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Does transcription-associated DNA damage limit lifespan?

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ABSTRACT

Small mammals undergo an aging process similar to that of larger mammals, but aging occurs at a dramatically faster rate. This phenomenon is often assumed to be the result of damage caused by reactive oxygen species generated in mitochondria. An alternative explanation for the phenomenon is suggested here. The rate of RNA synthesis is dramatically elevated in small mammals and correlates quantitatively with the rate of aging among different mammalian species. The rate of RNA synthesis is reduced by caloric restriction and inhibition of TOR pathway signaling, two perturbations that increase lifespan in multiple metazoan species. From bacteria to man, the transcription of a gene has been found to increase the rate at which it is damaged, and a number of lines of evidence suggest that DNA damage is sufficient to induce multiple symptoms associated with normal aging. Thus, the correlations frequently found between the rate of RNA synthesis and the rate of aging could potentially reflect an important role for transcription-associated DNA damage in the aging process.

1. Transcription accelerates the endogenous rate of damage to DNA

Inducing the transcription of a gene increases the rate at which it mutates, a phenomenon that occurs in bacteria, yeast, and humans [1–9] Transcription-associated mutagenesis (TAM) has been observed in experimental systems and its influence has been inferred from comparative genomics in yeasts and primates [9]. Genetic analysis of TAM in yeast indicates that transcription induces both base substitution mutations and deletions [10]. Most of the base substitution mutations are generated by Polζ, a translesion polymerase that incorporates nucleotides opposite damaged bases [10]. Deletions are generally caused by topoisomerase I mistakes made while the enzyme relaxes supercoils produced by RNA polymerases [10]. Thus, transcription induces multiple forms of DNA damage that in turn lead to genome instability.

Transcription-associated base damage is likely the result of the vulnerability of ssDNA to chemical attack. To transcribe a gene, RNA polymerase complexes must unwind the DNA double helix into single strands. Some chemical processes occur much faster in ssDNA than in double-stranded DNA. For instance, cytosine bases deaminate spontaneously to uracil 140- to 200-fold more rapidly [11]. When DNA containing uracil is used as a template for DNA replication or repair, C to T mutation can result. Genome sequencing has revealed that C to T mutation is the most frequent alteration in the human germline and in cancer cells, suggesting that the rate of cytosine deamination is an important determinant of the rate at which the genome loses information [12–14]. C to T mutation occurs more frequently in the non-transcribed strand of genes than in nearby intergenic regions that are not transcribed at all, consistent with the idea that the production of ssDNA during transcription increases the rate of spontaneous deamination [9,14].

G to T mutation also occurs frequently in the human germline and in cancer cells [12–14]. The mutation arises more frequently in the non-transcribed strand of genes than in nearby intergenic regions that are not transcribed [9,14], suggesting the involvement transcription-associated DNA damage. G to T mutation occurs when DNA containing 8-oxoguanine, a product of oxidative damage to guanine, is used as a template for DNA repair or replication [15]. The formation of 8-oxoguanine does not appear to be influenced by DNA conformation under physiological conditions or to correlate with the rate of transcription [16,17]. G to T mutation can also occur when the hydrolysis of guanine generates an abasic site. This hydrolysis reaction is one of the most rapidly occurring spontaneous chemical changes in DNA [11], and leads to G to T mutation when Polζ or other DNA polymerases insert dAMP opposite the resulting abasic site [18–20]. Guanine hydrolysis is known to occur about four times more rapidly in ssDNA than in dsDNA [11], so the elevated rate of G to T mutation in transcribed regions of the genome may be the result of ssDNA exposed during transcription.

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In addition to the transient production of ssDNA during routine transcription, secondary structures in DNA and extended RNA/DNA duplexes sometimes form as a result of transcription and trap the genome in a vulnerable ssDNA state for more extended periods [21–23]. Recent studies suggest that ssDNA is also vulnerable to base damage generated by cellular cytosine deaminases [24]. Thus, transcription is a double-edged sword that is essential for cellular function but causes DNA damage and a loss of genetic information over time.

2. DNA damage can cause symptoms of premature aging

DNA damage may be unique in its ability to induce multiple symptoms associated with old age. Repeated exposure of mice to near lethal doses of non-genotoxic poisons such as tetanus toxin, typhoid toxin, or turpentine causes debilitating stress but does not cause symptoms of premature aging [25]. Mice that lack adaptive immunity suffer from chronic inflammation, infection, and malnutrition but do not appear to age at an accelerated rate [26]. In contrast, exposure of rodents to ionizing radiation leads to the premature occurrence of a number of histological features of normal aging as well as a subset of symptoms associated with old age such as gray hair, kidney disease, cataracts, osteoporosis, neuronal atrophy, and muscle atrophy [27,28]. These aging phenotypes appear long after irradiation occurs and are therefore are not the immediate result of damage but are more consistent with a radiation-induced advancement of biological age [27,29]. This advancement occurs without a shortening of telomeres [28]. Humans exposed to ionizing radiation also exhibit a few symptoms of premature aging such as elevated rates of heart disease, cataracts, dementia, atherosclerosis, and reduced skin elasticity [28].

Ionizing radiation can cause multiple forms of cellular and extracellular damage, but the nuclear genome is particularly vulnerable because (1) it is present at extremely low levels in the cell – only four polynucleotide chains for each gene in non-diving cells – and (2) it cannot be replenished from precursors in the same way as other macromolecules because it provides the blueprint for the cell. The vulnerability of the nuclear genome to damage largely accounts for the cell death induced by ionizing radiation [30,31], and has led to the evolution of high fidelity DNA repair mechanisms. Excision repair mechanisms take advantage of redundant genetic information within the double helix, and homology-directed repair uses redundant information within the tethered sister chromatids [32]. However, high fidelity DNA repair is not possible or extremely inefficient when both strands of the double helix are damaged at the same time in G_1/G_0 phase [31]. Damage to both DNA strands destroys the redundant information within the double helix and prevents high fidelity excision repair [33], and cells in G_1/G_0 phase do not have a sister chromatid to provide a template for homology-directed repair [32]. This particular limitation of the body's capacity for DNA repair renders the genome vulnerable to ionizing radiation, which is highly effective at generating DNA double-strand breaks (DSBs) [31].

DSBs generated in G_1/G_0 phase cells are repaired by the mutagenic non-homologous end-joining pathway or left unrepaired [34]. Loss of genetic information during mutagenic repair could potentially induce symptoms of aging by reducing cellular function or causing cell death. In addition, there is mounting evidence that unrepaired DSBs accumulate during normal aging [35]. Unrepaired DSBs may lead to symptoms of aging by reducing gene function directly, or by exposing chromosome ends to the DNA damage checkpoint signaling system which induces apoptosis and cellular senescence [36]. Consistent with the idea that unrepaired DSBs can cause symptoms of aging, mice lacking the non-homologous end-joining factors Ku70 or Ku80, which function in telomere maintenance and in the repair of DSBs in nuclear DNA [37], exhibit multiple symptoms of premature aging in the absence of irradiation [26,38]. Furthermore, nuclear expression of an endonuclease in the mouse liver causes several histopathologies associated with normal liver aging [39].

Exposure of mice to nitrogen mustard induces mutations and cancer, but does not accelerate the rate of aging [25]. Likewise, defects in mismatch repair cause severe genome instability and cancer in both mice and humans but also do not lead to premature aging [40]. These observations could be taken to indicate that DNA damage or genome instability is not sufficient to accelerate aging. However, it is worth noting that nitrogen mustard and mismatch repair defects cause little or no genome instability in non-dividing cells [25]. To induce symptoms of aging, it might be particularly important to damage the DNA of non-dividing cells given that quiescent stem cells act as a reservoir of genetic information in proliferative tissues and that aging is associated with the degeneration of post-mitotic cells such as neurons and muscle cells [41,42]. Consistent with the idea that conserved aging processes can occur in non-dividing cells, aging in the roundworm *Caenorhabditis elegans*, where the adult has only post-mitotic somatic cells, shares significant similarities with aging in mammals such as common genetic pathways regulating lifespan and life extension induced by caloric restriction [43,44].

Werner syndrome (WS) is a rare genetic disease characterized by striking symptoms of accelerated aging. These include cataracts, gray hair, atherosclerosis, arteriosclerosis, hypermelanosis, osteoporosis, and atrophy of skeletal muscle, connective tissue, the cerebral cortex, and the thymus [45]. There is a general consensus that these symptoms are caused by DNA damage [46], but the exact nature of the damage is unclear. Initial studies of cells from patients with WS found no obvious DNA repair defect but revealed multiple abnormalities associated with cell proliferation such as chromosomal instability, slowed S phase, and reduced replicative capacity [47,48]. Despite these cellular phenotypes, highly proliferative tissues such as the gastrointestinal epithelium and the hematopoietic compartment appear to be relatively normal in WS patients, and cancers of epithelial or hematopoietic origin are not common [47]. Instead, less proliferative connective tissue cells are disproportionately affected by the disease and give rise to cancers at a dramatically elevated rate [45,47,49]. Thus, mutations in the *WRN* gene that cause WS may reduce genome stability in non-dividing cells as well as in proliferating cells. Consistent with this idea, knockdown of *WRN* in non-dividing primary human fibroblasts induces the formation of nuclear γH2AX and 53BP1 foci, markers of DNA damage [49]. Likewise, disruption of the yeast *WRN* ortholog *SGS1* causes severe genome instability in non-dividing cells [50].

The WRN protein contains a helicase domain related to bacterial RecQ that can unwind DNA and RNA/DNA duplexes in vitro [46,51]. In addition, the protein contains an exonuclease domain most closely related to that of bacterial ribonuclease D [52], as well as an HRDC domain common to ribonuclease D, RecQ, and components of the human exosome complex involved with processing ribosomal RNAs [53,54]. WRN is localized to the nucleolus, a phenomenon that requires active RNA polymerase I transcription [55]. WRN-defective cells synthesize RNA at a lower rate than normal cells [46,55–57], as do yeast cells defective in Sgs1 [58], suggesting a possible role for WRN in transcription or in the elimination of abnormal structures that impede transcription. Extended RNA/DNA duplexes referred to as R-loop structures sometimes form during transcription and can lead to genome instability [7]. WRN can degrade the RNA strand of RNA/DNA duplexes in vitro [59] as well as unwind these structures, suggesting a potential role for WRN in the elimination of R-loops. Thus, WRN may contribute to genome stability in non-dividing cells by preventing transcription-associated DNA damage.