307P CELLULAR MECHANISMS OF AGEING

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Evolutionary theories of ageing indicate that ageing results from the accumulation of unrepaired faults in somatic cells, and that there is unlikely to be any one, single mechanism of ageing.

Specific cellular mechanisms for which growing support exists include instability of the somatic nuclear genome (including progressive telomere loss), instability of the mitochondrial genome resulting in an intracellular proliferation of defective mitochondria, accumulation of general oxidative damage resulting from the actions of reactive oxygen species (including free radicals), the accumulation of aberrant proteins (mis-translated, mis-folded, incorrectly modified via post-translational modification systems), and the accumulation of damage in membranes. It is likely that most of these general mechanisms may contribute in some part to the build-up of senescent changes in most tissues of the body.

Nevertheless, in different cell types one or other mechanism may predominate. In dividing cells, accumulation of faults in proteins and membranes is checked by dilution through ongoing *de novo* synthesis. However, genomic instability, especially telomere loss may be particularly important in these cells. In post-mitotic cells such as neurones and muscle cells, genomic instability is less of a threat but a build-up of protease-resistant damaged products may be of major importance. We have demonstrated with theoretical models that it is important to consider interactions between mechanisms.

In general, the study of cellular mechanisms of ageing requires an integrative approach which also allows for the dynamics of cell deletion via apoptosis or necrosis and cell renewal by division. Our recent work on age changes in stem cells of the intestinal epithelium, and on the association of cellular stress response mechanisms with species life spans, provides illustrative examples.

308P REPAIR OF ENDOGENOUS DAMAGE TO DNA

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In each cell, several thousands of DNA base residues in the human genome are converted daily to potentially mutagenic or cytotoxic forms by spontaneous damage inflicted by water, active oxygen, or reactive metabolites. The different branches of the excision-repair pathway for removal of such endogenous DNA damage have been reconstituted with human enzymes overexpressed from cloned cDNAs. Recent work has concentrated on repair of oxidative DNA damage, which shows several differences from the repair pathway for removal of hydrolytic damage from DNA. Excision-repair usually occurs by a short patch, one-nucleotide gap pathway.

The two most abundant miscoding lesions generated by endogenous DNA damage are 8-oxoguanine derived by guanine oxidation, and uracil derived by hydrolytic deamination of cytosine. Each of these lesions is removed by a distinct DNA glycosylase. Viable -/- knockout mice, deficient in one or other DNA glycosylase, have been constructed. Studies on the levels of spontaneous mutagenesis in these animals are in progress. With regard to the uracil-DNA glycosylase knockout mice, small amounts of a different backup enzyme for uracil excision from DNA have been detected in cell extracts from certain tissues of these animals. In contrast, no compensatory backup glycosylase activity has been detected in the 8oxoguanine-DNA glycosylase deficient mice. The consequences of accumulation of mutagenic lesions in mammalian DNA will be described.

309P GENETICS AND TRANSGENICS: WHAT DO THEY TELL US ABOUT AGEING?

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For many years now our laboratory has been interested in *testing* the validity of the Oxidative Stress Hypothesis of which, in essence, postulates that accrual of macromolecular oxidative damage is a primary underlying cause of the physiological attrition associated with senescence. There are multiple lines of evidence which strongly implicate oxidative e stress as a major causal factor in senescence. For instance, studies in our lab and others consistently provide evidence for a correlation between the age-associated increase in oxidative molecular damage and losses in the efficiency of homeostatic mechanisms. More recently, we have provided evidence suggesting that the targets of damage that underlie this deterioration are not random but specific.

If oxidative stress is a causal factor in ageing, it would follow that the ageing process may be governed by genes involved in: (1) the regulation of the redox state, i.e., antioxidative defenses and ROS generation; (2) the repair or elimination of oxidized macromolecules and (3) the specific targets of oxidative damage. To test this idea more directly, it is advantageous to adopt a genetic approach, which permits one to establish a causal rather than a strictly correlational relationship. Thus once candidate genes are identified the isolation and analysis of appropriate mutants and transgenics permit their validation as genes that affect longevity.

The oxidative stress hypothesis predicts that increasing the antioxidative defenses would slow down the ageing process by protecting the tissues against oxidative damage and functional compromise, while reducing the antioxidative defenses would have the opposite effect. In *Drosophila*, the impact of reduced levels of catalase or Cu,Zn SOD is a significant reduction in life span and/or metabolic potential.

Although few effects on life span were noted in transgenics overexpressing either Cu,Zn SOD alone or catalase alone, in transgenic lines overexpressing both together, there was a reduction in the rate of accumulation of oxidative damage, accompanied by an increase in life span that was both chronological and physiological. In other words, there was a true extension of life span as measured by metabolic potential (in this case the overall lifetime consumption of O^2 of the experimental groups relative to the controls) as well as an up to 34% increase in mean and maximum life span. In a separate study, Parkes *et al.* (*Nature Genet* 19, 171, 1998) showed that overexpression of Cu,Zn SOD alone in motorneurons has a similar effect, increasing life span by up to 40% with a comparable effect on metabolic potential.

Thus, the genetic manipulation of antioxidative defenses can have a dramatic impact on the accumulation of oxidative damage and the rate of ageing. It will now be important to refine our mechanistic understanding of the antioxidative network by further genetic and biochemical analyses and to identify any selective targets of oxidative damage that could provide important clues to specific functional deterioration. Our preliminary efforts in this direction will be described.

310P THE AGEING MITOCHONDRION: A TARGET FOR THERAPEUTIC INTERVENTION?

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Free radicals are involved in ageing. Experimental evidence supports the free radical theory of ageing, especially the extension of mean lifespan obtained by increasing the antioxidant defense.

The role of mitochondria in the generation of oxidative stress associated with aging was postulated some twenty years ago. Several studies had shown, using isolated mitochondria, that mitochondrial function is impaired in aging. We will report experiments showing for the first time (Sastre *et al.*, 1996) the impairment of mitochondrial function within intact cells of old animals. This has been achieved using both a metabolic approach - the study of specific metabolic pathways that involve both cytosol and mitochondria - and a flow cytometric approach. Mitochondria from old animals have a lower membrane potential and produce more peroxides than those from young ones. Specific transport systems such as the one for malate are impaired with aging. Other important mitochondrial functions like oxidative phosphorylation are also affected.

These changes may be due to oxidation of key molecules such as mitochondrial DNA, which is affected in aging. Mitochondrial DNA (mtDNA) is specially susceptible to oxidative damage and mutation because it lacks protective histones or effective repair systems. Indeed, levels of oxidative damage to mtDNA are several times higher than those in nuclear DNA, and mtDNA mutates several times more frequently than nuclear DNA.

Glutathione is oxidized in aging. changes in GSSG/GSH with aging are an order of magnitude higher in mitochondria than in cytosol. We found that there is a relationship between oxidation of glutathione and oxidative damage to mtDNA. These changes are prevented by oral administration of antioxidant vitamins (Garcia de la Asuncion *et al*, 1996).

Ageing mitochondria can also be protected by other antioxidants, for example polyphenols contained in extracts of plants such as Gingko Biloba (Sastre *et al.*, 1998).

Mitochondria can also be protected from oxidative stress generated in other diseases. For instance, we recently found that administration of the antiretroviral drug AZT results in oxidative damage to muscle mitochondria. In this case, also, administration of vitamins C and E protected mitochondria (and muscle cells) from damage caused by AZT (García de la Asunción *et al.*, 1998).

A conclusion that can drawn from these studies is that mitochondria can be protected from oxidative stress by administration of antioxidant nutrients.

Garcia de la Asuncion *et al* (1996) *FASEB Journal* l0, 333-338

García de la Asunción *et al.* (1998) *J. Clin. Invest.*. 101, 1-6

Sastre *et al.* (1996) *Hepatology* 24, 1199-1205.

Sastre *et al* . (1998) *Free Rad Biol Med* 24, 298-304