A6. Oxidative Modification of DNA

Significant evidence suggests oxygen free radicals are linked to aging and diseases. Mutations caused by hydroxylation reactions (presumably from the generation of hydroxyl free radicals as shown above) can potentially lead to cancer. Recently it has been shown that mitochondrial DNA is more susceptible to oxidation than nuclear DNA. Human mitochondria has its own small genome (16.5 Kb compared to the nuclear genome of 3 Gb) which code 13 protein subunits involved in respiration, 22 tRNAs and two ribosomal RNAs. (The mitochondria presumably are vestiges of a bacteria which invaded an early cell and established a symbiotic relationship with the cell). A recent study has shown that there is an inverse correlation of oxidized mitochondrial DNA [8-oxoG] with maximal life span of an organism, but this correlation is not seen with nuclear DNA. Presumably the nuclear DNA is somewhat protected from oxidative damage since it is bound to histone proteins (which form nucleosome core particles with DNA) and by DNA repair enzymes. DNA repair enzymes that are encoded in the nucleus are found in the mitochondria and mitochondrial DNA is package with mitochondrial transcription factor A (TFAM). Examination of human bladder, head and neck and lung primary tumors reveals a high frequency of mitochondrial DNA mutations. In addition most dioxygen use by the cell occurs in the mitochondria. Hence this organelle probably faces the highest concentration of toxic oxygen reduction products. Recently, the crystal structure of an enzyme, adenine DNA glycosylase (MutY), that repairs 8-oxyG modified DNA has been determined in complex with the oxidatively damaged DNA. If not repaired, the 8-oxyG base pairs with adenine instead of cytosine, causing a GC to AT mutation on DNA replication.

Although oxidative damage in mitochondria clearly can promote premature aging, other independent mechanisms may also. In a recent study, Kujoth et al. developed a mouse model that expressed a mutant form of mitochondrial DNA polymerase that was defective in the proofreading activity of the enzyme. These mice displayed premature aging but...
showed no increased levels of oxidized mitochondrial lipids or hydroxylated G residues in mitochondrial DNA. They did show significant activation of a cytosolic enzyme called caspase-3, which when active lead to the programmed death of cells (a process called apoptosis). This calcium-activated aspartic acid proteases (with an active site Asp) is activated by binding mitochondrial cytochrome C that has "leaked" into the cytoplasm from its normal location in the intermembrane space in mitochondria. The process is usually associated with DNA damage (mutations, fragmentation) that would arise if the proofreading function of DNA polymerase was defective. This was indeed found in these mice.

Oxidative damage to biomolecules might not initiate aging and disease processes, but rather might be a secondary effect of other initiating events. Reversing or preventing oxidative damage might slow the progression of aging and disease. Aging is a complex feature of organisms and would be expected to have complex causes and biological effects. At the organismal level, aging has been studied in the round worm C. elegans which lives for only a few weeks. Genetic analyses can be easily used to find gene alterations associated with premature aging. One hormonal system that has recently been associated with aging in eukaryotes (and in C. elegans) involves the signaling pathways for insulin and insulin growth factor I (IGF-1), which regulate carbohydrate, lipid, and reproductive pathways in C. elegans. Mutations that decrease signaling from this pathway increase C. elegans life span. These mutations lead to increased activity of the DAF16 transcription factor, which upregulates the expression of many genes. In contrast, wild type organisms, when exposed to insulin or IGF-1, decrease the activity of DAF16. Using DNA microarrays, investigators determined which DAF16-controlled genes were upregulated in mutant worms in the mid-life point of the organism. These genes included, among others, peroxisomal and cytosolic catalase, Mn-superoxide dismutase, cytochrome P450s, metallothionein-related Cd-binding protein, and heat shock proteins. We will investigate the function of several of these gene products in the next section, but needless to say, they are all involved in cellular responses to stress, often involving dioxygen metabolites. Over expression of mitochondrial catalase in mice increased their lifespan by 20%. It has also been showed that decreased levels of insulin-like growth factor also promote longevity in mice, indicating again that mechanisms in addition to oxidative damage by ROS are involved in aging.

Contributors

- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)