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## Society for Experimental Biology Annual Main Meeting 31st March – 4th April 2007, Glasgow, Scotland

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### CSS–CROSS SECTIONAL SESSION — ANTIOXIDANTS AND AGING

#### CSS.1

##### **Why aren't babies born old and why doesn't mutton taste like lamb?**

R. Faragher, (University of Brighton, United Kingdom)

Ageing is a universal, progressive, intrinsic and degenerative process which probably evolved over a billion years ago. 40 years ago the Society for Experimental Biology held a special symposium to examine the similarities and differences between the ageing of plants and animals. In the spirit of that Symposium this lecture will review progress in the area of the cross-kingdom biology of ageing with an emphasis on what is known about why animals age and how they do it.

Ageing probably evolved in non-ageing organisms as the unintended consequences of selection for mutations which favour early life fecundity. This unprogrammed process, known as antagonistic pleiotropy, should be operative on any organism for which the chances of survival decline with increasing chronological time. However, there is little evidence that the concept has been widely applied to the evolution of ageing and mortality in plants.

Individual animals age through the progressive accumulation of subtle alterations in the functional capacity of tissues which compromises the ability of the animal to withstand physiological stress. These degenerative changes include damage to long lived macromolecules such as structural proteins, the death or altered function of post-mitotic cells and the senescence of cells which have a capacity for division during the lifespan of the animal. However, within these broad confines the mechanisms by which different species of animal may age seem to vary quite widely. Thus it is possible that at least some similarities in ageing mechanisms exist between some animal body forms and those of some plants.

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#### CSS.2

##### **Redox metabolism in plants and its role in programmed cell death and senescence**

C. Foyer, (University of Newcastle, United Kingdom)

Plants have optimized strategies for redox regulation particularly regarding the generation and accumulation of reactive oxygen species (ROS). Low molecular weight antioxidants such as ascorbate and glutathione are information-rich redox buffers that control ROS accumulation and interact with numerous cellular components. They provide essential information on cellular redox state and influence gene expression associated with biotic and abiotic stress responses to maximize defense. Ascorbate and glutathione act as barometers of plant health and are important in senescence. While it is widely accepted that senescence is accompanied by increased cellular oxidation that triggers activation of the 26S proteasome system, the relationship between protein oxidation and senescence in leaves is far from clear. Moreover, the onset of leaf senescence can be delayed by inhibition of cysteine proteinases. A characteristic transcriptome signature has been obtained for young and senescent maize leaves grown in air and with carbon dioxide enrichment. Surprisingly, although about 3000 transcripts were differentially expressed in senescent leaves compared to young leaves, only 18 high CO<sub>2</sub>-induced transcripts were common to both mature and senescent leaves.

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#### CSS.3

##### **Elucidating signaling pathways that control Arabidopsis leaf senescence**

V. Buchanan-Wollaston, E. Harrison, E. Breeze, (Warwick HRI, United Kingdom)

Leaf senescence is a programmed event responding to a wide range of external and internal signals. It requires de novo gene expression and protein synthesis and is controlled in a tightly regulated manner. Many different genes show enhanced expression during senescence and elucidation of the roles of these genes is throwing light on the mechanisms that occur during the senescence process. Elucidation of the genes that control senescence has been complicated by the complex combination of signalling pathways that appear to be involved in senescence. Cross talk exists between senescence and stress or pathogen responses and also hormonal and nutrient signals are implicated in the control of senescence. The role of oxidative stress in controlling senescence is likely to be significant but has not been clearly demonstrated.

We are using *Arabidopsis* as a model, to study the genes involved with the control of leaf senescence. Extensive microarray analysis over a detailed time course of development is being used to identify transcriptional networks that operate to control gene expression during leaf senescence. Cross talk between stress related pathways, oxidative stress responses and senescence is being elucidated by the use of mutants, treatments and comparative gene expression analysis.

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#### CSS.4

##### **Negative linear correlation between human lifespan and exercise intensity**

A. Brierley, (University of St. Andrews, Scotland)

Moderate levels of physical activity are generally considered to confer health benefits over a sedentary lifestyle, and playing sports may increase lifespan. Conversely, energy production in the mitochondria generates free radicals that can cause cellular damage and ageing, raising the possibility that high intensity exercise might shorten lifespan. Previous studies have, however, failed to demonstrate a negative correlation between exercise intensity and longevity. This has brought in to question the notion that free radicals are damaging to health and, in a surprising new twist, it has been theorised that they may actually contribute to longevity. Here the longevities of the winners of Europe's three cycling Grand Tours – the Tour de France, Giro d'Italia and Vuelta a España – are examined alongside winning speed cubed, a proxy for rider power development/exercise intensity. As races become more competitive over time, winning speed and the effort required to win increases, and the longevity of winners has decreased significantly over time during an era when lifespan generally in the European population has increased. A significant negative linear relationship ( $r^2 = 0.348$ ,  $P < 0.001$ ,  $n = 46$ ) between life deficit (difference between observed and expected lifespan) and exercise

intensity is apparent, providing evidence to support the contention that mitochondrial oxidative stress is in fact genotoxic. This analysis may guide the development of an optimal level of exercise intensity for humans that protects against obesity and the associated maladies but does not lead to premature ageing and death.

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#### CSS.5

##### **The functional involvement of lamin A and LAPa in human ageing: the role for lamina protein redox modifications in senescence signaling**

V. Pekovic, A. Benham, D. Dixon, R. Edwards, C. Hutchison, (University of Durham, UK); I. Kill, (Brunel University, UK); K. Bushby, (University of Newcastle-upon-Tyne, UK); R. Foisner, (Medical University of Vienna, Austria)

Mutations in the gene encoding a nuclear intermediate filament protein, lamin A, cause a range of age-associated and premature ageing diseases, collectively termed laminopathies. These findings prompted us to explore whether lamin A plays a role in normal ageing process. Here we show that human fibroblasts aged in vitro acquire a range of nuclear phenotypes reminiscent of progeroid-like fibroblasts. These include dysmorphic nuclei and defective nuclear assembly of lamin A and their binding partner LAP2a (lamina-associated polypeptide 2a), all of which strongly correlate with cell cycle arrest. The C-terminal domains of lamin A and LAP2a contain multiple cysteine residues, which undergo oxidative modifications in senescent fibroblasts. These modifications inhibit the formation of higher-order disulphide-linked structures of both proteins and led to a partial proteolysis of lamin A within its tail domain. Consequently, lamin A and LAP2a fail to tether the nucleoplasmic forms of retinoblastoma protein within the nuclei of late-passage fibroblasts, which we have recently reported to be required for maintaining a proliferative state in early-passage fibroblasts. Consistent with these findings, addition of extracts from senescent fibroblasts to a *Xenopus* in vitro nuclear assembly system caused oxidative modifications to C-terminal cysteine residues in *Xenopus* lamin LIII and inhibited nuclear lamina assembly and DNA replication. Our data show that lamin A and LAP2a act as oxidative stress sensors and are central components of senescence pathway. Our findings suggest a novel model for ageing of human fibroblasts in vitro whereby the accumulation of oxidative damage to lamin A and LAP2a contributes to senescence pathway by de-stabilising the nuclear architecture.

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**CSS.6****Membrane fatty acids and maximum lifespan in mammals — A reassessment**

T. Valencak, T. Ruf, (Research Institute of Wildlife Ecology, Veterinary University Vienna, Austria)

Although generally considered as beneficial dietary components, polyunsaturated fatty acids (PUFAs) are suspected to compromise lifespan in mammals. The negative correlation between maximum lifespan of mammals and the proportion of PUFAs in tissue membranes has been explained by biochemical properties of PUFAs. As polyunsaturated fatty acids are particularly prone to peroxidation, they are considered a source of potent damagers of other cellular molecules. The “membrane pacemaker theory of aging” proposes that the amount of polyunsaturated fatty acids in membranes is a determinant of lipid peroxidation and consequently of the rate of free radical production and aging. We re-analysed the relation between PUFAs and longevity in 42 mammalian species, but also incorporated the influences of body weight and phylogeny by using multivariate conventional and phylogenetic regression. We found that maximum lifespan significantly decreased as the ratio of n-3 PUFAs to n-6 PUFAs in muscle phospholipids increased. We could not confirm, however, the previously reported correlation between the unsaturation index and the maximum lifespan of mammals. Also, we found no support in our data for peroxidisability being the reason for the inverse relationship between lifespan and membrane unsaturation. The muscle phospholipid content of the very long chain and highly unsaturated fatty acid, docosahexaenoic acid (DHA), which is most susceptible for peroxidation, did not have an influence on maximum lifespan of mammals. The implications of these findings will be discussed.

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**CSS.7****The curiously complex role of mitochondria in ageing**

T. Kirkwood, (Newcastle University, United Kingdom)

Extensive evidence points to an important role for oxidative stress in ageing. We and others have shown that mitochondrial defects accumulate with age, which might either underpin age-related changes in exposure to oxidative stress or signal the cumulative consequences of stress-induced damage. Recently we also found that mitochondrial defects show important interactions with telomere-driven cell senescence and that heterogeneity in mitochondrial function is linked to cell-to-cell variation in cell division potential. In order to understand cause and effect in terms of the role of mitochondria in ageing it is important to take account of the dynamics within the cellular mitochondrial population. This requires a systems biology

approach in which we are combining mathematical modelling with experiments.

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**CSS.8****The free radical theory of ageing in the genome era**

J. de Magalhaes, (Harvard Medical School, Massachusetts, United States)

Proposed in 1956 by Denham Harman, the free radical theory of ageing is arguably the most accepted mechanistic explanation for ageing. Nonetheless, our understanding of ageing and of the role of reactive oxygen species (ROS) in this process has changed much in recent decades. We now know of many genes that can modulate ageing in model organisms, suggesting that gene networks regulate the ageing process. It also appears that ROS are not mere sources of damage but crucial signalling molecules in, for instance, development and growth. To help study and elucidate the increasingly complex gene networks of ageing, we developed GenAge (<http://genomics.senescence.info/genes/>), the first curated database of genes possibly related to human ageing. We also statistically evaluated whether genes, including antioxidants, regulating longevity in rodents influence ageing or rather solely impact on age-independent mortality. Although Harman's theory provided an elegant explanation for the negative correlation between metabolic rates and a species' longevity, using AnAge, an ageing-related database of over 3000 animal species (<http://genomics.senescence.info/species/>), we recently showed that metabolic rates, when corrected for body size and phylogeny, do not correlate with longevity in placental mammals. Trying to gather clues about species differences in ageing, we studied the evolutionary forces acting on ageing-related genes, yet such genes appear to evolve slowly since humans and chimpanzees diverged and we failed to find any evidence of selection on antioxidant defences. Finally, we have been integrating computational and evolutionary perspectives into testable hypotheses and are constructing stress-resistant mice.

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**CSS.9****Sulforaphane suppresses reactive oxygen species and induces transcriptional activation of antioxidant response element related genes expression in human BJ-5ta fibroblasts**

M. Xue, P. Thornalley, (University of Warwick, United Kingdom)

*Background and aims:* Many studies have assessed that sulforaphane (SFN), a bioactive isothiocyanate absorbed from a precursor in broccoli, is a potent inducer of antioxidant responsive element (ARE) linked gene expression. The effectiveness of SFN at concentrations associated with dietary

intake remains unclear. In this present study, the reactive oxygen species (ROS) and expression of 15 ARE-linked genes (GSR, TKT, GCLM, GCLC, PRDX1, TALDO1, AKR1C1, HMOX-1, TXN, TXNRD1, CAT, NQO1, SOD1, NRF2, KEAP1) were investigated in hTERT-immortalized human BJ-5ta fibroblasts. *Methods:* BJ-5ta fibroblasts were cultured in a 4:1 mixture of Dulbecco's medium and Medium 199 at 5%CO<sub>2</sub> and 37 °C. Intracellular ROS production was assessed by loading dihydro-fluorescein diacetate and measurement of ROS-induced fluorescence by microplate fluorimetry. ARE-linked gene expression was assessed by real-time PCR using SYBR Green fluorescent probe and appropriate primers after SFN (4 iM) treatment for 1–24 h.

*Results:* ROS-induced fluorescence was decreased in a dose-dependent manner (0.5 – 5 μM) after exposure 24 h. For gene expression, increased expression of 11 ARE-linked genes was induced by SFN. This effect appeared at 6 h; the increased expressions of GCLM, GCLC, and HMOX1 were earlier than of other ARE-linked genes. Only SOD1, HMOX1 and PRDX1 failed to show SFN-inducible expression.

*Conclusions:* Low concentrations of SFN (0.5–5 μM) induced the expression of ARE related-genes. This provides support for further work on chemoprevention to oxidative and glycation damage of proteins and genome by SFN in ageing and age-related diseases.

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### CSS.10

#### **Evidence of advanced glycation endproducts, oxidation and nitrosation damage in Arabidopsis leaves**

U. Bechtold, (University of Essex, United Kingdom); N. Rabbani, (University of Warwick, United Kingdom)

Oxidative stress causes damage to important biological structures such as proteins. In all aerobic organisms, a major loss of protein function occurs through oxidation and is implicated in a number of degenerative diseases and ageing in mammalian systems. Depending on the amino acid and the oxidising agent, products formed are varied and until now have been difficult to detect and/or discriminate in living organisms. This report is the first time that advanced glycation endproducts (AGEs), oxidation and nitrosation products have been detected simultaneously in total leaf extracts of Arabidopsis plants. Up to eleven different amino acid modifications are detected in plants grown under non-stress conditions. Analysis throughout a diurnal cycle showed clear differences in a number of oxidative modifications between the light and dark phases under non-stress conditions. A mutant (pmsr2-1) lacking a protein repair mechanism, showed an increase in a broad range of AGEs, oxidation and nitrosation products in comparison to wild-type. The pmsr2-1 mutant is known to undergo increased protein turnover during the dark period resulting in increased oxidative stress. Short term excess light exposure on the other hand, does not lead to a general

increase in protein oxidation. During excess light stress increases in tryptophan, arginine and tyrosine oxidation are noticeable suggesting that increases in ROS within the chloroplast may act on specific targets and thus may be involved in regulatory functions. The possibility of a signaling function of these protein oxidation products in the integration of seasonal and/or environmental changes is currently being investigated.

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### CSS.11

#### **Rebirth and death: Nitric oxide and reactive oxygen species in seeds**

P. Bethke, (USDA ARS University of Wisconsin Madison, United States); I. Libourel, R. Jones, (University of California Berkeley, United States)

Plant seeds provide excellent opportunities to study well-defined progressions of physiological changes at the single cell, tissue and whole organism levels. Most seeds are dormant at maturity, and dormancy must be lost before germination can occur. The emergence of the embryo and its early growth depend on the coordinated activities of the embryo itself and the tissues surrounding it. We have used Arabidopsis seeds and barley grains to study the roles of plant hormones, nitric oxide (NO), and reactive oxygen species in dormancy loss, germination and post-germinative growth. Our data support the following sequence of events for dormancy and germination in these species. (1) NO produced by the seed is perceived by the aleurone layer, a tissue that surrounds the embryo, and NO-perception promotes dormancy loss. (2) NO perception leads to the transcription in the embryo of genes for the biosynthesis of the plant hormone GA. (3) GA produced by the embryo up regulates the conversion of stored lipid to sugar in the embryo and aleurone. This nourishes the embryo and provides substrates in the aleurone layer for the synthesis and secretion of hydrolytic enzymes. (4) Cell wall degrading hydrolases weaken the aleurone cell wall and allow the embryonic root to break through. (4) Hydrogen peroxide is generated as a by-product of lipid breakdown. In barley GA represses the production of enzymes that metabolize reactive oxygen species. (5) Aleurone cell death occurs as a result of reactive oxygen species damage, but NO acts as an antioxidant and delays death.

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### CSS.12

#### **Mitochondrial ROS production**

M. Brand, (MRC Dunn Human Nutrition Unit, United Kingdom)

Reduction of oxygen by single low-potential electrons in the mitochondrial respiratory chain produces most of the reactive

oxygen species (ROS) in typical cells. Such ROS production may underlie ageing: mitochondria from long-lived species have lower ROS production than mitochondria from short-lived species. Understanding the sites and topology of ROS production can illuminate the mechanisms involved, and understanding how mitochondria regulate ROS production can suggest ways to attenuate cellular oxidative damage and combat degenerative diseases and ageing.

Different sites in the electron transport chain produce superoxide to different sides of the membrane. Complex I and ETF-Q oxidoreductase produce superoxide exclusively in the matrix, whereas glycerol phosphate dehydrogenase and centre o of Complex III produce it about equally in the matrix and intermembrane space. Mitochondrial ROS production is very sensitive to protonmotive force, so mild uncoupling, which reduces protonmotive force slightly, attenuates ROS production at the expense of slight inefficiency.

Uncoupling proteins (UCPs) appear to be specialised to cause mild uncoupling. Analysis of the activation of the proton conductance of UCPs by ROS and analogues leads to a model in which endogenous superoxide generates carbon-centred radicals on fatty acyl chains of membrane phospholipids. These initiate a cascade of lipid peroxidation reactions that form reactive alkenals, particularly 4-hydroxynonenal, which activates UCP proton conductance. This sets up a simple feedback loop that attenuates mitochondrial ROS production by mild uncoupling when it becomes dangerously high, and may protect against oxidative damage.

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### CSS.13

#### Is superoxide dismutase a determinant of longevity in the nematode *C. elegans*?

D. Gems, J. McElwee, R. Doonan, (University College London, United Kingdom)

Accrual of molecular damage and the mechanisms that protect against it appear to be central to ageing and longevity assurance, respectively. While there are many potential causes of molecular damage, much attention has focused on the role of superoxide, formed as a by-product of metabolic processes e.g. mitochondrial respiration. If superoxide causes ageing then experimentally induced increases in activity of the enzyme superoxide dismutase (SOD) should retard ageing. This prediction has been tested in model organisms (e.g. *Drosophila*) with mixed results.

We have conducted a systematic study of *C. elegans* SOD gene function. *sod-1* and *sod-5* encode cytosolic Cu/Zn SODs, while *sod-4* encodes a secreted Cu/Zn SOD. *sod-2* and *sod-3* both encode mitochondrial Mn SODs. The major Cu/Zn and Mn SODs are SOD-1 and SOD-2, respectively. Deletion of *sod-1* reduces adult lifespan, and deletion of *sod-2* results in

hypersensitivity to the life-shortening effects of hyperoxia. SOD-5 and SOD-3 are mainly expressed in the diapausal dauer larva stage.

We created multiple mutant strains entirely lacking Cu/Zn SOD or Mn SOD. The Cu/Zn SOD-less strain is short-lived but, remarkably, the Mn SOD-less strain is not. Thus, intramitochondrial superoxide seems unimportant for *C. elegans* ageing. Lifespan studies of strains over-expressing Cu/Zn SOD and Mn SOD are in progress, and the results will be presented.

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### CSS.14

#### Glycation and the enzymatic defence against glycation in the ageing process

P. Thornalley, (University of Warwick, United Kingdom)

Glycation is the spontaneous, non-enzymatic reaction of saccharides and saccharide derivatives with proteins, nucleotides and basic phospholipids.

It involves a complex series of parallel and sequential reactions collectively called the Maillard reaction. Early stage glycation of proteins with glucose forms fructosyl-lysine (FL) and other fructosamines. Advanced glycation endproducts (AGEs) are formed by the slow degradation of FL. Glyoxal, methylglyoxal and 3-deoxyglucosone are also potent glycating agents formed by the degradation of glycolytic intermediates, glycated proteins and lipid peroxidation. They react with proteins and nucleotides to form AGEs directly. Important AGEs quantitatively are hydroimidazolones formed by dicarbonyl modification of arginine residues. Other important widely-studied AGEs are *N*<sub>ε</sub>-carboxymethyl-lysine (CML), *N*<sub>ε</sub>-carboxyethyl-lysine (CEL) and the fluorescent crosslink pentosidine. The formation of AGEs is suppressed by the metabolism of dicarbonyl glycating agents by glyoxalase I and aldo-keto reductases and repair of FL-modified proteins by amadoriase and fructosamine 3-phosphokinase. Together, these enzymatic activities constitute the enzymatic defence against glycation. Glycation occurs in all tissues and body fluids. Glycation adducts accumulate in ageing leading to protein dysfunction and mutagenesis – often associated with dicarbonyl accumulation or dicarbonyl stress. Stable glycation adducts accumulate on long-lived proteins – such as CML and pentosidine residues of skin collagen. Glycation adducts with short chemical half-lives also accumulate on short-lived proteins in ageing because of an age-related decline in activities of the enzymes of the enzymatic defence against glycation. Anti-glycation enzymes are potential genetic determinants of ageing. Enhancing the enzymatic defence against glycation is a novel strategy for healthy ageing.

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**CSS.15****Genetic manipulation of glyoxalase pathway delays plant senescence under stress conditions**

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Glyoxalase pathway involving glyoxalaseI (glyI) and glyoxalaseII (glyII) enzymes is required for glutathione based detoxification of methylglyoxal (MG) which is a potent cytotoxic compound. The exact physiological role of the glyoxalase pathway in plants is not well worked out. Our studies have shown that under normal conditions plants maintain and tolerate a certain level of MG, however, under stress conditions MG concentration increased which seems to be one of the factor that induces the process of senescence and cell death. This process can be slowed down if the level of glyoxalases can be increased. Overexpression of both the genes in transgenic tobacco and rice showed delayed leaf senescence and improved capability to tolerate exposure to high NaCl, metal and drought stress and the plants were able to grow, flower and set seeds under stress Enhanced detoxification of MG and maintenance of glutathione homeostasis in the glyoxalase overexpressing transgenic plants seems to be one of the possible mechanisms behind this tolerance. Our preliminary work suggests that MG could also act as a signal molecule for regulating the expression of some of the genes including glyoxalase I and II.

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**CSS.16****Mitochondria and telomeres: Strange bedfellows in cell senescence**

T. von Zglinicki, J. Passos, G. Saretzki, (University of Newcastle, United Kingdom)

Telomeres shorten with ongoing age in replicating cells, and ultimately trigger replicative senescence, a permanent growth arrest. While telomeres are often regarded as a molecular clock, it is one that is dependent on the environment. Telomeres are deficient in DNA repair, and thus shorten faster in response to oxidative stress, some of which is generated internally.

Mitochondrial DNA damage and superoxide production increase with replicative age in human fibroblasts. This mitochondrial dysfunction is accompanied by compromised Ca<sup>2+</sup> handling and induction of a retrograde response in senescent cells. Replicative senescence of human fibroblasts is delayed by mild mitochondrial uncoupling.

Uncoupling reduces mitochondrial superoxide generation, slows down telomere shortening and delays formation of telomeric g-H2A X foci. Interestingly, immortalisation of primary human fibroblasts by hTERT overexpression improves

mitochondrial function especially under high stress conditions, where hTERT is largely re-located into mitochondria. This indicates mitochondrial ROS production as one cause of replicative senescence. However, mitochondrial dysfunction and cellular ROS production is also a consequence of cell senescence. Induction of growth arrest by either telomere uncapping or by telomere-independent DNA damage results in increased mitochondrial dysfunction including superoxide production. This ROS induction is downstream of p53/p21 and is dependent on growth factor signalling and on signalling via p38 MAPK and TGFb/NADPH oxidase pathways. Importantly, frequencies of nuclear DNA damage foci are significantly reduced by inhibition of the same signalling pathways, suggesting that secondary ROS generation contributes to long-term DNA damage signalling and, thus, stability of the senescent phenotype.

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**CSS.17****Roles of catalases during leaf senescence of *Arabidopsis thaliana***

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Free radicals are thought to play an essential role in senescence especially those derived from oxygen. In addition to their deleterious function, they might serve as signaling molecules. In *Arabidopsis thaliana* plants, a peak in the hydrogen peroxide content can be measured in leaves during the time of bolting. In accordance, CAT2 and APX1 activities decrease at this time point when coordinated senescence of all rosette leaves should be induced but the loss of chlorophyll can not yet be measured. However, treatment of cell cultures with H<sub>2</sub>O<sub>2</sub> led to an inactivation of APX1 activity indicating that the decrease of APX1 activity during bolting time might be a secondary effect and the down-regulation of CAT2 activity might be the initial step to produce an elevated level of hydrogen peroxide. Subsequently, CAT3 activity which can be induced by oxidative stress increases which then might lower the hydrogen peroxide level again and restore APX1 activity. The decrease of the CAT2 activity appears to be regulated predominantly on the transcription level. Therefore, we have characterized factors that regulate CAT2 expression and have analyzed their function in leaf senescence. A transcription factor and two proteins of unknown function could be isolated. If the gene for the transcriptions factor is knocked-out, the hydrogen peroxide peak during the bolting time is lost and plants exhibit a senescence phenotype indicating that transcriptional regulation of CAT2 is involved in senescence regulation.

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**CSS.18****Life extension in *Caenorhabditis elegans* by overexpression of Glyoxalase I — A mechanistic integration of protein damage by glycation, oxidation and nitration**

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*Background and aim:* Ageing is associated with accumulation of damaged and dysfunctional proteins. Damage to proteins by glycation, oxidation and nitration is increased in aged organisms. Mitochondrial dysfunction has been proposed as a source of increased reactive oxygen species (ROS) in aging. Glyoxalase 1 (Glo-1) metabolizes the dicarbonyl glycation agents, glyoxal and methylglyoxal (MG). Glyoxal and MG are major precursors of AGEs. The aim of this study was to assess if protection against dicarbonyl glycation of proteins could influence the ageing process.

*Methods:* The nematode *Caenorhabditis elegans* was the experimental model of aging employed. The *C. elegans* Glo-1 homologue was identified (CeGly) and transgenic animals with ubiquitous CeGly overexpression generated. In other experiments CeGly activity was suppressed by RNAi silencing. Effects on lifespan, mitochondrial function and protein damage were assessed. Markers of protein damage were determined by LC-MS/MS.

*Results:* Overexpression of CeGly increased lifespan by ca. 40% and RNAi silencing of CeGly decreased lifespan by ca. 40% ( $P < 0.001$ ). Overexpression of CeGly decreased the concentration of glyoxal and MG-derived AGEs and surprisingly also decreased the concentrations of markers of oxidative and nitrosative damage, methionine sulphoxide and 3-nitrotyrosine. Immunohistochemistry showed that MG-derived AGE residues accumulated in ageing localized to mitochondria and were associated with increased mitochondrial superoxide formation. Interpretation. These data suggest a link between dicarbonyl glycation and mitochondrial dysfunction in ageing, with downstream increased formation of ROS and reactive nitrogen species – an integrative mechanism of protein damage. Additionally, our data point to a central role of Glo-1 in the aging process.

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**CSS.19****Telomere shortening in subjects with impaired glucose tolerance and patients with diabetic macroangiopathy**

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*Objective:* Shortening of telomere length has been reported in several ageing-related diseases. The aims of this study were to: (i) assess whether telomere shortening occurs in impaired glucose tolerance (IGT) of human subjects, and (ii) whether telomere shortening is increased in subjects with type 2 diabetes with atherosclerosis.

*Methods:* Subjects with IGT ( $n=30$ ), non-diabetic control subjects ( $n=30$ ), subjects with type 2 diabetes without ( $n=30$ ) and with atherosclerotic plaques ( $n=30$ ) were selected from the Chennai Urban Rural Epidemiology Study (CURES). Southern-blot analysis was used to determine mean terminal restriction fragment (TRF) length in leukocyte DNA. Levels of thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCO) and high sensitive C-reactive protein (hs-CRP) were measured by standard methodologies. Carotid intima-media thickness (IMT) was assessed by high resolution B-mode ultrasonography.

*Results:* Mean ( $\pm$ SE) TRF lengths were significantly lower in IGT subjects ( $6.97 \pm 0.3$  kb;  $p=0.002$ ) and lower still in Type 2 diabetic subjects without plaques ( $6.21 \pm 0.2$ ;  $p=0.0001$ ) and lowest in Type 2 diabetic subjects with atherosclerotic plaques ( $5.39 \pm 0.2$ ;  $p=0.0001$ ) when compared to control subjects ( $8.7 \pm 0.5$ ). In IGT subjects, TRF length was positively correlated to HDL cholesterol and negatively correlated to glycated haemoglobin (HbA1c), TBARS, PCO, HOMA-IR and IMT. In regression analysis, presence of diabetes, HDL cholesterol and increased TBARS were significant correlates of telomere shortening.

*Conclusion:* Telomere shortening is seen even at the stage of IGT. Among subjects with Type 2 diabetes, those with atherosclerotic plaques had greater shortening of telomere length compared to those without plaques.

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**CSS.20****Insulin signalling, oxidative stress and aging**

Linda Partridge, (University College London, United Kingdom)

Ageing is a complex process during which many forms of damage and pathology accumulate. Furthermore, the ageing process is variable between individuals. This complexity and variability has led to the view that ageing will be intractable, both from the point of view of experimental scientists trying to understand its mechanisms and clinicians trying to improve health during ageing. Research into ageing has been galvanised by the discovery that this rather pessimistic view may be incorrect. Mutations in single genes can extend healthy lifespan in laboratory model organisms, and they do so by keeping the organisms healthy and youthful for longer. Furthermore, pathways with effects that are conserved during evolution seem to be involved. This talk will describe one of these, the insulin/IGF-like

signalling pathway, and the role of oxidative stress in mediating its effects upon ageing.

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### CSS.21

#### The perception of reactive oxygen species in plants: The road to signal transduction

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During normal life, substantial amounts of reactive oxygen species (ROS) are produced by organisms, and this is thought to contribute to the ageing process. ROS are likely to cause damage to a variety of bio-molecules, and such damage can lead to cellular dysfunction and disease, but could also be used to control programmed death, ie apoptosis. However, ROS are also known to control a wide variety of cellular activities, such as development and stomatal closure, and such control will not be through random oxidative damage. To activate a signal transduction cascade, the first event must be the perception of the ROS, followed by the activation or inhibition of the downstream signalling components, to bring about the desired effect. ROS perception may involve the oxidation of thiol groups on cysteines on proteins, with these proteins being at the head of signalling cascades. Such thiols are also likely to be targets for nitric oxide, adding a possible level of complexity to signalling. Therefore, proteins with susceptible thiols need to be identified. Using fluorescence tagging in conjunction with mass spectrometric identification, several such proteins have been identified in *Arabidopsis thaliana*, including glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a protein already characterised from animal cells as being controlled by ROS. The challenge now is to determine exactly how ROS affects the functioning of such proteins in cell signalling pathways, to determine those responses specifically downstream of these proteins, and to elucidate how such signalling controls the survival, or death, of cells.

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### CSS.22

#### Genetic, molecular and physiological mechanisms controlling cell death, defenses, and antioxidant network in response to abiotic and biotic stresses in plants

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Molecular, genetic and physiological mechanisms of cellular responses, such as cell death, signaling of systemic acquired

acclimation (SAA) and systemic acquired resistance (SAR), in response to environmentally induced oxidative stress in plants will be discussed. We identify that the redox status of the plastoquinone pool that signalizes in both, light acclimation and cell death responses, is controlled by LSD1, EDS1, EIN2 and PAD4. We also show that these genes regulate not only cellular homeostasis of salicylic acid (SA) and superoxide ion, but also ethylene (ET), auxin (IAA), and other reactive oxygen species (ROS). Furthermore we propose that the roles of LSD1 in light acclimation and in restricting pathogen-induced cell death are functionally linked. Through its regulation, LSD1 influences the effectiveness of photorespiration in dissipating excess excitation energy (EEE). The influence of SA on plant growth, on acclimation to EEE, and on the cellular redox homeostasis of *Arabidopsis thaliana* leaves is also assessed. These observations implied an essential role of SA in the light acclimation processes and in regulating the redox homeostasis of the cell. We also find that propagation of cell death depends on the plant defence regulators EDS1 and PAD4 operating upstream of ET production. Finally, we show that *Arabidopsis* hypocotyls form lysigenous aerenchyma in response to hypoxia and that this process involves H<sub>2</sub>O<sub>2</sub> and ET signalling, which is controlled by LSD1, EDS1 and PAD4 operating downstream of metabolic signals. We conclude that the balanced activities of LSD1, EDS1, PAD4 and EIN2 regulate chloroplast dependent acclimatory and defence responses.

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### CSS.23

#### Synthesis of novel nitron spin-traps for the investigation of oxidative stress

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Reactive oxygen species such as superoxide and hydroxyl radicals are natural side products of the respiratory cycle and are terminated by our inherent antioxidant defence system. However, during periods of oxidative stress the body's pro-oxidant activity exceeds its antioxidant activity leading to an excess of ROS.

These radicals can react with a variety of biomolecules including DNA, proteins and lipids. The damage caused can lead to cell malfunction and ultimately apoptosis. They are mainly produced in the electron transport chain located in the inner membrane of mitochondria and are therefore thought to be the major cause of mitochondrial dysfunction. It is hypothesised that the accumulative damage occurring, over time especially to the mitochondria, is a major contributor to a variety of age related diseases as well as the degenerative effects of ageing.

Due to the short half-life of ROS they cannot be directly analysed by Electron Paramagnetic Resonance (EPR) Spectroscopy and therefore a technique known as spin-trapping is employed. Novel mitochondrial spin-traps have been synthesised and their ability to trap oxygen-centred and carbon-centred

radicals determined with a view to further investigate the role of ROS and oxidative stress on ageing and age related diseases. Because such spin traps convert highly reactive ROS into stable nitroxyl radicals, they may also ameliorate oxidative stress itself.

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#### CSS.24

##### Diet, gastric nitrosation and stomach cancer

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The incidence of cancer of the proximal stomach has increased markedly over the past 20 years. Unlike cancer of the more distal stomach, it is not related to *Helicobacter pylori* infection and occurs in healthy non-atrophic acid secreting stomachs. While the responsible agent remains unidentified, it is likely that environmental factors, such as the diet, play a role in the rising incidence of these cancers. Saliva contains high concentrations of nitrite derived from the enterosalivary recirculation of dietary nitrate and its reduction by buccal bacterial. For many years, there has been interest in nitrite as a potential pre-carcinogen for gastric cancer. Acidification of nitrite in the stomach produces nitrosative species, which can form potentially carcinogenic *N*-nitroso compounds. Antioxidants such as ascorbic acid protect against this nitrosative chemistry by converting the nitrosative species to nitric oxide. However, nitric oxide diffuses rapidly to lipids, where it reacts with oxygen to form further nitrosative species ( $N_2O_3$ ).

Using gas chromatography — ion trap tandem mass spectrometry (GC-MS/MS), we have investigated the effect of lipids on the formation of *N*-nitroso compounds from nitrite in a model of the human stomach. While we successfully demonstrated the inhibitory effect of ascorbic acid on *N*-nitrosation in a single-phase system, *N*-nitrosation was not inhibited by ascorbic acid in presence of 10% lipids. These results indicate that the presence of lipids can markedly alter the protective effects of antioxidants with respect to potentially carcinogenic nitrosative chemistry occurring in the human stomach, and illustrate how diet can influence gastric biochemistry.

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#### CSS.25

##### A novel hydroxylamine spin probe

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The implication of oxidative stress in ageing, and various disease states, has resulted in great interest in understanding the processes associated with it (Halliwell and Gutteridge, 1999). Mitochondria are particularly susceptible to oxidative stress as

they are the major source of reactive oxygen species (ROS) via the electron transport chain, and mitochondrial dysfunction is causally related to neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and Huntington's disease. ROS may be free radicals, or molecules that rapidly form free radicals (Lin and Beal, 2006). These free radicals can undergo various rapid reactions with cellular components resulting in cell damage, and eventually cell death. Therefore, there is much interest in studying ROS production specifically from mitochondria.

Spin probes are molecules that react specifically with radical species to form stable products. They can be used to quantify the total oxidative stress using electron paramagnetic resonance (EPR) spectroscopy. As the products of reaction with spin probes are stable, they are also able to ameliorate the damaging effects of ROS.

We have synthesised a novel hydroxylamine spin probe that is specifically targeted to mitochondria in order to study the total mitochondrial derived ROS production.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine, 3rd ed., OUP.

Lin, M.T., Beal, M.F., 2006. Nature, 443, 787–795.

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#### CSS.26

##### Conserved and tissue-specific genic and physiologic responses to aging and caloric restriction in mitotic and postmitotic tissues

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Conserved and tissue-specific genic and physiologic responses to caloric restriction and altered IGF-I signaling in mitotic and postmitotic tissues drive the anti-cancer and health benefits of caloric restriction; S.R. Spindler<sup>1</sup>, J.D. Dhahbi<sup>2</sup>, P.L. Mote<sup>1</sup>; <sup>1</sup>University of California, Riverside, Department of Biochemistry, 5478 Boyce Hall, Riverside, CA 92521, U.S.A.; <sup>2</sup>Childrens Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland, CA 94609, USA. Dietary calorie restriction (CR), the consumption of fewer calories without malnutrition, and reduced insulin and/or IGF1 receptor signaling delay many age-related physiological changes and extend the lifespan of many model organisms. Evolutionary theory holds that these responses to CR evolved in metazoans an adaptation to variations in their food supply. However, cancer is primarily a post-reproductive disease, and thus its anticancer effects could not have been subject to direct selection. Thus, we propose that its anticancer effects evolved as a secondary consequence of selection for another trait, the role of many mitotic tissues as reservoirs of metabolic energy. We will present microarray and biochemical data from studies of liver, heart and skeletal muscle suggesting that the longevity effects of CR are derived from nutritionally driven cycles of apoptotic and autophagic cell death followed by insulin-driven

compensatory biosynthesis and cell division in mitotically competent tissues; and protein and lipid degradation, followed by resynthesis and repair in postmitotic tissues. We will present studies indicating these effects allow CR initiated late in life to rapidly induce many of the health and longevity benefits of life-long CR, including its anti-cancer effects.

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### CSS.27

#### **Enhancing the enzymatic defences: Antioxidant response element linked gene expression in animals**

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Redox stressors increase the expression of a range of cytoprotective genes that each contain an antioxidant response element (ARE, 5'-TGACnnnGC-3') in their promoters. Induction of ARE-driven genes is mediated by the Nrf2 cap'n'collar bZIP transcription factor, and involves redox-

dependent stabilization of the bZIP protein through antagonism of its interaction with the cullin-3:Rbx1 E3 ubiquitination substrate adaptor Keap1.

We have been interested in how Nrf2 and Keap1 interact because antagonism of this process could allow manipulation of antioxidant status. The Keap1 protein contains a BTB domain and a Kelch-repeat domain. The former domain ensures Keap1 exists as a dimeric protein, and the latter serves as a docking site for substrates like Nrf2 that are ubiquitylated. A region between the BTB and Kelch-repeat domains is referred to as the IVR (intervening region), and this contains reactive cysteine residues that can be modified by thiol-active agents and probably represent the target for redox stressors. Based on the facts that the N-terminal Neh2 domain of Nrf2 contains two separate binding sites for Keap1 (the low-affinity DLG motif and the high-affinity ETGE motif) and that Keap1 is a dimeric protein, we have developed a model for the complex between these proteins.

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