AGING, DNA REPAIR, AND POLY(ADP-RIBOSYL)ATION

Alexander Bürkle, María Moreno-Villanueva, Andrea Kunzmann, Maike Schmitz, Yvonne Rudigier, Raphael Hahn, and Malgorzata Debiak

Molecular Toxicology, University of Konstanz, Konstanz, Germany.
Email: alexander.buerkle@uni-konstanz.de

Poly(ADP-ribosyl)ation is a DNA strand break-driven posttranslational modification of proteins catalysed by poly(ADP-ribose) polymerase-1 (PARP-1), which uses NAD^+ as substrate [1]. Poly(ADP-ribosyl)ation is triggered by DNA strand breaks, is functionally associated with DNA repair pathways and is a survival factor for cells under low to moderate levels of genotoxic stress. We have previously described a positive correlation between poly(ADP-ribosyl)ation capacity of mononuclear blood cells with longevity of mammalian species. Our comparison of purified recombinant human and rat PARP-1 revealed that this correlation might be explained in part by evolutionary sequence divergence. We have also developed molecular genetic approaches to modulate the poly(ADP-ribosyl)ation status in living cells. Our results revealed that PARP-1 acts as a negative regulator of DNA damage-induced cytotoxicity and genomic instability, as assessed by cell survival, sister-chromatid exchange and micronucleus formation. Genomic instability is considered an important driving force for carcinogenesis as well as for the aging process. Recently we have also developed improved and highly sensitive methods to detect poly(ADP-ribose) formation in permeabilised [2] and intact cells by flow cytometry as well as an automated version of the fluorescence-detected alkaline DNA unwinding (FADU) assay to detect DNA strand breaks or DNA crosslinks formed in living cells. The usefulness of such assays for biogerontological research will be discussed and illustrated.

References

[1] Bürkle A (Ed.) (2006) Poly(ADP-Ribosyl)ation. Landes Bioscience, Georgetown, TX, USA. ISBN: 0-387-33371-1

[2] Kunzmann A, Liu D, Annett K, Malaise M, Thaa B, Hyland P, Barnett Y, Bürkle A. Flow-cytometric assessment of cellular poly(ADP-ribosyl)ation capacity in peripheral blood lymphocytes. *Immun Aging*. 2006 Jul 19;3:8.

PREMATURE CELL CYCLE ARREST OF CONGENITAL DM1 SATELLITE CELLS

A. Bigot, V. Mouly, Gillian Butler-Browne, D. Furling

UMR-S 787 Faculté de Médecine Pitié Salpetrière
105 boulevard de l'hôpital- 75013 Paris, France.Email: butlerb@ext.jussieu.fr

Myotonic dystrophy type 1 (DM1) is the most commonly inherited adult neuromuscular disorder. DM1 is caused by the expansion of a trinucleotide repeat (CTG) located in the 3'UTR of the DMPK gene. Impairment in skeletal muscle development represents one of the main features in the congenital form (CDM) that is associated with large expansions. Previously, we have showed that the satellite cells isolated from the CDM muscles are defective, including a reduction in their proliferating potential leading to a premature growth arrest. The aim of this study was to characterize this premature arrest of myotonic dystrophy satellite cells with large expansions.

Our results show that (1) human satellite cells stop dividing with the appearance of markers of the Rb pathway, (2) the premature cell cycle exit observed in CDM cultures is associated also with a disappearance of the hyper-phosphorylated form of Rb and an increase of p16 at the protein level. CDM cells adopt the same phenotype and express the same markers as the control senescent cells. Telomeric analyses show that CDM myoblasts exit prematurely of cell cycle with telomere lengths higher than control. However, an accelerated telomere shortening is also observed at each division in CDM myoblasts. These results suggest that large CTG expansions interfere with the regulation of the mitotic clock in CDM cells.

Finally, we show that, as observed in control cells, CDM cells may bypass the proliferative arrest by introduction of hTERT and CdK4, which leads to their immortalization. This model represents a new cellular tool for studying the pathological mechanisms involved in myotonic dystrophy.

IDENTIFICATION AND CHARACTERIZATION OF FOOD DEPRIVATION ACTIVATED GENES AND A NOVEL INSULIN RECEPTOR SUBSTRATE INTERACTING PROTEIN IN RAT HYPOTHALAMUS BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION ANALYSIS

Takuya Chiba, Daisuke Inoue, Satoshi Fujita, Haruaki Kubota, Maria Leticia Rassi Guimarães, Toshimitsu Komatsu, Takahiro Hayashida, Hiroko Hayashi, Haruyoshi Yamaza, Yoshikazu Higami, Isao Shimokawa

Department of Investigative Pathology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8523, Japan. Email: takuya@net.nagasaki-u.ac.jp

Hypothalamus is organized as a collection of distinct, autonomously active nuclei with discrete functions such as feeding activity and metabolism. Recent studies revealed hypothalamus might have important functions for the lifespan expression by caloric restriction. We applied the suppression subtractive hybridization (SSH) method to isolate food deprivation-activated and hypothalamus enriched genes from rat brain derived cDNA. We screened 160 clones each into two subtractive libraries, and identified four genes (UBE2D3, PKCβ, EAAC1, Fth1), which were up-regulated by fasting, and four genes (WDR6, ASS and two EST clones), which were enriched in the hypothalamus of the rat brain. Most of food deprivation up-regulated genes were implicated in the protective function of neuronal cell death from various neurodegenerative stresses such as oxidative stress. Gene expression of WDR6, a member of WD-repeat protein, was abundant in the hypothalamus and we found WDR6 protein could interact with insulin receptor substrate-4 (IRS-4) in both in vivo and in vitro. Moreover, WDR6 gene expression in the hypothalamic arcuate nucleus was decreased by caloric restriction and suppression of GH/IGF-I axis, both treatments are known to increase lifespan in many organisms. Our results might indicate WDR6 participates in the down stream of insulin/IGF-I signal being important for the regulation of feeding behaviour and longevity through the interaction with IRS-4 in the central nerves systems.

SUSCEPTIBILITY GROUPS FOR ALZHEIMER'S DISEASE AND ESTIMATION OF INHERITED RISK FOR INDIVIDUALS

Elizabeth H. Corder, SE Poduslo, F Licastro, DJ Lehmann, JA Prince

Center for Demographic Studies, Duke University, Durham NC, USA.
Email: elizabethcorder@hotmail.com

Age-related disorders such as Alzheimer's disease (AD) typically run in families, but it has proved difficult to identify the underlying genetic factors of importance even when plausible candidate genes have been identified. One interpretation is that there are multiple inputs derived from both parents that in combination influence an individual's level of risk and onset age. One exception is the type 4 allele for apolipoprotein E (APOE) which has been established as a risk factor for AD. However, it is neither necessary nor sufficient to predict AD. Since 1993 when APOE4 was identified a litany of candidate gene variants and environmental factors have been investigated but none convincingly identified via replication in independent samples. To address this problem, a series of studies has been undertaken to integrate gene variant information to identify high and low intrinsic risk sets using a fuzzy latent class analysis approach termed grade-of-membership analysis (GoM). The graded membership of individuals in these groups defines a gradient of risk from high to low. Four datasets composed of information on AD status, APOE genotype and other multiple markers have been investigated in this way. Relative risk for individuals are found to vary widely for each dataset. Major findings are that the polymorphic interaction of APOE with its LDL receptor is important; a parsimonious number of markers located within genes that modulate inflammation and cholesterol metabolism are highly predictive of risk; plasma folate and B12 levels are often low among very elderly ages; the often-investigated candidate genes are collectively of moderate importance; and, there are multiple important determinants located on chromosome 10q. These findings indicate that the sheer multiplicity of genetic and environmental determinants has been an obstacle to defining risk sets and quantitation of risk for individuals.

PRO-NGF AND THE SORTILIN RECEPTOR INFLUENCE AGE-RELATED NEURODEGENERATION

Raya Al-Shawi[1*], Angela Hafner[1], Jessica Olsen[1], Soyon Chun[1], Saba Raza[1], Christopher Thrasivoulou[1], Paul Simons[1*], Timothy Cowen[1*#]

**Centre for Biomedical Sciences, University College London, Hampstead Campus, Rowland Hill Street, London NW3 2PF, UK [1] Department of Anatomy and Developmental Biology, University College London,Hampstead Campus, Rowland Hill Street, London NW3 2PF, UK. Email: t.cowen@medsch.ucl.ac.uk*

Several studies have sought to demonstrate that neurodegeneration during disease and in old age is associated with reduced neurotrophic support. Little positive evidence has been forthcoming, either in relation to the availability of neurotrophins, or to expression and function of the relevant receptors. Recently, a novel way in which neurotrophins could contribute to neurodegeneration has been suggested. In contrast to the well-known neurotrophic functions of the mature β form of NGF (mNGF), its precursor, proNGF, has recently been shown to be abundant in the brain of patients with Alzheimer's disease and in the injured adult spinal cord. proNGF has been shown to be neurotoxic when bound in a heterotrimer with the p75 receptor and the receptor sortilin (identical to the neurotensin receptor NTS3). However, the contributions of proNGF and sortilin to known patterns of age- and disease-related neurodegeneration had not been investigated. We have recently shown that proNGF exhibits neurotoxicity in vitro in aged, but not young, basal forebrain and sympathetic neurons. Cell death is reversed by co-treatment with neurotensin, which competes with proNGF for the sortilin receptor, thus implicating sortilin as a mediator of the effects of proNGF. ProNGF induces neurite outgrowth in vitro in young adult sympathetic neurons but this response is much reduced in old age. Using Western blotting, we find a significant increase in proNGF in the projection areas of vulnerable BFN and SCG neurons. Vulnerable neurons therefore become exposed to an increasingly neurotoxic environment during aging. The age-shift in proNGF levels in peripheral targets was partly rescued by caloric restriction providing evidence of a physiological role for proNGF. Finally, we demonstrated significant increases in levels of sortilin in aging rodent BFN and SCG neurons. We therefore propose that increased proNGF in targets combined with increased sortilin expression in projecting neurons contributes to age-related neuronal atrophy and degeneration.

HOW MUCH REJUVENATION IS ENOUGH? QUANTIFYING LONGEVITY ESCAPE VELOCITY

Chris Phoenix and Aubrey D.N.J. de Grey

Methuselah Foundation, Lorton, VA, USA. Email: aubrey@sens.org

The physiological decline associated with aging derives ultimately from the accumulation of side-effects of metabolism. Here we model that accumulation starting from a biologically intuitive interpretation of the way in which those side-effects interact; we validate this model by showing that it accurately predicts the distribution of ages at death seen in typical populations that are protected from age-independent causes of death. We then exploit the mechanistic basis of this model to explore the impact on lifespans of interventions that combat aging, with an emphasis on interventions that repair the direct molecular or cellular consequences of metabolism and thus prevent them from translating into pathology. We establish that an indefinite extension of healthy and total life expectancy can be achieved by a plausible rate of progress in the development of such therapies, once a threshold level of efficacy of those therapies has been reached.

ALTERATIONS IN SUPRAMOLECULAR ARCHITECTURE AND POST-TRANSLATIONAL MODIFICATIONS OF RESPIRATORY CHAIN COMPLEXES: A CLUE IN AGING?

M. Frenzel, D. Dani, R. Hill[#], F. Krause, N. H. Reifschneider, H. Rommelspacher[#], M. Sugawa[#], and Norbert A. Dencher

Physical Biochemistry, Department of Chemistry, Darmstadt University of Technology, D-64287 Darmstadt, Germany, [#]Charité – University Medicine Berlin, Department of Psychiatry,Clinical Neurobiology, D-14050 Berlin, Germany.
Email: nad@pop.tu-darmstadt.de

The maintenance and regulation of cellular metabolism depend on the activity of numerous reaction pathways which might get affected during aging. Metabolic dysfunctions might even trigger or accelerate the aging process. In order to unravel the key steps involved in aging, we searched for the alterations in the mitochondrial proteome, with emphasis on membrane localized respiratory chain complexes. Besides the conventional proteomic approach to study changes in the abundance of proteins, we also looked for protein modifications and protein-protein interactions. The proteomes of three different brain areas and liver of rats were analysed by native polyacrylamide gel electrophoresis in combination with mass spectrometry. Native PAGE not only preserves the native protein structure but is an efficient technique to resolve multisubunit proteins and protein supercomplexes.In rat brain, age-modulated differences in the abundance of various non-mitochondrial and mitochondrial proteins such as the Na,K-ATPase, V-type ATPase, HSP60, mitochondrial aconitase-2, MF_oF_1 ATP synthase, and the OXPHOS complexes I – IV were detected. Noteworthy is the decrease in the amount of intact MF_oF_1 ATP synthase in the cortex during aging and especially the altered oligomeric assembly of the ATP synthases observed under the experimental conditions for the first time. This could be a clue for understanding the link between respiration and aging. The respiratory chain complexes I, III_2, and IV were also present as supramolecular stoichiometric assemblies such as $I_1III_2IV_{0-4}$. The abundance of these detergent-stable OXPHOS supercomplexes varied in brain mitochondria of young and older rats. Age-related changes in the supramolecular architecture of OXPHOS complexes could cause alterations in ROS generation. The age-related post-translational modifications, like phosphorylation, nitration, and carbonylation that are currently being investigated, might not only regulate the activity and stability of proteins, but also directly control the assembly of ATP synthase oligomers and OXPHOS supercomplexes.

AGE-ASSOCIATED DECLINE, GENETICS AND CHANCE IN A SIMPLE ANIMAL MODEL

Monica Driscoll

Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey, Nelson Biological Laboratories, 604 Allison Road, Piscataway NJ 08854, USA.
Email: Driscoll@Biology.Rutgers.Edu

No abstract was submitted

SENESCENCE, PROLIFERATION AND APOPTOSIS IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

Richard G.A. Faragher,[1] Dominic G.A. Burton,[1] Angela N. Sheerin,[1] Elizabeth L. Ostler,[1] Kaye Smith,[2] Peter J. Giles,[2] Jill Lowe,[1] William Rhys-Williams[3] & David G. Kipling.[2]

[1]School of Pharmacy and Biomolecular Sciences, University of Brighton, United Kingdom [2]Department of Pathology, School of Medicine, Cardiff University, Heath Park, Cardiff, United Kingdom [3]Destiny Pharma Ltd, Science Park Square, Falmer, Brighton, United Kingdom. Email: R.G.A.Faragher@bton.ac.uk

The senescence of mitotic cells is hypothesised to play a causal role in organismal aging. Cultures of normal human cells become senescent *in vitro* due to a continuous decline in the mitotic fraction. Although cardiovascular disease is a major feature of human aging, little systematic data on the senescence of vascular cell types currently exists.

One potential barrier to the evaluation of the frequency and distribution of senescent cells in tissues is the absence of a panel of robust markers for the senescent state. In parallel with an analysis of the growth kinetics of human vascular smooth muscle cells we have undertaken transcriptomic comparisons of early and late passage cultures in order to identify potential markers that can distinguish between cells in the growing and senescent states.

A wide range of genes are upregulated at senescence in human vascular smooth muscle cells. In particular, we have identified a 12-fold upregulation of expression in Cyclin D1 message, which is reflected in a concomitant upregulation at the protein level. Quantitative cytochemical analysis of senescent and growing vascular smooth muscle cells, indicates that cyclin D1 reactivity is a better predictor of replicative senescence than senescence associated beta-galactosidase activity. We have extended this analysis (in combination with Ki67, COMET and TUNEL staining) to the study of human vascular smooth muscle cells treated with resveratrol. Treatment with this compound produces a complex but consistent pattern of dose-dependent DNA damage, apoptosis and exit from the cell cycle. The significance of these observations for the proposed anti-atherosclerotic effects of resveratrol will be discussed.

GRO-ALPHA: A POTENTIAL MARKER FOR CANCER AND AGING SILENCED BY RNA INTERFERENCE

Lena Devermann[1], Sabine Fimmel[1], Anke Hermann[1], Christos C. Zouboulis[1,2]

[1]Research Group on Biogentology, Dermato-Pharmacology and Dermato-Endocrinology, Institute of Clinical Pharmacology and Toxicolgy, Charité–Universitaetsmedizin Berlin, Campus Benjamin Franklin, Berlin, and [1]Departments of Dermatology and Immunology, Medical Center, Dessau, Germany. Email: sabine.fimmel@charite.de

GRO-alpha (growth-related oncogene alpha; GROα), a member of the CXC chemokine subfamily, plays a major role in inflammation, tumorigenesis and angiogenesis. Additionally its expression has been found increased in divergent aging human organs. SZ95 sebaceous gland cells and A375 melanoma cells were transiently transfected in the presence of *in vivo*-tested cationic lipids (AtuPLEX®, 1-2µg/ml) with synthetic ribonucleotides with blunt ends and internal 2'-*O*-methyl modifications at the ribose (AtuRNA®, 10-25 nM). SZ95 sebocytes and A375 melanoma cells were seeded in 24-well plates 24 h before transfection. The efficient cell uptake of the synthetic ribonucleotides was shown with Cy3-labelled siRNA molecules using fluorescence microscopy. The knock-down of the target gene GROα on RNA level was shown by qPCR; the reduction on protein level was detected by GROα - ELISA. To achieve a required level of gene knock-down, the siRNA has to be transfected with high efficiency and low cytotoxicity. Transfection of SZ95 sebocytes and A375 melanoma cells was performed for 10-12 h in serum- and antibiotica-free medium, following different recovery times (1-15 h) in the culture medium. For relative quantification of GROα knock-down on RNA level, G6PDH housekeeping standards were analysed against the GROα dilution series and the ratio of siRNA to only AtuPLEX-treated cells. Both cell types secreted the chemokine GROα in their culture supernatant, whereas A375 melanoma cells produced more than 30-fold higher GROα levels than SZ95 sebocytes. The protein expression was reduced about 70% in SZ95 sebocytes and about 45 % in A375 melanoma cells compared with negative controls. A potential apoptotic effect of the GROα siRNA was excluded by Tunnel assay. Further investigation will show if the silence of GROα in normal aging cells has similar biological relevance to tumor cells.

SYSTEMS BIOLOGY AND GENETICS OF IMMUNOSENESCENCE IN HUMANS

Claudio Franceschi

Department of Experimental Pathology,Laboratory of Immunology, University of Bologna, Italy. Email: claudio.franceschi@unibo.it

No abstract was submitted

AGING OF PROTEINS: OXIDATION, DEGRADATION AND REPAIR

Bertrand Friguet, Filipe Cabreiro, Cédric Picot, Martine Perichon and Isabelle Petropoulos

Laboratoire de Biologie et Biochimie Cellulaire du Vieillissement,
Université Denis Diderot-Paris 7, Paris, France. Email: bfriguet@paris7.jussieu.fr

Cellular aging is characterized by the accumulation of oxidatively modified proteins that results, at least in part, from impaired protein turnover. Indeed, oxidized protein buildup with age may be due to increased protein damage, decreased elimination of oxidized protein (i.e. degradation and repair), or the combination of both mechanisms. Since the proteasome is in charge of both general protein turnover and removal of oxidized protein, its fate during aging has received considerable attention, and evidence has been provided for an impaired proteasome function with age in different cellular systems. In fact, depending on the cellular system investigated, the loss in proteasome activity observed during aging and upon oxidative stress appears to be due to either or both: i) decreased proteasome subunit expression and content, ii) inactivation upon modification of proteasome subunits, and iii) formation of endogeneous inhibitory proteins. However oxidative protein modifications can be eliminated not only through degradation but also repair. Cysteine and methionine are among the most sensitive amino acids to reactive oxygen species, but their oxidation products can be reversed within proteins. Indeed, repair is limited to the reversion of a few modifications such as the reduction of methionine sulfoxide by the methionine sulfoxide reductase (Msr) system. We have previously shown that Msr activity is impaired during aging and replicative senescence. In order to analyze the relationship between oxidative stress, protein oxidative damage and Msr, MsrA full-length cDNA has been overexpressed in SV40 T antigen-immortalized WI-38 human fibroblasts and MsrB2 full-length cDNA has been overexpressed in Molt-4 lymphoblastoid cells. After hydrogen peroxide-induced oxidative stress, both MsrA- and MsrB-overexpressing cells exhibit lower protein oxidative damage than control cells. These results indicate that the Msr system may play an important role in cellular defenses against oxidative stress by protecting proteins against oxidation and limiting the accumulation of oxidized proteins.

IS SUPEROXIDE DISMUTASE A DETERMINANT OF LONGEVITY IN THE NEMATODE *C. ELEGANS* ?

Joshua J. McElwee, Ryan Doonan, Glenda Walker, Filip Matthijssens, Koen Houthoofd, Jacques R. Vanfleteren and David Gems

Department of Biology, University College London, Gower Street, London WC1E 6BT, UK.
Email: david.gems@ucl.ac.uk

Accrual of molecular damage and the mechanisms that protect against it appear to be central to aging and longevity assurance, respectively. While there are many potential causes of molecular damage, much attention has focused on the role of superoxide, which is formed as an accidental by-product of metabolic processes such as mitochondrial oxidative phosphorylation. An experimental prediction of the theory that superoxide causes aging is that experimentally induced increases in activity of the enzyme superoxide dismutase (SOD) should lead to retardation of aging. This prediction has been tested in model organisms, particular the fruitfly *Drosophila*, with mixed results.

The SODs of *C. elegans* remain poorly characterized. *C. elegans sod-1* and *sod-5* encode cytosolic Cu/Zn SODs, while *sod-4* encodes a putative secreted Cu/Zn SOD. *sod-2* and *sod-3* both encode mitochondrial Mn SODs. We have conducted a systematic study of these genes, their expression and, by means of gene deletion and over-expression, their importance in viability, development, fertility, stress resistance and aging.

Our analysis of expression using GFP reporter lines and of SOD activity levels in deletion mutants implies that the major Cu/Zn and Mn SODs are SOD-1 and SOD-2, respectively. Consistent with this, deletion of *sod-1* reduces adult lifespan, and deletion of *sod-2* results in hypersensitivity to the life-shortening effects of hyperoxia. By contrast, SOD-5 and SOD-3 are mainly expressed in the diapausal dauer larva stage.

We have also constructed strains lacking Cu/Zn SOD (i.e. without *sod-1, sod-4* or *sod-5*), and lacking Mn SOD (i.e. without *sod-2* or *sod-3*). The Cu/Zn SOD-less strain is short-lived, but remarkably the Mn SOD-less strain is not. Thus, intra-mitochondrial superoxide is apparently unimportant for *C. elegans* aging. Lifespan studies of strains over-expressing Cu/Zn SOD and Mn SOD, alone or in combination with over-expression of catalase are in progress, and we shall present the results.

MORPHOLOGICAL CHANGES ASSOCIATED WITH AGING

Paolo U. Giacomoni

Clinique laboratories - Melville NY 11747 USA. Email: pgiacomo@Estee.com

Aging in humans is associated with morphological and shape modulations, such as wrinkle formation and accumulation of fat in specific anatomical sites. The paper will present a discussion of evidences, to understand the causes of such phenomena and the mechanisms involved.

LONGEVITY ASSURANCE MOLECULAR PATHWAYS IN HUMAN CELLS

Stathis Gonos

National Hellenic Research Foundation, Athens, Greece.
Email: sgonos@eie.gr

Aging and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have developed a clonal senescence induced system and we have cloned several senescence associated genes. Analysis of the function of one of the isolated genes, encoding for Clusterin/Apolipoprotein J (CLU), suggests that it is a novel survival factor. CLU is found over-expressed in vitro under a variety of stress conditions and in vivo in samples from patients suffering from various age-related diseases as well as in primary tumours which have acquired chemotherapeutic drug resistance. In addition, it has been demonstrated that inhibition of endogenous CLU expression by RNA interference induces growth retardation, higher rates of endogenous cellular death and sensitizes human cells to stress (Cancer Res 64, 1834-1842, 2004). Recent findings indicate that effective and sustained CLU depletion by siRNA induces late morphological alterations, growth arrest at the G_1/S checkpoint and activation of the mitochondrial axis of apoptosis that engages caspase-9. Moreover, CLU knock-down resulted in down regulation of the BH pro-survival (bcl-2 and bcl-X_L) proteins and activation of p53 and its downstream targets, namely $p21^{WAF1/CIP1}$ and bax.

We have also attempted an overall molecular and biochemical approach regarding proteasome function in replicative senescence and cell survival. We have observed reduced levels of proteasomal peptidase activities coupled with increased levels of oxidized and ubiquitinated proteins in senescent cells. We have found the catalytic subunits of the 20S complex and subunits of the 19S regulatory complex to be down-regulated in senescent cells. This is accompanied by a decrease in the level of both 20S and 26S complexes (J Biol Chem 278, 28026-28037, 2003). In support, partial inhibition of proteasomes in young cells by specific inhibitors induced a senescence-like phenotype. Stable over-expression of β subunits or POMP in human cell lines resulted in enhanced proteasome assembly and activities and increased cell survival following treatments with various oxidants. Moreover, stable over-expression of β_5 subunit delayed senescence in human fibroblasts (J Biol Chem 280, 11840-11850, 2005). Finally in search of natural compounds that may activate proteasome, we have identified that the main constituent of olives, oleuropein, exerts stimulatory effects on proteasome. Importantly, continuous treatment of human fibroblasts cultures with oleuropein delays senescence by approximately 15%.

$CD8^+$ T CELLS AND IMMUNOLOGICAL MEMORY IN AGED HUMANS

Beatrix Grubeck-Loebenstein,

Institute for Biomedical Aging Research of the Austrian Academy of Sciences, Innsbruck, Austria.Email: Beatrix.Grubeck@oeaw.ac.at

It is the goal of this talk to illustrate changes in the naïve and memory CD8 T cell pool that occur with aging in humans. Analysis of $CD45RACD28^+$ and $CD45RACD28CD62^+$ cells, which are generally considered as naïve, reveals that these populations are not only extremely small, but have an impaired homing receptor expression, a restricted diversity and shortened telomeres in comparison to young controls. The data demonstrate that these cells, even if antigen-inexperienced, have divided a lot, lost their diversity and are therefore unlikely to guarantee full immunological protection following exposure to neoantigens. Lack of fully functioning naïve T cells can still, at least partly, be compensated by an increase in the number of $CD8CD45RO^+$ cells, in particular a subset within this population that expresses CD25 constitutively without being regulatory. $CD45ROCD25^+$ T cells have lymph node homing receptors, contain $CD4^+CD8^+$CD40L-expressing cells, produce large amounts of IL-2 and IL-4, display a polyclonal T cell repertoire and contain a variety of cells of different antigenic specificity. Gene array analysis, however, shows that $CD45ROCD25^+$ cells from elderly persons greatly differ from CD45RO cells from young persons. We therefore conclude that memory T cells have age-specific properties in elderly persons, but may still protect this age group in the absence of fully functioning naïve T cells.

DISTRIBUTION OF OXIDIZED PROTEINS IN OXIDATIVE STRESS AND AGING

Tilman Grune

University of Hohenheim, Institute of Biological Chemistry and Nutrition Biofunctionality and Food Safety (140f), Germany. Email: grune@uni-hohenheim.de

The accumulation of oxidized proteins is one of the highlights of aging and oxidative stress. In general the bulk of oxidatively modified proteins is selectively recognized and degraded by the 20S proteasome. The proteasomal system is one of the major cytosolic proteolytic systems and it is responsible for the degradation of a large share of cytosolic proteins. This proteolytic system consists of several components including the 26S and the 20S proteasome. The role of the 20S proteasome was not acknowledged for a long time. Recent investigations demonstrated that the 20S proteasome seems to have a key role in the degradation of unfolded proteins. Several lines of evidence demonstrate that the recognition of oxidized proteins by this protease is due to oxidation-induced unfolding. Therefore, moderately oxidized proteins are degraded, whereas severe oxidized model proteins are poor substrates for proteolysis. The latter is the result of an aggregation of highly oxidized proteins.

However, protein oxidation and, therefore, degradation of oxidized proteins in the cell is not taking place in all areas of the cell to the same extent. Nuclear proteins seem to be well protected from oxidation and damaged nuclear proteins are rapidly removed. On the other hand oxidized and cross-linked protein aggregates are accumulating outside the nucleus. These protein aggregates in turn are disturbing the cellular metabolism and in particular the proteolytic systems.

Therefore, the maintenance of the proteasomal function seems to be one of the important features for a healthy aging process, whereas malfunction of the proteasomal system seems to play playing a major role in a premature aging process.

HUMAN PROGEROID DNA REPAIR DISORDERS AND MOUSE MODELS: IMPACT ON AGING AND LIFESPAN EXTENSION

Jan H.J. Hoeijmakers, I. van der Pluijm, G. Garinis, L. Niedernhofer[b)], K. Diderich, A. Lalai, J. Mitchell, R.B. Beems[a)], H. van Steeg[a)], and G.T.J. van der Horst.

MGC, CBG, Dept. of Cell Biol. and Genetics, Erasmus University, 3000 DR Rotterdam, The Netherlands.
[a)] RIVM, Bilthoven, The Netherlands; [b)] University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, PA 15213-1863, USA.
Email: j.hoeijmakers@erasmusmc.nl

Radiation and numerous chemicals as well as endogenous metabolism generating reactive oxygen species constantly damage cellular DNA. In proliferating cells DNA injury can be converted into mutations or chromosomal aberrations, leading to cancer. In addition, DNA lesions can cause cell death and cellular senescence. An elaborate genome maintenance machinery comprised of various intricate DNA repair pathways, cell cycle checkpoint and damage tolerance systems counteracts the deleterious consequences of damage to our genes. One of the most versatile DNA repair systems is nucleotide excision repair (NER), which removes a wide class of helix-distorting lesions in a multi-step 'cut and patch' reaction. Two sub-pathways exist: global genome NER operates genome-wide and mainly prevents mutations, transcription-coupled repair removes damage that blocks transcription counteracting cytotoxic effects of DNA injury. Inherited, UV-sensitive NER disorders include xeroderma pigmentosum (XP), characterized by high cancer predisposition, most prominently skin cancer and the very severe neuro-developmental conditions Cockayne syndrome (CS) and trichothiodystrophy (TTD), in which patients, curiously, seem to be protected from cancer. Also combined XP/CS occurs.

Mutations in NER helicases XPB and XPD are associated with all three disorders. XPD^{TTD} mice demonstrated that TTD is, like CS, in fact a premature aging syndrome. XPD^{TTD} mice also exhibit reduced spontaneous cancer incidence, which is explained by the idea that the transcription-coupled repair defect triggers cell death from endogenous DNA damage, that blocks transcription. This response prevents DNA damage induced cancer. $XPD^{XP/CS}$ mutant mice are highly predisposed to cancer, due to aberrant repair but also display premature aging, demonstrating that both phenotypes can co-exist. Complete repair deficiency in XPD^{TTD}/XPA or CS/XPA double mutant mice dramatically aggravates premature aging symptoms, including prominent neurodegeneration and an extremely short lifespan of ~3 weeks. Different mutants with mild to severe defects in the dual functional NER-crosslink repair endonuclease ERCC1 exhibit similar and in part distinct premature aging features over a period of 15 months to 4 weeks depending on the severity of the repair defect. The correlation between severity of compromised repair and rate of onset and severity of the clinical aging manifestations provides strong arguments for the DNA damage theory of aging. We also generated conditional mutants in which dramatic aging is triggered only in e.g. the brain. These mice display many signs of neurodegeneration and only mild aging features in the remainder of the body. We propose that endogenous oxidative lesions, including crosslinks and transcription-blocking damage hamper transcription/replication and trigger apoptosis-senescence and consequently aging. Thus, exaggerated cytotoxic and cytostatic responses to DNA damage may protect from cancer, but enhance aging. On the other hand, attenuated cytotoxic and cytostatic responses to DNA damage will favour cancer. Microarray, functional and physiological studies have revealed that persisting DNA damage triggers a systemic downregulation of the IGF1 somatotrophic axis, causing a shift towards energy storage (diapause) rather than energy production explaining the severe growth defect of the repair mutants.

This 'survival' response also maximizes anti-oxidant defence and is aimed at protecting from cancer and aging-related diseases and extending lifespan. Interestingly, long-lived dwarf mice and mice subjected to caloric restriction exhibit a *grosso modo* similar response. These data link accumulation of DNA damage and the IGF1 control of lifespan, cancer and aging.

References

Niedernhofer L.J., Garinis G.A., Raams A., Lalai S.A., Robinson A.R., Appeldoorn E., Odijk H., Oostendorp R., Ahmad A., van Leeuwen W., Theil A., Vermeulen W., van der Horst G.T., Meinecke P., Kleijer W., Vijg J., Jaspers N.G.J. and Hoeijmakers J.H.J. 2006. A novel progeria caused by a DNA repair defect reveals that genotoxic stress supresses the somatotroph axis. *Nature* **444:** 1038-1043.

van der Pluijm I., Garinis G.A., Brandt R.M.C., Gorgels T.G.M.F., Wijnhoven S.W., Diderich K.E.M., de Wit J., Mitchell J.R., van Oostrom C., Beems R., Niedernhofer L.J., Velasco S., Friedberg E.C., Tanaka K., van Steeg H., Hoeijmakers J.H.J. and van der Horst G.T.J. 2006. Impaired genome maintenance suppresses the GH/IGF1 axis in Cockayne syndrome mice. *PLOS*, **e436.**

van de Ven M., Andressoo J.O., Holcomb V.B., von Lindern M., Jong W.M., Zeeuw C.I., Suh Y., Hasty P., Hoeijmakers J.H., van der Horst, G.T., Mitchell J.R. 2006 Adaptive Stress Response in Segmental Progeria Resembles Long-Lived Dwarfism and Calorie Restriction in Mice. *PLoS Genet.* **12:** e192.

OXIDATIVE STRESS AND ENERGY FAILURE IN CELLULAR SENESCENCE AND AGING

Pidder Jansen-Dürr

Institute for Biomedical Aging Research, Innsbruck, Austria.
Email: P.Jansen-Duerr@oeaw.ac.at

The free radical theory of aging suggests that reactive oxygen species (ROS) are a driving force of the aging process. In many postmitotic tissues with high mitochondrial activity, the mitochondria are considered as main sources of ROS, which arise as by-products of oxidative metabolism. However, in recent years it has been shown that depending on the tissue type also nonmitochondrial enzymes, such as NADPH oxidases and other oxidative enzymes, can contribute significantly to the cellular load of ROS. The emerging picture suggests that various sources of ROS contribute in a different way to aging phenotypes in various tissues. We have addressed the role of reactive oxygen species in the aging of human tissues with high content of proliferating cells and also in postmitotic tissues. The data suggest that both mitochondrial and non-mitochondrial ROS sources contribute to the aging process, but a detailed understanding of tissue specific oxidative processes and the role of the individual ROS sources for this process remains to be established.

Changes in oxidative metabolism in aging cells and tissues also have an impact on the level of cellular energy production and availability.

A functional link between energy failure and cellular aging is considered as a new paradigm, which has received support from recent experiments carried out in various model systems of aging.

CELL INTRINSIC CHECKPOINTS AND ENVIRONMENTAL ALTERATIONS LIMIT STEM CELLS FUNCTION IN AGING TELOMERE DYSFUNCTIONAL MICE

Zhenyu Ju, Sonja Schaetzlein, Aaheli Roy Choudhury, K. Lenhard Rudolph

Department of Gastroenterology, Hepatology and Endocrinology, Medical School Hannover, Carl-Neuberg-Str.1, 30625 Hannover, Germany. Email: Ju.Zhenyu@MH-Hannover.De

During aging telomere shortening occurs in the vast majority of human tissues and organs including stem cells. The molecular mechanisms that limit stem cell function in response to telomere shortening are not well understood.

Here we studied stem cell function and aging in telomere dysfunctional mice. Our studies show that telomere dysfunction induces cell intrinsic checkpoints in stem and progenitor cells of the intestine and the hematopoietic system. Deletion of p21 elongates lifespan and stem cell function of telomere dysfunctional mice without affecting upstream telomere dysfunction signalling. Exonuclease-1 (Exo-1) deletion prevented the induction of DNA damage signals at dysfunctional telomeres and also improved survival and stem cell function of aging telomere dysfunctional mice indicating that the processing of dysfunctional telomeres in mammalian cells involves Exo-1.

In addition to these cell intrinsic checkpoints, telomere dysfunction induces an age associated secretory phenotype. Transplantation experiments revealed that the elevated cytokine profile associates with the host rather than the engrafted stem cells. Moreover, the altered hematopoietic environment limits stem cell engraftment and is responsible for the skewed hematopoiesis (impaired B-cell development and increased myeloid proliferation) in aging telomere dysfunctional mice.

SYSTEMS BIOLOGY AND THE ROLE OF MITOCHONDRIA IN AGING

Tom Kirkwood

Centre for Integrated Systems Biology of Aging and Nutrition,Institute for Aging and Health, Newcastle University, UK. Email: Tom.Kirkwood@newcastle.ac.uk

Extensive evidence points to an important role for oxidative stress in aging. 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 aging 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.

HEALTHY AGING: REGULATION OF THE METABOLOME BY CELLULAR REDOX MODULATION AND PROOXIDANT SIGNALING SYSTEMS. THE ESSENTIAL ROLES OF SUPEROXIDE ANION AND HYDROGEN PEROXIDE

Anthony W. Linnane

Centre for Molecular Biology and Medicine, Epworth Medical Centre, Richmond (Melbourne) Victoria, 3121, Australia. Email: tlinnane@cmbm.com.au

Reactive oxygen species (ROS) have long been posited as major deleterious contributors to the aging process. There is a huge literature concerned with proposed benefits arising from antioxidant therapy for the amelioration / prevention of the postulated ravages brought about by ROS. These claims are not based on well defined mammalian end point clinical trials but are almost exclusively based on in vitro studies inappropriately extrapolated to support hypothetical in vivo situations in order to support the hypothesis. Indeed there are no human clinical trials which substantiate the benefits of antioxidant therapy. On the contrary, evidence will be presented in support of the concept that superoxide anion and hydrogen peroxide constitute a regulated prooxidant second messenger system, whose localized sub-cellular production is essential for normal metabolome and physiological function. The role of this second messenger system in the regulation of the metabolome will be discussed in terms of their essential contribution to the normal function of

sub-cellular bioenergy systems

- transcription activation
- mitochondrial DNA changes
- cell differentiation

protein turn over regulation

- enzyme activation
- cellular redox regulation of cellular metabolism

Particular reference will be made to the function and essential prooxidant role of ascorbic acid. The formation of superoxide anion and hydrogen peroxide does not lead to random unregulated macromolecular damage as envisaged by the ROS aging hypothesis.

OXIDATIVE DAMAGE AND SUFFICIENT TELOMERE LENGTH ENABLE EMERGENCE OF PRETUMORAL CELLS FROM SENESCENT EPIDERMAL KERATINOCYTES

Sébastien Martien [1], Karo Gosselin [1], Laure Sabatier [2], Chantal Verkamer [1] and Corinne Abbadie [1]

[1]UMR8161, CNRS-Univ Lille1-Univ Lille2-Institut Pasteur de Lille, Institut de Biologie de Lille,1 rue du Pr. Calmette, 59021 Lille Cedex, France.
[2]CEA, DSV/DRR/Laboratoire de radiobiologie et oncologie, BP6, 92265 Fontenay-aux-Roses, France[1]. Email: sebastien.martien@ibl.fr

Senescence is considered as a barrier against tumorigenesis. However, we observed that epidermal keratinocytes are able to spontaneously escape senescence, leading to emergence of partially transformed cells. In contrast, dermal fibroblasts never display such an emergence.
To understand this difference, we first investigated the mechanisms leading to senescence in the two cell types. FISH experiments show an important telomere shortening during fibroblast senescence, but a much less important one in keratinocytes. In accordance, H_2AX foci formed on the shorten telomeres were observed principally in fibroblasts. Senescent keratinocytes and fibroblasts differ also on their level of oxidative stress. We have previously shown that NF-kB factors are involved in keratinocyte senescence *via* MnSOD induction. MnSOD is a redox enzyme that dismutates $O_2°$- in H_2O_2, leading to H_2O_2 accumulation that contributes to senescence (Bernard et al, Cancer Research 64, 472-481, 2004). In fibroblasts, in spite of an increase in MnSOD expression and ROS accumulation during senescence, antioxidant treatments do not delay senescence.

Since H_2O_2 was shown to be mutagenic, we next investigated whether the accumulation of H_2O_2 associated with keratinocyte senescence could cause the emergence of the partially transformed cells. AIP bridges (an oxidative damage) accumulate essentially in nucleus of keratinocytes, but in cytoplasm of fibroblasts, and 8-oxoguanines (mutagenic damage) are principally detected in keratinocytes. In keratinocytes, inhibiting NF-kB activity or reducing oxidative stress with antioxidant treatments decrease the emergence frequency. Conversely, treating young fibroblasts with subtoxic doses of H_2O_2 induces premature senescence and thereafter emergence.

Together, these results suggest that in keratinocytes, NF-kB activity, *via* MnSOD induction, may cause oxidative damage essentially in nucleus, leading to mutagenesis of some cells which could reproliferate owing to their sufficient telomere length. In opposite, the lack of nuclear oxidative damage and the too short telomeres of fibroblasts would not allow emergence.

CLONAL ATTENUATION OF SOMATIC CELLS IN AGING MAMMALS: A REVIEW OF SUPPORTIVE EVIDENCE AND ITS BIOMEDICAL SIGNIFICANCE.

George M. Martin

University of Washington, Seattle, WA, 98195, USA. Email: gmmartin@u.washington.edu

Clonal attenuation can be defined as the gradual depletion of the replicative potentials of individual clones of mammalian somatic cells (GM Martin et al., Am J Path 74:137, 1974). Publications from the author's lab and those of others will be reviewed that support the proposition that it is a continuous process throughout the life course and that it occurs in vivo in primates (U Herbig et al., Science 311:1257, 2006). The puzzling discordance between the mass culture results from the labs of the late Vincent Cristofalo (tissue from living subjects, Proc Natl Acad Sci USA. 95:10614, 1998 and those from the Martin lab (tissue predominately from autopsy subjects, Lab Invest 23:86, 1970) will be discussed. Finally, the implications of clonal attenuation and replicative senescence for such major age-related pathological processes as neoplasia, atherosclerosis, benign prostatic hyperplasia and osteoarthritis will be addressed; these and other disorders of aging can be characterized as a mixture of atrophy and hyperplasia, presumably related to a failure of homeostatic cell-cell interactions in aging tissues.

For the case of neoplasia, an argument can be made that such failures precede what is increasingly regarded as the most critical step in carcinogenesis, the evolution of a mutator phenotype (JH Bilelas et al., Proc Natl Acad Sci USA 103: 18238, 2006).

OXIDATIVE DNA DAMAGE PROCESSING AND CHANGES WITH AGING

Vilhelm A. Bohr, Penelope Mason

Laboratory of Molecular Gerontology, National Institute on Aging, NIH Baltimore, Maryland, USA. Email: masonpe@grc.nia.nih.gov

Oxidative modifications of macromolecules may be an important causal factor in aging and cancer. Since DNA is the master molecule, the DNA oxidation maybe most critical. DNA base oxidations are repaired via an elaborate DNA repair system called base excision repair. There are separate compartments for repair of the nuclear and the mitochondrial DNA, and understanding of the processes that occur in mitochondria may be of high importance as DNA damage accumulates in these organelles with aging. A number of human disorders of premature aging are associated with defects in oxidative DNA damage processing, and this will be discussed, in particular using the example with Werner syndrome, the hallmark progeria.

RHESUS MONKEYS: PARALLELS TO HUMAN AGING AND THE EFFECT OF DIETARY RESTRICTION

Julie A. Mattison[1], Jennifer E. Young[1], Peter Gouras[2], Lena Ivert[3], Janko Nikolich-Zugich[4], MaryAnn Ottinger[5], Donald K. Ingram[1]

[1]Laboratory of Experimental Gerontology, NIA, NIH, Baltimore, MD., USA.
[2]Dept. of Ophthalmology, Columbia University, New York, NY.
[3]St. Erik's Eye Hospital, Stockholm, Sweden
[4]Oregon Health Sciences University, Beaverton, OR. [5]Dept. of Animal Science, University of Maryland, College Park, MD. Email: mattisonj@mail.nih.gov

Progress in aging research has advanced with the use of numerous animal models which have helped identify possible mechanisms of aging and age-related disease as well as evaluate interventions. The rhesus monkey (*Macaca mulatta*) has a close genetic relationship to humans and a similar aging phenotype, including morphology, physiology, and behaviour, and provides an ideal model for the study of aging. The NIA has been conducting a study of aging and dietary restriction in rhesus monkeys since 1987 and thus has a unique opportunity to characterize aging in several cellular and organ systems. With an average lifespan of 25 years and maximum of 40 years, rhesus monkeys' rate of aging is estimated at 3 times that of humans. Age-related declines in sensory systems such as vision and hearing are comparable to humans. Additionally, the histopathology of age-related macular degeneration is similar between the species. Although occurring later relative to lifespan compared to humans, female rhesus monkeys undergo a similar menopause transition in an ovary driven manner with elevated follicle stimulating hormone and reduced inhibin. Because monkeys also experience immunosenescence, such as a depletion of naïve T cells and an increased production of inflammatory cytokines, they are an excellent model to study the response to vaccinations which is currently underway. Rhesus monkeys are well-suited for characterization of several components of behaviour and show general age-related declines in measures of learning and memory, generalized activity, and tests of motor function. Dietary restriction of calories attenuates many age-related changes in this long-lived model of aging suggesting enhanced survival as well.

ZINC, OXIDATIVE STRESS, GENETIC BACKGROUND AND IMMUNOSENESCENCE: IMPLICATIONS FOR HEALTHY AGING. ZINCAGE STUDY

Eugenio Mocchegiani

Immunology Ctr. (Section: Nutrition and Immunosenescence) Res. Dept. INRCA, Ancona, Italy. Email: e.mocchegiani@inrca.it

The relevance of zinc for proper functioning of the entire immune system is already well documented. However, the identification of individuals who really need zinc supplementation is still debated in view of the fact that excessive zinc may also be toxic. The risk of developing zinc deficiency in people from industrialized countries is relatively low, except for elderly subjects where zinc intake may be suboptimal and inflammation is chronic. Thus, the role of zinc on the immune system and on the health of European elderly people is becoming of paramount importance, considering also that the elderly population is rapidly increasing. In particular, the factors contributing to and the biochemical markers of zinc deficiency in the elderly are still remain to be established. Epidemiological, functional, and genetic studies aimed at formulating a rationale for the promotion of healthy aging through zinc supplementation was the subject of Zincage project. The main results obtained by Zincage project will be presented and discussed with special focus on the genetic background, zinc status and healthy aging in different European countries.

OVEREXPRESSION OF GLUTAMATE-CYSTEINE LIGASE EXTENDS LIFESPAN IN DROSOPHILA MELANOGASTER*

William C. Orr[1], Svetlana N. Radyuk[1], Leena Prabhudesai[1], Dikran Toroser[2], Judith J. Benes[1], James M. Luchak[1], Robin J. Mockett[2], Igor Rebrin[2], and Rajindar S. Sohal[2]

[1]From the Department of Biological Sciences, Southern Methodist University, Dallas, Texas 75275, USA. [2]From the Department of Molecular Pharmacology and Toxicology, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90089-9121, USA. Email: borr@smu.edu

The hypothesis that overexpression of glutamate-cysteine ligase (GCL), which catalyses the rate-limiting reaction in *de novo* glutathione biosynthesis, could extend lifespan was tested in the fruit fly, *Drosophila melanogaster*. The GAL4-UAS binary transgenic system was used to generate flies overexpressing either the catalytic (GCLc) or modulatory (GCLm) subunit of this enzyme, in a global or neuronally targeted pattern. The GCL protein content of the central nervous system was elevated dramatically in the presence of either global or neuronal drivers. GCL activity was increased in the whole body or in heads, respectively, of GCLc transgenic flies containing global or neuronal drivers. The glutathione content of fly homogenates was increased by overexpression of GCLc or GCLm, particularly in flies overexpressing either subunit globally, or in the heads of GCLc flies possessing neuronal drivers. Neuronal overexpression of GCLc and low-level global overexpression extended mean and maximum lifespans up to 50%, without affecting the rate of oxygen consumption by the flies. Further dissection, using additional tissue-specific drivers, has revealed that the mushroom body seems to play an important role in this long-lived phenotype. In a complementary study, we have generated transgenic knock-downs (up to 95%), and observed greater susceptibility to oxidative stress and reduced longevity.

In the series of overexpression studies involving GCLm, we have seen an extension in mean lifespan of up to 24%, but only when overexpression is driven by global drivers. Overall these results demonstrate that enhancement of the glutathione biosynthetic capability, particularly in neuronal tissues, can extend the lifespan of flies, and thus support the oxidative stress hypothesis of aging.

MOLECULAR NETWORKS CONTROLLING AGING IN *PODOSPORA ANSERINA*

Heinz D. Osiewacz, Diana Brust, J. Grief, Andrea Hamann, Karin Jezek, Birgit Kunstmann, Christian Scheckhuber

Johann Wolfgang Goethe University, Institute of Molecular Biosciences, Molecular Developmentall Biology, Max-von-Laue-Str. 9, D-60438 Frankfurt, Germany.
Email: osiewacz@em.uni-frankfurt.de

The ascomycete *Podospora anserina* is a eukaryotic model system to experimentally unravel the basis of aging. Over the last decades it became clear that a network of molecular pathways control aging and lifespan in this system. Mitochondria do play a central role in this network. More specifically, mitochondrial DNA (mtDNA) reorganization, respiration, the generation and scavenging of reactive oxygen species (ROS) have been demonstrated to be involved. More recently, we investigated the role of mitochondrial morphology, which was found to be dynamic, the impact of mtDNA repair, of protein quality control, and of apoptosis like processes as the final excecution program on the end of the lifespan of individual cultures. The genetic disruption of the underlying pathways was found to result in lifespan extension. Both, mitochondrial as well as non-mitochondrial componenents of an apoptotic machinery were identified to play a key role.

MITOCHONDRIAL DYSFUNCTION AND TELOMERES IN CELLULAR SENESCENCE

Joao Passos, G. Saretzki, S. Ahmed, G. Nelson, T. von Zglinicki

Institute for Aging and Health, University of Newcastle, Newcastle upon Tyne, UK.
Email: Joao.Passos@newcastle.ac.uk

Mitochondrial DNA damage and superoxide production increase with replicative age in human fibroblasts despite an adaptive UCP-2-dependent mitochondrial uncoupling. This mitochondrial dysfunction is accompanied by compromised Ca^{2+} 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 gamma-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 production of reactive oxygen species (ROS) as one of the causes of replicative senescence.

However, to a large extent 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, genome-wide DNA damage results in increased mitochondrial biogenesis and dysfunction including mitochondrial superoxide production, cellular ROS increase and compromised calcium dynamics. This ROS induction is downstream of p53/p21 and is dependent on growth factor signalling and on signalling via p38 MAPK and TGF-beta 1-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.

HUMAN T CELL SENESCENCE

Graham Pawelec

Center for Medical Research, University of Tübingen Medical School, Tübingen, Germany.
Email: graham.pawelec@uni-tuebingen.de

Aging affects all parts of the immune system but adaptive immunity, especially T cell responses, seem to be most severely compromised. Long-term cultured T cells, particularly monoclonal populations, have demonstrated that both major subsets undergo progressive changes with increasing population doublings (PD) in vitro which may model those seen under the conditions of chronic antigenic stress and low-grade inflammation prevalent in the elderly in vivo. The CD8 subset shows changes characteristic of senescent processes established in other cell types, eg. decreased telomere lengths, increased resistance to apoptosis, altered patterns of secretion of soluble mediators etc. In contrast, the CD4 subset does not undergo replicative senescence in vitro, but shows increased susceptibility to apoptosis with increasing PD, eventually resulting in clonal deletion. If similar events occur in vivo, one would predict that maintaining necessary immune responses to chronic infectious agents or against antigens derived from other sources which cannot be eliminated from the organism would eventually result in "exhaustion" of immunity reflected by decreased numbers of antigen-specific CD4 cells and increased numbers of apoptosis-resistant antigen-specific CD8 cells (manifesting as an inverted CD4:CD8 cell ratio). In longitudinal studies of the very elderly, conducted in collaboration with Prof. Anders Wikby, University of Jönköping, Sweden, under the aegis of the EU project "T-CIA", we have established that an inverted CD4:8 cell ratio is a crucial marker of the Immune Risk Profile" (IRP) which predicts mortality at 2, 4 and 6-yr. follow-up. A major source of chronic antigen in the majority of these subjects are persistent ß-Herpes viruses, especially Cytomegalovirus (CMV), which drive clonal expansions and contractions of T cells and impact materially on longevity in this population. Using in vitro T cell cultures to model clonal expansion and contraction under these conditions enables us to better understand and eventually to modulate these processes in order to maintain appropriate immune function in the very elderly. Furthermore, these findings will also be applicable to other immune-compromising conditions of chronic antigenic stress, such as cancer and parasitic infections.

MUSCLE STEM CELL AGING AND TISSUE REGENERATIVE POTENTIAL

Thomas A. Rando

Stanford University, Stanford, California, USA. Email: rando@stanford.edu

One focus of our work has been to understand age-related changes in tissue regenerative potential by studying adult stem cells and the environment in which they reside. We have focused primarily on the adult muscle stem cell, the "satellite cell". The Notch signaling pathway plays an important role in different phases of the activation of satellite cells and their progeny. Inhibition of Notch signaling profoundly impairs muscle regenerative potential. With age, there is a failure of activation of this pathway in satellite cells in response to injury because of failure of upregulation of the Notch ligand, Delta. However, when Notch signaling is directly stimulated, aged satellite cells are as effective in mediating regeneration as are young satellite cells. Furthermore, when muscles of aged mice are exposed to the systemic milieu of younger animals by parabiotic pairings, the upregulation of Delta following injury and the regeneration of the aged muscle appear similar to what is observed in young animals. Clearly, factors in serum are capable of modifying the progenitor cells or their niche such that, in response to injury, aged muscle can be induced to adopt a youthful phenotype. In vitro studies suggest that old serum contains a suppressive activity that inhibits effective satellite cell activation and lineage progression. Recently, we have found that activated aged satellite cells have an increased propensity to adopt a fibroblast-like fate. This appears to be due to increased levels of Wnts in aged tissues and the ability of Wnt signaling to alter the lineage properties of activated satellite cells. Inhibition of Wnt signaling in aged tissue leads to a reduction in this "myogenic-to-fibrogenic" conversion, a reduction in the fibrotic response in aged muscle after injury, and an enhancement of the regenerative response of aged muscle. These studies suggest that the molecular pathways that lead to the phenotypic characteristics of aging may be related to an overall increase in Wnt signaling, and that such signaling may be acutely modulated to ameliorate the age-related decline in tissue regenerative potential.

AGING INTERVENTION, PREVENTION AND THERAPY THROUGH MILD STRESSED-INDUCED HORMESIS IN HUMAN CELLS

Suresh Rattan

Laboratory of Cellular Aging, Department of Molecular Biology, University of Aarhus, Denmark. Email: rattan@mb.au.dk

Progressive accumulation of molecular damage is a hallmark of cellular aging, which is amenable to intervention and prevention by hormesis through mild stress. Our studies have shown that repeated mild heat stress (RMHS) has anti-aging effects on growth and various other cellular and biochemical characteristics of normal human skin fibroblasts undergoing aging *in vitro*. RMHS at 41°C, for 1 hr twice a week, increased the basal levels of various chaperones, reduced the accumulation of oxidatively and glycoxidatively damaged proteins, stimulated proteasomal activities for the degradation of abnormal proteins, improved cellular resistance to ethanol, hydrogenperoxide and UV-B rays, enhanced the levels of various antioxidant enzymes, and increased the phosphorylation-mediated activities of various stress kinases. RMHS-exposed human fibroblasts are also better protected against glucose, fructose and glyoxal-induced growth inhibition and apoptosis. We have also observed various hormetic effects of RMHS on normal human epidermal keratinocytes, which include increased replicative lifespan, increased proteasomal activity, and enhanced levels of Na,K-ATPase pump. We are also testing the above effects of RMHS in combination with potential hormetic molecules, such as curcumin on aging, longevity and differentiation of human cells in culture.

GENOMIC INSTABILITY ON LOSS OF DROSOPHILA WRN EXONUCLEASE

Lynne S. Cox, Robert D. C. Saunders

Department of Biological Sciences, The Open University, Milton Keynes, Buckinghamshire MK7 6AA, UK. Email: r.d.saunders@open.ac.uk

Human progeroid Werner's syndrome provides the current best model for analysis of human aging; many aspects of normal aging occur as a result of mutation of the WRN gene encoding a helicase/exonuclease. Previous RNAi studies have emphasised the importance of WRN to DNA metabolism, but RNAi results in ablation of both helicase and exonuclease activities. Whilst biochemical studies can inform on the substrate specificities of the WRN exonuclease and helicase, it has not been possible to dissociate the two key enzyme activities *in vivo*, even using point mutations as these may act as dominant negatives. We report the identification of a *Drosophila* homologue of the human WRN exonuclease, which is encoded on a separate genetic locus from the helicase component. We have obtained a PiggyBac insertional mutant in the first exon of the *DmWRNexo* gene. Flies homozygous for this mutation in *DmWRNexo* show marked genomic instability as evidenced by a very high rate of mitotic recombination. The characterisation of the *DmWRNexo* gene and fly phenotype will be discussed. These allow us to conclude that the exonuclease activity of WRN is critical in maintaining genomic integrity. Excitingly, *Drosophila* is amenable to extensive genetic manipulation, allowing us to investigate regulatory pathways and the impact of WRNexo loss at an organismal level.

SIGNALING NETWORKS OF STRESS-INDUCED CELLULAR AGING

John M. Sedivy

Department of Molecular Biology, Cell Biology and Biochemistry, Division of Biology and Medicine , Brown University , Providence, USA. Email: john_sedivy@brown.edu

In response to hyperproliferative signaling elicited by transforming oncogenes some normal human cells can enter replicative senescence as a tumor defense mechanism. We recently found that human fibroblasts or endothelial cells with genetically-engineered reduction of proto-oncogene c-Myc expression switched with an increased frequency to a senescent state by a telomere-independent mechanism involving the polycomb group repressor Bmi-1 and the cyclin-dependent kinase inhibitor p16INK4a. The same regulatory circuit was triggered upon exposure to mild oxidative stress. Reactive oxygen species (ROS), such as superoxide and H2O2, are the products of normal oxidative processes and have been implicated in cancer and other diseases related to aging. It has been well documented that organismal aging is correlated with an increase in ROS-caused damage. Our findings point to the existence of a mechanism for monitoring hypoproliferative signaling, whose function may be to limit the proliferation and accretion of physiologically compromised cells. This mechanism may be another example of antagonistic pleiotropy leading to organismal aging.

IS CELLUAR AGING DUE TO DNA DAMAGE SIGNALING FROM SHORT TELOMERES, TELOMERE POSITION EFFECTS OR BOTH?

Jerry W. Shay

University of Texas Southwestern Medical Center at Dallas, Department of Cell Biology, Dallas, TX USA 75390-9039. Email: Jerry.Shay@UTSouthwestern.edu

The onset of replicative senescence depends on telomere status. There is not a sentinel short telomere that triggers senescence but a group of ~10% of the shortest telomeres that are involved. The first stage of cellular senescence (M1) occurs when some telomeres have shortened sufficiently to form telomere dysfunction induced foci that contain DNA damage response factors. The DNA-damage response observed in senescent cells is not a transient phenomenon, but consists of a permanent activation of the DNA damage checkpoint machinery. The long-term growth arrest at senescence may be thought of as an initial anti-tumor protection mechanism. In some situations cells can bypass M1 and continue to proliferate until they reach another growth arrest state known as crisis or the M2 stage of senescence. In crisis telomeres are terminally short, end-fusions occur, leading to subsequent breakage-fusion-bridge cycles, mitotic catastrophe, and apoptosis. In rare cells M2 is bypassed resulting in cellular immortalization. Bypasse of M2 is almost universally accompanied by the up-regulation/reactivation of the enzyme telomerase. The common link between between M1 and M2 are telomeres since ectopic introduction of the catalytic subunit of telomerase (hTERT) into cells prior to M1 or between M1 and M2 results in direct immortalization. This demonstrates that telomeres are important in both replicative senescence (M1) and crisis (M2). In addition, we have recently shown that endogenous genes next to telomeres are regulated by telomere length. Young cells with long telomeres have low expression of specific subtelomeric genes that greatly increase in expression when telomeres are short and nearing senescence. In additon, elongation of telomeres in old cells with short telomeres results in telomere elongation and reduced expression of the subtelomeric genes.

In summary, both DNA damage signaling from a too short telomere and telomere position effects may be important molecular mechanisms regulating cellular aging.

GHRELIN RECEPTOR (GHS-R1A) AGONISTS SHOW POTENTIAL AS INTERVENTIVE AGENTS DURING AGING

Roy G. Smith, Yuxiang Sun, Hong Jiang, Rosie Albarran-Zeckler, and Nik Timchenko

Huffington Center on Aging, Baylor College of Medicine, Houston, Texas, USA.
Email: rsmith@bcm.tmc.edu

Daily administration of an orally active agonist (MK-0677) of the growth hormone secretagogue receptor (GHS-R1a) to elderly subjects for 12-18 months rejuvenated their growth hormone (GH)/insulin-like growth factor (IGF-1) axis by restoring the amplitude of episodic GH release to that of young adults. This was accompanied by increases in lean mass and bone density, modest improvements in strength, and prevention of continued loss of strength. A similar agonist partially restored function to the thymus in old mice and resulted in inhibition of tumor growth and metastasis. We next tested the restorative effects of the endogenous GHS-R1a agonist, ghrelin, on the liver. Ghrelin treatment of 24 month old mice for 7 days reversed the molecular changes associated with reduced regenerative capacity. Reduced regenerative capacity is associated with formation of a C/EBPα-Brm nuclear complex that represses E2F-dependent promoters in the liver. Formation of this complex requires phosphorylation of C/EBPα; ghrelin treatment of old mice inhibited cyclin D3-cdk4 activity and increased phosphatase PP2A activity, thereby reducing phosphorylation of C/EBPα and derepressing E2F target gene expression. Besides being expressed in neurons that regulate pulsatile GH release, GHS-R1a is expressed in brain areas that regulate memory and cognitive function. Because aging is associated with decline in dopamine function, we investigated the potential neuromodulatory roles of GHS-R1a on dopamine action. We generated *Ghsr-IRES-tauGFP* mice by gene targeting and by combining GFP fluorescence and immunohistochemistry identified neurons that coexpress dopamine receptor subtype-1 (D1R) and GHS-R in the hippocampus, cortex, substantia nigra and ventral tegmental areas. In vitro studies showed that GHS-R and D1R form heterodimers causing modification of G-protein signal transduction and amplification of dopamine signaling. We speculate that aging is associated with deficient endogenous ghrelin signaling that can be rescued by intervention with GHS-R agonists to improve quality of life and maintain independence.

PROTEIN SYNTHESIS IS A NOVEL DETERMINANT OF AGING IN *C. ELEGANS*

Popi Syntichaki, Kostoula Troulinaki and Nektarios Tavernarakis

Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Crete, Greece. Email: synticha@imbb.forth.gr

Protein synthesis is a tightly regulated cellular process that affects growth, reproduction and survival in response to both intrinsic and extrinsic cues such as nutrient availability and energy levels. A pronounced age-related decline of total protein synthesis rate has been observed in many organisms, including humans. The molecular mechanisms underlying this decline and their role in the aging process remain unclear. We examined the role of the eukaryotic initiation factor 4E (eIF4E), a key regulator of protein synthesis rate, in aging of the nematode *Caenorhabditis elegans*. Five eIF4E isoforms with different cap-binding specificity and anatomical expression are encoded in the *C. elegans* genome (Keiper et al., JBC, 275: 10590). We found that loss of a specific eIF4E isoform, IFE-2 that functions in the soma, extends nematode lifespan. Lifespan extension by IFE-2 depletion is independent of the forkhead transcription factor DAF-16, the downstream effector of the insulin-like signaling pathway. In addition, knockdown of *ife-2* further extends the lifespan of long-lived mutants carrying genetic lesions affecting the insulin/IGF signaling (*age-1*and *daf-2*), mitochondrial electron transport chain (*clk-1*), dietary restriction (*eat-2*) and nutrient-sensing (TOR) pathways. Long-lived *ife-2* mutant worms are more resistant to oxidative stress, compared with wild-type worms. Knockdown of *ife-2* extends lifespan of short-lived *mev-1(kn1)* mutants and increases their resistance to paraquat. Protein synthesis rates are lower in worms lacking IFE-2, compared to wild-type animals. Taken together, our results suggest that down-regulation of protein synthesis modulates aging in the worm by regulating somatic maintenance.

SMALL HSP CHAPERONES, AGING, AND RESISTANCE TO OXIDATIVE STRESS IN DROSOPHILA.

Robert M. Tanguay, Geneviève Morrow, Hyun-Ju Kim, Sébastien Michaud.

Lab Cellular Developmental Genetics, CREFSIP, Dept Medecine, Pav. Marchand, Université Laval, Québec, Canada G1K 7P4. Email: Robert.tanguay@rsvs.ulaval.ca

According to the free radical theory, lifespan is determined by the ability of organisms to cope with random damages induced by reactive oxygen species, the natural by-products of energy metabolism. Cells from the nervous system are particularly sensitive to ROS and studies in worms and flies argue for a neuroendocrine regulation of lifespan.

Small chaperones have been shown to be involved in the refolding or disposal of protein aggregates, a feature of many age-associated diseases. In *Drosophila melanogaster*, there are 4 main small Hsps each residing in a different intracellular compartment. Targeting the expression of the mitochondrial Hsp22, to different cell types can increase lifespan by more than 30%. Long-lived flies expressing Hsp22 in motorneurons have an increased resistance to oxidative stress and maintain their locomotor activity longer. Over expressing the cytosolic Hsp23 in a pan-neuronal fashion (transgenic GAL4/UAS system) also increases longevity by 15%. Conversely, a strain carrying an insertion in the promoter of *hsp23* which downregulates its expression in specific cells of the embryonic CNS and in adults has a decreased lifespan. The action of these chaperones on lifespan likely involves different pathways as suggested by the longevity curves, and their respective site and developmental pattern of expression. Microarrays analysis was used to unveil the mechanisms involved in lifespan. Results show that overexpression of mitochondrial Hsp22 affects, among others, genes of the insulin/IGF signaling pathway, one of the key elements in lifespan determination. The relation between this pathway and the heat shock response has been examined using flies with mutations in the heat shock factor HSF and dFOXO. Altogether, these results confirm a beneficial role of the expression of small chaperones and corroborate the pivotal role of the nervous system and the insulin/IGF pathway in the aging process.

Supported by CIHR (Canada) and the EU 6th Framework Programme MiMage.

STRESS-INDUCED SENESCENCE AND HUMAN AGING

Olivier Toussaint and Florence Chainiaux

Univ. of Namur, Research Unit on Cellular Biology Rue de Bruxelles, 61, B-5000 Namur, Belgium / http://www.fundp.ac.be/urbc /Email: olivier.toussaint@fundp.ac.be

Stress-induced premature senescence (SIPS) cell biology background.

SIPS occurs in several proliferative cell types like skin fibroblasts, lung fibroblasts, endothelial cells, etc. after subcytotoxic exposure(s) to tert-butylhydroperoxide (t-BHP), H_2O_2, ethanol, UV, etc. Cells in SIPS display features of replicatively senescent cells.

Firstly we have studied the cell signaling and found that H_2O_2–induced phosphorylation of Mitogen-Activated Protein Kinase p38MAPK triggers a sustained overexpression of Transforming Growth Factor-ß1 (TGF-ß1) via activation of ATF-2 transcription factor, and that a regulatory loop establishes between TGF-ß1 overexpression and sustained p38MAPK phosphorylation. At 24 hrs after stress, ATF-2 interacts with hypophosphorylated retinoblastoma protein, which allows the biomarkers of RS to appear (Frippiat et al., 2002, Chen et al., 2000, Frippiat et al., 2001, Chainiaux et al., 2005). Secondly we have shown that H_2O_2- and UVB-induced SIPS also takes place in fibroblasts with ectopically induced telomerase activity. Several p38MAPK-dependent genes were found in H_2O_2–induced senescence of human lung fibrobalsts (Zdanov et al., 2006). Very limited mean telomere shortening is observed in these conditions (Magalhaes et al., 2002).

Technological platforms.

Firstly proteomic studies (high resolution 2-D gels & mass spectrometry) identified 30 proteins differentially expressed in SIPS induced by ethanol and t-BHP and/or in RS (Dierick et al., 2002). Secondly we performed transcriptomic studies using dedicated low density cDNA arrays developed in-house in collaboration with a major european company (Magalhaes et al., 2004a). Thirdly we undertake functional studies for investigating the role of proteins that are differentially expressed in SIPS and/or RS like clusterin (apo J) or peroxiredoxin VI (Dumont et al., 2002, Salmon et al, 2004). Forthly, we have developed a digital database on aging and computational tools (presented in *Nature Review Genetics*, 2004; and in http://sageke.sciencemag.org , see also Magalhaes et al. FEBS Lett. (2004b).

References

Frippiat et al. (2002) Free Radic. Biol. Med. 33 : 1334-46 / Chen et al. (2000) J. Cell Sci. 113 (Pt 22) : 4087-97 / Frippiat et al. (2001) J. Biol. Chem. 276 : 2531-7 / Chainiaux et al. (2005) J Cell Sci, 118, 743-58 / Magalhaes et al. (2002) FEBS Lett. 523 : 157-62 / Dierick et al. (2002) FEBS Lett. 531 : 499-504 / Magalhaes et al. (2004a) Exp. Gerontol. 39 : 1379-1389 / Dumont et al. (2002) Cell Stress & Chaperones 7 , 23-35 / Salmon et al. (2004) FEBS Lett, 557, 25-32 / Magalhaes et al. (2004b) FEBS Lett. 571 : 243-7 / Zdanov et al. (2006) FEBS Lett. 580, 6455-63.

CARF IS INVOLVED IN SENESCENCE AND APOPTOTIC RESPONSE OF HUMAN CELLS

Sunil C. Kaul, Md. Kamrul Hasan, Tomoko Yaguchi and Renu Wadhwa

National Institute of Advanced Industrial Science & Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan. Email: renu-wadhwa@aist.go.jp

CARF, a collaborator of ARF, was first cloned as a novel ARF-binding protein by a yeast interaction screen. It also interacts with p53 and HDM2 leading to ARF-independent enhancement of p53 function. It undergoes a negative feedback regulation by an HDM2-dependent proteasome pathway and in turn regulates this pathway by acting as a transcriptional repressor of HDM2. By overexpression and silencing studies, we demonstrate that CARF exerts a vital control on the p53-HDM2-p21WAF1 pathway. CARF expression was upregulated during cellular senescence and its overexpression caused premature senescence in human fibroblasts. Whereas its overexpression caused senescence, its repression led cancer cells to the apoptosis route. We demonstrate that CARF exerts a vital control on the senescence and apoptotic pathway.

THE COMPLEX REGULATION OF HUMAN LIFESPAN

Rudi G.J. Westendorp

Dept. of Gerontology and Geriatrics Leiden University Medical Center,
PO box 9600, 2300 RC Leiden, The Netherlands. Email: r.g.j.westendorp@lumc.nl

The uninterrupted increase of life expectancy in developed countries was largely unforeseen and our societies will witness epidemics of diseases in older people. Current medical research should thus, in part, shift from understanding mid-life associated diseases to understanding the specifics of old age diseases. We are especially ignorant of the biological processes that explain malfunction in old age, referred to as "frailty" or "normal aging". If we strive to age healthily there is an urgent need for a better understanding of healthy aging to postpone the occurrence of disease and disability in old age.

The aging process is determined by genetic, environmental and stochastic factors. Clear candidate genes and mechanisms have emerged from mutant and genetically manipulated model organisms under laboratory conditions. These include the Insulin / Insulin-like Growth Factor Signalling (IIS) pathway, DNA repair systems, metabolic disorders, and oxidative stress biology. However, it has rarely been addressed whether the genetic variants of these model systems contribute to the standing genetic variation in natural populations and whether these experimental data can be extrapolated to humans.

We challenge experimental data from distinct model systems with observations in exceptional human cohort studies. To this end we combine basic and clinical research in ongoing genomics projects. In these experimental models and humans with different aging trajectories we will compare amongst others circulating levels of markers of the ISS pathway, somatotrophic axis, and metabolism. The potential outcome of this unique endeavour, experimental models and human observations pointing to the same signalling pathways, provides us with key mechanisms in which interventions may slow down the rate of aging.