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Chapter 9

The Role of WRN Helicase/Exonuclease in DNA Replication

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1. Introduction

1.1. WRN is a RecQ helicase/exonuclease required for genome stability and to prevent premature ageing

1.1.1. Clinical phenotype of Werner's syndrome

Humans possess five distinct RecQ helicases (see Figure 1), all of which possess a hallmark RecQ helicase domain. Mutation or loss in any one of three human RecQ helicases give rise to genetic instability syndromes: WRN mutation gives Werner's syndrome (WS), BLM loss results in Bloom syndrome (BS), and Rothmund-Thomson syndrome (RTS) is caused by mutation of RECQL4[1]. WRN has come to prominence because its loss of function results in human Werner's syndrome, a segmental progeria (premature ageing) characterised by many signs and symptoms of normal ageing at both the organismal and cellular levels, with shortened lifespan (median age of death 47 years [2]). In particular WS patients suffer from osteoporosis, athero-and arterio-sclerosis and a high cancer incidence (particularly sarcoma) together with metabolic disorders normally associated with increased age, especially type II diabetes and lipodystrophy. Furthermore, patients show outwardly recognisable signs of ageing such as cataracts, greying hair and skin wrinkling, while female WS patients suffer premature menopause and both sexes show hypogonadism, with decreased fertility (reviewed in ref. [2]).

1 RTS is found in a subset of patients with RECQL4 mutation; different mutations in the same gene give rise to RAPADILLINO syndrome [1]

1.2. Cellular phenotype on WRN loss

This premature ageing phenotype is also observed at the cellular level: fibroblasts from WS patients undergo highly premature replicative senescence in culture, failing to proliferate after only 9-11 population doublings, compared with the 50-60 doublings characteristic of wild type fibroblasts [3]. Transcriptomic studies have demonstrated that >90% gene expression changes associated with normal ageing are seen in young WS cells [4], while glycosylation of blood albumin (a biomarker of ageing) in young WS patients is equivalent to levels detected in normal centenarians [5]. Importantly, loss of function of WRN is associated with significant genome instability with a high frequency of chromosomal translocations and deletions [6, 7], which is thought to contribute to the increased cancer risk. Genome instability is a hallmark of defective S phase checkpoint proteins (reviewed in ref. [8]), suggesting either than WRN is directly involved in the checkpoint, or that it normally serves downstream of the checkpoint such that its loss prevents correct execution of the arrest and recovery pathways. Notably, it is not only WS patients who are more susceptible to cancer on WRN loss: epigenetic inactivation by methylation of CpG islands in the WRN gene promoter has been reported in epithelial and mesenchymal cancers with value in prognosis in colorectal cancer [9], while specific WRN SNPs have been correlated with breast cancer incidence [10], even though such genetic changes do not alter the helicase or exonuclease activities of the protein or modulate the levels expressed. WRN is therefore of interest not only to those attempting to understand the molecular basis of human ageing, but also to cancer biologists – indeed WRN knockdown is likely to promote cancer cell death and hypersensitise cells to current chemotherapeutic agents such as camptothecin that impact on DNA replication [9, 11, 12]. Small molecules that specifically inhibit WRN but not other RecQ helicases are therefore likely to have therapeutic potential [13].

1.3. WRN protein

The wide range of ageing-associated phenotypes in WS patients and their cells indicates a fundamental role for WRN in preventing premature ageing, but how can loss of one protein lead to the pleiotropic outcomes of human ageing? The most important clue came from cloning the WRN gene [14], which showed for the first time that the human WRN gene encodes a large protein of 1432 amino acid (~162kDa) with an amino terminal exonuclease domain conserved with proteins of the DnaQ family, and a central helicase domain of the RecQ family. In addition, DNA binding (RQC) and protein interaction (HRDC) domains exist distal to the helicase domain (Figure 1A). Immunofluorescence and mutational studies have demonstrated that WRN is a nuclear protein with both NLS and NoLS sequences situated at the C terminus [15]), that appears to be sequestered in the nucleolus [16] except during S phase or upon DNA damage, when it is redistributed to sites of DNA replication or repair ([17-19].

Of the five human RecQ proteins, WRN is the only one to possess exonuclease activity [20]. Acting in a 3′-5′ direction (as shown using 3′- or 5′-end labelled substrates), WRN exonuclease has been demonstrated to bind onto overhanging 5′ ends of the guide strand of duplex DNA and cleave the target strand sequentially, though with relatively low processivity [21]. While it cannot cleave blunt ended substrates, nor those where ends are blocked by

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bulky lesions [22], WRN exonuclease degrades substrates that are likely to be found both during DNA repair and as intermediates in DNA replication, including forks and bubble substrates [23] (see Table 1). Despite early reports of lack of activity on short single-stranded DNA (e.g. [21]), WRN exonuclease can digest single stranded oligonucleotides over 50 bases in length [24, 25].

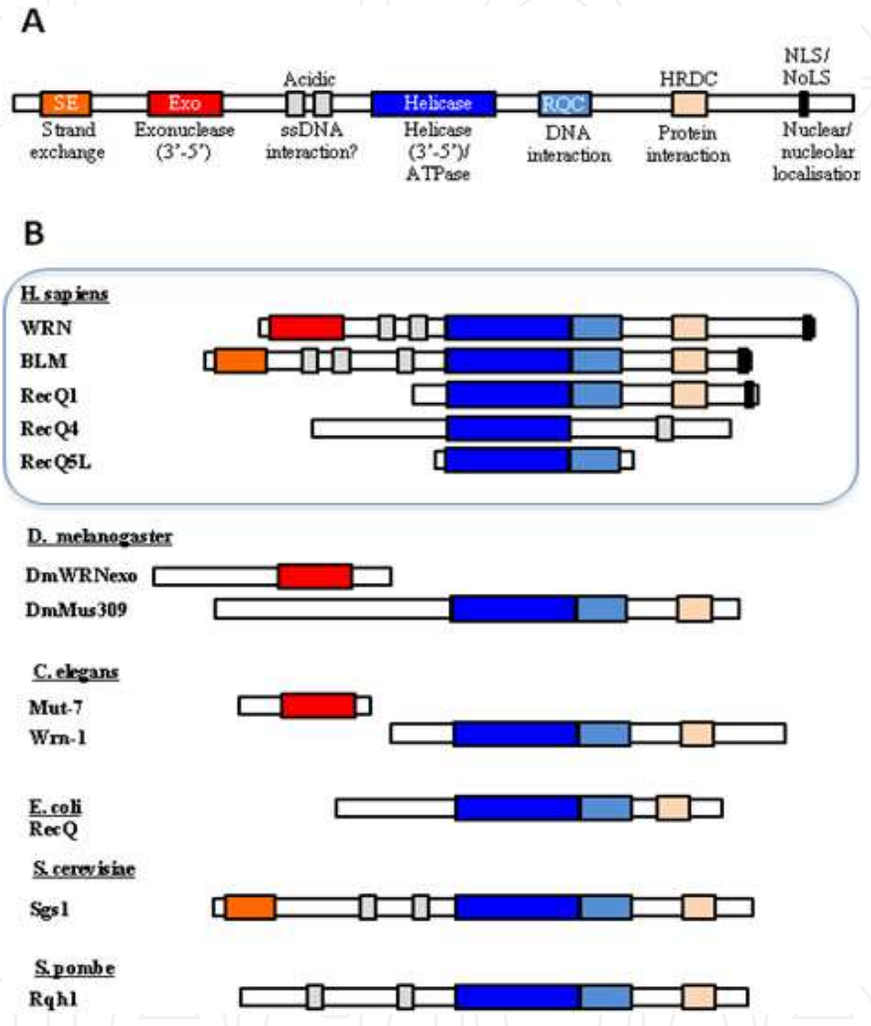

Figure 1. The RecQ helicase family. (A) Domain organization of human WRN. Note that for human WRN, the RQC serves in DNA binding and the HRDC is probably involved in protein-protein interaction, though these roles may be reversed in other RecQs. (B) Humans have 5 RecQ helicases (boxed), named after the archetypal RecQ of *E. coli*. Human WRN is unique in the family in possessing an exonuclease domain. In invertebrates such as *Drosophila* and C. *elegans*, the exonuclease (red) and helicase (blue) activities are encoded by separate genes.

Helicase substrates	Exonuclease substrates
Holliday junction	Holliday junction
Bubble duplex	Bubble duplex
3'-recessed duplex	3'-recessed duplex

Helicase substrates	Exonuclease substrates
D-loop duplex	D-loop duplex
Duplex with 5′-flaps	Looped duplex
G-quadruplex	Nicked looped duplex
Partial duplex	
Forked duplex	
Triplex	
NOT blunt duplex	
NOT 5′-recessed duplex	

Table 1. Substrates unwound by WRN helicase or degraded by WRN exonuclease. (Note that BLM also unwinds the same substrates as WRN helicase)

The helicase activity of WRN is highly conserved with other RecQ helicase family members, acting 3′-5′to unwind duplex DNA in an ATP-dependent manner [26]. Within the helicase domain are seven conserved motifs characteristic of the RecQ family. In general, RecQ helicases are adept at unwinding unusual DNA structures that can inhibit the course of normal DNA replication. Examples are tailed and forked duplexes, small gaps and flaps (commonly found as DNA repair and recombination intermediates), bubble substrates and displacement-loop triplex and Holliday junctions (common at telomeres and during recombinational repair and sister chromatid exchange), and G-quadruplexes which are often found at tracts rich in guanine such as at the telomere (e.g. [27], reviewed in ref. [28], see Table 1). It is important to note that the helicase and exonuclease activities do not simply act as independent entities in cells, but that their actions are almost certainly co-ordinated and interlinked. For example, co-operation between them is required during telomere maintenance ([29]; see section 4 below for more detail).

WRN helicase template specificity requires DNA binding that is probably mediated through the conserved RQC domain. X-ray crystallographic analysis has shown some unusual features, in that binding of WRN to DNA does not occur through a standard 'recognition helix', but instead through a beta wing of the RQC domain that inserts like a wedge between the terminal bases of blunt duplex DNA to unwind one base even in the absence of ATP [30]. How this binding correlates with WRN's lack of unwinding of blunt ended substrates remains to be determined. In addition to binding to DNA, WRN binds to many different proteins at the replication fork, the telomere and during fork recovery after stalling. Protein interaction with WRN may occur through the helicase-and-ribonuclease D/C-terminal (HRDC) domain; while this region is through to be important for DNA binding in *E. coli* RecQ and yeast Sgs1, the conserved for DNA interaction surface is lacking in human WRN, and the domain is unable to bind DNA *in vitro*, but that reveals many exposed alpha helicases that are likely to bind to protein partners [31].

1.4. WRN orthologues

While the exonuclease and helicase activities are both encoded by the same gene in vertebrates, giving rise to one multifunctional protein, the enzymes are encoded by separate genetic loci in plants, invertebrates and prokaryotes (Figure 1B, reviewed in ref. [32]), with physical and/or functional interaction between the helicase and nuclease proposed *in vivo*. (Figure 1, reviewed in ref. [32, 33] For example, in the fruit fly *Drosophila melanogaster*, we have cloned and characterised the orthologue of human WRN exonuclease encoded by the fly locus *CG7670* [34]. *Drosophila* WRN exonuclease (DmWRNexo) is a 3′-5′ exonuclease [35] that shows remarkable substrate conservation with human WRN exonuclease and utilises conserved residues at the active site for nucleic acid cleavage [36]. Flies homozygous for a strong hypomorphic mutation in *CG7670* have greatly elevated levels of recombination that appears to occur through reciprocal exchange, and are hypersensitive to the topoisomerase poison camptothecin, that leads to replication fork collapse [37]. Hence loss of only the WRN exonuclease activity in flies results in many features characteristic of human WS, suggesting a key role for the exonuclease in preventing premature ageing. We consider the possible role(s) of WRN exonuclease in replication fidelity, restart of stalled forks and telomere maintenance in more detail below (see sections 2.2, 3.4 and 4 below).

A limitation to studying WRN in flies is the lack of a fully characterised WRN helicase orthologue. However, the nematode worm *C. elegans* has a highly conserved WRN-like helicase, encoded by the *wrn-1* gene, and two candidate exonucleases, at loci *ZK1098.8* (*mut-7* [38]) and adjacent *ZK1098.3*. RNAi knockdown of *wrn-1* results in shortened lifespan [39] and perturbation of the S phase checkpoint via ATM/R kinases [40], suggesting both that WRN is important during DNA replication, and that its role is critical in maintaining normal longevity of the organism. These outcomes are of particular interest since they so closely echo the findings in humans, but in a genetically tractable and short-lived lower eukaryotic model organism. In plants, WRN has been most studied in *Arabidopsis*, where physical and function interaction has been described between the exonuclease (AtWEX) and helicase (AtWRN) orthologues [41]. In budding yeast and fission yeast, there is only one RecQ helicase (Sgs1 and Rqh1, respectively); whether these proteins interacts directly with an exonuclease to reconstitute human WRN-like activity is yet to be determined, though genetic interaction between Rqh1 and Mus81/Eme1 has been reported [42].

Because of the phenotypes resulting from WRN loss or mutation, it has been implicated in many aspects of DNA metabolism, including transcription, DNA repair, recombination and telomere maintenance. Its role in DNA replication will be discussed in this chapter, including not only a direct role in normal processive DNA replication, and replication of the telomeres, but also in preventing replication fork stalling or assisting fork recovery after arrest.

1.5. S phase defects in WS cells

Fibroblasts and lymphoblastoid cells from Werner's syndrome patients show a defect in progression through S phase [17, 43]. FACS analysis demonstrates both a longer duration of S phase and an overall significant increase in cell cycle time in primary fibroblasts from WS patients ([17] and in normal primary fibroblasts in which WRN was depleted by shRNAi by

80-90% [44]. Early studies on replication rates in WS fibroblasts used alkaline sucrose gradients to detect the size of nascent DNA, demonstrating slower replication in WS cells compared with normal controls [45]. The ability of WS cells to incorporate Texas-red-dUTP into nascent DNA is also significantly impaired [46]. Interestingly, while acute shRNAi-mediated WRN depletion in SV40 T antigen-transformed cells had no impact on cell cycle progression in the absence of imposed replication stress, primary fibroblasts depleted of WRN did show an S phase delay [44]. Hence it appears that loss of WRN protein results in an S phase phenotype.

WRN has been isolated within a large multi-protein replication complex [47]and found to interact *in vitro* with purified PCNA. The binding region has been localised to a PIP-like motif on WRN towards the amino terminus [18], which is likely to bind within the hydrophobic pocket of PCNA, as described for other PIP-containing proteins (see section 2.1). Studies on *Xenopus* egg cell-free extracts depleted of the frog orthologue of WRN, called FFA-1 (focus-forming activity-1) initially suggested that the protein was required for establishment of replication foci and thus served a central role in DNA synthesis [48]. (Note however that immunoprecipitation from *Xenopus* egg extracts is fraught with difficulties and accidental removal of other components such as membranes may inadvertently lead to loss of replication capacity). Subsequently, FFA-1 was shown to localise to sites of DNA synthesis coincident with RPA, and expression of a dominant negative GST-FFA-1 fusion protein blocked replication activity [49]. Similar immunofluorescence studies in both HeLa cells and primary human fibroblasts, supported by high-resolution immuno-electron microscopy, also showed WRN present at a subset (~60%) of replication foci, colocalising with PCNA [18]. This localisation is in the absence of replication stress, while on HU arrest, the majority of WRN relocates from the nucleolus to RPA-containing foci that are suggested to represent stalled forks [19]. Hence WRN is present at replication sites, and in its absence, cell cycle and DNA synthesis phenotypes are consistent with a replication defect.

2. WRN at the replication fork

In order to appreciate where WRN acts during DNA replication, it is necessary to understand the core structure of the DNA replication fork during the elongation stage of DNA replication. During elongation, processive polymerisation of the leading strand is carried out by DNA polymerase epsilon (pol ε) and the leading strand by DNA polymerase delta (pol δ) (based on mutational studies of the proof-reading domains of each in yeast) [50-52]. The replicative polymerases are tethered to the template by association with the homotrimeric sliding clamp protein PCNA (proliferating cell nuclear antigen) [53]. Co-ordination between leading and lagging strands may be achieved through the action of the GINS/Cdc45 complex that has been proposed to act as a replisome progression complex (RPC) [54]. On the lagging strand, repeated cycles of priming by DNA pol α-primase results in synthesis of 7-10 nucleotide of RNA primer followed by ~20 nucleotides of initiator DNA (with error rates of 10^{-2} and 10^{-4} respectively), followed by switching to the higher fidelity and more processive DNA pol δ on the lagging strand and pol ε on the leading strand. This switch occurs through a multistep loading process essentially requiring recognition of the

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primer-template junction (where RPA is bound to the unwound single-stranded parental DNA) by RFC, an AAA+ ATPase that serves to load the sliding clamp PCNA. Pol δ is then recruited to PCNA through its p66 subunit to synthesise approximately 200 nucleotides of the Okazaki fragment. (For a more detailed discussion of fork establishment, see ref. [55]).

2.1. Okazaki fragment processing

Because of the low fidelity of pol α-primase, it is essential to remove both the RNA primer and iDNA during Okazaki fragment processing (OFP) This is coincident with continued synthesis of nascent DNA on the lagging strand; processive replication by pol δ results in displacement of the RNA-iDNA primer as a 5′ flap and its removal by one of a range of postulated pathways involving RNase H1, FEN1, Dna2 (on long RPA-coated flaps) and other helicases/nucleases including Pif1 and possibly a RecQ helicase (Sgs1 in yeast, WRN in humans) (reviewed in ref. [56]). Pol δ synthesises DNA to fill the gap and DNA ligase seals the nick in the phosphodiester backbone. These steps in Okazaki fragment processing (OFP) may be co-ordinated through differential binding of the separate enzymes to PCNA, which has been suggested to act as a molecular 'toolbelt' in OFP [57]. Association of the OFP proteins[2] with PCNA occurs through a conserved PCNA-interacting peptide (PIP) of the general motif QxxL/M/IxxFF to the hydrophobic pocket of PCNA formed at the interdomain connector loop (e.g. [58, 60], reviewed in ref. [61]). Each PIP is likely to bind by an induced fit mechanism, since the crystal structures of PCNA bound by its various partners shows variation in this loop region [62]. Notably, WRN has a conserved PIP, and peptide ELISA studies showed that this region is sufficient for PCNA binding *in vitro* [18]. Additionally, WRN binds to and stimulates the nuclease activity of Fen1, which may contribute to efficiency of Okazaki fragment processing [63]; as WRN binds to Fen1 immediately adjacent to its PCNA binding site, it is likely that there is some interplay between the three proteins [64] that may be important in Okazaki fragment processing, though this has not been fully explored.

2.2. Proof-reading during processive DNA synthesis

DNA replication overall has an extremely low error rate of 10^{-9}, achieved in part by the very high fidelity of the processive replicative polymerase ε and δ, and also by additional 'extrinsic' proofreading activities together with mismatch repair (MMR) to remove incorrectly incorporated bases. The high fidelity DNA polymerases ε and δ achieve an error rate of ~2 $x10^{-5}$ (reviewed in ref. [65]) through two key structural features. Firstly, the active site is only fully formed upon acceptance of the correct incoming dNTP to create a solvent–inaccessible site that is partially specified by correct helical geometry of duplex DNA, thus increasing enthalpy and decreasing entropy for correct nucleotides and allowing high discrimination over incorrect nucleotides. Secondly, these polymerases each possesses a 3′-5′ exonuclease active site whereby the nascent DNA swings through ~40° to present to this site [66], and where incorrect nucleotides are removed by hydrolysis of the phosphodiester backbone just created. X-ray crystal structures of the isolated WRN exonuclease domain have shown that

2 Many other proteins also bind to PCNA in this manner – some regulate PCNA's activity (e.g. p21) [58] while others are regulated by such binding (e.g. Cdt1 degradation is PIP-dependent) [59].

WRN shares structural homology with exonuclease domains of the high fidelity DnaQ family of replication polymerases, suggesting a possible role for WRN in editing DNA, either during DNA synthesis or in processing free ends, in collaboration with and stimulated by the end-binding protein Ku [67]. Very recently, it has been shown that WRN assists pol δ (possibly on the lagging strand during Okazaki fragment synthesis) by removing 3′ mismatches, thus allowing the polymerase to extend primers [68]. This supports a direct role for WRN in Okazaki fragment synthesis.

3. Replication fork stalling – the role of WRN

3.1. High rates of replication fork stalling in WS

Early electron microscopy studies of ^{3}H-T labelled DNA in fibre autoradiographs suggested a problem with replication origin spacing in WS [69, 70], though subsequent higher resolution studies using fluorescent antibodies to halogenated nucleotides suggest rather that it is replication fork rate, not inter-origin distance, which is abnormal in WS cells [17, 44]. Indeed, these DNA combing studies, that analyse individual DNA molecules labelled during replication, have demonstrated a problem with replication fork progression in WS cells, resulting in a high degree of replication fork asymmetry from what should be bidirectional origins [17]. Such studies led to the proposal that replication forks stall at high frequency in cells lacking WRN protein. Why should WS cells be particularly prone to fork stalling?

3.2. Causes of fork stalling

The replication fork encounters barriers during normal replication, such as unusual DNA structures arising at G-rich regions (G4-quadruplex) or fragile sites. These structures must be unwound to present a single stranded template suitable for copying; a high incidence of replication fork stalling is likely if the normal mechanisms for tackling the unusual structures is lacking. Alteration in nucleotide pools through treatment with hydroxyurea (HU), or polymerase inhibition with the dCTP mimic aphidicolin results in replication fork arrest in the absence of template abnormalities or lesions. In addition, exogenous agents can cause formation of lesions in the DNA that the replication fork cannot easily pass over – for example, methylated or oxidized bases.

Replication fork pausing or stalling is therefore likely to be a common occurrence, and the cell has mechanisms to stabilise the fork, deal with the unusual structure or repair the damaged region, and allow fork restart. Where DNA synthesis pauses but the MCM replicative helicases proceed to unwind the duplex template, regions of single stranded parental DNA arise, that are rapidly coated with RPA. This forms a signal to the S phase checkpoint machinery, particularly the kinase ATR, that, together with other checkpoint kinases such as Mec1, Chk1 and Chk2 (Rad53) and mediator Mrc1, leads both to recruitment of proteins to deal with the particular fork progression barrier, and to stabilisation of the replisome at the stalled fork, reviewed in ref. [8]. Indeed, DNA pol ε has been shown to stay associated with stalled forks in yeast [71]

under the influence of Rad53 signalling. Replication fork restart then occurs once the damage has been resolved and the checkpoint lifted. More serious to the cell is the collapse of replication forks as they traverse regions of the template containing single strand breaks – single-stranded breaks are converted to double-strand breaks (DSBs) by the passage of the replication fork, forming highly cytotoxic and potentially recombinogenic lesions. Hence surveillance and rescue mechanisms must exist in the cell to deal both with stalled and collapsed forks. The RecQ helicase family has been implicated as key in this mechanism.

3.3. Dealing with unusual structures before they arrest the fork

The most efficient mode of replication involves the removal of barriers to fork progression before they lead to fork stalling. Importantly, WRN has been shown to be required by DNA pol δ (but not α or ε) to unwind G4 DNA [72], bubbles and D loops [68] to allow pol δ-mediated synthesis over such template sequences without leading to fork stalling. In addition, the helicase activity of WRN is also required to limit the formation of single stranded DNA regions and gaps during replication of common fragile sites (CFS) [73, 74] and enhances processivity of DNA pol δ on fragile site FRA16D over hairpins and microsatellite regions, requiring either the helicase or DNA binding activities of WRN [75]. Hence one important role of WRN in DNA replication is to present the replisome with a template that is easy to replicate, but does it act at any other point to ensure efficient replication?

3.4. Is WRN involved in fork restart or progression following restart?

Where replication forks have stalled, replication restart can occur in one of a number of ways: (i) the block may be repaired (or removed); (ii) it may be bypassed using error-prone translesional synthesis (TLS), or (iii) it may be avoided by using an alternative template (e.g. the newly synthesised region on the opposite strand, resulting from fork regression or generated by recombination). The first option is usually the easiest and the least likely to have mutational consequences; translesional synthesis is inherently more likely to cause mutation (pol iota (ι), for example, has an error rate of 0.72 i.e. it incorporates nucleotides almost at random, irrespective of the template sequence [76, 77]), whilst recombination requires a suitable donor template that is not always available. The type of lesion, whether it is on the leading or lagging strand, and the surrounding environment all contribute to how the replication block is dealt with. For example, nucleotide depletion following HU treatment imposes replication stress and can lead to fork stalling, but such stalling may be 'seen' differently by the checkpoint and restart machinery to forks that stall at physical barriers caused by damaging agents such as MMS.

It appears that RecQ helicases may aid in pathway 'choice', although the mechanisms that dictate which pathway is utilised are not fully understood. For instance, yeast complementation studies in *rad50* mutants have demonstrated that BLM is important in resistance to ionising radiation that causes double-strand breaks [78], while WRN confers resistance to drugs such as MMS that lead to replication fork stalling [79, 80]. In human cells, dual labelling of DNA before and after either HU or MMS treatment and analysis by fibre spreading (DNA

combing) has shown that cells acutely depleted of WRN using shRNAi were still able to preserve replisome integrity upon HU- or MMS-induced fork stalling, though following recovery, replication fork rates were slower in WRN-depleted cells than controls, as evidenced by much shorter tracts of labelled DNA post-treatment compared with those synthesised before treatment [44]. It has been proposed [44] that WRN leads to rapid elimination of single-stranded DNA tracts by promoting recombination (using the sister chromatid as template), by enhancing translesion polymerase-mediated gap filling, or by removing DNA immediately after fork passage. It has therefore been suggested that the genome instability in WS results from a defective response to stalled replication forks.

3.5. Error-prone translesional synthesis to relieve the replication block

Some lesions such as those caused by MMS or 4NQO present an insurmountable barrier to templating for the high fidelity B family DNA polymerases, but error-prone replication through these small lesions is often less costly for the cell than replication pausing and recruitment of repair complexes. Such error-prone synthesis is conducted by the Y family translesion DNA polymerases (TLS pols). These can pair nucleotides opposite modified and unusual bases, but at the cost of fidelity (ranging from error rates of ~6 x 10^{-3} for pol kappa (κ), through 3.5 x 10^{-2} for pol eta (η) to the essentially random 0.72 error rate for pol ι [76, 77, 81, 82]). The active site of such polymerasis is much larger than that of the proofreading polymerasis, allowing for unusual base pairing geometry, helical distortion of the template DNA, and solvent access [83]. Consistent with an important role for WRN in replication fork progression after pausing, WRN has been found to promote the processivity of Y-family TLS pols on a wide range of substrates including oxidized bases, abasic sites, and thymine dimers [84]. This activity is specific to WRN, and appears to increase the apparent V_{max} of polymerisation. This does not require either catalytic activity of WRN, as proteins with point mutations that ablate both helicase and exonuclease activities can still promote pol η polymerisation, although neither catalytically-active BLM nor RecQ5 can substitute [84].

3.6. WRN suppresses illegitimate recombination at stalled forks

Whilst the experiments described above strongly support the assertion that WRN is required for fork progression after restart, others have suggested that WRN is itself required to promote restart, possibly through preventing either the accumulation of recombinogenic substrates or in suppressing recombination itself. High levels of spontaneous Rad51 foci in WS cells indicate the presence of an increased number of DNA double-strand breaks (DSBs) and elevated recombination when WRN is absent, supporting the assertion that WRN blocks excessive and illegitimate recombination. Indeed, stalled forks are thought to regress to ‘chicken foot; structures with 4-way Holiday junctions that can either be removed by exonuclease degradation of the free ends, by branch migration to a point at which replication can simply restart, or by recombination at the junction (see Figure 2). WRN is likely to suppress the recombinational route, as shown by partial complementation of yeast cells defective in Sgs1 by expression of human WRN. Accumulation and persistence of Holliday junctions is likely, since ectopic expression of the bacterial RusA resolvase allows WS cells to

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proliferate as rapidly as control cells, and to resist treatment with CPT or 4NQO (fork collapse and fork stalling agents) to which WS are normally hypersensitive [46].

WRN helicase may branch migrate the chicken foot to 'fold back' the regressed form and thus re-establish a normal fork structure (Figure 2). Indeed, fork regression by WRN on RPA-coated DNA has recently been reported [85]. Alternatively, WRN exonuclease may degrade regions of the chicken foot and allow reformation of a normal replication fork. In addition to its own exonuclease activity, WRN associates with human Exonuclease 1 (Exo1), stimulating its activity [86]. It may therefore be the case that the two nuclease activities combine to remove regressed forks. It has been suggested that in the absence of WRN, the recombinational route is used to process the accumulated HJs, and that this requires the action of the nuclease Mus81; fission yeast Rqh1 suppresses Mus81 mutation [42] and human WRN suppresses Mus81-mediated recombination [87].

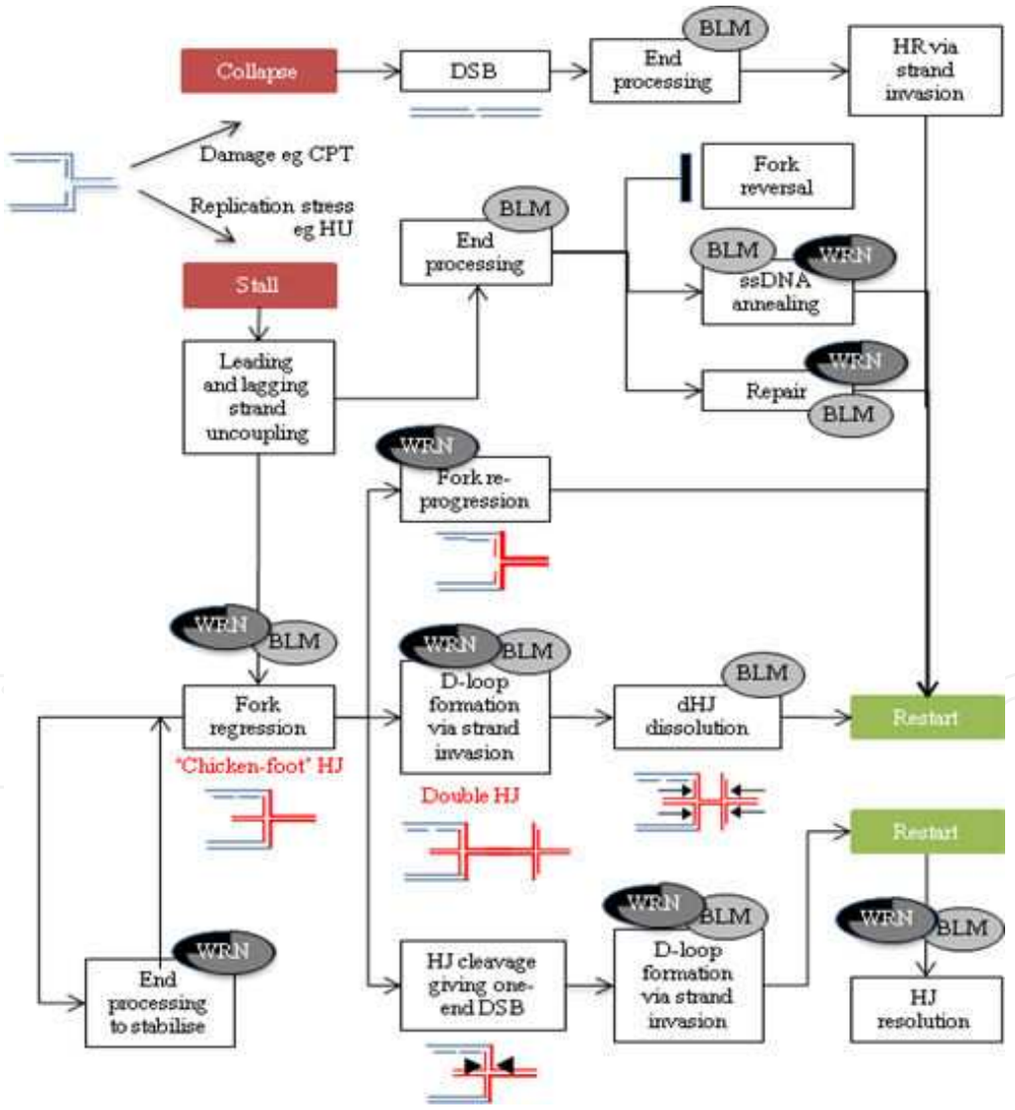

Figure 2. Possible roles of WRN in replication restart after fork stalling (see text for details)

3.7. Template switching at stalled forks

Leading strand blockage often uncouples the replicative helicases from the rest of the replisome, allowing significant unwinding to form long tracts of single-stranded DNA, with lagging strand synthesis continuing for a distance [88]. The accumulated long single stranded loop of leading strand DNA is highly susceptible to damage. Replication fork restart on the leading strand might simply utilise new priming by RPA-mediated recruitment of pol α-primase to the region of transition between singe stranded and duplex DNA (i.e. where the previous polymerase ceased synthesis), in much the same way that it normally reassociates with the primer-template junction in Okazaki fragment synthesis. Alternatively, regression of the replication fork may permit annealing to the new lagging strand using 'template switching' to give a Holliday junction that can then be reversed past the lesion [89, 90]. In bacteria, this can be done by RecQ helicase, with RecJ exonuclease to remove the protruding lagging strand flap [91].

In mammals this is likely to require WRN and the flap endonuclease activity of FEN-1 [92]. WRN (and BLM) can induce fork regression over the lesion by local unwinding, and can lead to the formation of the chicken-foot. WRN can also reverse a regressed fork. Both BLM and WRN helicase activities can also catalyse branch migration of the DNA leading to recovery of the template daughter strand annealing via Rad51 [93], formation of a double Holliday junction and strand exchange. If the product here is a hemicatenenes, it can be resolved into either a chicken foot or a HJ and processed the same way. Ultimately, functional replication forks may be reformed [94]. Alternatively, the Holliday junction can then be cleaved by a resolvase and DSB repair as before. See Figure 2 (above) for a schematic of replication fork restart.

3.8. How is WRN recruited to stalled forks?

Stalling of replication forks initiates the caffeine-sensitive S phase checkpoint, mediated by RPA, ATR and Rad53. WRN recruitment to, or retention at, stalled forks may be direct through binding to RPA [85], but it also appears to require phosphorylation by the checkpoint kinase ATR [95]. When such phosphorylation is prevented, WRN cannot accumulate at repair sites and DNA strand breaks are detected [73]. That WRN is an *in vivo* as well as *in vitro* target of ATR has been confirmed by phosphoproteomic studies [96]. However, it is still the subject of research and debate as to whether WRN is an upstream sensor or downstream effector in the S phase checkpoint that responds to replication stress or stalled forks. For example, shRNAi-mediated WRN knockdown abrogated the S phase checkpoint on CPT treatment but did not affect checkpoint induction on HU exposure [97], suggesting that WRN may be an important 'sensor' of collapsed but not stalled forks, although the mechanism has yet to been defined. Perhaps fork collapse (e.g. upon CPT treatment) requires ATM, with its double-strand break sensing activity through recruitment by Ku and activation by DNA-PKcs (DNA-dependent protein kinase catalytic subunit), while fork stalling (e.g. on HU) uses the ATR pathway. This is consistent with differential regulation of WRN by the two kinases [73], and with a requirement for WRN not only in replication fork progression after stalling (see above) but also in directing recombination in concert with RAD51

and RAD54 [93]. Recently, it has been shown that WRN also interacts with the repair sliding clamp 9-1-1 (homologous structurally and functionally to PCNA, though acting in repair rather than replication), and that upon fork arrest, the 9-1-1 complex recruits TopBP1 that in turn recruits ATR which phosphorylates WRN [98]. Perhaps the initial type of damage that leads to fork arrest is therefore a deciding factor in the pathways of WRN recruitment and post-translational modification.

3.9. Role of WRN at stalled forks on the lagging strand

Lagging strand blocks do not uncouple the replication fork; rather, lagging strand polymerase merely stutters to the next primer to restart synthesis of the next Okazaki fragment [99, 100]. The resulting single-stranded gap is repaired by translesional synthesis as above (which may be error-prone) or by homologous recombination with the sister chromatid (which is more likely to retain fidelity). In *E. coli*, this requires formation of a double Holliday junction and resolution via non-crossover [101]. In mammals, BLM has the ability to mobilise double Holliday junctions and the resulting catenated DNA is resolved by topoisomerase III without crossover [102]. WRN does not interact with TopoIII and cannot migrate a double Holliday junction [103], although structures involved in intermediate formation (D-loops, G-quadruplex) might require either WRN or BLM. WRN can process a mobile D-loop [104] using the co-ordinate action of both helicase and exonuclease.

However, WRN is also linked to the functionality of the lagging strand polymerase, pol δ. WRN stimulates the base incorporation of pol δ (but not α or ε) even in the absence of PCNA [105]. Pol δ is slowed at fragile sites and repetitive runs likely to cause hairpin or bubble structures, but this can be alleviated by the helicase functionality of WRN [75]. Like WRN, pol δ has a 3′-5′ exonuclease capability which it can use to proofread bases after insertion [106]. WRN can substitute at this proofreader, and cells with low levels of WRN show increased mutation of the lagging strand [107]. Interestingly, the exonuclease activity of pol δ is active on WRN-preferred DNA substrates such as Holliday junctions, D-loops and bubble duplex, and can form a complex with WRN [107] that increases the degradation of these substrates. WRN exonuclease is blocked by many common lesions [22, 108]; it will be interesting to find out whether the nuclease activity of pol δ is complementary to this, and might suggest why the two would functionally substitute within the lagging strand complex.

Ultimately, fork restart requires proximal repositioning of the replication complex; this remodelling may make use of WRN nuclease activities to further process DNA ends and allow removal of damage. Interactions with PCNA and either strict (pol δ, pol α) or promiscuous (TLS pathway) repair polymerases and FEN1 flap removal activity can allow bypass of nicks and modified DNA bases at the same time as restart positioning, allowing many lesions to be handled.

4. Involvement of WRN in telomere maintenance

4.1. Telomere structure and replication

Mammalian telomeres consist of a few kilobases of repetitive non-coding G-rich sequence (the human sequence is (TTAGGG)n) which must be 'capped' rather like a bootlace in order to stop the DNA end being recognised as a DSB via p53/p21 signalling [109] and instigating profligate double-strand break (DSB) repair [110]. Functional capping forms a lasso-like structure [111] called the telomere-loop (T-loop) where the repetitive telomere sequence folds back upon itself to displace a short segment of proximal sequence with a 3' single stranded end to give a displacement-loop (the D-loop)[112]. The proteins that make up the telosome (or core shelterin complex [112]) include TRF1 and TRF2 [113], which bind and stabilise telomeric duplex DNA at the T-loop [114], and POT1 [115], a DNA-binding protein which coats and protects the tracts of single stranded telomeric sequence that occur at the telomeric D-loop and during telomeric replication and processing. Figure 3 shows the T and D loop structure with associated proteins.

Telomeres are replicated by passage of a replication fork that initiated upstream of the chromosome end: obviously it is not possible to load the replisome or prime DNA synthesis beyond the end of the chromosome. At each round of replication, the telomeric sequence is unwound from the D (and possibly also the T) loops, and passively replicated by an incoming fork. While early reports suggested that priming on the lagging strand was defective at the very end of the chromosome, it has become apparent that both leading and lagging strands are normally replicated but that regeneration of a 3' overhang for strand invasion to form the D loop involves end resection of the leading strand, thus removing sequence information and shortening the telomere at each round of replication.

4.2. Telomere shortening leads to replicative senescence and genome instability

Telomere shortening acts as a counting mechanism to indicate the number of cell divisions a somatic cell has passed through, and normal fibroblasts generally arrest at the Hayflick limit of 55-60 population doublings [3] under the influence of this telomere attrition. Hence cellular ageing is in a large part caused by progressive telomere loss – cells that lose telomeres more rapidly senesce more quickly that those with long telomeres, and people with prematurely short telomeres (e.g. mothers of chronically sick children[116], carers of partners with dementia and low paid workers experiencing
work-related stress) age prematurely [117, 118]. (Note that this is not the case in mice, where lab strains have extremely long telomeres and cells senesce prior to telomeres reaching a critical length).

To overcome this cellular ageing, it is vital that immortal cells such as those of the germline have a mechanism to restore telomeric DNA at every round of replication. Such cells express active telomerase, a reverse transcriptase which utilises its endogenous RNA template to regenerate telomeric sequence [119], but telomerase levels are extremely low or absent in most somatic cells [120]. Notably, immortalisation of cancer cells is accompanied by re-expression

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of telomerase [121] in about 85% of all human cancers, while the remaining 15% are able to maintain their telomere lengths in the absence of telomerase, by alternatives mechanisms, reviewed in ref. [122] (see section 4.6).

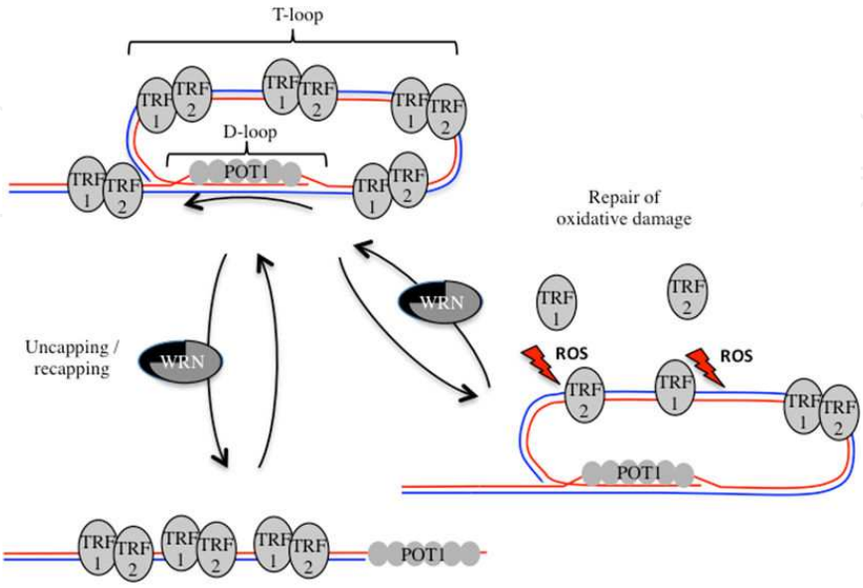

Figure 3. The structure of the telomere, showing the large telomere (T) loop and the smaller displacement (D) loop. Proteins TRF1, TRF2 and POT1 are critically important in stabilising the telomeric structure. WRN binds to all of these proteins

Dysfunctional telomeres that become uncapped are liable to degradation or immediate repair by homologous recombination (HR) or non-homologous end-joining (NHEJ), the latter causing chromosome fusions that are usually catastrophic for the cell. However, the tightly capped telomere cannot serve as a template during replication, so regulated disassembly of the shelterin complex and unwinding of the D (and possibly T) loop is necessary for efficient copying of telomeric regions. The transient uncapping that occurs during replication is recognised by repair proteins as DNA damage [123], and the correct reformation of the T-loop requires correct handling and processing by repair enzymes. Uncontrolled uncapping is therefore a powerful cause of genomic instability, and loss of telomeres shortens replicative lifespan; both are hallmarks of WS.

4.3. Are telomeres defective in WS?

The major clinical characteristics of WS are premature ageing, presumably resulting from the highly premature replicative senescence, and elevated cancer risk, which is caused by excess genome instability. Since replicative senescence is caused, at least in part, by telomere shortening, and chromosome fusions result from telomere loss, it has been of major importance to determine whether telomeres are indeed defective in WS cells, and whether WRN plays any role in telomere maintenance. Human WS cells in culture show elevated rates of telomere loss [124]. Contradictory to this, however, are data from single telomere length analysis (STELA) that suggest WS cells do not experience exceptional rates of telomere

shortening, at least in clonal populations, though in bulk cultures of WS fibroblast, telomere loss ranges from a normal 99bp/PD to a four fold increase at 355 bp/PD [125].

Support for the importance of telomeric dysfunction in WS replicative senescence comes from studies of mouse models that are null for WRN. However, mice lacking WRN do not exhibit the premature ageing symptoms seen in humans [126] because laboratory mouse strains possess much longer telomeres than humans (40-80kb compared to 2-10kb) and detectable levels of telomerase even in somatic cells [127]. When mice deficient in telomerase are bred for several generations to reduce their telomere lengths to that approaching the normal human mean, removal of WRN gives similar premature ageing characteristics to those seen in human WS [128, 129]. Crucially, later-generation telomerase-null mice that still retain longer telomeres do not show this phenotype even though premature senescence is seen in their littermates that have short telomeres. Hence short telomeres combined with lack of WRN results in premature ageing.

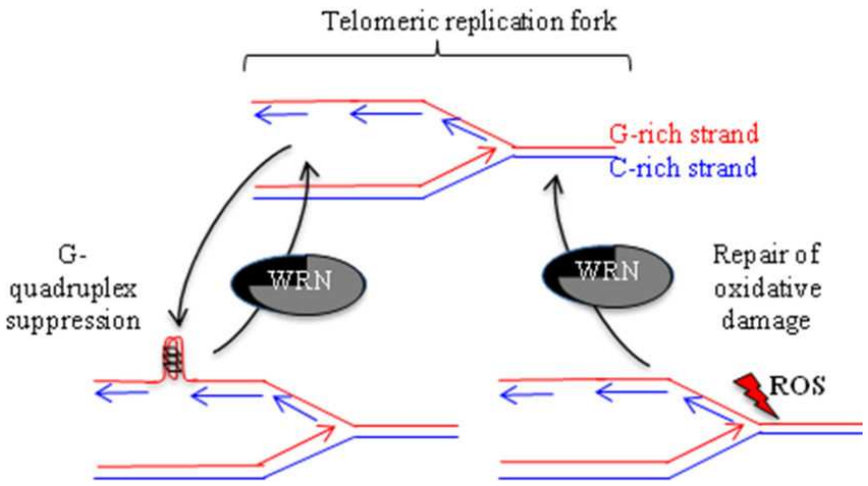

Figure 4. Roles of WRN at the telomere include unwinding of G4 DNA, that would otherwise lead to replication fork stalling, and repair of oxidative damage to which the telomeric DNA is exquisitely sensitive.

4.4. WRN helicase and exonuclease co-operate at the telomere

The repetitive nature of telomeric DNA arises as a consequence of the short RNA template within telomerase; this, combined with the G-rich nature leads to these sequences forming secondary structures called G-quadruplexes, which stall replication machinery much as any bulky lesion or DNA gap or break will. It is therefore essential for cells to unwind telomeric DNA ahead of the replication fork to prevent stalling, or worse, collapse. As discussed above, D-loops, recombination intermediates and G-quadruplexes may all require WRN and other RecQ helicases to remove these blockages (Figure 4). Under experimental conditions *in vitro*, WRN localises to a sub-set of telomeres during S-phase without the induction of stress [29], and is enriched when cells are subjected to damaging agents that cause replication stress such as CPT. Thus WRN catalysis is needed to police both endogenous replication fork blocks and induced damage.

WRN interacts with many of the proteins making up the shelterin complex or telosome [130-133] (see also Figure 3, above). Such interactions are likely to have functional consequences: for example, POT1 stimulates WRN helicase activity on linear and D-loop structures *in vitro* [134], whilst the presence of TRF1 and TRF2 can modulate their activity. TRF2 recruits WRN to D-loops and therefore stimulates unwinding [134], but it inhibits the helicase activity of WRN if binding to telomeric HJ substrates [135].

There are fewer pathways for replication fork recovery at telomeric ends because of the lack of downstream origins [136]. This obviously increases the need for proteins such as WRN that can dissolve or resolve replication blocks and promote fork progression before irreversible fork collapse occurs. One such block is G-quadruplex DNA: it has been shown to stall the major replicative polymerase δ [72]. G-quadruplex structures can arise spontaneously in single-stranded telomeric sequence [137] and can be suppressed by the binding of POT1 to release single stranded telomeric sequence during uncapping [138]. WRN preferentially unwinds G-quadruplex DNA [139] and its presence will suppress polymerase δ stalling [72], suggesting it is a good candidate for this role.

Interestingly, WRN – the only human RecQ helicase to also have exonuclease activity – unwinds D-loops *in vitro* in the absence of other proteins, using co-ordinate activity of both its helicase and exonuclease functions (RecQ helicase activity on these substrates is not particularly processive without stimulation for example by RPA [140]). The catalytic subunit of DNA-PK has also been shown to interact with WRN at telomeres [141], acting to suppress its exonuclease function and allow longer tracts to be unwound by the helicase activity. Therefore in the presence of DNA-PKcs, WRN processing of telomeric DNA does not shorten telomeric ends.

4.5. WRN acts on the lagging strand during telomere replication

Despite these detailed studies, the exact catalytic role(s) of WRN in telomere maintenance are still not fully defined. There is good evidence that cells lacking WRN have defective lagging strand synthesis at the telomere [142], as metaphase chromosomes in WRN helicase-deficient cells show a characteristic (if low-level) loss of telomeric sequence on one but not both sister chromatids. This is called sister telomere loss (STL), and suggests dysfunctional processing of one strand of the telomere during replication. The sister telomere lost is always the one resulting from lagging strand synthesis [142] This phenotype is thought to arise because in the absence of WRN activity, G-quadruplexes accumulate in the G-rich template strand and cause failure of lagging-telomere replication. Expression of active (but not inactive) telomerase suppresses STLs in cells lacking WRN [142], suggesting that sister telomere loss occurs during WRN-dependent processing of telomeres at times other than normal S phase when telomeres are uncapped for replication elongation.

The low levels of STL that occur (if the experimental data from chromosomal FISH reflect the underlying levels) suggest that the events that cause the telomere loss might be difficult to process or close to irreparable, or are merely rare; they might be alternatively-processed in a pathway that does not induce loss. Conversely, the catalytic activity supplied by WRN might be substituted by other enzymes – its helicase role by one of the other RecQs, or its

exonuclease function by another appropriate 3′-5′ exonuclease such as ExoI [143]. Whilst this has not yet been determined, however it is notable that cells deficient in BLM also show telomeric defects, although these are not the end-fusions arising from DSB repair as seen with WRN, but seem to be catenated associations possibly from aberrant HR [131]. BLM may thus have a role in resolving late-replicating DNA intermediates at telomeres distinct from WRN, as the rate of telomere dysfunction seen in cells with either single-null genotype is exacerbated in a double null [144].

Since the processing of Okazaki fragments during lagging strand synthesis gives rise to regions of ssDNA, and G-rich sequences have a tendency to form G-quadruplex structures spontaneously, at the telomere there is increased likelihood of G-quadruplex formation in the single-stranded tracts. POT1 cannot actively dissociate the structure by binding, strongly suggesting that the G-quadruplex must first be dissociated before POT1 can bind and protect the telomeric sequence, and implicating a role for WRN in removing the replication block before problems arise (see review [145]). Interestingly, the available levels of POT1 may modulate the coupling of the leading and lagging strands at telomeres in the absence of WRN, allowing uncoupled synthesis of leading strand without processing of the lagging strand block [146].

Supporting this hypothesis, recent research in yeast suggests an alternative protein that may function to suppress G-quadruplex formation at telomeres, but this time on the leading strand. Pif1 is a 5′-3′ helicase that negatively regulates telomere length [147]. Loss of Pif1 leads to slow replication fork progression, and *in vitro* Pif1 can unwind replication substrates [148]. Recently it was shown that cells without Pif1 have chromosome breakage at sites of G-tracts, and Pif1 can unwind G-quadruplex DNA that forms in the leading strand [149]. The higher eukaryote *C. elegans* also possesses a helicase (DOG-1) that is able to inhibit loss of guanine tracts, presumably by suppression of G-quadruplex structures [150]. It is tempting to speculate that genome surveillance utilises Pif1 on the leading strand and WRN on the lagging strand to suppress G-quadruplex formation and subsequent replication fork blockage at sites of high guanine content such as fragile sites and telomeric sequence.

The loss of WRN in this putative mechanism inherently implies loss specifically of lagging-strand DNA at the telomeres. In this model, the rarity of STL may be explained by a low rate of G-quadruplex formation at single-stranded telomeric tracts during Okazaki fragment replication, the ability of BLM (or another RecQ) to substitute for WRN, or the specific need for WRN in a small subset of these events – perhaps because exonuclease processing is also required. The WRN exonuclease activity is itself specifically implicated in processing of the 3′-end of the telomere, although other nucleases such as ExoI or perhaps FEN-1 [151] might possess the capability to substitute for WRN. Addition of exogenous DNA oligonucleotides homologous to the 3′-overhang structure of an uncapped telomeric end to cells lacking WRN results in an increase in DNA damage responses and ultimately cell senescence [152].

The loss of WRN in telomerase-positive cells *in vivo* causes the generation of extrachromosomal telomeric structures [153], [154] and this requires both helicase and exonuclease activities. WRN has exonuclease activity here that requires telomeric sequence in both double-stranded and single-stranded portions, and shows a characteristic limited degradation

pattern [155]. TRF2 recruits WRN to telomeric sequence and *in vitro* it synergistically enhances the ability of WRN to degrade the G-rich 3'-overhangs of telomeric D-loops substrates [132, 156]. POT1 inhibits WRN exonuclease activity here [155]. TRF2 or WRN alone exhibit little or no stimulation on these substrates. Non-telomeric substrates show similarly little WRN-dependent degradation, presumably because TRF2 does not bind/recruit and stimulate WRN exonuclease, whilst TRF2 bound to telomeric sequence completely inhibits the activity of other nucleases such as ExoIII [132]. WRN helicase and exonuclease, together with TRF2, POT1, and Ku therefore probably act together to prevent telomeric free ends from becoming substrates for HR or other aberrant pathways. Taken together, these results support the specificity of WRN exonuclease in reducing the length of the telomeric 3'-end to the optimal length for regeneration of the T-loop after replication, and suppression of extrachromosomal telomeric circles.

4.6. WRN may be important in ALT

Telomeres can be lengthened without the use of telomerase using recombination to generate the template DNA needed. In yeast, this ALT[3] pathway requires Sgs1 (the RecQ homologue in *S. cerevisiae*), for which WRN and BLM may both partially substitute [131, 157, 158]. Both WRN and BLM have also been seen to interact with telomeric DNA in human cells that utilize the ALT pathway [29, 130, 131], albeit only a small proportion. Although the ALT pathways are not yet elucidated, most models suggest recombinational mechanisms where strand invasion into telomeric DNA of the same (or different) chromosome or chromatid is utilized as template for resynthesis (e.g. see [159, 160]). BLM-deficient cells show elevated rates of sister chromatid exchange [161] that were not detected in cells lacking WRN, however finer resolution experiments suggest that WS cells do show elevated SCE, but only at telomeres [162, 163]. The WS mouse models with shortened telomeres (described in section 4.3 above) show elevated levels of this telomere-specific SCE [164], as do cells deficient in POT1, or Ku and TRF2 together [165]. Ku stimulates both helicase and exonuclease activities of WRN [166, 167], and suppresses telomeric recombination brought on by the absence of TRF2 and consequent telomeric uncapping [110]. Taken together, these data suggest that WRN is prominent in a pathway that specifically suppresses telomeric recombination or dissolves junctions, and it is at least partially distinct from the role of BLM.

4.7. Telomeric DNA is hypersensitive to oxidative lesions – a further role for WRN

The G-rich nature of telomeric sequence means it is a rich target for oxidative damage[4] [168], and oxidative stress and mitochondrial dysfunction often give rise to concomitant telomeric dysfunction [169], which can be reduced using antioxidants. Notably, artificial replicative senescence can be induced with a burst of oxidative damage [170, 171]. Oxidation of telomeric bases can disrupt DNA binding of TRF1 and TRF2, and presumably therefore telo-

3 ALT = alternative lengthening of telomeres

4 8-oxoG is a common product of oxidative attack of DNA

some and T-loop assembly [166], whilst over-expression of TRF2 protect cells with shortened telomeres from early senescence [172].

WRN is a central component of base excision repair (BER) of oxidative lesions, interacting with most of the key proteins in the pathway such as pol beta (β) and FEN1 [173, 174]. Consistent with an important role for WRN in removing oxidative lesions, WS cells show increased oxidative damage [175, 176].

It has been shown that D-loops containing oxidised bases can be bound by POT1 and are a preferred substrate for WRN [177]. The strand-displacement activity of pol β, the repair polymerase in BER, is also stimulated by TRF2 [178], and TRF1, TRF2 and POT1 can enhance all the constituent steps of long patch BER [179]. As previously mentioned, WRN itself can also stimulate TLS pols to replicate past an oxidative block [84]. This suggests active recruitment and stimulation of anti-oxidative damage processes at telomeres involving RecQ helicases. These findings partly illustrate how the activities of RecQ helicases are tightly controlled by the surrounding milieu in order to differentiate their roles in replication and repair.

Although the wider significance of all these data is yet to be determined, it is obvious that WRN is active at multiple points in telomere replication and repair.

5. Conclusions

The helicase/exonuclease WRN has been shown to be critically important in DNA replication, acting to enhance fidelity, regulate template unwinding to prevent fork stalling at unusual structures, assist with replication fork restart and/or enhance processivity post-restart, aid translesion synthesis over otherwise unreplicatable lesions, promote regression of stalled replication forks to allow error-free restart, modulate recombination at collapsed replication forks, and aid telomere replication. It is recruited to sites of DNA synthesis, possibly through association with the sliding clamp PCNA, and to sites of stalled/collapsed forks probably by RPA in concert with the S phase checkpoint kinase ATR and its downstream effectors and mediators Chk1, Rad53, Mec1 and Mrc1. Loss of WRN results in high levels of chromosomal instability and elevated cancer risk, and the defects in DNA replication on WRN loss also results in premature onset of replicative senescence with concomitant organismal ageing, manifest as progeroid Werner's syndrome. While much has been discovered as to WRN's mode of action, there is still an enormous amount to learn as to how its activities are co-ordinated with the cell during DNA replication.

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References

[1] Larizza, L., Roversi, G., & Volpi, L. Rothmund-Thomson syndrome. *Orphanet J Rare Dis*, vol. 5, 2.

[2] Goto, M. (2001). Clinical characteristics of Werner syndrome and other premature aging syndromes: pattern of aging in progeroid syndromes *From premature gray hair to helicase- Werner syndrome: implications for aging and cancer, M. Goto and R. W. Miller eds., Tokyo: Japan Scientific Societies Press*, 27-39.

[3] Hayflick, L. (1979). The cell biology of aging. *J Invest Dermatol*, 73(1), 8-14.

[4] Kyng, K. J., May, A., Kolvraa, S., & Bohr, V. A. (2003). Gene expression profiling in Werner syndrome closely resembles that of normal aging. *Proc Natl Acad Sci U S A*, 100(21), 12259-12264.

[5] Vanhooren, V., Desmyter, L., Liu, X. E., Cardelli, M., Franceschi, C., Federico, A., Libert, C., Laroy, W., Dewaele, S., Contreras, R., & Chen, C. (2007). N-glycomic changes in serum proteins during human aging. *Rejuvenation Res*, 10(4), 521-531a.

[6] Fukuchi, K., Martin, G. M., & Monnat, R. J. (1989). Mutator phenotype of Werner syndrome is characterized by extensive deletions. *Proc Natl Acad Sci U S A*, 86(15), 5893-5897.

[7] Scappaticci, S., Cerimele, D., & Fraccaro, M. (1982). Clonal structural chromosomal rearrangements in primary fibroblast cultures and in lymphocytes of patients with Werner's Syndrome,. *Hum Genet*, 62(1), 16-24.

[8] Segurado, M., & Tercero, J. A. (2009). The S-phase checkpoint: targeting the replication fork. *Biol Cell*, 101(11), 617-627.

[9] Agrelo, R., Cheng, W. H., Setien, F., Ropero, S., Espada, J., Fraga, M. F., Herranz, M., Paz, M. F., Sanchez-Cespedes, M., Artiga, M. J., Guerrero, D., Castells, A., von, C., Kobbe, V. A., & Esteller, M. (2006). Epigenetic inactivation of the premature aging Werner syndrome gene in human cancer. *Proc Natl Acad Sci U S A*, 103(23), 8822-8827.

[10] Ding, S. L., Yu, J. C., Chen, S. T., Hsu, G. C., & Shen, C. Y. (2007). Genetic variation in the premature aging gene WRN: a case-control study on breast cancer susceptibility. *Cancer Epidemiol Biomarkers Prev*, 16(2), 263-269.

[11] Bird, J. L., Jennert-Burston, K. C., Bachler, M. A., Mason, P. A., Lowe, J. E., Heo, S. J., Campisi, J., Faragher, R. G., & Cox, L. S. (2012). Recapitulation of Werner syndrome sensitivity to camptothecin by limited knockdown of the WRN helicase/exonuclease. *Biogerontology*, 13(1), 49-62.

[12] Futami, K., Takagi, M., Shimamoto, A., Sugimoto, M., & Furuichi, Y. (2007). Increased chemotherapeutic activity of camptothecin in cancer cells by siRNA-induced silencing of WRN helicase. *Biol Pharm Bull*, 30(10), 1958-1961.

[13] Aggarwal, M., Sommers, J. A., Shoemaker, R. H., & Brosh, R. M. (2011). Inhibition of helicase activity by a small molecule impairs Werner syndrome helicase (WRN) function in the cellular response to DNA damage or replication stress. *Proc Natl Acad Sci U S A*, 108(4), 1525-1530.

[14] Yu, C. E., Oshima, J., Fu, Y. H., Wijsman, E. M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S., Martin, G. M., Mulligan, J., & Schellenberg, G. D. (1996). Positional cloning of the Werner's syndrome gene,. *Science*, 272(5259), 258-262.

[15] von Kobbe, C., & Bohr, V.A. (2002). A nucleolar targeting sequence in the Werner syndrome protein resides within residues 949-1092. *J Cell Sci*, 115, 3901-3907.

[16] Marciniak, R. A., Lombard, D. B., Johnson, F. B., & Guarente, L. (1998). Nucleolar localization of the Werner syndrome protein in human cells. *Proc Natl Acad Sci U S A*, 95(12), 6887-6892.

[17] Rodriguez-Lopez, A. M., Jackson, D. A., Iborra, F., & Cox, L. S. (2002). Asymmetry of DNA replication fork progression in Werner's syndrome. *Aging Cell*, 1(1), 30-39.

[18] Rodriguez-Lopez, A. M., Jackson, D. A., Nehlin, J. O., Iborra, F., Warren, A. V., & Cox, L. S. (2003). Characterisation of the interaction between WRN, the helicase/exonuclease defective in progeroid Werner's syndrome, and an essential replication factor, PCNA. *Mech Ageing Dev*, 124(2), 167-174.

[19] Constantinou, A., Tarsounas, M., Karow, J. K., Brosh, R. M., Bohr, V. A., Hickson, I. D., & West, S. C. (2000). Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. *EMBO Rep*, 1(1), 80-84.

[20] Huang, S., Li, B., Gray, M. D., Oshima, J., Mian, I. S., & Campisi, J. (1998). The premature ageing syndrome protein, WRN, is a 3'-->5' exonuclease. *Nat Genet*, 20(2), 114-116.

[21] Huang, S., Beresten, S., Li, B., Oshima, J., Ellis, N. A., & Campisi, J. (2000). Characterization of the human and mouse WRN 3'-->5' exonuclease. *Nucleic Acids Res*, 28(12), 2396-2405.

[22] Harrigan, J. A., Fan, J., Momand, J., Perrino, F. W., Bohr, V. A., & Wilson, D. M. (2007). WRN exonuclease activity is blocked by DNA termini harboring 3′ obstructive groups. *Mech Ageing Dev*, 128(3), 259-266.

[23] Ozgenc, A., & Loeb, L. A. (2005). Current advances in unraveling the function of the Werner syndrome protein. *Mutat Res*, 577(1-2), 237-251.

[24] Xue, Y., Ratcliff, G. C., Wang, H., Davis-Searles, P. R., Gray, M. D., Erie, D. A., & Redinbo, M. R. (2002). A minimal exonuclease domain of WRN forms a hexamer on DNA and possesses both 3′- 5′ exonuclease and 5′-protruding strand endonuclease activities. *Biochemistry*, 41(9), 2901-2912.

[25] Machwe, A., Xiao, L., & Orren, D. K. (2006). Length-dependent degradation of single-stranded 3′ ends by the Werner syndrome protein (WRN): implications for spatial orientation and coordinated 3′ to 5′ movement of its ATPase/helicase and exonuclease domains. *BMC Mol Biol*, 7, 6.

[26] Gray, M. D., Shen, J. C., Kamath-Loeb, A. S., Blank, A., Sopher, B. L., Martin, G. M., Oshima, J., & Loeb, L. A. (1997). The Werner syndrome protein is a DNA helicase. *Nat Genet*, 17(1), 100-103.

[27] Brosh, R. M., Waheed, J., & Sommers, J. A. (2002). Biochemical characterization of the DNA substrate specificity of Werner syndrome helicase. *J Biol Chem*, 277(26), 23236-23245.

[28] Shen, J., & Loeb, L. A. (2001). Unwinding the molecular basis of the Werner syndrome. *Mech Ageing Dev*, 122(9), 921-944.

[29] Opresko, P. L., Otterlei, M., Graakjaer, J., Bruheim, P., Dawut, L., Kolvraa, S., May, A., Seidman, M. M., & Bohr, V. A. (2004). The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2. *Mol Cell*, 14(6), 763-774.

[30] Kitano, K., Kim, S. Y., & Hakoshima, T. (2010). Structural basis for DNA strand separation by the unconventional winged-helix domain of RecQ helicase WRN. *Structure*, 18(2), 177-187.

[31] Kitano, K., Yoshihara, N., & Hakoshima, T. (2007). Crystal structure of the HRDC domain of human Werner syndrome protein, WRN. *J Biol Chem*, 282(4), 2717-2728.

[32] Hartung, F., & Puchta, H. (2006). The RecQ gene family in plants. *J Plant Physiol*, 163(3), 287-296.

[33] Opresko, P. L., Cheng, W. H., & Bohr, V. A. (2004). Junction of RecQ helicase biochemistry and human disease. *J Biol Chem*, 279(18), 18099-18102.

[34] Cox, L. S., Clancy, D. J., Boubriak, I., & Saunders, R. D. (2007). Modeling Werner Syndrome in Drosophila melanogaster: hyper-recombination in flies lacking WRN-like exonuclease. *Ann N Y Acad Sci*, 1119, 274-288.

[35] Boubriak, I., Mason, P. A., Clancy, D. J., Dockray, J., Saunders, R. D., & Cox, L. S. (2009). DmWRNexo is a 3′-5′ exonuclease: phenotypic and biochemical characterization of mutants of the Drosophila orthologue of human WRN exonuclease. *Biogerontology*, 10(3), 267-277.

[36] Mason, P. A., Boubriak, I., Robbins, T., Lasala, R., Saunders, R., & Cox, L. S. (2012). The Drosophila orthologue of progeroid human WRN exonuclease, DmWRNexo, cleaves replication substrates but is inhibited by uracil or abasic sites : Analysis of DmWRNexo activity in vitro. *Age (Dordr)*.

[37] Saunders, R. D., Boubriak, I., Clancy, D. J., & Cox, L. S. (2008). Identification and characterization of a Drosophila ortholog of WRN exonuclease that is required to maintain genome integrity. *Aging Cell*, 7(3), 418-425.

[38] Ketting, R. F., Haverkamp, T. H., van Luenen, H. G., & Plasterk, R. H. (1999). Mut-7 of C. elegans, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNaseD. *Cell*, 99(2), 133-141.

[39] Lee, S. J., Yook, J. S., Han, S. M., & Koo, H. S. (2004). A Werner syndrome protein homolog affects C. elegans development, growth rate, life span and sensitivity to DNA damage by acting at a DNA damage checkpoint. *Development*, 131(11), 2565-2575.

[40] Lee, S. J., Gartner, A., Hyun, M., Ahn, B., & Koo, H. S. (2010). The Caenorhabditis elegans Werner syndrome protein functions upstream of ATR and ATM in response to DNA replication inhibition and double-strand DNA breaks. *PLoS Genet*, 6(1), e1000801.

[41] Hartung, F., Plchova, H., & Puchta, H. (2000). Molecular characterisation of RecQ homologues in Arabidopsis thaliana. *Nucleic Acids Res*, 28(21), 4275-4282.

[42] Doe, C. L., Ahn, J. S., Dixon, J., & Whitby, M. C. (2002). Mus81-Eme1 and Rqh1 involvement in processing stalled and collapsed replication forks. *J Biol Chem*, 277(36), 32753-32759.

[43] Poot, M., Hoehn, H., Runger, T. M., & Martin, G. M. (1992). Impaired S-phase transit of Werner syndrome cells expressed in lymphoblastoid cell lines. *Exp Cell Res*, 202(2), 267-273.

[44] Sidorova, J. M., Li, N., Folch, A., & Monnat, R. J. (2008). The RecQ helicase WRN is required for normal replication fork progression after DNA damage or replication fork arrest. *Cell Cycle*, 7(6), 796-807.

[45] Fujiwara, Y., Higashikawa, T., & Tatsumi, M. (1977). A retarded rate of DNA replication and normal level of DNA repair in Werner's syndrome fibroblasts in culture. *J Cell Physiol*, 92(3), 365-374.

[46] Rodriguez-Lopez, A. M., Whitby, M. C., Borer, C. M., Bachler, M. A., & Cox, L. S. (2007). Correction of proliferation and drug sensitivity defects in the progeroid Werner's Syndrome by Holliday junction resolution. *Rejuvenation Res*, 10(1), 27-40.

[47] Lebel, M., Spillare, E. A., Harris, C. C., & Leder, P. (1999). The Werner syndrome gene product co-purifies with the DNA replication complex and interacts with PCNA and topoisomerase I. *J Biol Chem*, 274(53), 37795-37799.

[48] Yan, H., & Newport, J. (1995). FFA-1, a protein that promotes the formation of replication centers within nuclei. *Science*, 269(5232), 1883-1885.

[49] Chen, C. Y., Graham, J., & Yan, H. (2001). Evidence for a replication function of FFA-1, the Xenopus orthologue of Werner syndrome protein. *J Cell Biol*, 152(5), 985-996.

[50] Garg, P., & Burgers, P. M. (2005). DNA polymerases that propagate the eukaryotic DNA replication fork. *Crit Rev Biochem Mol Biol*, 40(2), 115-128.

[51] Pursell, Z. F., Isoz, I., Lundstrom, E. B., Johansson, E., & Kunkel, T. A. (2007). Yeast DNA polymerase epsilon participates in leading-strand DNA replication. *Science*, 317(5834), 127-130.

[52] McCulloch, S.D., & Kunkel, T.A. (2008). The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. *Cell Res*, 18(1), 148-161.

[53] Podust, V. N., Tiwari, N., Stephan, S., & Fanning, E. (1998). Replication factor C disengages from proliferating cell nuclear antigen (PCNA) upon sliding clamp formation, and PCNA itself tethers DNA polymerase delta to DNA. *J Biol Chem*, 273(48), 31992-31999.

[54] Gambus, A., Jones, R. C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R. D., & Labib, K. (2006). GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat Cell Biol*, 8(4), 358-366.

[55] Cox, L.S. (2009). Molecular Themes in DNA Replication in Book Molecular Themes in DNA Replication. *Series Molecular Themes in DNA Replication*, Editor ed.^eds., City: Royal Society of Chemistry, 2009, 443.

[56] Budd, M. E., Cox, L. S., & Campbell, J. L. (2009). Coordination of nucleases and helicases during DNA replication and double-strand break repair. *Molecular Themes in DNA Replication, L. S. Cox ed., Cambridge: Royal Society of Chemistry*, 112-155.

[57] Beattie, T.R., & Bell, S.D. (2012). Coordination of multiple enzyme activities by a single PCNA in archaeal Okazaki fragment maturation. *EMBO J*, 31(6), 1556-1567.

[58] Warbrick, E., Lane, D. P., Glover, D. M., & Cox, L. S. (1995). A small peptide inhibitor of DNA replication defines the site of interaction between the cyclin-dependent kinase inhibitor p21WAF1 and proliferating cell nuclear antigen. *Curr Biol*, 5(3), 275-282.

[59] Guarino, E., Shepherd, M. E., Salguero, I., Hua, H., Deegan, R. S., & Kearsey, S. E. (2011). Cdt1 proteolysis is promoted by dual PIP degrons and is modulated by PCNA ubiquitylation. *Nucleic Acids Res*, 39(14), 5978-5990.

[60] Warbrick, E., Lane, D. P., Glover, D. M., & Cox, L. S. (1997). Homologous regions of Fen1 and p21Cip1 compete for binding to the same site on PCNA: a potential mechanism to co-ordinate DNA replication and repair. *Oncogene*, 14(19), 2313-2321.

[61] Cox, L. S. (1997). Who binds wins: Competition for PCNA rings out cell-cycle changes. *Trends Cell Biol*, 7(12), 493-498.

[62] Cox, L. S., & Kearsey, S. (2009). Ring structures and six-fold symmetry in DNA replication. *Molecular Themes in DNA Replication, L. S. Cox ed., Cambridge: Royal Society of Chemistry*, 47-85.

[63] Brosh, R. M., von Kobbe, C., Sommers, J. A., Karmakar, P., Opresko, P. L., Piotrowski, J., Dianova, I., Dianov, G. L., & Bohr, V. A. (2001). Werner syndrome protein interacts with human flap endonuclease 1 and stimulates its cleavage activity. *EMBO J*, 20(20), 5791-5801.

[64] Sharma, S., Sommers, J. A., Gary, R. K., Friedrich-Heineken, E., Hubscher, U., & Brosh, R. M. (2005). The interaction site of Flap Endonuclease-1 with WRN helicase suggests a coordination of WRN and PCNA. *Nucleic Acids Res*, 33(21), 6769-6781.

[65] Nick, S. A., Mc Elhinny, Z. F., Pursell, S., & Kunkel, T. A. (2009). Mechanisms for high fidelity DNA replication. *Molecular Themes in DNA Replication, L. S. Cox ed., Cambridge, UK: Royal Society of Chemistry*, 86-111.

[66] Swan, M. K., Johnson, R. E., Prakash, L., Prakash, S., & Aggarwal, A. K. (2009). Structural basis of high-fidelity DNA synthesis by yeast DNA polymerase delta. *Nat Struct Mol Biol*, 16(9), 979-986.

[67] Perry, J. J., Yannone, S. M., Holden, L. G., Hitomi, C., Asaithamby, A., Han, S., Cooper, P. K., Chen, D. J., & Tainer, J. A. (2006). WRN exonuclease structure and molecular mechanism imply an editing role in DNA end processing. *Nat Struct Mol Biol*, 13(5), 414-422.

[68] Kamath-Loeb, A. S., Shen, J. C., Schmitt, M. W., & Loeb, L. A. (2012). The Werner syndrome exonuclease facilitates DNA degradation and high fidelity DNA polymerization by human DNA polymerase delta. *J Biol Chem*, 287(15), 12480-12490.

[69] Takeuchi, F., Hanaoka, F., Goto, M., Akaoka, I., Hori, T., Yamada, M., & Miyamoto, T. (1982). Altered frequency of initiation sites of DNA replication in Werner's syndrome cells. *Hum Genet*, 60(4), 365-368.

[70] Hanaoka, F., Yamada, M., Takeuchi, F., Goto, M., Miyamoto, T., & Hori, T. (1985). Autoradiographic studies of DNA replication in Werner's syndrome cells. *Adv Exp Med Biol*, 190, 439-457.

[71] Foiani, M., Ferrari, M., Liberi, G., Lopes, M., Lucca, C., Marini, F., Pellicioli, A., Muzi, M., Falconi, , & Plevani, P. (1998). S-phase DNA damage checkpoint in budding yeast. *Biol Chem*, 379(8-9), 1019-1023.

[72] Kamath-Loeb, A. S., Loeb, L. A., Johansson, E., Burgers, P. M., & Fry, M. (2001). Interactions between the Werner syndrome helicase and DNA polymerase delta specifi-

cally facilitate copying of tetraplex and hairpin structures of the d(CGG)n trinucleotide repeat sequence. *J Biol Chem*, 276(19), 16439-16446.

[73] Ammazzalorso, F., Pirzio, L. M., Bignami, M., Franchitto, A., & Pichierri, P. (2010). ATR and ATM differently regulate WRN to prevent DSBs at stalled replication forks and promote replication fork recovery. *EMBO J*, 29(18), 3156-3169.

[74] Murfuni, I., De Santis, A., Federico, M., Bignami, M., Pichierri, P., & Franchitto, A. (2012). Perturbed replication induced genome-wide or at common fragile sites is differently managed in the absence of WRN. *Carcinogenesis*, 33(9), 1655-63.

[75] Shah, S. N., Opresko, P. L., Meng, X., Lee, M. Y., & Eckert, K. A. (2010). DNA structure and the Werner protein modulate human DNA polymerase delta-dependent replication dynamics within the common fragile site FRA16D,. *Nucleic Acids Res*, 38(4), 1149-1162.

[76] Johnson, R. E., Washington, M. T., Haracska, L., Prakash, S., & Prakash, L. (2000). Eukaryotic polymerases iota and zeta act sequentially to bypass DNA lesions. *Nature*, 406(6799), 1015-1019.

[77] Choi, J.Y., & Guengerich, F.P. (2006). Kinetic evidence for inefficient and error-prone bypass across bulky N2-guanine DNA adducts by human DNA polymerase iota. *J Biol Chem*, 281(18), 12315-12324.

[78] Nimonkar, A. V., Ozsoy, A. Z., Genschel, J., Modrich, P., & Kowalczykowski, S. C. (2008). Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc Natl Acad Sci U S A*, 105(44), 16906-16911.

[79] Aggarwal, M., Sommers, J. A., Morris, C., & Brosh, R. M. (2010). Delineation of WRN helicase function with EXO1 in the replicational stress response. *DNA Repair (Amst)*, 9(7), 765-776.

[80] Aggarwal, M., & Brosh, R. M. (2010). Genetic mutants illuminate the roles of RecQ helicases in recombinational repair or response to replicational stress. *Cell Cycle*, 9(16), 3139-3141.

[81] Matsuda, T., Bebenek, K., Masutani, C., Rogozin, I. B., Hanaoka, F., & Kunkel, T. A. (2001). Error rate and specificity of human and murine DNA polymerase eta. *J Mol Biol*, 312(2), 335-346.

[82] Zhang, Y., Yuan, F., Xin, H., Wu, X., Rajpal, D. K., Yang, D., & Wang, Z. (2000). Human DNA polymerase kappa synthesizes DNA with extraordinarily low fidelity. *Nucleic Acids Res*, 28(21), 4147-4156.

[83] Vasquez Del, R., Carpio, T. D., Silverstein, S., Lone, M. K., Swan, J. R., Choudhury, R. E., Johnson, S., Prakash, L., & Aggarwal, A. K. (2009). Structure of human DNA polymerase kappa inserting dATP opposite an 8-OxoG DNA lesion. *PLoS One*, 4(6), e5766.

[84] Kamath-Loeb, A. S., Lan, L., Nakajima, S., Yasui, A., & Loeb, L. A. (2007). Werner syndrome protein interacts functionally with translesion DNA polymerases. *Proc Natl Acad Sci U S A*, 104(25), 10394-10399.

[85] Machwe, A., Lozada, E., Wold, M. S., Li, G. M., & Orren, D. K. (2011). Molecular cooperation between the Werner syndrome protein and replication protein A in relation to replication fork blockage. *J Biol Chem*, 286(5), 3497-3508.

[86] Sharma, S., Sommers, J. A., Driscoll, H. C., Uzdilla, L., Wilson, T. M., & Brosh, R. M. (2003). The exonucleolytic and endonucleolytic cleavage activities of human exonuclease 1 are stimulated by an interaction with the carboxyl-terminal region of the Werner syndrome protein. *J Biol Chem*, 278(26), 23487-23496.

[87] Franchitto, A., Pirzio, L. M., Prosperi, E., Sapora, O., Bignami, M., & Pichierri, P. (2008). Replication fork stalling in WRN-deficient cells is overcome by prompt activation of a MUS81-dependent pathway. *J Cell Biol*, 183(2), 241-252.

[88] Byun, T. S., Pacek, M., Yee, M. C., Walter, J. C., & Cimprich, K. A. (2005). Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes Dev*, 19(9), 1040-1052.

[89] Singleton, M. R., Scaife, S., Raven, N. D., & Wigley, D. B. (2001). Crystallization and preliminary X-ray analysis of RecG, a replication-fork reversal helicase from Thermotoga maritima complexed with a three-way DNA junction. *Acta Crystallogr D Biol Crystallogr*, 57(11), 1695-1696.

[90] Singleton, M. R., Scaife, S., & Wigley, D. B. (2001). Structural analysis of DNA replication fork reversal by RecG. *Cell*, 107(1), 79-89.

[91] Courcelle, J., Donaldson, J. R., Chow, K. H., & Courcelle, C. T. (2003). DNA damage-induced replication fork regression and processing in Escherichia coli. *Science*, 299(5609), 1064-1067.

[92] Sharma, S., Otterlei, M., Sommers, J. A., Driscoll, H. C., Dianov, G. L., Kao, H. I., Bambara, R. A., & Brosh, R. M. (2004). WRN helicase and FEN-1 form a complex upon replication arrest and together process branchmigrating DNA structures associated with the replication fork. *Mol Biol Cell*, 15(2), 734-750.

[93] Otterlei, M., Bruheim, P., Ahn, B., Bussen, W., Karmakar, P., Baynton, K., & Bohr, V. A. (2006). Werner syndrome protein participates in a complex with RAD51, RAD54, RAD54B and ATR in response to ICL-induced replication arrest. *J Cell Sci*, 119, 5137-5146.

[94] Machwe, A., Karale, R., Xu, X., Liu, Y., & Orren, D. K. (2011). The Werner and Bloom syndrome proteins help resolve replication blockage by converting (regressed) holliday junctions to functional replication forks. *Biochemistry*, 50(32), 6774-6788.

[95] Pichierri, P., & Rosselli, F. (2004). The DNA crosslink-induced S-phase checkpoint depends on ATR-CHK1 and ATR-NBS1-FANCD2 pathways. *EMBO J*, 23(5), 1178-1187.

[96] Bennetzen, M. V., Marino, G., Pultz, D., Morselli, E., Faergeman, N. J., Kroemer, G., & Andersen, J. S. (2012). Phosphoproteomic analysis of cells treated with longevity-related autophagy inducers. *Cell Cycle*, 11(9), 1827-1840.

[97] Patro, B. S., Frohlich, R., Bohr, V. A., & Stevnsner, T. (2011). WRN helicase regulates the ATR-CHK1-induced S-phase checkpoint pathway in response to topoisomerase-I-DNA covalent complexes. *J Cell Sci*, 124, 3967-3979.

[98] Pichierri, P., Nicolai, S., Cignolo, L., Bignami, M., & Franchitto, A. (2011). The RAD9-RAD1-HUS1 (9.1.1) complex interacts with WRN and is crucial to regulate its response to replication fork stalling. *Oncogene*, 31(23), 2809-2823.

[99] Svoboda, D.L., & Vos, J.M. (1995). Differential replication of a single, UV-induced lesion in the leading or lagging strand by a human cell extract: fork uncoupling or gap formation. *Proc Natl Acad Sci U S A*, 92(26), 11975-11979.

[100] Mc Inerney, P., & O'Donnell, M. (2004). Functional uncoupling of twin polymerases: mechanism of polymerase dissociation from a lagging-strand block. *J Biol Chem*, 279(20), 21543-21551.

[101] Heller, R.C., & Marians, K.J. (2006). Replisome assembly and the direct restart of stalled replication forks. *Nat Rev Mol Cell Biol*, 7(12), 932-943.

[102] Wu, L., & Hickson, I. D. (2003). The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature*, 426(6968), 870-874.

[103] Wu, L., Chan, K. L., Ralf, C., Bernstein, D. A., Garcia, P. L., Bohr, V. A., Vindigni, A., Janscak, P., Keck, J. L., & Hickson, I. D. (2005). The HRDC domain of BLM is required for the dissolution of double Holliday junctions. *EMBO J*, 24(14), 2679-2687.

[104] Opresko, P. L., Sowd, G., & Wang, H. (2009). The Werner syndrome helicase/exonuclease processes mobile D-loops through branch migration and degradation. *PLoS One*, 4(3), e4825.

[105] Kamath-Loeb, A. S., Johansson, E., Burgers, P. M., & Loeb, L. A. (2000). Functional interaction between the Werner Syndrome protein and DNA polymerase delta. *Proc Natl Acad Sci U S A*, 97(9), 4603-4608.

[106] Kunkel, T. A., Sabatino, R. D., & Bambara, R. A. (1987). Exonucleolytic proofreading by calf thymus DNA polymerase delta. *Proc Natl Acad Sci U S A*, 84(14), 4865-4869.

[107] Kamath-Loeb, A., Loeb, L. A., & Fry, M. (2012). The Werner syndrome protein is distinguished from the Bloom syndrome protein by its capacity to tightly bind diverse DNA structures. *PLoS One*, 7(1), e30189.

[108] Bukowy, Z., Harrigan, J. A., Ramsden, D. A., Tudek, B., Bohr, V. A., & Stevnsner, T. (2008). WRN Exonuclease activity is blocked by specific oxidatively induced base lesions positioned in either DNA strand. *Nucleic Acids Res*, 36(15), 4975-4987.

[109] Maser, R.S., & DePinho, R.A. (2004). Telomeres and the DNA damage response: why the fox is guarding the henhouse,. *DNA Repair (Amst)*, 3(8-9), 979-988.

[110] d'Adda, F., di Fagagna, S. H., & Jackson, S. P. (2004). Functional links between telomeres and proteins of the DNA-damage response. *Genes Dev*, 18(15), 1781-1799.

[111] Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H., & de Lange, T. (1999). Mammalian telomeres end in a large duplex loop. *Cell*, 97(4), 503-514.

[112] de Lange, T. (2005). Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev*, 19(18), 2100-2110.

[113] Broccoli, D., Smogorzewska, A., Chong, L., & de Lange, T. (1997). Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat Genet*, 17(2), 231-235.

[114] Liu, D., O'Connor, M. S., Qin, J., & Songyang, Z. (2004). Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins. *J Biol Chem*, 279(49), 51338-51342.

[115] Baumann, P., & Cech, T. R. (2001). Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science*, 292(5519), 1171-1175.

[116] O'Donovan, A., Tomiyama, A. J., Lin, J., Puterman, E., Adler, N. E., Kemeny, M., Wolkowitz, O. M., Blackburn, E. H., & Epel, E. S. (2012). Stress appraisals and cellular aging: a key role for anticipatory threat in the relationship between psychological stress and telomere length. *Brain Behav Immun*, 26(4), 573-579.

[117] Epel, E. S., Lin, J., Dhabhar, F. S., Wolkowitz, O. M., Puterman, E., Karan, L., & Blackburn, E. H. (2010). Dynamics of telomerase activity in response to acute psychological stress. *Brain Behav Immun*, 24(4), 531-539.

[118] Damjanovic, A. K., Yang, Y., Glaser, R., Kiecolt-Glaser, J. K., Nguyen, H., Laskowski, B., Zou, Y., Beversdorf, D. Q., & Weng, N. P. (2007). Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J Immunol*, 179(6), 4249-4254.

[119] Smogorzewska, A., & de Lange, T. (2004). Regulation of telomerase by telomeric proteins. *Annu Rev Biochem*, 73, 177-208.

[120] Harley, C. B., Futcher, A. B., & Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature*, 345(6274), 458-460.

[121] Harley, C. B., Kim, N. W., Prowse, K. R., Weinrich, S. L., Hirsch, K. S., West, M. D., Bacchetti, S., Hirte, H. W., Counter, C. M., Greider, C. W., et al. (1994). Telomerase, cell immortality, and cancer,. *Cold Spring Harb Symp Quant Biol*, 59, 307-315.

[122] Cesare, A.J., & Reddel, R.R. (2010). Alternative lengthening of telomeres: models, mechanisms and implications. *Nat Rev Genet*, 11(5), 319-330.

[123] Verdun, R. E., & Karlseder, J. (2007). Replication and protection of telomeres. *Nature*, 447(7147), 924-931.

[124] Schulz, V. P., Zakian, V. A., Ogburn, C. E., Mc Kay, J., Jarzebowicz, A. A., Edland, S. D., & Martin, G. M. (1996). Accelerated loss of telomeric repeats may not explain accelerated replicative decline of Werner syndrome cells. *Hum Genet*, 97(6), 750-754.

[125] Baird, D. M., Davis, T., Rowson, J., Jones, C. J., & Kipling, D. (2004). Normal telomere erosion rates at the single cell level in Werner syndrome fibroblast cells. *Hum Mol Genet*, 13(14), 1515-1524.

[126] Lombard, D. B., Beard, C., Johnson, B., Marciniak, R. A., Dausman, J., Bronson, R., Buhlmann, J. E., Lipman, R., Curry, R., Sharpe, A., Jaenisch, R., & Guarente, L. (2000). Mutations in the WRN gene in mice accelerate mortality in a p53-null background. *Mol Cell Biol*, 20(9), 3286-3291.

[127] Chang, S. (2005). A mouse model of Werner Syndrome: what can it tell us about aging and cancer ? *Int J Biochem Cell Biol*, 37(5), 991-999.

[128] Du, X., Shen, J., Kugan, N., Furth, E. E., Lombard, D. B., Cheung, C., Pak, S., Luo, G., Pignolo, R. J., De Pinho, R. A., Guarente, L., & Johnson, F. B. (2004). Telomere shortening exposes functions for the mouse Werner and Bloom syndrome genes. *Mol Cell Biol*, 24(19), 8437-8446.

[129] Chang, S., Multani, A. S., Cabrera, N. G., Naylor, M. L., Laud, P., Lombard, D., Pathak, S., Guarente, L., & De Pinho, R. A. (2004). Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet*, 36(8), 877-882.

[130] Stavropoulos, D. J., Bradshaw, P. S., Li, X., Pasic, I., Truong, K., Ikura, M., Ungrin, M., & Meyn, M. S. (2002). The Bloom syndrome helicase BLM interacts with TRF2 in ALT cells and promotes telomeric DNA synthesis. *Hum Mol Genet*, 11(25), 3135-3144.

[131] Lillard-Wetherell, K., Machwe, A., Langland, G. T., Combs, K. A., Behbehani, G. K., Schonberg, S. A., German, J., Turchi, J. J., Orren, D. K., & Groden, J. (2004). Association and regulation of the BLM helicase by the telomere proteins TRF1 and TRF2. *Hum Mol Genet*, 13(17), 1919-1932.

[132] Machwe, A., Xiao, L., & Orren, D. K. (2004). TRF2 recruits the Werner syndrome (WRN) exonuclease for processing of telomeric DNA. *Oncogene*, 23(1), 149-156.

[133] Opresko, P. L., von, C., Kobbe, J. P., Laine, J., Harrigan, I. D., & Hickson, V. A. (2002). Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases. *J Biol Chem*, 277(43), 41110-41119.

[134] Opresko, P. L., Mason, P. A., Podell, E. R., Lei, M., Hickson, I. D., Cech, T. R., & Bohr, V. A. (2005). POT1 stimulates RecQ helicases WRN and BLM to unwind telomeric DNA substrates. *J Biol Chem*, 280(37), 32069-32080.

[135] Nora, G. J., Buncher, N. A., & Opresko, P. L. (2010). Telomeric protein TRF2 protects Holliday junctions with telomeric arms from displacement by the Werner syndrome helicase. *Nucleic Acids Res*, 38(12), 3984-3998.

[136] Lee, J. Y., Kozak, M., Martin, J. D., Pennock, E., & Johnson, F. B. (2007). Evidence that a RecQ helicase slows senescence by resolving recombining telomeres. *PLoS Biol*, 5(6), e160.

[137] Duquette, M. L., Handa, P., Vincent, J. A., Taylor, A. F., & Maizels, N. (2004). Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4 DNA. *Genes Dev*, 18(13), 1618-1629.

[138] Zaug, A. J., Podell, E. R., & Cech, T. R. (2005). Human POT1 disrupts telomeric G-quadruplexes allowing telomerase extension in vitro. *Proc Natl Acad Sci U S A*, 102(31), 10864-10869.

[139] Mohaghegh, P., & Hickson, I. D. (2001). DNA helicase deficiencies associated with cancer predisposition and premature ageing disorders. *Hum Mol Genet*, 10(7), 741-746.

[140] Opresko, P. L., Laine, J. P., Brosh, R. M., Seidman, M. M., & Bohr, V. A. (2001). Coordinate action of the helicase and 3′ to 5′ exonuclease of Werner syndrome protein. *J Biol Chem*, 276(48), 44677-44687.

[141] Kusumoto-Matsuo, R., Opresko, P. L., Ramsden, D., Tahara, H., & Bohr, V. A. (2010). Cooperation of DNA-PKcs and WRN helicase in the maintenance of telomeric D-loops. *Aging (Albany NY)*, 2(5), 274-284.

[142] Crabbe, L., Verdun, R. E., Haggblom, C. I., & Karlseder, J. (2004). Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. *Science*, 306(5703), 1951-1953.

[143] Dewar, J. M., & Lydall, D. (2010). Pif1- and Exo1-dependent nucleases coordinate checkpoint activation following telomere uncapping. *EMBO J*, 29(23), 4020-4034.

[144] Barefield, C., & Karlseder, J. (2012). The BLM helicase contributes to telomere maintenance through processing of late-replicating intermediate structures. *Nucleic Acids Res*, 40(15), 7358-7367.

[145] Opresko, P. L. (2008). Telomere ResQue and preservation--roles for the Werner syndrome protein and other RecQ helicases. *Mech Ageing Dev*, 129(1-2), 79-90.

[146] Arnoult, N., Saintome, C., Ourliac-Garnier, I., Riou, J. F., & Londono-Vallejo, A. (2009). Human POT1 is required for efficient telomere C-rich strand replication in the absence of WRN. *Genes Dev*, 23(24), 2915-2924.

[147] Schulz, V.P., & Zakian, V.A. (1994). The saccharomyces PIF1 DNA helicase inhibits telomere elongation and de novo telomere formation. *Cell*, 76(1), 145-155.

[148] George, T., Wen, Q., Griffiths, R., Ganesh, A., Meuth, M., & Sanders, C. M. (2009). Human Pif1 helicase unwinds synthetic DNA structures resembling stalled DNA replication forks. *Nucleic Acids Res*, 37(19), 6491-6502.

[149] Paeschke, K., Capra, J. A., & Zakian, V. A. (2011). DNA replication through G-quadruplex motifs is promoted by the Saccharomyces cerevisiae Pif1 DNA helicase. *Cell*, 145(5), 678-691.

[150] Cheung, I., Schertzer, M., Rose, A., & Lansdorp, P. M. (2002). Disruption of dog-1 in Caenorhabditis elegans triggers deletions upstream of guanine-rich DNA. *Nat Genet*, 31(4), 405-409.

[151] Vallur, A. C., & Maizels, N. (2010). Distinct activities of exonuclease 1 and flap endonuclease 1 at telomeric g4 DNA. *PLoS One*, 5(1), e8908.

[152] Eller, M. S., Liao, X., Liu, S., Hanna, K., Backvall, H., Opresko, P. L., Bohr, V. A., & Gilchrest, B. A. (2006). A role for WRN in telomere-based DNA damage responses. *Proc Natl Acad Sci U S A*, 103(41), 15073-15078.

[153] Li, B., Jog, S. P., Reddy, S., & Comai, L. (2008). WRN controls formation of extrachromosomal telomeric circles and is required for TRF2DeltaB-mediated telomere shortening. *Mol Cell Biol*, 28(6), 1892-1904.

[154] Reddy, S., Li, B., & Comai, L. (2010). Processing of human telomeres by the Werner syndrome protein. *Cell Cycle*, 9(16), 3137-3138.

[155] Li, B., Reddy, S., & Comai, L. (2009). Sequence-specific processing of telomeric 3′ overhangs by the Werner syndrome protein exonuclease activity. *Aging (Albany NY)*, 1(3), 289-302.

[156] Orren, D. K., Theodore, S., & Machwe, A. (2002). The Werner syndrome helicase/exonuclease (WRN) disrupts and degrades D-loops in vitro. *Biochemistry*, 41(46), 13483-13488.

[157] Johnson, F. B., Marciniak, R. A., Mc Vey, M., Stewart, S. A., Hahn, W. C., & Guarente, L. (2001). The Saccharomyces cerevisiae WRN homolog Sgs1p participates in telomere maintenance in cells lacking telomerase. *EMBO J*, 20(4), 905-913.

[158] Cohen, H., & Sinclair, D. A. (2001). Recombination-mediated lengthening of terminal telomeric repeats requires the Sgs1 DNA helicase. *Proc Natl Acad Sci U S A* [6], 3174-3179.

[159] Neumann, A.A., & Reddel, R.R. (2002). Telomere maintenance and cancer-- look, no telomerase. *Nat Rev Cancer*, 2(11), 879-884.

[160] Dunham, M. A., Neumann, A. A., Fasching, C. L., & Reddel, R. R. (2000). Telomere maintenance by recombination in human cells. *Nat Genet*, 26(4), 447-450.

[161] Hickson, I. D. (2003). RecQ helicases: caretakers of the genome. *Nat Rev Cancer*, 3(3), 169-178.

[162] Bailey, S. M., Cornforth, M. N., Ullrich, R. L., & Goodwin, E. H. (2004). Dysfunctional mammalian telomeres join with DNA double-strand breaks. *DNA Repair (Amst)*, 3(4), 349-357.

[163] Bailey, S. M., Brenneman, M. A., & Goodwin, E. H. (2004). Frequent recombination in telomeric DNA may extend the proliferative life of telomerase-negative cells. *Nucleic Acids Res*, 32(12), 3743-3751.

[164] Laud, P. R., Multani, A. S., Bailey, S. M., Wu, L., Kingsley, J. C., Lebel, M., Pathak, S., De Pinho, R. A., & Chang, S. (2005). Elevated telomere-telomere recombination in WRN-deficient, telomere dysfunctional cells promotes escape from senescence and engagement of the ALT pathway. *Genes Dev*, 19(21), 2560-2570.

[165] Celli, G. B., Denchi, E. L., & de Lange, T. (2006). Ku70 stimulates fusion of dysfunctional telomeres yet protects chromosome ends from homologous recombination. *Nat Cell Biol 8* [8], 885-890.

[166] Opresko, P. L., Fan, J., Danzy, S., Wilson, D. M., & Bohr, V. A. (2005). Oxidative damage in telomeric DNA disrupts recognition by TRF1 and TRF2. *Nucleic Acids Res*, 33(4), 1230-1239.

[167] Cooper, M. P., Machwe, A., Orren, D. K., Brosh, R. M., Ramsden, D., & Bohr, V. A. (2000). Ku complex interacts with and stimulates the Werner protein. *Genes Dev 14* [8], 907-912.

[168] Petersen, S., Saretzki, G., & von Zglinicki, T. (1998). Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Exp Cell Res*, 239(1), 152-160.

[169] von, T., Zglinicki, G., Saretzki, J., Ladhoff, F., d'Adda di, Fagagna., & Jackson, S. P. (2005). Human cell senescence as a DNA damage response. *Mech Ageing Dev*, 126(1), 111-117.

[170] Passos, J. F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., Wappler, I., Birket, M. J., Harold, G., Schaeuble, K., Birch-Machin, M. A., Kirkwood, T. B., & von Zglinicki, T. (2007). Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS Biol*, 5(5), e110.

[171] Passos, J. F., Saretzki, G., & von Zglinicki, T. (2007). DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? *Nucleic Acids Res*, 35(22), 7505-7513.

[172] Karlseder, J., Smogorzewska, A., & de Lange, T. (2002). Senescence induced by altered telomere state, not telomere loss. *Science*, 295(5564), 2446-2449.

[173] Harrigan, J. A., Wilson, D. M., 3rd Prasad, R., Opresko, P. L., Beck, G., May, A., Wilson, S. H., & Bohr, V. A. (2006). The Werner syndrome protein operates in base excision repair and cooperates with DNA polymerase beta. *Nucleic Acids Res*, 34(2), 745-754.

[174] Harrigan, J. A., Opresko, P. L., von, C., Kobbe, P. S., Kedar, R., Prasad, S. H., Wilson, , & Bohr, V. A. (2003). The Werner syndrome protein stimulates DNA polymerase beta strand displacement synthesis via its helicase activity. *J Biol Chem*, 278(25), 22686-22695.

[175] Pagano, G., Zatterale, A., Degan, P., d'Ischia, M., Kelly, F. J., Pallardo, F. V., & Kodama, S. (2005). Multiple involvement of oxidative stress in Werner syndrome phenotype. *Biogerontology*, 6(4), 233-243.

[176] Labbe, A., Turaga, R. V., Paquet, E. R., Garand, C., & Lebel, M. (2010). Expression profiling of mouse embryonic fibroblasts with a deletion in the helicase domain of the Werner Syndrome gene homologue treated with hydrogen peroxide. *BMC Genomics*, 11, 127.

[177] Ghosh, A., Rossi, M. L., Aulds, J., Croteau, D., & Bohr, V. A. (2009). Telomeric D-loops containing 8-oxo-2'-deoxyguanosine are preferred substrates for Werner and Bloom syndrome helicases and are bound by POT1. *J Biol Chem*, 284(45), 31074-31084.

[178] Muftuoglu, M., Wong, H. K., Imam, S. Z., Wilson, D. M., 3rd Bohr, V. A., & Opresko, P. L. (2006). Telomere repeat binding factor 2 interacts with base excision repair proteins and stimulates DNA synthesis by DNA polymerase beta. *Cancer Res*, 66(1), 113-124.

[179] Miller, A., Balakrishnan, L., Buncher, N. A., Opresko, P. L., & Bambara, R. A. (2012). Telomere proteins POT1, TRF1 and TRF2 augment long-patch base excision repair in vitro. *Cell Cycle*.

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