The role of DNA exonucleases in protecting genome stability and their impact on ageing

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Abstract

Exonucleases are key enzymes involved in many aspects of cellular metabolism and maintenance, and are essential to genome stability, acting to cleave DNA from free ends. Exonucleases can act as proof-readers during DNA polymerisation in DNA replication, to remove unusual DNA structures that arise from problems with DNA replication fork progression, and they can be directly involved in repairing damaged DNA. Several exonucleases have been recently discovered, with potentially critical roles in genome stability and ageing. Here we discuss how both intrinsic and extrinsic exonuclease activities contribute to the fidelity of DNA polymerases in DNA replication. The action of exonucleases in processing DNA intermediates during normal and aberrant DNA replication is then assessed, as is the importance of exonucleases in repair of double strand breaks and interstrand crosslinks. Finally we examine how exonucleases are involved in maintenance of mitochondrial genome stability. Throughout the review, we assess how nuclease mutation or loss predisposes to a range of clinical diseases and particularly ageing.

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Introduction

Maintenance of an intact genome is a key requisite both for evolutionary fitness and for health of the individual organism. While cells have evolved protective DNA packaging, such as eukaryotic chromatin, DNA is an active molecule involved in replication and transcription, and it can be modified by direct covalent changes such as CpG methylation during epigenetic regulation. It is also subject to mutation from both internal and external sources. Highly conserved surveillance and repair mechanisms have therefore evolved to keep pace with the daily insults that the genome

undergoes. Most, if not all, of these mechanisms require cleavage of the DNA's sugar-phosphate backbone in a controlled and accurate way by enzymes collectively called nucleases. Nucleases act in multiple pathways of DNA metabolism, including during the normal course of DNA replication, providing proofreading capacity to enhance polymerase fidelity, in mismatch repair (MMR), in tackling ends and unusual structures at stalled DNA replication forks, during repair of damaged bases (BER) or larger lesions (NER and recombinational repair) and in telomere maintenance (Figure 1). Here, we describe the action of exonucleases that are most likely to be associated with DNA metabolism important in preventing premature ageing (Table 1).

Nucleases are highly evolutionarily conserved, and can be classified into families based on both sequence and functional homology. Of particular relevance to this review are the 5'-3' exo C terminal domain (CTD) superfamily and the RNaseH domain superfamily, the latter of which includes the DnaQ-like family and other 3'-5 exonucleases (Table 2). While endonucleases cleave DNA internally by cutting the phosphodiester backbone, exonucleases act biochemically to catalyse the removal of a single nucleotide monophosphate (dNMP) from the end of one strand of DNA; a critical characteristic of such nucleases is thus their ability to bind to the relevant DNA substrate, and most exonucleases do so in a non-sequence specific manner, though they generally show some degree of structure specificity. For example, the 5'-3' exo CTD superfamily share a common helix hairpin helix (HhH) motif (Doherty et al, 1996; Thayer et al, 1995) that permits broad specificity DNA binding, probably through hydrogen bonding between the sugar-phosphate backbone of DNA and the protein backbone.

The functions of exonucleases are many and varied, reflected in the large number of independent nucleases found in many species. For example, of the DnaQ-like family (Table 2), humans possess 25 different family members, mice have 23, and other experimentally relevant organisms also have

multiple DnaQ-like exonucleases (seven in fruit flies, eight in *Arabidopsis* and three in each of *S. pombe* and *S cerevisiae*)[#]. Within a single nuclease, there may reside multiple specificities: the human APE1 nuclease has both 3'-5' exonuclease and abasic-site endonuclease activities, while FEN1 is both a structure-specific flap endonuclease and a 5'-3' exonuclease. Similarly, the RAD2 domain of human EXO1 contains both 5'-3' exonuclease and flap endonuclease activities (Lee and Wilson, 1999), (See Table 1 and references therein).

Nucleases may also be partially or fully redundant, depending upon pathway, and such redundancy may complement functional losses. For example, in the yeast *S. cerevisiae*, 5' end resection in double strand DNA break repair can be catalysed either by MRE11/Rad50/Xrs2, Exo1 or Rad27 (yeast FEN1) (Moreau et al, 2001). The general trend observed on nuclease mutation, however, is decreased viability and repair as more exonuclease activities are lost, suggesting overlapping but not identical roles.

Nucleases involved in DNA replication

Intrinsic proofreading activity of DNA polymerases

DNA replication is a highly accurate and precise process for copying the genome prior to segregation to daughter cells; accumulation of errors occurring during DNA replication may contribute to both carcinogenesis and cellular ageing. Accuracy in the first instance depends on high fidelity of the replicative DNA polymerases, resulting from a combination both of a solvent-inaccessible polymerisation active site that allows 10,000 fold discrimination for correct over incorrect Watson-Crick base-pairing of substrate with template, and editing which involves a 40^{o} shift of the new duplex DNA into the 3'-5' exonuclease active site for proof-reading (Franklin et al, 2001). This results in a base misincorporation rate during DNA replication in yeast of ~$1x10^{-5}$ for DNA polymerase δ and approximately double that for DNA polymerase ε (Nick McElhinny et al,

[#] see http://supfam.cs.bris.ac.uk/SUPERFAMILY/index, which also includes detailed phylogeny

2009). Note that human polymerase ε has a significantly higher fidelity of nucleotide incorporation (5.5x) (Korona et al, 2010) than its orthologue in yeast.

Replication fidelity rates vary slightly depending upon the base incorporated, suggesting that levels of individual nucleotides in the environment can modulate fidelity (Chen et al, 2000), thus replication stress occurs when nucleotide pools are imbalanced e.g. following treatment with hydroxyurea. Translesion DNA polymerases can take over from faithful DNA polymerases in cases of DNA damage where correct templating is not possible, but these have solvent-accessible active sites and lack proofreading capacity, leading to very high error rates (Kunkel, 2009) and accounting for the highly mutagenic effects of lesions that block the bulk replicative polymerases.

Since the high degree of replicative DNA polymerase accuracy requires intrinsic proofreading activity whereby incorrectly incorporated bases are excised by a 3'-5' exonuclease subunit of the polymerase (Hubscher et al, 2002), it is not surprising that mutation of the proofreading domains of either pol δ or pol ε increases the error rate for base substitution 12-13 fold in yeast (Nick McElhinny et al, 2009) or seven-fold for mutated human pol (Korona et al, 2010). Such loss of fidelity can have major biological consequences, as seen by the development of cancer in mice which have lost of the proofreading function of polymerase δ *in vivo* (Goldsby et al, 2001). While there have yet been no studies directly linking *nuclear* DNA polymerase fidelity loss with ageing[∞], it is notable that polymerase ε is required to prevent replicative senescence in budding yeast that lack telomerase activity (Deshpande et al, 2011). Moreover, instability of the nuclear genome can trigger either apoptosis, neoplastic change or senescence, leading to organismal ageing.

[∞] However, loss of fidelity of the mitochondrial replicative DNA polymerase γ is strongly associated with premature ageing phenotypes in mice – see later.

Extrinsic proofreaders that act in concert with DNA polymerases

Proofreading during DNA synthesis does not necessarily have to proceed via intramolecular activity of intrinsic exonuclease domains of the DNA polymerase, since priming from a mismatch may be orders of magnitude slower than from paired bases (Huang et al, 1992), allowing time for intermolecular intervention. A number of exonucleases may supply extrinsic proofreading activity for DNA polymerases that do not possess their own capacity such as DNA polymerase α, which synthesises initiator DNA (iDNA) at the start of each new Okazaki fragment, and DNA polymerase β, important in repair. Yeast studies with DNA polymerases bearing point mutations in their exonuclease sites provide evidence that the proofreading component of polymerase δ can lend its exonuclease function to polymerase α to correct errors during lagging strand replication; mutation of pol δ exonuclease prevented correction of the large number of errors generated by a mutant pol α (L868M which *in vivo* confers a mutator phenotype), while mutation of pol ε exonuclease had no effect (Nick McElhinny et al, 2006; Nick McElhinny et al, 2009; Pavlov et al, 2006; Perrino and Loeb, 1990).

The high error rate of non-proofreading DNA pol α may also be corrected *in vivo* by the action of ExoN, a 45.3 kDa 3'-5' exonuclease with the ability to remove 3' mismatched termini from dsDNA, including the nucleotide analogues 1-beta-D-arabinofuranosylcytosine monophosphate (araC) and 9-beta-d-arabinofuranosyl-2-fluoroadenine 5'-monophosphate. ExoN is reported to interact with DNA pol α and may supply it with proofreading capability (Brown et al, 2002; Perrino and Loeb, 1990; Skalski et al, 2000). ExoN up-regulation by approximately six-fold in araC-resistant leukemia cells suggests that these cancer cells overcome drug treatment through efficient removal of drug-induced mismatches, and hence ExoN is likely to be a clinically relevant target in cancer (Skalski et al, 2000), and possibly age-related accumulation of genetic damage. It is important to note, however, that the genetic locus of ExoN has not yet been specified, so it might

prove to be a known exonuclease, or possibly a novel isoform or duplication of one already described.

Autonomous proofreaders

Autonomous 3'-5' exonucleases are, as their name suggests, nucleases that are not necessarily intrinsic to DNA polymerases. They may be fairly non-specific and may act with overlapping functionality depending upon the DNA polymerase involved and the type of proofreading required.

TREX1 and TREX2

The mammalian proteins TREX1 and TREX2 (three prime repair exonucleases) are small homodimeric exonucleases that cleave DNA non-processively from the 3' end (Hoss et al, 1999; Mazur and Perrino, 1999; Mazur and Perrino, 2001a; Mazur and Perrino, 2001b). The TREX proteins make up the bulk of nuclear 3'-5' exonuclease activity in mammals (Mazur and Perrino, 2001a), suggesting that they serve an important role in metazoan nuclear genome maintenance, possibly acting as autonomous proofreaders (note there are no TREX homologues in yeast). For example, TREX1 can remove mismatched DNA at a strand end (Mazur and Perrino, 2001a), a required activity of a proofreader nuclease during DNA replication.

TREX1, whilst predominantly a cytoplasmic protein, is upregulated via the AP-1/ Fos transcriptional pathway and relocated to the nucleus under conditions of genotoxic stress such as UV damage (Christmann et al). This may reflect both a role for TREX1 in DNA repair and in dealing with damage during DNA replication. However, TREX1$^{-/-}$ null mice show no increase in spontaneous mutation (although induced mutation has not been tested) or increase in cancer, but this might merely show that the nuclear function of TREX1 may be complemented by one of the other autonomous proofreaders, perhaps TREX2 (see below).

TREX1 in the cytoplasm appears to degrade single DNA strands arising from processing of aberrant replication intermediates that migrate to the endoplasmic reticulum (ER) after S-phase (Yang et al, 2007). Furthermore, it participates in Granzyme-A mediated cell death in concert with the NM23-H1 nuclease (Chowdhury et al, 2006), resulting in DNA fragmentation characteristic of apoptosis. As loss-of-function mutations in the TREX1 gene cause the human disease Aicardi-Goutieres syndrome (a neurological syndrome that has symptomatic overlap with systemic lupus erythematosis - Crow et al, 2006; Lindahl et al, 2009), this suggests that non-degradation of ER/cytoplasmic DNA moieties might lead to immune dysfunction based upon an immune response to self-DNA, particularly if the DNA enters the secretory pathway via the ER. Notably, release of DNA from damaged cells is known to trigger inflammatory responses through 'damage-associated molecular patterns' (DAMPS) which are likely to trigger an innate immune response (Zhang et al, 2010), as such factors closely resemble 'pathogen associated molecular patterns' (PAMPS) to which the innate immune system is tuned. Indeed, mice null for Trex1 only live for one-fifth as long as wild type mice, and develop inflammatory myocarditis (Morita et al, 2004), consistent with autoimmunity and/or premature ageing, since high levels of inflammation are associated with premature ageing (the 'inflamm-aging' hypothesis e.g. (Franceschi et al, 2007)). Removal of the aberrant cytoplasmic DNA by TREX1 probably mutes DNA damage signalling and checkpoint activation so that the inflammatory response cannot mount.

Defective TREX1 is associated not only with Aicardi-Goutieres syndrome, but also with Ataxia Telangiectasia-like symptoms (Lindahl et al, 2009) and autosomal dominant retinal vasculopathy with cerebral leukodystrophy (Richards et al, 2007), caused by C-terminal truncation and accompanied by loss of perinuclear localisation (See Table 3). This gives rise to the onset of stroke, dementia, loss of visual acuity and other pathologies prematurely in middle-age, with death occurring within ten years of disease onset (Richards et al, 2007). Pathologies of this type become

more common in old age; early onset might thus suggest defects in TREX1 or similar exonuclease activities.

Thus TREX1 has both nuclear and cytoplasmic roles, possibly as an autonomous proof-reader in replication but also in removing potential inflammatory triggers. Similarly, human TREX2, which is 44% homologous to TREX1, shows punctate nuclear staining with some cytoplasmic localisation. It is cell cycle regulated, being down-regulated during G2/M; like TREX1, its levels are highest in S-phase and lowest at mitosis (Chen et al, 2007). HeLa cells in which TREX2 has been knocked out show reduced cellular proliferation (Chen et al, 2007), suggesting an as yet undetermined role in proliferative DNA metabolism for TREX2 but consistent with action as an extrinsic proof-reader.

p53

The tumour suppressor protein p53 has been suggested to possess 3'-5' exonuclease activity (Mummenbrauer et al, 1996), in addition to its better-characterised roles as a transcriptional activator and an inducer of apoptosis (reviewed in Cox and Lane, 1995; Green and Kroemer, 2009; Vousden and Prives, 2009). The 3'-5' exonuclease activity of p53 is active on both ss and dsDNA, and has a preference for removing mismatches from replicating strand DNA, while paired bases inhibit the exonuclease activity (Huang et al, 1998). p53 may act by enhancing a further nuclease, AEN (apoptosis enhancing nuclease), to result in the DNA fragmentation seen in p53-dependent apoptosis (Kawase et al, 2008). However, p53 is found to co-localise with DNA synthesis during S-phase, and might itself provide proofreading functionality for DNA polymerase α, since it enhances the replication fidelity of error-prone pol α but not of high fidelity proofreading pol ε (Hollstein et al, 1996). Thus a novel way in which p53 might safeguard the genome is by increasing replication fidelity when necessary, for example under conditions of genomic stress following genotoxic

damage. It is notable that over half of all human cancers have p53 mutations, frequently in the DNA binding and exonuclease domain of the protein[#]. p53 is also known to be critical in establishing cell senescence, though it is likely that this is primarily through its transcriptional activation of p21 (el-Deiry et al, 1993; Noda et al, 1994) rather than through its nuclease activity.

APE1 and APE2

A novel class of proteins that may also act as autonomous proofreaders are the AP endonucleases. There are four types of AP endonucleases, based upon the sites of incision: Class I and class II incise such that 3´-hydroxyl and 5´-phosphate ends arise. Class III and class IV instead generate the reverse - a 3´-phosphate and a 5´-hydroxyl (Myles and Sancar, 1989). Human APE1 belongs to the most common class II. Both APE1 and APE2 (Hadi and Wilson, 2000) have been shown to possess specific, non-processive but nevertheless possibly important abilities to cleave 3'- termini from DNA. APE1 removes nucleotides from matched, mismatched and beta-l-dioxolane-cytidine nicked DNA (Chou and Cheng, 2002; Chou and Cheng, 2003), with a marked preference for 3'-mismatched and nicked substrates. APE2 also has a preference for removal of mismatched bases (Burkovics et al, 2006). It remains to be determined whether and how much these proteins contribute to proofreading during replication, or whether they utilise this functionality only within the context of DNA repair.

Replication fidelity is enhanced through nucleases acting in mismatch repair

In addition to proofreading by intrinsic or extrinsic exonucleases, a further mechanism that can increase replication fidelity by up to a thousand-fold involves the mismatch repair (MMR) machinery. Errors in mismatch repair are linked to microsatellite instability and cancer. Eukaryotic MMR requires not only the well-characterised MSH (MutS homologue) proteins which survey the

[#] see http://p53.free.fr

genome for both mismatches and small insertion/deletion loops, but also exonucleases such as EXO1 (see Hsieh and Yamane, 2008 and references therein).

Eukaryotic EXO1 is a member of the Rad2/FEN1 family of nucleases (Lee and Wilson, 1999) (Table 2) and is one of four nucleases that may be involved in MMR, although it is not (as originally reported) an orthologue of the *E. coli* MMR protein EXO1 (Genschel et al, 2002; Tishkoff et al, 1998; Tishkoff et al, 1997). There are some reports that loss of EXO1 gives rise to one form of the MMR-deficient syndrome human nonpolyposis colon cancer, HNPCC (Wu et al, 2001), but a direct association has been questioned (Thompson et al, 2004). Eukaryotic EXO1 is involved in various DNA metabolic pathways other than MMR, including meiosis (Fiorentini et al, 1997) and recombination (Tsubouchi and Ogawa, 2000). EXO1 may also play a role in resecting ends at broken replication forks to prevent generation of recombinogenic intermediates.

Human EXO1 has 5'-3' activity on both ds and ssDNA, although its activity is greater on 3'-overhang and blunt duplex substrates, including those containing gaps or nicks, than on 5' overhang duplex or ssDNA (Lee and Wilson, 1999); it can also degrade RNA *in vitro*. EXO1 is also involved in 3'-nick directed repair, suggesting either that it might have a cryptic 3'-5' activity, or that it is required to activate another as yet uncharacterised 3'-5' nuclease (Genschel et al, 2002). EXO1 has been shown to resect DNA *in vitro*, with DNA affinity increased by the RecQ helicase BLM, and loading and processivity by the MRN complex and RPA (Nimonkar et al, 2011). In yeast, the flap endonuclease activity of EXO1 has been suggested to be a functional backup for the major Okazaki fragment processing flap endonuclease FEN1 (see below), again showing the overlapping redundancies of many cellular nucleases; moreover human EXO1 can complement its yeast homologue (Qiu et al, 1999).

Notably, it has recently been shown that a SNP in the EXO1 promoter leading to higher levels of EXO1 expression is enriched in female centenarians (Nebel et al, 2009), suggesting a possible role for EXO1 activity in prevention of premature ageing, possibly by reducing cancer risk. By contrast, *deletion* of the nuclease activity of EXO1 in short-lived late-generation telomerase-deficient mice rescues lifespan (Schaetzlein et al, 2007). This rather unexpected finding may result from repression of the cyclin-kinase inhibitor p21, which leads to the increased lifespan in terc-/- mice, as observed in mice lacking other components of MMR such as PMS2 (Siegl-Cachedenier et al, 2007) and which directly phenocopies p21-/- Terc-/- mice (Choudhury et al, 2007). Thus it is possible that rescue of longevity upon EXO1 loss in telomerase null mice was effected by suppression of the DNA damage response checkpoint - perhaps via lack of EXO1 end-processing and recruitment of downstream response elements - to allow proliferation in cells with uncapped telomeres and other DNA damage. Taken together, these data might suggest that upregulation of EXO1 is only useful for long lifespan in the presence of robust telomeres and DNA proofreading/repair.

Processing DNA intermediates at DNA replication forks

During the maturation step of DNA replication, Okazaki fragments on the lagging strand of the replication fork must be processed to remove both the RNA primers generated by primase and the initiator iDNA synthesised by error-prone DNA pol α, prior to ligation into high molecular weight DNA. Several models of Okazaki fragment processing (OFP) have been proposed (reviewed in Budd et al, 2009), all requiring exonucleases, particularly the flap endonuclease/exonuclease 1, FEN1. The DNA flaps and similar structures arising during OFP are likely also to be formed during DNA lesion processing, and the nucleases important in OFP have also been shown to play roles in DNA repair.

FEN1

FEN1 is a structure-specific endo/exonuclease originally cloned from mouse cells (Harrington and Lieber, 1994) and subsequently humans (Hiraoka et al, 1995), and is essential for DNA replication

in vitro (Waga et al, 1994). The FEN1 homologue in budding yeast is encoded by the *rad27* gene (Budd and Campbell, 2009). It acts during Okazaki fragment processing in DNA replication to remove the terminal deoxyribonucleotide (or ribonucleotide) by endonucleolytic cleavage at the elbow of DNA junctions bearing a 5' flap generated by incoming DNA pol δ on the lagging strand (Budd and Campbell, 2009). In addition, FEN1 can act exonucleolytically in a 5'-3' direction on flap substrates.

Recruitment of FEN1 to sites of exonuclease action involves direct binding to the sliding clamp PCNA through a conserved PCNA-interacting motif (PIP, Cox, 1997; Warbrick et al, 1997), the interaction positioning FEN1 to act preferentially as an exonuclease rather than an endonuclease (Hosfield et al, 1998a; Hosfield et al, 1998b). The mode of action has been elucidated by X-ray crystallography, showing that association with PCNA allows flexibility around a hinge region in FEN1 that brings the enzyme's nuclease active site in direct contact with the DNA substrate (Sakurai et al, 2005).

Furthermore, FEN1 has recently been crystallised both alone {Sakurai, 2008 #396; (Tsutakawa et al, 2011) and in association with the Rad9-Rad1-Hus1 (9-1-1) DNA damage checkpoint complex (Dore et al, 2009). Analysis of the structures of FEN1 (Tsutakawa et al, 2011) and EXO1 (Orans et al, 2011), which were solved at similar times, suggest a unified mechanism for this type of nuclease involving DNA bending, and two nucleotide 'fraying' of one strand (Orans et al, 2011).

Mutation of FEN1's nuclease active site (E160D) makes cells more susceptible to chemically-induced cancers (Xu et al, 2011). Loss of only one copy of FEN1 increases colon cancer incidence in mice heterozygous for mutation of the Apc tumour suppressor, with Fen1 halpoinsufficiency leading to rapid tumour progression (Kucherlapati et al, 2002). FEN1 also shows gap endonuclease

(GEN) activity, which is critical for the resolution of stalled replication forks following DNA damage (Zheng et al, 2005). Notably, these authors showed that a mutation in FEN1 that abolished GEN activity (E178A) removed FEN1's ability to rescue camptothecin and UV sensitivity in a yeast Fen1 mutant[∞]. Moreover, transgenic mice bearing a point mutation in FEN1 that abolished the majority of 5' exonuclease and GEN activity developed significant inflammation and autoimmunity, together with high cancer incidence (Zheng et al, 2007). An inability to recruit FEN1 to sites of action through mutation critical aromatic residues within its PCNA-interacting peptide (F343A F344A) (Warbrick et al, 1997) also results in loss of DNA processing and aneuploid cancers (Zheng et al, 2011).

Mutational studies in yeast have suggested that FEN1 restrains recombination between short DNA sequences (Negritto et al, 2001) such as the flaps arising during strand displacement DNA synthesis on the lagging strand of the replication fork, or during DNA repair. These findings are also consistent with the triplet repeat expansion phenotype observed on FEN1/Rad27 mutation (Liu et al, 2004; White et al, 1999) and with its involvement in long patch BER along with its partner PCNA (Frosina et al, 1996). (Note that FEN1 is important for removal of oxidative damage in both the nucleus (Asagoshi et al) and in mitochondria (Liu et al, 2008)). Mutation of pol ε in a Rad27 null background led to elevated +1 frameshifts on homonucleotide runs (Kirchner et al, 2000) suggesting a role for FEN1 either in mismatch repair or proofreading, in addition to its roles in OFP and BER. Notably, mutation of FEN1 leads to decreased yeast lifespan (Hoopes et al, 2002). Hence loss of function of FEN1 results in a mutator phenotype that predisposes to cancer and signs of premature ageing.

[∞] Note that the related human GEN1 (yeast Yen1) appears to act exclusively as an endonuclease (Ip et al. 2008, Nature 456, 357-361), and so is not discussed in this review

WRN

WRN is a multifunctional replication/repair RecQ-helicase family member (Brosh et al, 2001b), that in deficiency causes the premature ageing Werner's syndrome, an adult-onset progeria phenocopying early many normal ageing diseases such as athersclerosis, cancer, cataracts and grey hair. WRN association with FEN1 may be important in Okazaki fragment processing during DNA replication as FEN1's nuclease activity is stimulated by binding to the helicase domain of WRN (Brosh et al, 2002a).

Unlike other RecQ helicases, in addition to helicase activity WRN has a well-characterised 3'-5' exonuclease domain (Huang et al, 2000) which is highly conserved and of the DnaQ exonuclease family (Perry et al, 2006) (Table 2). In *Drosophila*, and other invertebrate and plant species, the exonuclease appears as an autonomous protein (Boubriak et al, 2009; Cox et al, 2007; Plchova et al, 2003; Saunders et al, 2008; Sekelsky et al, 1999).

While WRN exonuclease acts on both single stranded (Machwe et al, 2006) and duplex DNA with overhanging ends, it is also active on substrates that resemble either DNA replication intermediates, or structures found during the processing of DNA damage (Brosh et al, 2002b). However, *in vitro* without other factors, WRN cannot cleave blunt-ended duplex DNA. In DNA replication, WRN is implicated in processing unusual DNA structures that, if left unprocessed, would result in replication fork regression and formation of recombinogenic intermediates including Holliday junctions (HJs). This is supported by the accumulation of stalled replication forks in cells lacking WRN protein (Rodriguez-Lopez et al, 2002; Sidorova et al, 2008), and it is likely that this reflects a need for the nuclease activity of WRN, since many of the defects observed in WS cells, including reduced proliferative capacity, low S phase fraction and drug sensitivity can be corrected by ectopic expression of a bacterial Holliday junction resolvase (Rodriguez-Lopez et al, 2007). Recruitment of

WRN to stalled replication forks may occur through binding to PCNA via a classical PIP (Lebel et al, 1999; Rodriguez-Lopez et al, 2003).

Thus WRN may be involved in directing the appropriate pathway during recombinational repair, for example in response to collapsed replication forks or other sources of double strand breaks (Plchova et al, 2003; Rodriguez-Lopez et al, 2002; Saintigny et al, 2002; Swanson et al, 2004). It may, like other RecQs such as yeast Rqh1 (in combination with EXO1 or the cross-over junction nuclease MUS81-EME1 (Doe et al, 2002) direct non-cross over pathways, perhaps by removing a potentially invasive DNA strand arising from replication fork stalling or dissociation of leading and lagging strand polymerases. Indeed, *Rqh1* null yeast are hyperrecombinant, while ectopic expression of human WRN in such cells represses recombination (Yamagata et al, 1998). That this suppression of recombination results from the DNA exonuclease activity of WRN is supported by the finding that fruit flies deficient in the orthologue of human WRN exonuclease, DmWRNexo, show extremely high levels of mitotic recombination (Saunders et al, 2008) and marked developmental abnormalities (R. Lasala *et al.*, manuscript in preparation). The DNA-end binding Ku70/86 dimer specifically and strongly stimulates WRN's exonuclease activity (Comai and Li, 2004; Li and Comai, 2002; Li et al, 2005; Li et al, 2004), changing its specificity to allow degradation of blunt and even 5'-protruding ends (Cooper et al, 2000), suggesting a particular role for WRN during Ku-directed repair such as non-homologous end joining (NHEJ) or in an alternative pathway to degrade free ends at broken replication forks.

WRN exonuclease is implicated in other DNA metabolic pathways in addition to its likely role as an accessory protein at the replication fork. For example, WRN may play an important role at the telomere, including telomeric D-loop resolution (Opresko et al, 2004). It is likely that this requires intramolecular co-operation between the intrinsic helicase and the exonuclease activities of WRN

such that the helicase unwinds unusual DNA structures (including G4 quadruplexes which mimic telomeric structures) and is followed by exonuclease activity to process the resulting ends: indeed, a K577M point mutation within the helicase domain has a dominant negative effect on any subsequent exonuclease activity and prevents lagging strand Okazaki fragment processing at the telomere (Crabbe et al, 2004). (Note that WRN also binds to telomere proteins POT1 and TRF2 (Opresko et al, 2005; Opresko et al, 2002)). Interestingly, WRN also interacts physically with and stimulates EXO1 (Aggarwal et al, 2010; Sharma et al, 2003), a nuclease that is implicated both in Okazaki fragment processing and in telomere processing (see above). This interaction may also be important for either WRN or EXO1, or both, to process stalled or regressed replication forks and to maintain the T and D loops at telomeres. Los of telomere integrity drives replicative senescence and ageing, and prolonged replication stress can similarly result in 'deep' senescence and contribute to organismal ageing.

WRN exonuclease can also function as an extrinsic proofreader for DNA polymerase β during base excision repair (BER) (Harrigan et al, 2006); studies based upon the X-ray crystal structure of human WRN exonuclease domain give a firm assignment of editing functionality (Perry et al, 2006), akin to end-processing activities of other members of the DnaQ family. Moreover, deletion of the exonuclease domain results in cells that are hypersensitive to DNA damage (Kashino et al, 2005). Confusingly, p53, itself a nuclease, strongly inhibits the exonuclease activity of WRN (Brosh et al, 2001a), highlighting the complex synergistic/antagonistic interplay between cellular nucleases. Since loss of WRN causes the segmental progeroid Werner's syndrome (Huang et al, 1998; Yu et al, 1996), this provides a direct evidential link between genome instability and ageing.

Dna2

Dna2 is a combined helicase/nuclease that interacts with FEN1 and is likely to be involved in Okazaki fragment processing, as yeast cells with a mutant allele of *Dna2* (*dna2-1*) can only synthesize short stretches of DNA under restrictive conditions (Budd et al, 2009). It is likely that Dna2 is recruited to process Okazaki fragments under conditions when long flaps arise during pol δ-mediated strand displacement of the primer DNA and iDNA, as these flaps become coated with RPA and resistant to cleavage by FEN1 – indeed, recruitment of Dna2 is likely to occur through direct interaction with RPA. Exonuclease cleavage of the flap DNA by Dna2 eventually results in flaps short enough to be processed by FEN1 (reviewed in Kao and Bambara, 2003; MacNeill, 2001; Budd 2009).

In addition to a helicase domain in the C terminus, Dna2 possesses 3'-5' exonuclease activity with further 5' flap endo-exonuclease activity (Fortini et al, 2011) present in the amino terminal half of the protein. The gene is itself essential with deletion mutants being inviable, and yeast cells mutant for both Fen1 and Dna2 are inviable (dna2-1 rad27Δ), while Fen1 overexpression can complement Dna2-1 phenotypes (reviewed in Budd and Campbell, 2009), although Dna2 stimulates Fen1's activity (Kao et al, 2004). Consistent with a role in preventing ageing, presumably by ensuring genome stability especially of the repetitive rDNA or at the telomeres, Dna2 mutants have a short lifespan (Weitao et al, 2003a; Weitao et al, 2003b).

C. elegans lacking worm Dna2 show compromised genome maintenance and shortened lifespan (Lee et al, 2011). The human homologue of Dna2 is also a helicase/nuclease, which does not have an obvious nuclear localisation signal but in deficiency causes nuclear genome instability (Duxin et al, 2009) and colocalises with DNA (nucleoids) in the mitochondrion. It interacts with and stimulates mitochondrial DNA polymerase γ, and functions with FEN1 in mitochondrial long-patch

BER (Copeland and Longley, 2008; Zheng et al, 2008). *In vitro* reconstituted DSB repair complexes show that in the nucleus, end resection proceeds via two pathways (Nimonkar et al, 2011). One involves human DNA2 nuclease activity and the RecQ helicase activity of BLM, stimulated by RPA. RPA also ensures that DNA2 exonuclease activity occurs in the correct 5'-3' polarity. The MRN complex is found to stimulate activity by recruiting BLM to the site of resection (Nimonkar et al, 2011). DNA2 therefore probably suppresses genome instability via both DNA repair (BER, DSB repair) and recombination pathways.

Nucleases in Nucleotide Excision Repair and Transcription

Bulky lesions such as UV-induced pyrimidine dimers that prevent replication fork progression or transcription elongation are generally removed through the nucleotide excision repair (NER) pathway, involving many proteins of the Xeroderma Pigmentosam (XP) complementation group. Nucleases including XPG[∞] are required to make incisions either side of the lesion, hence mutation of the *incision* activity of XPG gives rise to symptoms of XP (Tian et al, 2004), conferring sensitivity to UV damage and consequent great susceptibility to epithelial cancers via defects in nucleotide excision repair (NER) (Table 3). By contrast, total absence of XPG protein causes symptoms of Cockayne's syndrome (Arenas-Sordo Mde et al, 2006), with developmental defects and premature ageing. These phenotypes are thought to arise through loss of XPG's role in stabilising transcription, rather than its activity in DNA repair (Friedberg and Wood, 2007). Hence XPG mutation can give rise to various symptoms of both CS and XP including premature ageing (Friedberg and Wood, 2007; Wijnhoven et al, 2007). In addition to its incision activity (O'Donovan et al, 1994, Habraken et al, 1994b), XPG belongs to the FEN1/RAD2 family because of its conserved 5'-3' exonuclease activity (Habraken et al, 1994a, see Table 2) Of note, XPG has recently been shown to interact with the WRN helicase/exonuclease (Trego et al, 2011) via the C-terminal domain of both; XPG co-localises with WRN during S-phase and stimulates its helicase

[∞] also known as ERCC5

activity (Trego et al, 2011). Whether this association is important in preventing premature ageing is not yet clear.

Exonucleases involved in double strand DNA break repair

Mre11

The first nuclease recruited in repair of DNA double stranded breaks is MRE11 (Stracker et al, 2004), a component of the MRN complex (Mre11-Rad50-Nbs1) which plays a key role early in the DNA damage response (DDR) (Berkovich et al, 2007; Lisby et al, 2004; Mirzoeva and Petrini, 2001). MRN detects and localises rapidly to DSBs after damage, and secures and shields the frayed DNA ends (Williams and Tainer, 2005) whilst activating checkpoint signalling via ATM (Lee and Paull, 2007; Williams and Tainer, 2005). Two Mre11 and two Rad50 molecules form a heterotetramer that interacts with Nbs1 (Xrs2 in yeast) and can bind both ends of a DSB, although Mre11 can itself form homomultimers (reviewed in Budd and Campbell, 2009). Yeast and human Mre11 have multiple nuclease activities (Furuse et al, 1998; Moreau et al, 1999; Paull and Gellert, 1998) and in addition have helicase-like activities in that they can cause both strand-annealing (de Jager et al, 2001) and strand dissociation.

Mre11 has 3'-5' dsDNA exonuclease activity *in vitro*, as well as ds and ssDNA endonuclease activity, but is specific for blunt or 3'-recessed ends, or, very weakly, for hairpin structures (D'Amours and Jackson, 2002). Whilst the 3'-5' exonuclease activity is necessary for DSB repair (Paull and Gellert, 1998), experiments on *S. cerevisiae* mutants show that functions of Mre11 are separable and distinct, with the N-terminal nuclease domain required for DSB repair, and the C-terminal dsDNA-binding domain needed for meiotic functions such as chromatin modification (Furuse et al, 1998).

Although removal of the nuclease activities of Mre11 in yeast results in only slight radiation sensitivity (Moreau et al, 1999), Mre11 mutation in mammals results in more severe phenotypes. Inherited hypomorphs cause Ataxia Telangiectasia-like symptoms such as extreme sensitivity to IR and cerebellar degeneration (Stewart et al, 1999, see Table 3), even though the nuclease-dead Mre11 mutant retains the ability to associate with the other members of the MRN complex, and to activate ATM (Buis et al, 2008). This suggests that Mre11 nuclease activity may be more important in mammals than in yeast, and this importance is not based upon the DNA damage checkpoint acting through ATM, but occurs via other roles of Mre11. It is possible that Mre11 is involved in preventing replicative senescence caused by telomere attrition, since in yeast, it has been implicated in telomere maintenance acting as a block to replicative senescence (Joseph et al, 2010). Additionally, defective DSB repair has been reported in the progeroid Hutchinson Gilford progeria syndrome (HGPS), with a delay in localization of Mre11 to sites of DSBs suggesting that defects in Mre11 targeting may be associated with premature ageing characteristic of this syndrome (Constantinescu et al, 2010). Hence Mre11 nuclease activity may be necessary to prevent premature ageing.

hRAD9

The hRAD9 protein was discovered by homology to the *S. pombe* rad9 protein that is involved in the early DNA damage response checkpoint. Modified hRAD9, along with hRAD1 and hHus1, form the human 9-1-1 complex (Volkmer and Karnitz, 1999), a heterotrimeric clamp complex structurally (Xu et al, 2009) and functionally analogous to PCNA that is loaded onto damaged DNA tracts (Burtelow et al, 2000; Carr, 2002) by an RF-C like complex and is hyperphosphorylated in a damage-dependent manner, for example by ATM after ionising radiation (IR) (reviewed in Cox, and Kearsey, 2009). Such phosphorylation is required for IR-induced G1/S checkpoint activation (Chen et al, 2001a) but also after hydroxyurea-induced replication stress or UV-induced DNA damage. hRAD9 has 3'-5' exonuclease activity (Bessho and Sancar, 2000); as hRAD9 quickly

localises to double-strand breaks, this suggests that the nuclease activity is important during early DNA processing. The 9-1-1 complex can also bind to and stimulate FEN1 nuclease activity (Wang et al, 2004) and also interacts with and enhances the function of DNA polymerase β, although not pol δ nor pol α (Toueille et al, 2004). hRad9 alone (not as part of the 9-1-1 complex) is also implicated in numerous other pathways; for example in ribonucleotide synthesis via stimulation of the carbamoyl phosphate synthetase activity of CAD (Lindsey-Boltz et al, 2004), and in apoptosis by interaction with members of the Bcl-2 family of proteins (Komatsu et al, 2000). Mouse rad9 nulls are not viable (Hopkins et al, 2004) presumably because Rad9 has so many different roles that there cannot be full complementation of all its activities. The exact role(s) of hRad9 nuclease activity have not yet been elucidated, nor its link with ageing, but in the nematode worm *C. elegans*, the 9-1-1 complex is implicated in telomere maintenance (Meier et al, 2006). Whether this is the case in higher organisms with an overt effect on ageing has yet to be determined.

Interstrand crosslink (ICL) repair

Interstrand crosslinks (ICLs) are chemical links between the bases (or backbones) on opposite strands of duplex DNA. They block progress of the replication fork, causing stalling; ICLs must therefore be removed in order for cells to proliferate and for their long-term survival. Deficiencies in ICL repair give rise to Fanconi's anaemia (FA), a genome instability syndrome characterized by increased sensitivity to agents that promote ICLs in DNA (e.g. mitomycin C) and FA patients show many gross phenotypic abnormalities (Auerbach, 1995), with greatly elevated predisposition to cancer (Alter, 1996) associated with marked chromosomal instability. The FA protein core complex monoubiquitinates the ID complex comprising FANCI and FANCD2 (Garcia-Higuera et al, 2001), which localises to BRCA-containing nuclear DNA repair foci along with the recombination protein RAD51 (Taniguchi et al, 2002), and promotes removal of the DNA tract containing the cross-link.

FAN1

Genetic (shRNA) screens to identify novel factors that confer resistance to ICL-inducing mitomycin C very recently identified FAN1 (Fanconi-associated nuclease 1) as a novel but important exonuclease involved in the ICL repair pathway (MacKay et al, 2010; Smogorzewska et al, 2010). Furthermore, FAN1 was independently described as a putative nuclease that interacts with FANCD2; absence of FAN1 was found to increase cellular sensitivity to ICL-inducing agents such as mitomycin C and cisplatin (Liu et al, 2010; MacKay et al, 2010).

FAN1 is a protein containing an N-terminal ubiquitin-binding domain and a C-terminal VRR_nuc nuclease domain (ancient lineage, see Table 2), and localises to interstrand crosslinks (ICL) along with monoubiquitinated FANCD2 (Kratz et al, 2010; Liu et al, 2010), a process requiring its ubiquitin-binding domain. Under mitomycin C treatment, FAN1 co-localises with FANCD2 at sites of damaged DNA, but since FAN1 depletion does not affect the ability of FANCD2 to become monoubiquitinated or localise to damage, FAN1 is likely to act downstream of, and may be recruited to sites of damage by, monoubiquitinated FANCD2.

Purified FAN1 is a structure-specific endonuclease capable of cleaving branched structures such as replication fork-like structures, splayed DNA arms and 5' or 3' flaps (Liu et al, 2010), but not duplex DNA. Like FEN1, FAN1 is also a 5'-3' exonuclease as well as a flap endonuclease. Monoubiquitination of FANCD2 - and thus the concomitant recruitment of FAN1 to damage foci - is required for the 'unhooking' step in removal of the ICL (Knipscheer et al, 2009). During removal, dsDNA excisions generating double strand breaks are made either side of the crosslink in order to remove the crosslinked portion of the DNA. At least one of these incisions is catalysed by MUS81-EME1, a heterodimeric endonuclease (Ciccia et al, 2003) that can cleave branched structures and resolve Holliday junctions (Chen et al, 2001b). It is possible that MUS81-EME1 may also make the second incision, though the excision repair factor XPF-ERCC1 is another candidate

(Fekairi et al, 2009). Alternatively, the structure arising from the first incision may resemble a 5' flap, which could act as a substrate for the endonuclease activity of FAN1. While there is no difference in the rate of formation of cisplatin- or mitomycin C-induced DSBs in a FAN1-depleted background, their disappearance (removal) is slower than in controls (Kratz et al, 2010; MacKay et al, 2010), suggesting that FAN1 is not required to introduce DSBs, but rather is involved in their repair. Since DSB repair often occurs via homologous recombination (HR), this strongly implies that the FAN1 nuclease is involved in DNA processing during the latter stages of HR, but does not preclude the possibility that FAN1 may act in other as yet undiscovered pathways. The VRR_nuc domain is associated with the PD-(D/E)XK nuclease superfamily (including type III restriction enzymes and the replication/repair helicase/nuclease DNA2); the multiple nucleolytic abilities of FAN1 imply a multifunctional capacity similar to ExoG or Mre11. Whether the primary role of FAN1 is indeed in ICL repair (Kratz et al, 2010; Liu et al, 2010; MacKay et al, 2010; Smogorzewska et al, 2010), or lies in other DNA transactions such as removal of flaps at stalled replication forks remains to be determined.

EXDL2

The genetic screen that isolated FAN1 (Smogorzewska et al, 2010) also identified a further putative exonuclease, EXDL2, which has sequence homology to the 3'-5' exonuclease domain of WRN helicase/exonuclease, a DnaQ family nuclease (see above). While there is little published information concerning EXDL2, bioinformatics analysis shows that it has the DEDDy motif conserved in WRN and many related exonucleases. Various different isoforms of EXDL2 are reported; there is also a possible association with cancer[#]. RNAi depletion of EXDL2 has no effect on ubiquitination of the Fanconi's ID complex, leading Smogorzewska and colleagues (Smogorzewska et al, 2010) to suggest that EXDL2 may act in an ICL repair pathway either

[#] http://www.genecards.org/cgi-bin/carddisp.pl?gene=EXD2&gc_id=GC14P069658&rf=/home/genecards/current/website/carddisp.pl&asd=17#asd

downstream of, or parallel to, the FA repair complex. In *Drosophila*, flies with an insertional mutation in the EXDL2 orthologue, encoded by *CG6744*, show slightly elevated rates of chromosomal recombination (Cox et al, 2007), implicating EXDL2 in nuclear DNA metabolism consistent with a role in repair. Intriguingly, EXDL2 in early *Xenopus* development has been localised by *in situ* hybridisation to the mitochondrial cloud (Cuykendall and Houston), suggesting that it may also be involved in mitochondrial DNA metabolism, consistent with bioinformatics predictions of mitochondrial localisation of at least some of the isoforms of human EXDL2 (Mason and Cox, unpublished). The similarity between EXDL2 and WRN, its possible involvement in not only nuclear genome stability, perhaps in the ICL repair pathway, but also in mtDNA maintenance make EXDL2 an interesting candidate for further research into possible links with ageing.

Mitochondrial nucleases

Mitochondria have their own highly conserved DNA replication and maintenance apparatus, which includes mitochondrial-specific nucleases. In humans, the circular mitochondrial genome is only 16.569 kb, and encodes some components of the electron transport chain together with rRNAs and tRNAs required for mitochondrial m0RNA translation (the majority of gene products used in the mitochondria are, however, encoded by nuclear genes and the proteins subsequently imported across the mitochondrial membrane(s)). The mitochondrial genome has a mode of DNA replication unlike that of the nuclear genome, involving large regions of single stranded DNA and use of several possible origins that may act bidirectionally or unidirectionally (Falkenberg et al, 2007; Yasukawa et al, 2009) and mediated by the single mitochondrial DNA polymerase γ, POLG. DNA repair pathways within mitochondria include MMR, BER and possibly DSB repair. Interestingly, nuclear repair factors including the nuclease FEN1 are implicated in maintaining mtDNA integrity (Kalifa et al, 2009). Accumulation of mitochondrial genome mutations and deletions has been reported with increasing chronological age, resulting in mitochondria with defects in metabolism,

leading to the mitochondrial hypothesis of ageing (Harman, 1972; Miquel et al, 1980). Links between telomere attrition resulting in mtDNA damage and loss of mitochondrial function have recently been drawn (Passos et al, 2010; Sahin and Depinho, 2010). Thus maintenance of mtDNA integrity may be critical in preventing premature ageing, but until recently, little has been reported on the importance of nucleases in this process.

Proofreading nuclease of mitochondrial DNA POLG

As is the case for the nuclear genome, mitochondrial DNA requires high fidelity replication, which is conducted by POLG. The importance of exonucleolytic proofreading during mtDNA replication is clear from the phenotype of mice lacking mitochondrial polymerase proofreading through mutation of POLG, which show early-onset of many age-related phenotypes (Trifunovic et al, 2004)[#]. Mice carrying high levels of mitochondrial DNA mutation show sarcopenia and mitochondrial dysfunction in skeletal muscle (Hiona et al, 2010), although this can be blunted by endurance exercise (Safdar et al, 2011). They also show an accelerated age-related loss of retinal function (Kong et al). Interestingly, this high mitochondrial mutation load alters the ability of hematopoietic stem cell lineages to undergo appropriate differentiation, leading to defects such as anaemia and lymphopenia similar to those caused by the premature senescent of this compartment (Norddahl et al, 2011). Hence loss of mtDNA integrity via loss of the proofreading function of polymerase γ is strongly associated with ageing.

EXOG/EndoG

In addition to the intrinsic proofreading nuclease activity of POLG, in *S. cerevisiae* the endo/exonuclease Nucp1 (Dake et al, 1988; Vincent et al, 1988) is the major nuclease in yeast mitochondria, and is involved in a number of functions including mitochondrial DNA metabolism,

[#] (although the absolute cause of this phenotype has been questioned - see Kraytsberg Y, Simon DK, Turnbull DM, Khrapko K (2009) Do mtDNA deletions drive premature aging in mtDNA mutator mice? Aging Cell 8**:** 502-506)

recombination, and cell death (Buttner et al, 2007; Zassenhaus and Denniger, 1994). Nucp1 can degrade ssRNA and has both DNA endonuclease activity (on both ss and dsDNA) and 5'-3' exonuclease activity (on dsDNA). The mammalian homologue, EndoG, was discovered as a nuclease involved in apoptosis (Li et al, 2001), where it is translocated to the nucleus and degrades chromatin in a caspase-independent manner; active EndoG outside the mitochondria quickly induces cell death (Schafer et al, 2004). Like its yeast homologue, human EndoG is required for normal cellular proliferation (Huang et al, 2006).

Interestingly, the mammalian EndoG acts only as an endonuclease; unlike the multifunctional Nuc1p, it notably lacks the 5'-3' exonuclease activity used to generate gaps in dsDNA during recombination and repair. Recently, a novel EndoG paralogue, named EXOG, was discovered in mitochondria, which is thought to have arisen during ancestral gene duplication (Cymerman et al, 2008). Human EXOG homodimer possesses the 5'-3' exonuclease activity that is missing in EndoG, as well as its own endonuclease functionality with some preference towards ssDNA, and it can nick supercoiled DNA. Thus EXOG has the activity necessary to generate ssDNA gaps. Together, mammalian EndoG and EXOG recapitulate all the functions of yeast Nuc1p. EndoG has been reported to lie in the mitochondrial inter-membrane space away from the DNA (Ohsato et al, 2002), whilst EXOG is located in the inner membrane where the mitochondrial DNA attaches (Cymerman et al, 2008), suggesting a functional as well as a physical split, with EndoG responsible for the cell death function performed by Nuc1p in yeast, and EXOG involved in cellular proliferation. Both exonic (Cymerman et al, 2008) and intronic SNPs have been reported in the ExoG gene; the latter is associated with type 2 diabetes (Moritani et al. 2007), a disease linked with age.

Conclusions

Many different mechanisms have been proposed to account for cellular and organismal ageing, including the adverse impact of oxidative damage on proteins, lipids and DNA, genome instability arsing from defects in maintenance mechanisms, mitochondrial dysfunction, defects in autophagy, aberrant metabolic signalling through the IGF1 axis and mTOR, degradation of the stem cell compartment and telomere attrition. That these mechanisms are important in ageing have been demonstrated in several recent experiemtnal models. For example, Jaskelioff and colleagues have shown that short term telomerase reactivation can restore normal tissue integrity and function even to aged tissues in mice (Jaskelioff et al, 2011), demonstrating the importance of correct telomere maintenance (which may involve not only telomerase but also nucleases including EXO1 and WRN – see above) in preventing tissue homeostasis collapse and ageing. Telomere integrity is also thought to be important in preventing premature ageing of stem cells populations (Sahin and DePinho 2010). Inhibition of the IGF-1/mTOR axis through rapamycin administration significantly increased lifespan in adult mice (Harrison et al, 2009), verifying the importance of IGF1-mTOR in ageing, while nutrient restriction had a similar impact in increasing primate lifespan (Colman et al, 2009), reviewed in (Cox, 2009; Cox and Mattison, 2009).

It would at first sight seem obvious that deficiency of exonucleases, whose normal role is to promote genome stability, would result in premature cell senescence through accumulation of DNA damage. The exonucleases discussed above do show the importance of correct genome maintenance in preventing the onset of premature ageing or age-associated disease such as diabetes and cancer. However, in addition to the direct role in DNA stability, there appears to be much more complex interplay between nucleases and other pathways of ageing, with significant cross-talk between metabolic signalling and DNA damage. Perhaps nowhere is this exemplified more clearly in the recent description of a severe progeroid phenotype resulting from mutation of the nucleotide

excision repair nuclease, XPF, with a patient showing not only signs of both CS and XP (deficient global genomic NER and transcription coupled NER), but also severe effects on liver, blood, muscle and neurological systems (Niedernhofer et al, 2006). Mice engineered to lack ERCC1 similarly showed profound systemic progeroid symptoms and died before sexual maturity. These results suggest that XPF-ERCC1 are involved not only in NER but also in other critical systems, perhaps including interstrand crosslink repair (see FAN1, above). Perhaps most interestingly, in this mouse model, the IGF1 nutrient response axis was altered, presumably in response to the presence of unrepaired DNA damage (Niedernhofer et al, 2006). Hence ageing may be triggered by initial loss of a single nuclease activity but with impacts on multiple downstream regulatory pathways resulting in the loss of tissue homeostasis characteristic of whole organismal ageing.

The very tight association between mitochondrial genome maintenance defects and ageing strongly supports the idea that precise processing of mtDNA is critical to stave off ageing; nucleases including POLG, EXOG and possibly also EXDL2 are important to the cell to prevent premature accumulation of mtDNA mutations. Multiple nucleases have evolved to fulfill the functions required for each separate pathway, although there is often redundancy and overlap in functionality, shown by the dearth of human diseases that arise from a nuclease deficiency. However, it is suggestive of the importance of exonucleases that the diseases that do arise from their deficiency are systemic – premature ageing, genome-wide destabilization and general inflammation are all hallmark phenotypes. It is highly probable that a combination of mitochondrial nucleases, together with nucleases acting on the genomic DNA within the nucleus (particularly the progeroid-associated WRN), play a central role in preventing accumulation of damage that would otherwise give rise to a senescent phenotype. Such nucleases therefore present promising targets for further study and in developing agents either to promote precocious senescence (e.g. in cancer therapy) or anti-ageing therapies based on supporting or augmenting the role of these nucleases.

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References

Aggarwal M, Sommers JA, Morris C, Brosh RM, Jr. (2010) Delineation of WRN helicase function with EXO1 in the replicational stress response. DNA Repair (Amst) 9: 765-776

Alter BP (1996) Fanconi's anemia and malignancies. Am J Hematol 53: 99-110

Arenas-Sordo Mde L, Hernandez-Zamora E, Montoya-Perez LA, Aldape-Barrios BC (2006) Cockayne's syndrome: a case report. Literature review. Med Oral Patol Oral Cir Bucal 11: E236-238

Asagoshi K, Tano K, Chastain PD, 2nd, Adachi N, Sonoda E, Kikuchi K, Koyama H, Nagata K, Kaufman DG, Takeda S, Wilson SH, Watanabe M, Swenberg JA, Nakamura J (2010) FEN1 functions in long patch base excision repair under conditions of oxidative stress in vertebrate cells. Mol Cancer Res 8: 204-215

Auerbach AD (1995) Fanconi anemia. Dermatol Clin 13: 41-49

Berkovich E, Monnat RJ, Jr., Kastan MB (2007) Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. Nat Cell Biol 9: 683-690

Bessho T, Sancar A (2000) Human DNA damage checkpoint protein hRAD9 is a 3' to 5' exonuclease. J Biol Chem 275 7451-7454

Boubriak I, Mason PA, Clancy DJ, Dockray J, Saunders RD, Cox LS (2009) DmWRNexo is a 3'-5' exonuclease: phenotypic and biochemical characterization of mutants of the Drosophila orthologue of human WRN exonuclease. Biogerontology 10: 267-277

Brosh RM, Jr., Driscoll HC, Dianov GL, Sommers JA (2002a) Biochemical characterization of the WRN-FEN-1 functional interaction. Biochemistry 41: 12204-12216

Brosh RM, Jr., Karmakar P, Sommers JA, Yang Q, Wang XW, Spillare EA, Harris CC, Bohr VA (2001a) p53 Modulates the exonuclease activity of Werner syndrome protein. J Biol Chem 276: 35093-35102

Brosh RM, Jr., von Kobbe C, Sommers JA, Karmakar P, Opresko PL, Piotrowski J, Dianova I, Dianov GL, Bohr VA (2001b) Werner syndrome protein interacts with human flap endonuclease 1 and stimulates its cleavage activity. EMBO J 20: 5791-5801

Brosh RM, Jr., Waheed J, Sommers JA (2002b) Biochemical characterization of the DNA substrate specificity of Werner syndrome helicase. J Biol Chem 277: 23236-23245

Brown KR, Weatherdon KL, Galligan CL, Skalski V (2002) A nuclear 3'-5' exonuclease proofreads for the exonuclease-deficient DNA polymerase alpha. DNA Repair (Amst) 1 795-810

Budd ME, Campbell JL (2009) Interplay of Mre11 nuclease with Dna2 plus Sgs1 in Rad51-dependent recombinational repair. PLoS One 4: e4267

Budd ME, Cox LS, Campbell JL (2009) Coordination of nucleases and helicases during DNA replication and double-strand break repair. In: Cox LS (ed) Molecular Themes in DNA Replication, Royal Society of Chemistry Cambridge. pp 112-155

Buis J, Wu Y, Deng Y, Leddon J, Westfield G, Eckersdorff M, Sekiguchi JM, Chang S, Ferguson DO (2008) Mre11 nuclease activity has essential roles in DNA repair and genomic stability distinct from ATM activation. Cell 135 85-96

Burkovics P, Szukacsov V, Unk I, Haracska L (2006) Human Ape2 protein has a 3'-5' exonuclease activity that acts preferentially on mismatched base pairs. Nucleic Acids Res 34: 2508-2515

Burtelow MA, Kaufmann SH, Karnitz LM (2000) Retention of the human Rad9 checkpoint complex in extraction-resistant nuclear complexes after DNA damage. J Biol Chem 275: 26343-26348

Buttner S, Eisenberg T, Carmona-Gutierrez D, Ruli D, Knauer H, Ruckenstuhl C, Sigrist C, Wissing S, Kollroser M, Frohlich KU, Sigrist S, Madeo F (2007) Endonuclease G regulates budding yeast life and death. Mol Cell 25: 233-246

Carr AM (2002) DNA structure dependent checkpoints as regulators of DNA repair. DNA Repair (Amst) 1: 983-994

Chen MJ, Lin YT, Lieberman HB, Chen G, Lee EY (2001a) ATM-dependent phosphorylation of human Rad9 is required for ionizing radiation-induced checkpoint activation. J Biol Chem 276: 16580-16586

Chen MJ, Ma SM, Dumitrache LC, Hasty P (2007) Biochemical and cellular characteristics of the 3' -> 5' exonuclease TREX2. Nucleic Acids Res 35 2682-2694

Chen X, Zuo S, Kelman Z, O'Donnell M, Hurwitz J, Goodman MF (2000) Fidelity of eucaryotic DNA polymerase delta holoenzyme from Schizosaccharomyces pombe. J Biol Chem 275: 17677-17682

Chen XB, Melchionna R, Denis CM, Gaillard PH, Blasina A, Van de Weyer I, Boddy MN, Russell P, Vialard J, McGowan CH (2001b) Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. Mol Cell 8: 1117-1127

Chou KM, Cheng YC (2002) An exonucleolytic activity of human apurinic/apyrimidinic endonuclease on 3' mispaired DNA. Nature 415 655-659

Chou KM, Cheng YC (2003) The exonuclease activity of human apurinic/apyrimidinic endonuclease (APE1). Biochemical properties and inhibition by the natural dinucleotide Gp4G. J Biol Chem 278: 18289-18296

Choudhury AR, Ju Z, Djojosubroto MW, Schienke A, Lechel A, Schaetzlein S, Jiang H, Stepczynska A, Wang C, Buer J, Lee HW, von Zglinicki T, Ganser A, Schirmacher P, Nakauchi H, Rudolph KL (2007) Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. Nat Genet 39: 99-105

Chowdhury D, Beresford PJ, Zhu P, Zhang D, Sung JS, Demple B, Perrino FW, Lieberman J

(2006) The exonuclease TREX1 is in the SET complex and acts in concert with NM23-H1 to degrade DNA during granzyme A-mediated cell death. Mol Cell 23 133-142

Christmann M, Tomicic MT, Aasland D, Berdelle N, Kaina B (2010) Three prime exonuclease I (TREX1) is Fos/AP-1 regulated by genotoxic stress and protects against ultraviolet light and benzo(a)pyrene-induced DNA damage. Nucleic Acids Res 38: 6418-6432

Ciccia A, Constantinou A, West SC (2003) Identification and characterization of the human mus81-eme1 endonuclease. J Biol Chem 278: 25172-25178

Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325: 201-204

Comai L, Li B (2004) The Werner syndrome protein at the crossroads of DNA repair and apoptosis. Mech Ageing Dev 125: 521-528

Constantinescu D, Csoka AB, Navara CS, Schatten GP (2010) Defective DSB repair correlates with abnormal nuclear morphology and is improved with FTI treatment in Hutchinson-Gilford progeria syndrome fibroblasts. Exp Cell Res 316: 2747-2759

Cooper MP, Machwe A, Orren DK, Brosh RM, Ramsden D, Bohr VA (2000) Ku complex interacts with and stimulates the Werner protein. Genes Dev 14 907-912

Copeland WC, Longley MJ (2008) DNA2 resolves expanding flap in mitochondrial base excision repair. Mol Cell 32: 457-458

Cox LS (1997) Multiple pathways control cell growth and transformation: overlapping and independent activities of p53 and p21Cip1/WAF1/Sdi1. J Pathol 183: 134-140

Cox LS (2009) Live fast, die young: new lessons in mammalian longevity. Rejuvenation Res 12: 283-288

Cox LS, Clancy DJ, Boubriak I, Saunders RD (2007) Modeling Werner Syndrome in Drosophila melanogaster: hyper-recombination in flies lacking WRN-like exonuclease. Ann N Y Acad Sci 1119: 274-288

Cox LS, Kearsey S (2009) Ring structures and six-fold symmetry in DNA replication. In: Cox LS (ed) Molecular Themes in DNA Replication, Royal Society of Chemistry Cambridge. pp 47-85

Cox LS, Lane DP (1995) Tumour suppressors, kinases and clamps: how p53 regulates the cell cycle in response to DNA damage. Bioessays 17: 501-508

Cox LS, Mattison JA (2009) Increasing longevity through caloric restriction or rapamycin feeding in mammals: common mechanisms for common outcomes? Aging Cell 8: 607-613

Crabbe L, Verdun RE, Haggblom CI, Karlseder J (2004) Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. Science 306: 1951-1953

Crow YJ, Hayward BE, Parmar R, Robins P, Leitch A, Ali M, Black DN, van Bokhoven H,

Brunner HG, Hamel BC, Corry PC, Cowan FM, Frints SG, Klepper J, Livingston JH, Lynch SA, Massey RF, Meritet JF, Michaud JL, Ponsot G, Voit T, Lebon P, Bonthron DT, Jackson AP, Barnes DE, Lindahl T (2006) Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus. Nat Genet 38 917-920

Cuykendall TN, Houston DW (2010) Identification of germ plasm-associated transcripts by microarray analysis of Xenopus vegetal cortex RNA. Dev Dyn 239: 1838-1848

Cymerman IA, Chung I, Beckmann BM, Bujnicki JM, Meiss G (2008) EXOG, a novel paralog of Endonuclease G in higher eukaryotes. Nucleic Acids Res 36 1369-1379

D'Amours D, Jackson SP (2002) The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. Nat Rev Mol Cell Biol 3 317-327

Dake E, Hofmann TJ, McIntire S, Hudson A, Zassenhaus HP (1988) Purification and properties of the major nuclease from mitochondria of Saccharomyces cerevisiae. J Biol Chem 263: 7691-7702

de Jager M, Dronkert ML, Modesti M, Beerens CE, Kanaar R, van Gent DC (2001) DNA-binding and strand-annealing activities of human Mre11: implications for its roles in DNA double-strand break repair pathways. Nucleic Acids Res 29: 1317-1325

Deshpande AM, Ivanova IG, Raykov V, Xue Y, Maringele L (2011) Polymerase epsilon is required to maintain replicative senescence. Mol Cell Biol 31: 1637-1645

Doe CL, Ahn JS, Dixon J, Whitby MC (2002) Mus81-Eme1 and Rqh1 involvement in processing stalled and collapsed replication forks. J Biol Chem 277: 32753-32759

Doherty AJ, Serpell LC, Ponting CP (1996) The helix-hairpin-helix DNA-binding motif: a structural basis for non-sequence-specific recognition of DNA. Nucleic Acids Res 24: 2488-2497

Dore AS, Kilkenny ML, Rzechorzek NJ, Pearl LH (2009) Crystal structure of the rad9-rad1-hus1 DNA damage checkpoint complex--implications for clamp loading and regulation. Mol Cell 34: 735-745

Duxin JP, Dao B, Martinsson P, Rajala N, Guittat L, Campbell JL, Spelbrink JN, Stewart SA (2009) Human Dna2 is a nuclear and mitochondrial DNA maintenance protein. Mol Cell Biol 29: 4274-4282

el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B (1993) WAF1, a potential mediator of p53 tumor suppression. Cell 75: 817-825

Falkenberg M, Larsson NG, Gustafsson CM (2007) DNA replication and transcription in mammalian mitochondria. Annu Rev Biochem 76: 679-699

Fekairi S, Scaglione S, Chahwan C, Taylor ER, Tissier A, Coulon S, Dong MQ, Ruse C, Yates JR, 3rd, Russell P, Fuchs RP, McGowan CH, Gaillard PH (2009) Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. Cell 138: 78-89

Fiorentini P, Huang KN, Tishkoff DX, Kolodner RD, Symington LS (1997) Exonuclease I of

Saccharomyces cerevisiae functions in mitotic recombination in vivo and in vitro. Mol Cell Biol 17 2764-2773

Fortini BK, Pokharel S, Polaczek P, Balakrishnan L, Bambara RA, Campbell JL (2011) Characterization of the Endonuclease and ATP-dependent Flap Endo/Exonuclease of Dna2. J Biol Chem 286: 23763-23770

Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S (2007) Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev 128: 92-105

Franklin MC, Wang J, Steitz TA (2001) Structure of the replicating complex of a pol alpha family DNA polymerase. Cell 105: 657-667

Friedberg EC, Wood RD (2007) New insights into the combined Cockayne/xeroderma pigmentosum complex: human XPG protein can function in transcription factor stability. Mol Cell 26: 162-164

Frosina G, Fortini P, Rossi O, Carrozzino F, Raspaglio G, Cox LS, Lane DP, Abbondandolo A, Dogliotti E (1996) Two pathways for base excision repair in mammalian cells. J Biol Chem 271: 9573-9578

Furuse M, Nagase Y, Tsubouchi H, Murakami-Murofushi K, Shibata T, Ohta K (1998) Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J 17: 6412-6425

Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, D'Andrea AD (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Mol Cell 7: 249-262

Genschel J, Bazemore LR, Modrich P (2002) Human exonuclease I is required for 5' and 3' mismatch repair. J Biol Chem 277 13302-13311

Goldsby RE, Lawrence NA, Hays LE, Olmsted EA, Chen X, Singh M, Preston BD (2001) Defective DNA polymerase-delta proofreading causes cancer susceptibility in mice. Nat Med 7 638-639

Green DR, Kroemer G (2009) Cytoplasmic functions of the tumour suppressor p53. Nature 458: 1127-1130

Habraken Y, Sung P, Prakash L, Prakash S (1994a) A conserved 5' to 3' exonuclease activity in the yeast and human nucleotide excision repair proteins RAD2 and XPG. J Biol Chem 269: 31342-31345

Habraken Y, Sung P, Prakash L, Prakash S (1994b) Human xeroderma pigmentosum group G gene encodes a DNA endonuclease. Nucleic Acids Res 22: 3312-3316

Hadi MZ, Wilson DM, 3rd (2000) Second human protein with homology to the Escherichia coli abasic endonuclease exonuclease III. Environ Mol Mutagen 36: 312-324

Harman D (1972) The biologic clock: the mitochondria? J Am Geriatr Soc 20: 145-147

Harrigan JA, Wilson DM, 3rd, Prasad R, Opresko PL, Beck G, May A, Wilson SH, Bohr VA (2006) The Werner syndrome protein operates in base excision repair and cooperates with DNA polymerase beta. Nucleic Acids Res 34: 745-754

Harrington JJ, Lieber MR (1994) The characterization of a mammalian DNA structure-specific endonuclease. EMBO J 13 1235-1246

Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460: 392-395

Hiona A, Sanz A, Kujoth GC, Pamplona R, Seo AY, Hofer T, Someya S, Miyakawa T, Nakayama C, Samhan-Arias AK, Servais S, Barger JL, Portero-Otin M, Tanokura M, Prolla TA, Leeuwenburgh C (2010) Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice. PLoS One 5: e11468

Hiraoka LR, Harrington JJ, Gerhard DS, Lieber MR, Hsieh CL (1995) Sequence of human FEN-1, a structure-specific endonuclease, and chromosomal localization of the gene (FEN1) in mouse and human. Genomics 25: 220-225

Hollstein M, Shomer B, Greenblatt M, Soussi T, Hovig E, Montesano R, Harris CC (1996) Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. Nucleic Acids Res 24: 141-146

Hoopes LL, Budd M, Choe W, Weitao T, Campbell JL (2002) Mutations in DNA replication genes reduce yeast life span. Mol Cell Biol 22: 4136-4146

Hopkins KM, Auerbach W, Wang XY, Hande MP, Hang H, Wolgemuth DJ, Joyner AL, Lieberman HB (2004) Deletion of mouse rad9 causes abnormal cellular responses to DNA damage, genomic instability, and embryonic lethality. Mol Cell Biol 24: 7235-7248

Hosfield DJ, Frank G, Weng Y, Tainer JA, Shen B (1998a) Newly discovered archaebacterial flap endonucleases show a structure-specific mechanism for DNA substrate binding and catalysis resembling human flap endonuclease-1. J Biol Chem 273: 27154-27161

Hosfield DJ, Mol CD, Shen B, Tainer JA (1998b) Structure of the DNA repair and replication endonuclease and exonuclease FEN-1: coupling DNA and PCNA binding to FEN-1 activity. Cell 95: 135-146

Hoss M, Robins P, Naven TJ, Pappin DJ, Sgouros J, Lindahl T (1999) A human DNA editing enzyme homologous to the Escherichia coli DnaQ/MutD protein. EMBO J 18 3868-3875

Hsieh P, Yamane K (2008) DNA mismatch repair: molecular mechanism, cancer, and ageing. Mech Ageing Dev 129: 391-407

Huang KJ, Ku CC, Lehman IR (2006) Endonuclease G: a role for the enzyme in recombination and

cellular proliferation. Proc Natl Acad Sci U S A 103: 8995-9000

Huang MM, Arnheim N, Goodman MF (1992) Extension of base mispairs by Taq DNA polymerase: implications for single nucleotide discrimination in PCR. Nucleic Acids Res 20: 4567-4573

Huang S, Beresten S, Li B, Oshima J, Ellis NA, Campisi J (2000) Characterization of the human and mouse WRN 3'-->5' exonuclease. Nucleic Acids Res 28: 2396-2405

Huang S, Li B, Gray MD, Oshima J, Mian IS, Campisi J (1998) The premature ageing syndrome protein, WRN, is a 3'-->5' exonuclease. Nat Genet 20 114-116

Hubscher U, Maga G, Spadari S (2002) Eukaryotic DNA polymerases. Annu Rev Biochem 71 133-163

Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadinanos J, Horner JW, Maratos-Flier E, Depinho RA (2011) Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature 469: 102-106

Joseph IS, Kumari A, Bhattacharyya MK, Gao H, Li B, Lustig AJ (2010) An mre11 mutation that promotes telomere recombination and an efficient bypass of senescence. Genetics 185: 761-770

Kalifa L, Beutner G, Phadnis N, Sheu SS, Sia EA (2009) Evidence for a role of FEN1 in maintaining mitochondrial DNA integrity. DNA Repair (Amst) 8: 1242-1249

Kao HI, Bambara RA (2003) The protein components and mechanism of eukaryotic Okazaki fragment maturation. Crit Rev Biochem Mol Biol 38: 433-452

Kao HI, Veeraraghavan J, Polaczek P, Campbell JL, Bambara RA (2004) On the roles of Saccharomyces cerevisiae Dna2p and Flap endonuclease 1 in Okazaki fragment processing. J Biol Chem 279: 15014-15024

Kashino G, Kodama S, Suzuki K, Matsumoto T, Watanabe M (2005) Exogenous Expression of Exonuclease Domain-deleted WRN Interferes with the Repair of Radiation-induced DNA Damages. J Radiat Res (Tokyo) 46: 407-414

Kawase T, Ichikawa H, Ohta T, Nozaki N, Tashiro F, Ohki R, Taya Y (2008) p53 target gene AEN is a nuclear exonuclease required for p53-dependent apoptosis. Oncogene 27: 3797-3810

Kirchner JM, Tran H, Resnick MA (2000) A DNA polymerase epsilon mutant that specifically causes +1 frameshift mutations within homonucleotide runs in yeast. Genetics 155: 1623-1632

Knipscheer P, Raschle M, Smogorzewska A, Enoiu M, Ho TV, Scharer OD, Elledge SJ, Walter JC (2009) The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. Science 326: 1698-1701

Komatsu K, Miyashita T, Hang H, Hopkins KM, Zheng W, Cuddeback S, Yamada M, Lieberman HB, Wang HG (2000) Human homologue of S. pombe Rad9 interacts with BCL-2/BCL-xL and promotes apoptosis. Nat Cell Biol 2: 1-6

Kong YX, Van Bergen N, Trounce IA, Bui BV, Chrysostomou V, Waugh H, Vingrys A, Crowston JG (2011) Increase in mitochondrial DNA mutations impairs retinal function and renders the retina vulnerable to injury. Aging Cell 10: 572-583

Korona DA, Lecompte KG, Pursell ZF (2010) The high fidelity and unique error signature of human DNA polymerase epsilon. Nucleic Acids Res 39: 1763-1773

Kratz K, Schopf B, Kaden S, Sendoel A, Eberhard R, Lademann C, Cannavo E, Sartori AA, Hengartner MO, Jiricny J (2010) Deficiency of FANCD2-associated nuclease KIAA1018/FAN1 sensitizes cells to interstrand crosslinking agents. Cell 142: 77-88

Kraytsberg Y, Simon DK, Turnbull DM, Khrapko K (2009) Do mtDNA deletions drive premature aging in mtDNA mutator mice? Aging Cell 8**:** 502-506

Kucherlapati M, Yang K, Kuraguchi M, Zhao J, Lia M, Heyer J, Kane MF, Fan K, Russell R, Brown AM, Kneitz B, Edelmann W, Kolodner RD, Lipkin M, Kucherlapati R (2002) Haploinsufficiency of Flap endonuclease (Fen1) leads to rapid tumor progression. Proc Natl Acad Sci U S A 99: 9924-9929

Kunkel TA (2009) Evolving views of DNA replication (in)fidelity. Cold Spring Harb Symp Quant Biol 74: 91-101

Lebel M, Spillare EA, Harris CC, 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: 37795-37799

Lee BI, Wilson DM, 3rd (1999) The RAD2 domain of human exonuclease 1 exhibits 5' to 3' exonuclease and flap structure-specific endonuclease activities. J Biol Chem 274 37763-37769

Lee JH, Paull TT (2007) Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. Oncogene 26: 7741-7748

Lee MH, Hollis SE, Yoo BH, Nykamp K (2011) Caenorhabditis elegans DNA-2 helicase/endonuclease plays a vital role in maintaining genome stability, morphogenesis, and life span. Biochem Biophys Res Commun 407: 495-500

Li B, Comai L (2002) Displacement of DNA-PKcs from DNA ends by the Werner syndrome protein. Nucleic Acids Res 30: 3653-3661

Li B, Conway N, Navarro S, Comai L (2005) A conserved and species-specific functional interaction between the Werner syndrome-like exonuclease atWEX and the Ku heterodimer in Arabidopsis. Nucleic Acids Res 33: 6861-6867

Li B, Navarro S, Kasahara N, Comai L (2004) Identification and biochemical characterization of a Werner's syndrome protein complex with Ku70/80 and poly(ADP-ribose) polymerase-1. J Biol Chem 279: 13659-13667

Li LY, Luo X, Wang X (2001) Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 412: 95-99

Lindahl T, Barnes DE, Yang YG, Robins P (2009) Biochemical properties of mammalian TREX1 and its association with DNA replication and inherited inflammatory disease. Biochem Soc Trans 37: 535-538

Lindsey-Boltz LA, Wauson EM, Graves LM, Sancar A (2004) The human Rad9 checkpoint protein stimulates the carbamoyl phosphate synthetase activity of the multifunctional protein CAD. Nucleic Acids Res 32: 4524-4530

Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell 118: 699-713

Liu P, Qian L, Sung JS, de Souza-Pinto NC, Zheng L, Bogenhagen DF, Bohr VA, Wilson DM, 3rd, Shen B, Demple B (2008) Removal of oxidative DNA damage via FEN1-dependent long-patch base excision repair in human cell mitochondria. Mol Cell Biol 28: 4975-4987

Liu T, Ghosal G, Yuan J, Chen J, Huang J (2010) FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair. Science 329 693-696

Liu Y, Zhang H, Veeraraghavan J, Bambara RA, Freudenreich CH (2004) Saccharomyces cerevisiae flap endonuclease 1 uses flap equilibration to maintain triplet repeat stability. Mol Cell Biol 24: 4049-4064

Longley MJ, Nguyen D, Kunkel TA, Copeland WC (2001) The fidelity of human DNA polymerase gamma with and without exonucleolytic proofreading and the p55 accessory subunit. J Biol Chem 276: 38555-38562

Machwe A, Xiao L, Orren DK (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

MacKay C, Declais AC, Lundin C, Agostinho A, Deans AJ, MacArtney TJ, Hofmann K, Gartner A, West SC, Helleday T, Lilley DM, Rouse J (2010) Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. Cell 142 65-76

MacNeill SA (2001) DNA replication: partners in the Okazaki two-step. Curr Biol 11: R842-844

Mazur DJ, Perrino FW (1999) Identification and expression of the TREX1 and TREX2 cDNA sequences encoding mammalian 3'-->5' exonucleases. J Biol Chem 274: 19655-19660

Mazur DJ, Perrino FW (2001a) Excision of 3' termini by the Trex1 and TREX2 3'-->5' exonucleases. Characterization of the recombinant proteins. J Biol Chem 276 17022-17029

Mazur DJ, Perrino FW (2001b) Structure and expression of the TREX1 and TREX2 3' --> 5' exonuclease genes. J Biol Chem 276 14718-14727

Meier B, Clejan I, Liu Y, Lowden M, Gartner A, Hodgkin J, Ahmed S (2006) trt-1 is the Caenorhabditis elegans catalytic subunit of telomerase. PLoS Genet 2: e18

Miquel J, Economos AC, Fleming J, Johnson JE, Jr. (1980) Mitochondrial role in cell aging. Exp Gerontol 15: 575-591

Mirzoeva OK, Petrini JH (2001) DNA damage-dependent nuclear dynamics of the Mre11 complex. Mol Cell Biol 21: 281-288

Moreau S, Ferguson JR, Symington LS (1999) The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. Mol Cell Biol 19: 556-566

Moreau S, Morgan EA, Symington LS (2001) Overlapping functions of the Saccharomyces cerevisiae Mre11, Exo1 and Rad27 nucleases in DNA metabolism. Genetics 159 1423-1433

Morita M, Stamp G, Robins P, Dulic A, Rosewell I, Hrivnak G, Daly G, Lindahl T, Barnes DE (2004) Gene-targeted mice lacking the Trex1 (DNase III) 3'-->5' DNA exonuclease develop inflammatory myocarditis. Mol Cell Biol 24 6719-6727

Moritani M, Nomura K, Tanahashi T, Osabe D, Fujita Y, Shinohara S, Yamaguchi Y, Keshavarz P, Kudo E, Nakamura N, Yoshikawa T, Ichiishi E, Takata Y, Yasui N, Shiota H, Kunika K, Inoue H, Itakura M (2007) Genetic association of single nucleotide polymorphisms in endonuclease G-like 1 gene with type 2 diabetes in a Japanese population. Diabetologia 50: 1218-1227

Mummenbrauer T, Janus F, Muller B, Wiesmuller L, Deppert W, Grosse F (1996) p53 Protein exhibits 3'-to-5' exonuclease activity. Cell 85 1089-1099

Myles GM, Sancar A (1989) DNA repair. Chem Res Toxicol 2: 197-226

Nebel A, Flachsbart F, Till A, Caliebe A, Blanche H, Arlt A, Hasler R, Jacobs G, Kleindorp R, Franke A, Shen B, Nikolaus S, Krawczak M, Rosenstiel P, Schreiber S (2009) A functional EXO1 promoter variant is associated with prolonged life expectancy in centenarians. Mech Ageing Dev 130: 691-699

Negritto MC, Qiu J, Ratay DO, Shen B, Bailis AM (2001) Novel function of Rad27 (FEN-1) in restricting short-sequence recombination. Mol Cell Biol 21: 2349-2358

Nick McElhinny SA, Pavlov YI, Kunkel TA (2006) Evidence for extrinsic exonucleolytic proofreading. Cell Cycle 5: 958-962

Nick McElhinny SA, Pursell ZF, Kunkel TA (2009) Mechanisms for high fidelity DNA replication. In: Cox LS (ed) Molecular Themes in DNA Replication, Royal Society of Chemistry Cambridge, UK. pp 86-111

Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E, Odijk H, Oostendorp R, Ahmad A, van Leeuwen W, Theil AF, Vermeulen W, van der Horst GT, Meinecke P, Kleijer WJ, Vijg J, Jaspers NG, Hoeijmakers JH (2006) A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature 444: 1038-1043

Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, Wyman C, Modrich P, Kowalczykowski SC (2011) BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. Genes Dev 25: 350-362

Noda A, Ning Y, Venable SF, Pereira-Smith OM, Smith JR (1994) Cloning of senescent cell-

derived inhibitors of DNA synthesis using an expression screen. Exp Cell Res 211: 90-98

Norddahl GL, Pronk CJ, Wahlestedt M, Sten G, Nygren JM, Ugale A, Sigvardsson M, Bryder D (2011) Accumulating mitochondrial DNA mutations drive premature hematopoietic aging phenotypes distinct from physiological stem cell aging. Cell Stem Cell 8: 499-510

O'Donovan A, Davies AA, Moggs JG, West SC, Wood RD (1994) XPG endonuclease makes the 3' incision in human DNA nucleotide excision repair. Nature 371: 432-435

Ohsato T, Ishihara N, Muta T, Umeda S, Ikeda S, Mihara K, Hamasaki N, Kang D (2002) Mammalian mitochondrial endonuclease G. Digestion of R-loops and localization in intermembrane space. Eur J Biochem 269: 5765-5770

Opresko PL, Mason PA, Podell ER, Lei M, Hickson ID, Cech TR, Bohr VA (2005) POT1 stimulates RecQ helicases WRN and BLM to unwind telomeric DNA substrates. J Biol Chem 280: 32069-32080

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

Opresko PL, von Kobbe C, Laine JP, Harrigan J, Hickson ID, Bohr VA (2002) Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases. J Biol Chem 277: 41110-41119

Orans J, McSweeney EA, Iyer RR, Hast MA, Hellinga HW, Modrich P, Beese LS (2011) Structures of human exonuclease 1 DNA complexes suggest a unified mechanism for nuclease family. Cell 145: 212-223

Parker AE, Van de Weyer I, Laus MC, Oostveen I, Yon J, Verhasselt P, Luyten WH (1998) A human homologue of the Schizosaccharomyces pombe rad1+ checkpoint gene encodes an exonuclease. J Biol Chem 273: 18332-18339

Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, Miwa S, Olijslagers S, Hallinan J, Wipat A, Saretzki G, Rudolph KL, Kirkwood TB, von Zglinicki T (2010) Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol Syst Biol 6: 347

Paull TT, Gellert M (1998) The 3' to 5' exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. Mol Cell 1: 969-979

Pavlov YI, Frahm C, Nick McElhinny SA, Niimi A, Suzuki M, Kunkel TA (2006) Evidence that errors made by DNA polymerase alpha are corrected by DNA polymerase delta. Curr Biol 16: 202-207

Perrino FW, Loeb LA (1990) Hydrolysis of 3'-terminal mispairs in vitro by the 3'----5' exonuclease of DNA polymerase delta permits subsequent extension by DNA polymerase alpha. Biochemistry 29: 5226-5231

Perry JJ, Yannone SM, Holden LG, Hitomi C, Asaithamby A, Han S, Cooper PK, Chen DJ, Tainer JA (2006) WRN exonuclease structure and molecular mechanism imply an editing role in DNA end

processing. Nat Struct Mol Biol 13: 414-422

Plchova H, Hartung F, Puchta H (2003) Biochemical characterization of an exonuclease from Arabidopsis thaliana reveals similarities to the DNA exonuclease of the human Werner syndrome protein. J Biol Chem 278: 44128-44138

Qiu J, Qian Y, Chen V, Guan MX, Shen B (1999) Human exonuclease 1 functionally complements its yeast homologues in DNA recombination, RNA primer removal, and mutation avoidance. J Biol Chem 274: 17893-17900

Richards A, van den Maagdenberg AM, Jen JC, Kavanagh D, Bertram P, Spitzer D, Liszewski MK, Barilla-Labarca ML, Terwindt GM, Kasai Y, McLellan M, Grand MG, Vanmolkot KR, de Vries B, Wan J, Kane MJ, Mamsa H, Schafer R, Stam AH, Haan J, de Jong PT, Storimans CW, van Schooneveld MJ, Oosterhuis JA, Gschwendter A, Dichgans M, Kotschet KE, Hodgkinson S, Hardy TA, Delatycki MB, Hajj-Ali RA, Kothari PH, Nelson SF, Frants RR, Baloh RW, Ferrari MD, Atkinson JP (2007) C-terminal truncations in human 3'-5' DNA exonuclease TREX1 cause autosomal dominant retinal vasculopathy with cerebral leukodystrophy. Nat Genet 39: 1068-1070

Rodriguez-Lopez AM, Jackson DA, Iborra F, Cox LS (2002) Asymmetry of DNA replication fork progression in Werner's syndrome. Aging Cell 1: 30-39

Rodriguez-Lopez AM, Jackson DA, Nehlin JO, Iborra F, Warren AV, Cox LS (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: 167-174

Rodriguez-Lopez AM, Whitby MC, Borer CM, Bachler MA, Cox LS (2007) Correction of proliferation and drug sensitivity defects in the progeroid Werner's Syndrome by Holliday junction resolution. Rejuvenation Res 10: 27-40

Safdar A, Bourgeois JM, Ogborn DI, Little JP, Hettinga BP, Akhtar M, Thompson JE, Melov S, Mocellin NJ, Kujoth GC, Prolla TA, Tarnopolsky MA (2011) Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. Proc Natl Acad Sci U S A 108: 4135-4140

Sahin E, Depinho RA (2010) Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 464: 520-528

Saintigny Y, Makienko K, Swanson C, Emond MJ, Monnat RJ, Jr. (2002) Homologous recombination resolution defect in werner syndrome. Mol Cell Biol 22: 6971-6978

Sakurai S, Kitano K, Yamaguchi H, Hamada K, Okada K, Fukuda K, Uchida M, Ohtsuka E, Morioka H, Hakoshima T (2005) Structural basis for recruitment of human flap endonuclease 1 to PCNA. EMBO J 24: 683-693

Saunders RD, Boubriak I, Clancy DJ, Cox LS (2008) Identification and characterization of a Drosophila ortholog of WRN exonuclease that is required to maintain genome integrity. Aging Cell 7: 418-425

Schaetzlein S, Kodandaramireddy NR, Ju Z, Lechel A, Stepczynska A, Lilli DR, Clark AB, Rudolph C, Kuhnel F, Wei K, Schlegelberger B, Schirmacher P, Kunkel TA, Greenberg RA,

Edelmann W, Rudolph KL (2007) Exonuclease-1 deletion impairs DNA damage signaling and prolongs lifespan of telomere-dysfunctional mice. Cell 130: 863-877

Schafer P, Scholz SR, Gimadutdinow O, Cymerman IA, Bujnicki JM, Ruiz-Carrillo A, Pingoud A, Meiss G (2004) Structural and functional characterization of mitochondrial EndoG, a sugar non-specific nuclease which plays an important role during apoptosis. J Mol Biol 338: 217-228

Sekelsky JJ, Brodsky MH, Rubin GM, Hawley RS (1999) Drosophila and human RecQ5 exist in different isoforms generated by alternative splicing. Nucleic Acids Res 27: 3762-3769

Sharma S, Sommers JA, Driscoll HC, Uzdilla L, Wilson TM, Brosh RM, Jr. (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: 23487-23496

Sidorova JM, Li N, Folch A, Monnat RJ, Jr. (2008) The RecQ helicase WRN is required for normal replication fork progression after DNA damage or replication fork arrest. Cell Cycle 7: 796-807

Siegl-Cachedenier I, Munoz P, Flores JM, Klatt P, Blasco MA (2007) Deficient mismatch repair improves organismal fitness and survival of mice with dysfunctional telomeres. Genes Dev 21: 2234-2247

Skalski V, Brown KR, Choi BY, Lin ZY, Chen S (2000) A 3'-5' exonuclease in human leukemia cells: implications for resistance to 1-beta -D-arabinofuranosylcytosine and 9-beta -D-arabinofuranosyl-2-fluoroadenine 5'-monophosphate. J Biol Chem 275: 25814-25819

Smogorzewska A, Desetty R, Saito TT, Schlabach M, Lach FP, Sowa ME, Clark AB, Kunkel TA, Harper JW, Colaiacovo MP, Elledge SJ (2010) A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. Mol Cell 39 36-47

Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM (1999) The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell 99: 577-587

Stracker TH, Theunissen JW, Morales M, Petrini JH (2004) The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. DNA Repair (Amst) 3: 845-854

Swanson C, Saintigny Y, Emond MJ, Monnat RJ, Jr. (2004) The Werner syndrome protein has separable recombination and survival functions. DNA Repair (Amst) 3: 475-482

Taniguchi T, Garcia-Higuera I, Andreassen PR, Gregory RC, Grompe M, D'Andrea AD (2002) S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51. Blood 100: 2414-2420

Thayer MM, Ahern H, Xing D, Cunningham RP, Tainer JA (1995) Novel DNA binding motifs in the DNA repair enzyme endonuclease III crystal structure. EMBO J 14: 4108-4120

Thompson E, Meldrum CJ, Crooks R, McPhillips M, Thomas L, Spigelman AD, Scott RJ (2004) Hereditary non-polyposis colorectal cancer and the role of hPMS2 and hEXO1 mutations. Clin

Genet 65 215-225

Tian M, Jones DA, Smith M, Shinkura R, Alt FW (2004) Deficiency in the nuclease activity of xeroderma pigmentosum G in mice leads to hypersensitivity to UV irradiation. Mol Cell Biol 24: 2237-2242

Tishkoff DX, Amin NS, Viars CS, Arden KC, Kolodner RD (1998) Identification of a human gene encoding a homologue of Saccharomyces cerevisiae EXO1, an exonuclease implicated in mismatch repair and recombination. Cancer Res 58 5027-5031

Tishkoff DX, Boerger AL, Bertrand P, Filosi N, Gaida GM, Kane MF, Kolodner RD (1997) Identification and characterization of Saccharomyces cerevisiae EXO1, a gene encoding an exonuclease that interacts with MSH2. Proc Natl Acad Sci U S A 94 7487-7492

Toueille M, El-Andaloussi N, Frouin I, Freire R, Funk D, Shevelev I, Friedrich-Heineken E, Villani G, Hottiger MO, Hubscher U (2004) The human Rad9/Rad1/Hus1 damage sensor clamp interacts with DNA polymerase beta and increases its DNA substrate utilisation efficiency: implications for DNA repair. Nucleic Acids Res 32: 3316-3324

Tran HT, Gordenin DA, Resnick MA (1999) The 3'-->5' exonucleases of DNA polymerases delta and epsilon and the 5'-->3' exonuclease Exo1 have major roles in postreplication mutation avoidance in Saccharomyces cerevisiae. Mol Cell Biol 19: 2000-2007

Trego KS, Chernikova SB, Davalos AR, Perry JJ, Finger LD, Ng C, Tsai MS, Yannone SM, Tainer JA, Campisi J, Cooper PK (2011) The DNA repair endonuclease XPG interacts directly and functionally with the WRN helicase defective in Werner syndrome. Cell Cycle 10: 1998-2007

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlof S, Oldfors A, Wibom R, Tornell J, Jacobs HT, Larsson NG (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429 417-423

Tsubouchi H, Ogawa H (2000) Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in Saccharomyces cerevisiae. Mol Biol Cell 11 2221-2233

Tsutakawa SE, Classen S, Chapados BR, Arvai AS, Finger LD, Guenther G, Tomlinson CG, Thompson P, Sarker AH, Shen B, Cooper PK, Grasby JA, Tainer JA (2011) Human flap endonuclease structures, DNA double-base flipping, and a unified understanding of the FEN1 superfamily. Cell 145: 198-211

Vincent RD, Hofmann TJ, Zassenhaus HP (1988) Sequence and expression of NUC1, the gene encoding the mitochondrial nuclease in Saccharomyces cerevisiae. Nucleic Acids Res 16: 3297-3312

Volkmer E, Karnitz LM (1999) Human homologs of Schizosaccharomyces pombe rad1, hus1, and rad9 form a DNA damage-responsive protein complex. J Biol Chem 274: 567-570

Vousden KH, Prives C (2009) Blinded by the Light: The Growing Complexity of p53. Cell 137: 413-431

Waga S, Bauer G, Stillman B (1994) Reconstitution of complete SV40 DNA replication with

purified replication factors. J Biol Chem 269: 10923-10934

Wang W, Brandt P, Rossi ML, Lindsey-Boltz L, Podust V, Fanning E, Sancar A, Bambara RA (2004) The human Rad9-Rad1-Hus1 checkpoint complex stimulates flap endonuclease 1. Proc Natl Acad Sci U S A 101: 16762-16767

Warbrick E, Lane DP, Glover DM, Cox LS (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: 2313-2321

Weitao T, Budd M, Campbell JL (2003a) Evidence that yeast SGS1, DNA2, SRS2, and FOB1 interact to maintain rDNA stability. Mutat Res 532: 157-172

Weitao T, Budd M, Hoopes LL, Campbell JL (2003b) Dna2 helicase/nuclease causes replicative fork stalling and double-strand breaks in the ribosomal DNA of Saccharomyces cerevisiae. J Biol Chem 278: 22513-22522

White PJ, Borts RH, Hirst MC (1999) Stability of the human fragile X (CGG)(n) triplet repeat array in Saccharomyces cerevisiae deficient in aspects of DNA metabolism. Mol Cell Biol 19: 5675-5684

Wijnhoven SW, Hoogervorst EM, de Waard H, van der Horst GT, van Steeg H (2007) Tissue specific mutagenic and carcinogenic responses in NER defective mouse models. Mutat Res 614: 77-94

Williams RS, Tainer JA (2005) A nanomachine for making ends meet: MRN is a flexing scaffold for the repair of DNA double-strand breaks. Mol Cell 19: 724-726

Wu Y, Berends MJ, Post JG, Mensink RG, Verlind E, Van Der Sluis T, Kempinga C, Sijmons RH, van der Zee AG, Hollema H, Kleibeuker JH, Buys CH, Hofstra RM (2001) Germline mutations of EXO1 gene in patients with hereditary nonpolyposis colorectal cancer (HNPCC) and atypical HNPCC forms. Gastroenterology 120 1580-1587

Xu H, Zheng L, Dai H, Zhou M, Hua Y, Shen B (2011) Chemical-induced cancer incidence and underlying mechanisms in Fen1 mutant mice. Oncogene 30: 1072-1081

Xu M, Bai L, Gong Y, Xie W, Hang H, Jiang T (2009) Structure and functional implications of the human rad9-hus1-rad1 cell cycle checkpoint complex. J Biol Chem 284: 20457-20461

Yamagata K, Kato J, Shimamoto A, Goto M, Furuichi Y, Ikeda H (1998) Bloom's and Werner's syndrome genes suppress hyperrecombination in yeast sgs1 mutant: implication for genomic instability in human diseases. Proc Natl Acad Sci U S A 95: 8733-8738

Yang YG, Lindahl T, Barnes DE (2007) Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease. Cell 131 873-886

Yasukawa K, Oshiumi H, Takeda M, Ishihara N, Yanagi Y, Seya T, Kawabata S, Koshiba T (2009) Mitofusin 2 inhibits mitochondrial antiviral signaling. Sci Signal 2: ra47

Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, Matthews S, Nakura J, Miki T, Ouais S, Martin GM, Mulligan J, Schellenberg GD (1996) Positional cloning of the Werner's syndrome

gene. Science 272: 258-262

Zassenhaus HP, Denniger G (1994) Analysis of the role of the NUC1 endo/exonuclease in yeast mitochondrial DNA recombination. Curr Genet 25: 142-149

Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ (2010) Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 464: 104-107

Zheng L, Dai H, Hegde ML, Zhou M, Guo Z, Wu X, Wu J, Su L, Zhong X, Mitra S, Huang Q, Kernstine KH, Pfeifer GP, Shen B (2011) Fen1 mutations that specifically disrupt its interaction with PCNA cause aneuploidy-associated cancer. Cell Res 21: 1052-1067

Zheng L, Dai H, Zhou M, Li M, Singh P, Qiu J, Tsark W, Huang Q, Kernstine K, Zhang X, Lin D, Shen B (2007) Fen1 mutations result in autoimmunity, chronic inflammation and cancers. Nat Med 13: 812-819

Zheng L, Zhou M, Chai Q, Parrish J, Xue D, Patrick SM, Turchi JJ, Yannone SM, Chen D, Shen B (2005) Novel function of the flap endonuclease 1 complex in processing stalled DNA replication forks. EMBO Rep 6: 83-89

Zheng L, Zhou M, Guo Z, Lu H, Qian L, Dai H, Qiu J, Yakubovskaya E, Bogenhagen DF, Demple B, Shen B (2008) Human DNA2 is a mitochondrial nuclease/helicase for efficient processing of DNA replication and repair intermediates. Mol Cell 32: 325-336

Figure legends

Fig. 1 DNA transactions involving exonucleases

Exonucleases are central to many processes of DNA metabolism. They are important in DNA replication in providing proofreading capacity both intrinsic to the replicative DNA polymerases δ and ε and extrinsic (e.g. ExoN) or autonomous proof-readers (e.g. TREX1 and 2). During the process of Okazaki fragment processing on the lagging strand, nucleases FEN1 and Dna2 (with RNaseH) are important for cleaving the primer and iDNA. Where replication forks stall or collapse, exonucleases are important in resolving aberrant DNA structures to permit fork restart. Such nucleases include the progeroid WRN protein, and EXO1. Telomere maintenance also requires the action of exonucleases, possibly also using the same nucleases WRN and EXO1. DNA repair takes many forms depending on the lesion (on one or both strands); nucleases are involved in base excision repair (BER) (e.g. FEN1), in nucleotide excision repair, mismatch repair (e.g. EXO1) and in repair of DNA double strand breaks (e.g. Mre11). Finally, repair of interstrand cross links (ICL) requires the action of a newly discovered nuclease FAN1, which is thought to be recruited by association with the Fanconi anaemia protein complex FANC ID (see text)

colour figure

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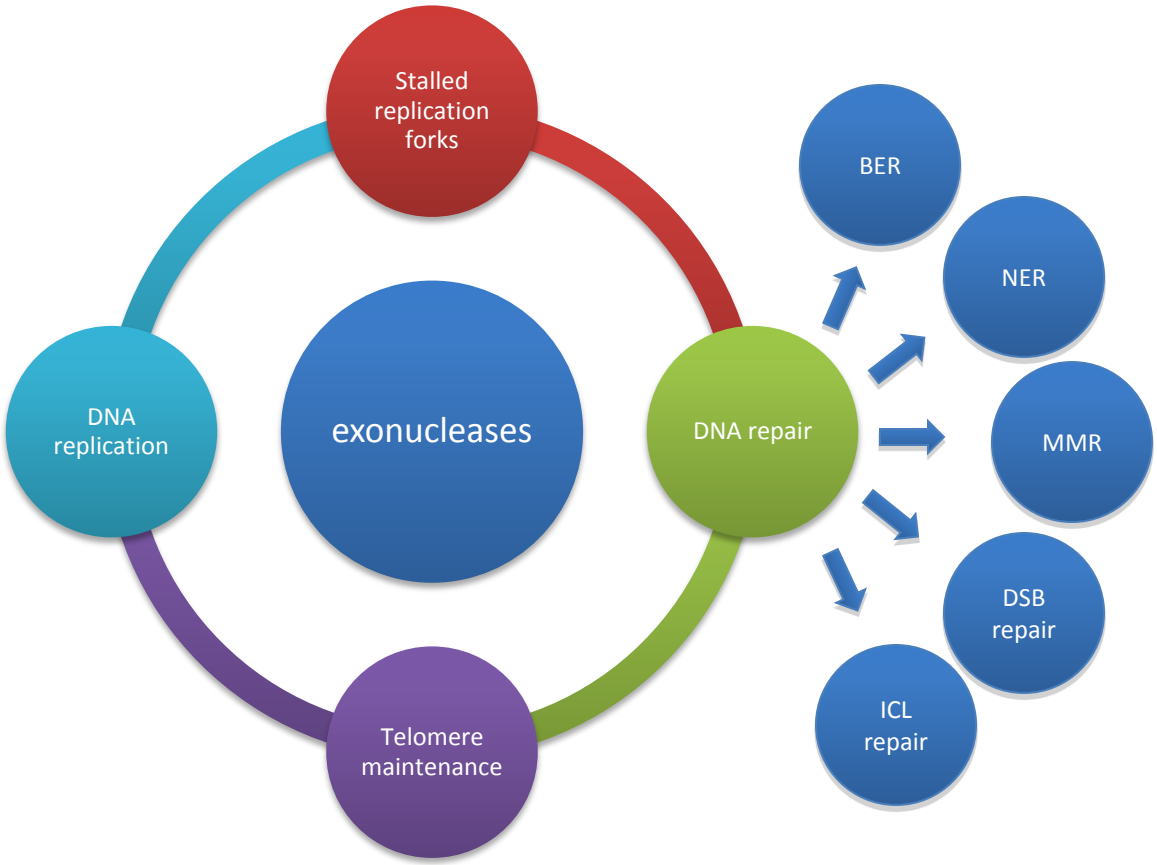

Mason and Cox Figure 1

Table 1
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EXONUCLEASE	POLARITY	METABOLIC PATHWAY	REFERENCES
Polymerase δ, ε, γ	3'-5'	Replication (proofreading)	(Hubscher et al, 2002; Tran et al, 1999; Longley et al, 2001)
ExoN	3'-5'	Unknown - proofreading?	(Brown et al, 2002)
TREX1/TREX2	3'-5'	Aberrant ssDNA removal / proofreading?	(Chen et al, 2007; Yang et al, 2007)
p53	3'-5'	Multiple; proofreading?	(Mummenbrauer et al, 1996)
APE1/APE2*	3'-5'	BER, replication	(Wilson, 2003; Burkovics, et al. 2006)
FEN1*	5'-3'	Okazaki fragment processing, BER	(Harrington and Lieber, 1994)
XPG/ERCC5	5'-3'	NER; transcription stabilisation	(Habraken 1994a;b)
EXO1*	5'-3'	Multiple: MMR, DSBR, meiosis	(Fiorentini et al, 1997; Lee and Wilson, 1999; Tishkoff et al, 1998; Tsubouchi and Ogawa, 2000)
WRN**	3'-5'	Multiple: DNA repair, telomeres	(Cooper et al, 2000; Huang et al, 1998, 2000)
Dna2	3'-5'	Replication – Okazaki fragment processing	(Reviewed in Budd et al 2009)
MRE11[§]/RAD50/NBS1	3'-5'	DSBR; replication restart	(Buis et al, 2008; D'Amours and Jackson, 2002)
hRAD9	3'-5'	Checkpoint	(Bessho and Sancar, 2000; Parker et al, 1998)
EXDL2	3'-5' [∞]	Unknown – ICLR?	(Smogorzewska et al 2010)
FAN1	5'-3'	ICLR?	(Kratz et al 2010; MacKay et al 2010; Smogorzewska et al 2010))
EXOG*	5'-3'	Multiple in mitochondria	(Cymerman et al, 2008)

* EXO1 also has (flap) endonucleolytic activity and 3'-5' polarity, whilst the major activities of FEN1 and APE1 are as Flap endonucleases. EXOG also has endonucleolytic activity. **WRN has 3'-5' exonuclease and a reported 5'-3' activity in conjunction with Ku70/86. [§]MRE11 contains the exonuclease activity. [∞]EXDL2 exonuclease activity is assumed by homology. BER – Base excision repair, DSBR – double-strand DNA break repair, MMR – mismatch repair, ICLR – interstrand crosslink repair

Table 1. Exonucleases discussed in this article

Table 2

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SF	**IPR002297** DNA-directed DNA-polymerase, family A, mitochondria						**IPR020045** 5'-3' exonuclease C terminal domain (CTD)		**IPR012547** PD-(D/E)xK	
	IPR012337 RNaseH-like			**IPR016265** DNA-directed DNA polymerase, family A, mitochondria, subgroup	**IPR001604** DNA/RNA_non sp_Endonuclease		**IPR004808** ExoDNase_II	IPR004808 ExoDNase_III	**IPR014808** DNA_replication_fac_Dna2	**IPR014883** VRR_NUC
F	**IPR013520** Exonuclease, RNase T/DNA polymerase III	**IPR002562** 3'-5' exonuclease	**IPR006133** DNA-directed DNA polymerase, family B, exonuclease domain		**IPR020821** Extracellular Endonuclease subunit A	**IPR018524** DNA/RNA non-specific endonuclease active site	**IPR006086** RAD2/XPG family **IPR000097** AP endonuclease family 1			
P	TREX1	WRN	Pol delta	Pol gamma	EXOG	ENDOG	FEN1	APE1	Dna2	FAN1
	TREX2	EXDL2	Pol epsilon		ENDOG		EXO1			
							XPG/ ERCC5			
							RAD2			

Table 2: Relationship of nuclease superfamilies, families and individual proteins. Note that some proteins (eg Fen1) constitute their own family or subgroup. SF = Superfamily F = family P – protein (see also http://www.ebi.ac.uk/interpro/)

Table 3
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Protein	Disease/dysfunction	Notes
Polymerase proofreader	Cancers	Lack of proofreading by replicative or repair polymerases leads to increased DNA mutation and cancer predisposition
TREX1	Aicardi-Goutieres syndrome, Lupus, Cree enchephalitis	Deleterious DNA mutation leads to immune system dysfunction by disruption of the inflammatory response
P53	Cancers	Most p53 mutations found in cancers are in the DNA-binding core region, which contains the exonuclease activity, suggesting loss of the proofreading functionality promotes cancers via mutation (cf. polymerase proofreaders)
APE1	Cancers, Alzheimer's disease, Parkinson's disease	Deficient BER causes genome instability and predisposition to cancers
FEN1	Cancer progression; triplet repeat expansion disease	Elevated mutation from compromised long patch BER facilitates cancer predisposition – triplet repeat expansion is common
XPG/ERCC5	Xeroderma Pigmentosum (XP), Cockayne syndrome (CS), Trichothiodystrophy, cancer	Dysfunctional NER leading to sensitivity to sunlight (UV) and increased genome instability. Some mutations can cause symptoms of Cockayne syndrome or TTD (premature ageing; neural and physical defects, skin and hair dysfunction). Note that mutation of the related XPF (endonuclease) results in severe progeroid phenotypes.
EXO1	Cancer, Immune deficiency	Cancer from compromised mismatch repair (HNPCC - Human non-polyposis colon cancer). Compromised immunoglobulin production owing to lack of class switching and somatic hypermutation
WRN	Werner syndrome (WS)	Segmental adult-onset progeroid syndrome from loss of the RecQ helicase/exonuclease WRN, which functions in multiple genome stability pathways (notably BER, replication restart and telomere maintenance); causes many symptoms associated with normal ageing to present early (for example, cancer, atherosclerosis and heart disease, physical deficiencies)
DNA2	None described	Defective DNA replication might cause genome instability and a rise in cancer predisposition
MRE11 (as part of MRN)	Nijmegen breakage syndrome (NBS); Ataxia Telangiectasia-like (AT-L)	A member of the MRN complex (with NBS1) and associates with ATM/ATR. Compromised DSBR and HJ resolution causes microcephaly and other physical defects, immunodeficiency, UV sensitivity and lymphoma predisposition
hRAD9	None described	Loss of the RAD9 DNA damage checkpoint might cause aberrant cellular division/loss of apoptosis presumably allowing cellular dysfunction and possible cancer predisposition
FAN1	Fanconi Anaemia	Nuclease associated with FA; symptoms are early

	(FA)	cancer (mainly acute myelogenous leukaemia) and bone marrow failure. Also associated with congenital defects, including physical and developmental disabilities, and early death.
EXDL2	None described	Putative association with cancer (see FAN1); may have a mitochondrial maintenance function
POLG	Deficiencies in mitochondrial polymerase gamma proofreading lead to premature ageing and multiple clinical features (see notes)	The human mitochondrial genome is densely-coded meaning that base substitution has a high chance of causing defective mitochondrial enzymology. Direct analysis of the importance of the proofreading function of polymerase gamma shows this - loss-of-function mutation of the proofreading exonuclease causes accelerated ageing symptoms in mice. Mitochondrial gene disruption causes a number of neuro-muscular disorders owing to the disruption of ATP production (for example Leigh's syndrome, NARP, MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes), MERRF (myoclonic epilepsy with ragged red fibres), LHON (Leber's hereditary optic neuropathy)
EXOG/ ENDOG	DNA editing and processing defects	None specifically associated, but loss gives rise to mitochondrial DNA instability and possible loss of genetic material. Mitochondrial DNA deficiency disease; mutations may cause mitochondrial diseases as listed above

Table 3: Clinical features associated with exonuclease mutation