S. Selig, M. Kammori, R. Pourvali, S. Poon, I. Vulto, E. Chavez, P.P. Tam, A. Nagy, P.M. Lansdorp, Regulation of murine telomere length by Rtel: an essential gene encoding a helicase-like protein, Cell 117 (2004) 873–886.
[44] S. Chang, A.S. Multani, N.G. Cabrera, M.L. Naylor, P. Laud, D. Lombard, S. Pathak, L. Guarente, R.A. DePinho, Essential role of limiting telomeres in the pathogenesis of Werner syndrome, Nat. Genet. 36 (2004) 877–882.
[45] L. Crabbe, R.E. Verdun, C.I. Haggblom, J. Karlseder, Defective telomere lagging strand synthesis in cells lacking WRN helicase activity, Science 306 (2004) 1951–1953.
[46] Z.T. von, Oxidative stress shortens telomeres, Trends Biochem. Sci. 27 (2002) 339–344.
[47] A. Tchirkov, P.M. Lansdorp, Role of oxidative stress in telomere shortening in cultured fibroblasts from normal individuals and patients with ataxia-telangiectasia, Hum. Mol. Genet. 12 (2003) 227–232.
[48] S. Oikawa, S. Tada-Oikawa, S. Kawanishi, Site-specific DNA damage at the GGG sequence by UVA involves acceleration of telomere shortening, Biochemistry 40 (2001) 4763–4768.
[49] P.A. Kruk, N.J. Rampino, V.A. Bohr, DNA damage and repair in telomeres: relation to aging, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 258–262.
[50] P.L. Opresko, M. Otterlei, J. Graakjaer, P. Bruheim, L. Dawut, S. Kolvraa, A. May, M.M. Seidman, V.A. Bohr, The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2, Mol. Cell 14 (2004) 763–774.