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Emerging Links Between Premature Ageing and Defective DNA Repair

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We begin with the general question: Is mammalian ageing programmed through a molecular clock that is an integral component in normal development or is it simply the inevitable consequence of cumulative wear and tear on cellular genomes? The latter could encompass accumulated (un-repairable?) endogenous and environmental damage to DNA in addition to faulty processing of damage, and even accumulated alterations acquired during metabolic processing of *undamaged* DNA. That “metabolic processing” includes DNA replication and recombination in dividing cells, as well as transcription in all cells. Thus, to put it bluntly (and facetiously!), too much transcription might be bad for our health and longevity. It may be very difficult to distinguish genetically programmed causes of ageing from the impact of particular genes involved in DNA repair and maintenance of genomic stability.

An inborn deficiency in DNA repair *per se* does not result in premature aging. In particular, that is true for the ubiquitous pathway of nucleotide excision repair (NER). Young victims of xeroderma pigmentosum (XP) are deficient in NER and remarkably susceptible to cancer caused by unrepaired DNA damage, but while cancer is generally an age-related disease the XP patients do not otherwise exhibit symptoms of aging. The childhood victims of the hereditary progeroid disease, Werner's syndrome (WS), on the other hand, clearly present many of the hallmarks of ageing, but without significant loss of NER capacity. So what, if any, could be the connections between ageing and DNA repair deficiency? Although a number of studies have claimed to show a modest decline in overall repair capacity with age, a similar number have reported no significant differences between DNA repair in cells from newborn infants and in those from the elderly (cf. Hanawalt, 1987). In fact, if there were such differences, then all elderly people should express the high frequency of sunlight induced skin cancers and tumors characteristic of XP. Of course, the above remarks are restricted to NER; there are other important repair pathways to consider as well. These include mismatch repair, base excision repair (BER) that deals with oxidative base lesions, and the various schemes for rejoining exposed DNA ends at double-strand breaks; homologous recombination (ER), single strand annealing (SSA) and non-homologous end joining (NHEJ). Is there convincing evidence that any of these DNA repair pathways decline as a function of age, and/or that they are deficient in hereditary diseases of premature aging such as WS? As a start we can accept that defects in double-strand break repair and single-strand break repair have been implicated in neurodegenerative disease, but that these repair defects are not accompanied by premature ageing (Rass et al., 2007).

If we resolve the complexities of ageing into its segmental components, there are some plausible scenarios implicating reduced DNA repair at the organ level. Since neurons do not

proliferate and therefore do not need to pass the rigorous challenge of high fidelity DNA replication, they might be unusually susceptible to an accumulation of unrepaired damage that could eventually compromise cell function as well as trigger apoptosis. For the case of attenuated mental function, which is one of the hallmarks of advanced age (but not a universal one!), it is frankly worrisome that terminally differentiated neurons are remarkably deficient in global genomic nucleotide excision repair (GGR) (Nospikel and Hanawalt, 2000). Reduced GGR efficiency has also been reported for other types of post-mitotic cells such as myocytes differentiated to myotubes, and monocytes differentiated to macrophages (reviewed in (Nospikel and Hanawalt, 2002)). The “good news” is that genomic domains encompassing expressed genes are still proficiently repaired, and the subpathway of transcription-coupled repair (TCR) appears fully operational in terminally differentiated cells (Nospikel et al., 2006; Nospikel and Hanawalt, 2006)). As long as integrity of the essential active genes is maintained, the lack of efficient processing of damage in silent genomic domains should have no deleterious effects, because these post-mitotic cells are not expected to replicate their DNA. However, in the case of late-onset Alzheimer's disease, there is evidence that some terminally differentiated neurons attempt to reenter the cell cycle and replicate their DNA, and that reactivated expression of dormant genes occurs. Extensive amounts of accumulated DNA damage in those genes might overwhelm TCR and contribute to the neuronal apoptosis, which is observed in these patients (cf. Nospikel and Hanawalt, 2003). Another problem with accumulating DNA damage throughout much of the genome is that of the eventual certainty of closely spaced lesions, which can yield double strand breaks and chromosomal fragmentation; the consequent loss of genomic integrity would surely compromise cell function. It should be noted that about 20% of the XP patients, generally those with the most severe repair defects, exhibit neurological problems that include progressive neurodegeneration with peripheral neuropathy, ataxia and dementia, suggesting that NER can be important for normal brain function and development. The clinical hallmarks in Cockayne syndrome (CS) also include serious developmental and neurological defects, which might be attributed to the documented lack of TCR in these patients, with consequential high levels of cellular apoptosis due to transcription-arresting lesions, in particular those due to endogenous reactive oxygen species. Rass et al. (2007) point out that no direct correlation between unrepaired oxidative lesions in DNA and the observed neurological defects has yet been established in these repair deficient diseases. However, there exists another disease, UV sensitive syndrome (UVSS) in which TCR of UV-induced DNA damage is just as defective as it is in CS; the patients are exceedingly sensitive to sunlight, but in this case there are none of the neurological deficiencies or developmental problems seen in CS. Indirect evidence from host cell reactivation assays suggests that CS but not UVSS may be compromised for the repair of oxidative damage in expressed genes (Spivak and Hanawalt, 2006). This observation deserves further biochemical approaches to define the role of TCR in the repair of oxidative DNA damage in comparison to that for other types of damage.

The connection between oxidative stress and the processes of ageing was proposed half a century ago (Harman, 1956), and one of the popular models for ageing invokes the genomic accumulation of endogenous oxidative damage as a predominant cause. While this is an attractive model it has not yet been established as a significant contributor to mammalian ageing. It has been argued that a reduction in the efficiency of base excision repair (BER) could be responsible for the reported effects; relevant to that model is the report of reduced activities of the BER pathway enzymes, OGG1, DNA polymerase beta, and uracil DNA glycosylase in brain tissue from Alzheimer patients (Weissman et al., 2007). Moreover, some oxidative lesions, generated at the same time as those oxidized bases repairable by BER, are in fact repaired by NER. These include the cyclopurines (Brooks, 2007) and the products of lipid peroxidation, such as malondialdehyde (Cline et al., 2004). There is also a problem with intermediates in BER such as abasic sites, since abasic sites can be oxidized and then form covalent complexes with DNA polymerase beta during attempted BER and this complex lesion

would require NER for its resolution (Demple and Demott, 2002). These repair intermediates are also potent blocks to transcription and therefore may initiate apoptosis (Tornaletti et al., 2006; Wang et al., 2006). Cellular apoptosis triggered by whatever cause could certainly be a serious contributor to organ failure. One simple way to look at ageing is to consider it as the accumulated failure of individual organs. Cell death portends organ failure and subsequent organism demise.

An age-dependent increase in deletions in mitochondrial DNA has been shown in a number of studies and seemed to support a model of mitochondrial dysfunction leading to increased reactive oxygen production, which could be responsible for the mutagenesis. However, studies with proof-reading deficient mitochondrial DNA polymerase gamma in mice also led to mutations and deletions, with decreased lifespan, but no significant increase in oxidative stress (For review see: Passos et al., 2007). More recently Vermulst et al. (2008) have shown in a similar mitochondrial mutator model that while mitochondrial point mutations do not significantly contribute to lifespan of wt mice the deletions appear to be the “driving force” behind the premature ageing phenotype in the mutator mice, and furthermore that different tissues varied with respect to the rate at which the mutations reached phenotypic expression. It is a challenge to relate models for premature ageing involving mitochondrial dysfunction to those that arise from the phenotype of defective WRN.

The clinical presentation of WS is remarkably similar to that of normal human ageing in many respects. We know that the gene, *WRN*, when mutated can be responsible for the disease and that this gene codes for a RecQ family helicase with an associated 3'–5' exonuclease. No one could have anticipated that basic research leading to the discovery of a gene controlling thymineless death in *Escherichia coli*, would reveal a new helicase, RecQ, for which there are 5 homologues known in humans, 3 in *C.elegans* and 7 in *Arabidopsis* (Nakayama, 2005; Bachrati and Hickson, 2008). Furthermore, although these homologues are remarkably similar in their helicase domains, only one of them turns out to be the product of *WRN*. Note that a mutation in the *WRN* gene does not shorten lifespan in *C. elegans*, but this organism does not necessarily follow the same paradigm as mammals with respect to ageing mechanisms. In fact, that could also be said for the mechanism (and definition!) of ageing in the single-cell eukaryote, *Saccharomyces cerevisiae*. The next question is what, if anything, does WRN have to do with DNA repair, or more broadly, with DNA metabolism? In *E. coli* we know that the prototype RecQ operates at an arrested replication fork and that in conjunction with the RecJ single-strand 5'–3' exonuclease, it is responsible for the selective degradation of the lagging strand nascent DNA, and thought to thus participate in a RecA-controlled NER pathway involving fork regression, to reveal and remove the blocking lesion (Courcelle and Hanawalt, 2003). One of the other RecQ homologues, responsible for Bloom's syndrome (without premature aging), is also thought to operate in the resolution of arrested replication forks. However, the RecQ helicase in *E. coli* has also been implicated in the resolution of non-canonical DNA structures, such as G-4 rafts, that can pose blocks to the translocation of RNA polymerases (Bachrati and Hickson, 2008; Shen and Loeb, 2000; Duquette et al., 2004; Tornaletti et al., 2008). There could be important roles for WRN in the processing of other transcription-arresting DNA secondary structures as well. WRN has been shown to effectively resolve four-stranded DNA structures such as those found in telomeres, and the tetrahelical structures of the triplet repeat of d(GGG)n in fragile X syndrome (Fry and Loeb, 1999). Does it also participate in the repair of lesions, such as oxidized bases, that may be generated in sequences that can form these non-canonical structures? If so, then defects in this protein might be expected to compromise the maintenance of telomeres, which have been definitively implicated in cell senescence and organism aging. It has been reported that telomere DNA is less efficiently repaired than the bulk DNA, perhaps as a consequence of the unusual secondary structures of these chromosome ends. WRN interacts with a remarkable number of other proteins including p53, which in turn interacts with a phenomenally large assortment of proteins

and is an important transcription regulatory factor – yet loss of p53 has no significant impact upon ageing! WRN interacts with Ku, which would implicate it in NHEJ of double-strand breaks. Yet WRN does not appear to be absolutely required for repair of double strand breaks in general... but how about breaks in telomeres compared to breaks elsewhere in the genome? A defect in WRN might result in some subclasses of double-strand breaks being inefficiently repaired.

With respect to a role for WRN in delaying the processes of ageing there is a provocative “guilt by association” reflected in its “choice” of interacting partners, beginning with its association with BER proteins and its evident involvement in the repair of 8oxoG in particular. Although WRN does not appear to have an essential role in BER in general, it is worth noting that oxidative base damage accumulates in the cells from WS patients (Brosh and Bohr, 2007; Von et al., 2004). As with the discussion of double strand break repair above, WRN may assist with the BER pathway for certain classes, or subcellular localizations, of base damage. WRN is localized to telomeres where it likely plays a role in telomere maintenance and replication, particularly with regard to its well-known ability to unwind G4 tetraplex structures that are found in telomeres as noted above. The associations of WRN with particular proteins and structures are all seemingly consistent with an importance of oxidative DNA damage and telomere maintenance to longevity, again suggesting that the locale for the significant damage may be in the unusual DNA structures found in telomeres. In that regard it is of interest that WRN-deficient mammalian cells are notably sensitive to 4-nitroquinoline-1-oxide (a hallmark of WS), and that this carcinogen reacts strongly with the B-Z junctions at the ends of Z-DNA structures (Rodolfo et al., 1994)

The operation of WRN in its various roles may be modulated through post-translational processing of the protein (Kusumoto et al., 2007). Post-translational processing is an emerging theme in the discovery of mechanisms that coordinate the essential processes of DNA replication, recombination and repair (Hanawalt, 2007), and the study of such processing may offer further clues to the complexities of ageing. In conclusion it would appear that there is substantial evidence in support of a significant role for reactive oxygen species in ageing, but the processing of the resulting DNA damage is complex (and can involve combinations of the pathways of BER, NER, NHEJ, and perhaps others?) Damage in particular regions of the genome, such as those containing unusual DNA secondary structures, may also be of particular importance. All of this is compounded by the additional complexities of post-transcriptional modifications of the relevant proteins as a mechanism of control. Not to be overlooked as we complete this “Opinion” could be the contributions from epigenetic modifications of the genome. But could some of those modifications constitute the expression of instructions from a molecular clock that ultimately controls the processes of ageing?

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