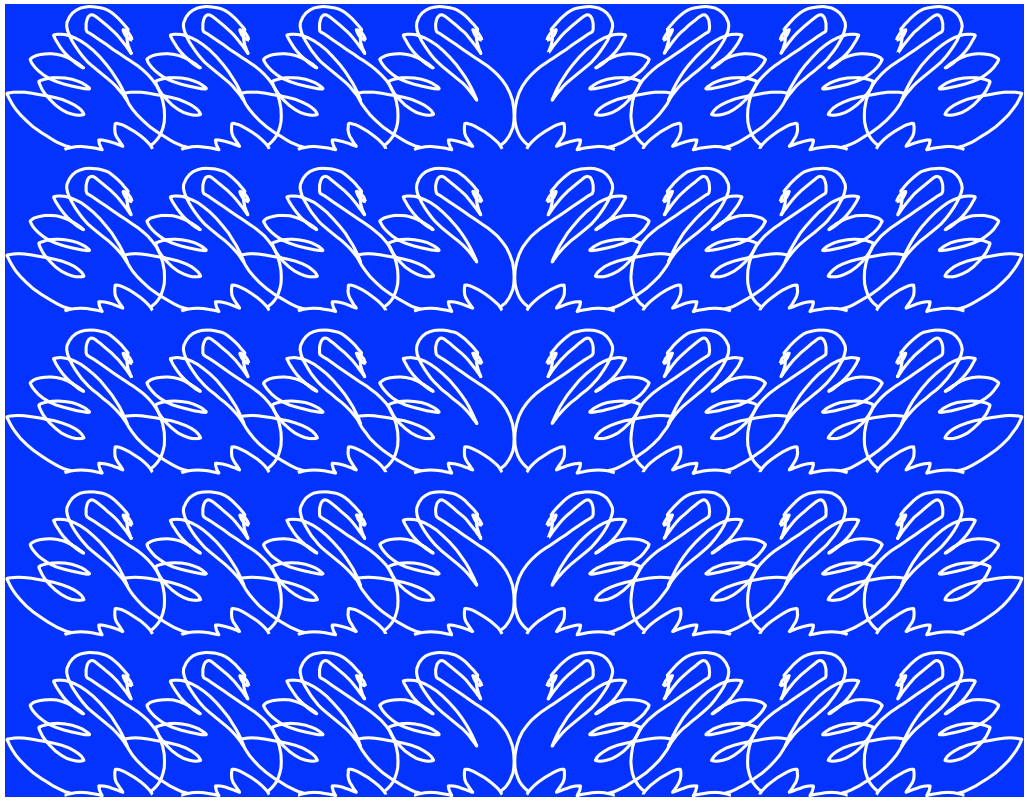
BENZON SYMPOSIUM
No. 62
GENOME INSTABILITY AND NEURODEGENERATION

AUGUST 22-25, 2016
COPENHAGEN, DENMARK



Organizing committee:
Lene Juel Rasmussen (Copenhagen), Ian D. Hickson (Copenhagen), Niels Borregaard (Copenhagen), and
Finn Cilius Nielsen (Copenhagen)

MONDAY, AUGUST 22, 2016

09:00-09:05 Welcome

Opening Keynote Lecture
Chair *Lene Juel Rasmussen*

09:05-09:55 ***Matthias Mann*:** Mass spectrometry-based proteomics in cell biology and biomedicine
09:55-10:05 Discussion

Session I ***Epidemiology of aging and neurodegeneration***
Chair *Lene Juel Rasmussen*

10:05-10:35 ***Nir Barzilai***: Targeting the Biology of Aging to Prevent Neurodegenerative diseases
10:35-10:45 Discussion

10:45-11:15 **Coffee Break**

11:15-11:45 ***Kaare Christensen***: Aging and Neurodegeneration in the Oldest-Old
11:45-11:55 Discussion

11:55-12:05 ***Rudi Westendorp***: Flow in Old Age (*Poster number I-1*)
12:05-12:10 Discussion

12:10-13:10 **LUNCH**

Session II ***The basic biology of DNA damage and repair in brain I***
Chair *Björn Schumacher*

13:10-13:40 ***Judith Campisi***: Cellular senescence links the DNA damage response to tissue degeneration
13:40-13:50 Discussion

13:50-14:20 ***Yosef Shiloh***: Linking ATM Functions to the Cerebellar Degeneration in Ataxia-Telangiectasia
14:20-14:30 Discussion

14:30-14:40 ***Daryl Shanley***: Computational modelling the cellular response to DNA damage: short term dynamics and long term consequences (*Poster number I-2*)
14:40-14:45 Discussion

14:45-15:15 **Coffee Break**

15:15-15:45 ***Lene Juel Rasmussen:*** Mitochondrial function regulates nucleotide metabolism and affects genomic stability: mechanisms and biomarker for cognitive function
15:45-15:55 Discussion

15:55-16:05 ***Victoria Meltser:*** Specific accumulation of p73 protein in irradiation-induced micronuclei (*Poster number I-3*)
16:05-16:10 Discussion

16:10-18:25 Authors present posters Nos. I-1 to I-19

19:30-21:30 **Reception at Café Mazzolis in Tivoli (old amusement park)**
(walking time from hotel to Tivoli is about 15 min.)

TUESDAY, AUGUST 23 2016

Session III ***The basic biology of DNA damage and repair in brain II***
Chair *Jan Vijg*

09:00-09:30 ***Ian D. Hickson***: Chromosome instability driven by fragile sites in the human genome
09:30-09:40 Discussion

09:40-10:10 ***Keith Caldecott***: DNA Single-Strand Break Repair and Human Neurological Disease
10:10-10:20 Discussion

10:20-10:50 **Coffee Break**

10:50-11:20 ***Björn Schumacher***: DNA damage responses in ageing and disease: an organismal perspective
11:20-11:30 Discussion

11:30-11:40 ***Sherif El-Khamisy***: Defective chromosomal break repair in spinal muscular atrophy *(Poster number II-1)*
11:40-11:45 Discussion

11:45-11:55 ***Victoria Alexandra Bjerregaard***: Folic Acid Deficiency Induces Anaphase DNABridges At The Fragile X Locus (*Poster number II-2*)
11:55-12:00 Discussion

12:00-13:00 **LUNCH**

Session IV ***Homeostasis in brain I***
Chair: *Vilhelm Bohr*

13:00-13:30 ***Karl Herrup***: ATM and ATR in neurons -- functions beyond the DNA damage response
13:30-13:40 Discussion

13:40-14:10 ***Jan Vijg***: Genome instability: a conserved mechanism of aging?
14:10-14:20 Discussion

14:20-14:30 ***Tom Kirkwood***: Aging, damage and repair: how complicated can it be? *(Poster number II-3)*
14:30-14:35 Discussion

14:35-15:00 **Coffee Break**

15:00-15:30 ***Karl Deisseroth***: Integrated brainwide structural and functional analysis
15:30-15:40 Discussion

15:40-15:50 ***Tinna Stevnsner:*** Regulation of base excision repair in the aging human brain (*Poster number II-4*)
15:50-15:55 Discussion

15:55-16:05 ***Claus Desler:*** Increased deoxythymidine triphosphate levels is a feature of relative cognitive decline (Poster number II-5)
16:05-16:10 Discussion

16:10-18:00 Authors present posters Nos. II-1 to II-15

WEDNESDAY, AUGUST 24, 2016

Session V	***Homeostasis in brain II***
Chair	*Jan Hoeijmakers*
09:00-09:30	***Vilhelm Bohr***: Nuclear to mitochondrial DNA damage signaling in neurodegeneration
09:30-09:40	Discussion
09:40-10:10	***Nils-Göran Larsson***: The role of Mitochondria in Parkinson´s disease
10:10-10:20	Discussion
10:20-10:50	**Coffee Break**
Session VI	***The pathogenesis of neurodegenerative disorders I***
Chair	*Nathaniel Heintz*
10:50-11:20	***Tony Wyss-Coray***: Systemic modulators of brain aging and plasticity
11:20-11:30	Discussion
11:30-11:40	***Kanagaraj Radhakrishnan***: Resolving RNA-DNA Damage-Induced Genomic Instability: Where There Is SETX, There Is a Way *(Poster number III-1)*
11:40-11:45	Discussion
11:45-11:55	***Hana Hanzlikova***: XRCC1 Mutations in Human Neurological Disease *(Poster number III-2)*
11:55-12:00	Discussion
12:00-13:00	**LUNCH**
Chair:	*Karl Herrup*
13:00-13:30	***Hongjun Song***: Dynamic DNA demethylation via base-excision repair regulates neuronal flexibility
13:30-13:40	Discussion
13:40-14:10	***Andre Nussenzweig***: The role of DNA breaks in neuronal gene expression determined by END-seq
14:10-14:20	Discussion
14:20-14:30	***Rami Aqeilan:*** Role of tumour suppressosr WWOX, gene product of a common fragile site, in brain development and neurodegeneration *(Poster number III-3)*
14:30-14:35	Discussion
14:35-15:00	**Coffee Break**
15:00-15:30	***Cynthia McMurray:*** Age–dependent expansion in human Huntington Disease: from genetics to metabolism
15:30-15:40	Discussion
15:40-15:50	***Morten Scheibye-Knudsen***: Neurodegeneration in Accelerated Aging (*Poster number III-4*)
15:50-15:55	Discussion
15:55-16:05	***Meryl Sønderby Lillenes:*** Altered DNA base excision repair profile in brain tissue and blood in Alzheimer's disease (*Poster number III-5*)

15:05-16:10 Discussion

16:10-17:40 Authors present posters Nos III-1 to III-19

19:30-23:30 **Banquet at The University of Copenhagen**
(walk 19:25; time from hotel to University is about 3 min.)

THURSDAY, AUGUST 25, 2015

Session VII ***The pathogenesis of neurodegenerative disorders II***
Chair *Cynthia McMurray*

09:00-09:30 ***Nathaniel Heintz:*** Exploring the Molecular Landscapes of CNS Cell Types: 5hmC, MeCP2 and Stabilization of Neuronal Phenotypes
09:30-09:40 Discussion

09:40-10:10 ***Martin Lavin:*** Role of oxidative stress in the ataxia-telangiectasia phenotype
10:10-10:20 Discussion

10:20-10:50 **Coffee Break**

10:50-11:00 ***Tone Tønjum*****:** On the Brain-Gut axis: Differential expression of DNA repair pathways in human brain and mucosal gut tissue *(Poster number III-6)*
11:05-11:05 Discussion

11:05-11:15 ***Cecilie Morland:*** A novel mechanism for exercise-induced angiogenesis in the brain (*Poster number III-7*)
11:15-11:20 Discussion
11:20-11:50 ***Maiken Nedergaard***: The Glymphatic system and its importance in amyloid clearance
11:50-12:00 Discussion

12:00-13:00 **LUNCH**

Chair *Ian D. Hickson*

13:00-13:30 ***Zhao-Qi Wang:*** Canonical and non-canonical functions of DDR molecules in neuro(de)generation
13:30-13:40 Discussion

13:40-14:10 ***Peter McKinnon:*** Maintaining genome stability to prevent neurologic Disease
14:10-14:20 Discussion

14:20-14:50 **Coffee Break**

14:50-15:00 ***Nabieh Ayoub***: NELF-E Facilitates Transcription Silencing at DNA Double-Strand Breaks and Promotes DNA Repair *(Poster number III-8)*
15:00-15:05 Discussion

15:05-15:10 ***Pier Giorgio Mastroberardino:*** DNA repair is an aging-related modifier of Parkinson's disease *(Poster number III-9)*
15:10-15:15 Discussion

Final Keynote Lecture

15:15-16:05 ***Jan Hoeijmakers:*** The impact of DNA damage on neurodegeneration and the potential of nutritional interventions
16:05-16:15 Discussion
16:15-16:25 Concluding Remarks

MONDAY, AUGUST 22, 2016

OPENING LECTURE

MASS SPECTROMETRY-BASED PROTEOMICS IN CELL BIOLOGY AND BIOMEDICINE

Matthias Mann; Max Planck Institute of Biochemistry, Munich, Germany and Novo Nordisk Foundation Center for Protein Research, Copenhagen

E-mail: mmann@biochem.mpg.de

Mass spectrometry has a long and illustrious history as an analytical science, even before the development of electrospray and MALDI made MS easily applicable to the study of proteins. Developments over the last few years have steadily increased the scope of MS-based studies in molecular biology but important challenges remain, chief among them the lack of comprehensiveness compared to oligonucleotide based systems. However, this limitation is now falling away, as shown by deep proteomic analysis of human cancer cell lines. More than 10,000 different proteins can now be identified in such systems, in a relatively short time, shedding new light on similarities and differences to each other and to in vivo cells. Developments in sample preparation make these capabilities available in clinically relevant material as well, such as formalin-fixed, paraffin embedded samples.

This included streamlined and highly efficient sample preparation, analysis with very high sequencing speed using modern mass spectrometers and bioinformatic analysis using the MaxQuant and Perseus platforms. Efforts in our group have focused on 'single shot' analysis and we demonstrate very high coverage in this mode (Mann et al., Mol. Cell, 2013). We have also extended this concept to the analysis of cellular interactomes (Hein et al. Cell 2015) and transcription factor complexes. There has likewise been much progress in the analysis of DNA bound complexes, for instance in the context of DNA repair (Raeschle et al. Science 2015). For post-translational modifications such as phosphorylation, the 'EasyPhos' method now allows acquiring large numbers of phosphoproteomes, for instance for the analysis of in vivo signaling (Humphrey et al. Nat. Biotech, 2015). Together such developments make proteomics increasingly relevant to translational research.

TARGETING THE BIOLOGY OF AGING TO PREVENT NEURODEGENERATIVE DISEASES

Nir Barzilai; Institute for Aging Research at Albert Einstein College of Medicine, Bronx NY, USA

E-mail: Nir.Barzilai@einstein.yu.edu

Aging is the major risk not only for AD but also for other diseases such as cancer, type 2 Diabetes mellitus and cardiovascular disease. We hypothesize that a progress in preventing these diseases will occur only if we can understand the reason people age at different rates, and develop strategy to delay aging. We present 2 examples.

We study the genome of centenarians, whose aging and onset of its diseases have been delayed. We have implicated a longevity genotype, cholesterol ester transfer protein (CETP), in the preservation of cognitive function in centenarians and their families. CETP inhibitor is in phase III trial to prevent CVD but offers an approach for prevention of AD.

Mutations that reduce somatotropic signaling result in improved lifespan and health-span in model organisms and humans. We have discovered several functional genomic changes in the

GH/IGF-1 pathways in centenarians. Furthermore, higher circulating IGF-1 is associated with worse cognitive function in females with exceptional longevity, without impairment in skeletal muscle mass and function.

These examples suggest an approach of delaying aging and several of its disease, rather than focus on one organ-specific drug at a time. We are in the process of establishing a study that will show a proof of principal to this idea by targeting aging with metformin (TAME).

AGING AND NEURODEGENERATION IN THE OLDEST-OLD

Kaare Christensen; Institute of Public Health, University of Southern Denmark, Odense, Denmark

E-mail: KChristensen@health.sdu.dk

A rapidly increasing proportion of individuals in the Western world are surviving into their tenth decade. While there is no doubt that we are doing well in making the elderly survive longer than previously, the key question is whether we are also doing good for the oldest-old. There is widespread concern that the basis for the survival success is better survival of frail and disabled elderly into the highest ages. To investigate this issue we have collected data on aging trajectories for Danish twins born since 1900 as well as for the entire Danish birth cohorts 1895, 1905 and 1915. These studies show that more people are living to the highest ages with better overall cognitive and physical functioning. So we are doing good, and there are reasons to expect the positive trajectory of cognitive functioning among the oldest-old to continue in the coming decades.

FLOW IN OLD AGE

(Poster number I-1)

Rudi GJ Westendorp; Department of Public Health, and, Center for Healthy Ageing, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

E-mail: westendorp@sund.ku.dk

Perfusion is the process by which arterial blood is delivered to a capillary bed in tissue. A constant level of perfusion is necessary to secure the adequate and timely supply of the building blocks of cellular metabolism, i.e. oxygen and glucose. Our success to prevent fatal atherosclerotic events leaves older people susceptible for different outcomes of vascular disease, as there are heart- and kidney-failure and dementia. Blood vessels, both large and small, tend to lose elasticity and narrow, causing biomechanical difficulties for maintaining levels of flow. There may also be age-related changes in the efficiency of (i) the heart as a pumping engine, (ii) maintaining a sufficiently high mean arterial pressure, (iii) neuroendocrine signaling pathways optimizing capillary flow to metabolic needs. Impaired perfusion and the resulting hypoxia and energy depletion at the cellular level give rise to stress responses involving, inter alia, the hypoxia-inducible factor 1α that regulates a complex array of physiological functions including: angiogenesis, erythropoiesis, metabolism, autophagy, and apoptosis. Too little attention has been paid to the homeostatic regulation of perfusion across the life course and what can be done to minimize the adverse consequences for maintaining the health of the body-brain-mind axis.

CELLULAR SENESCENCE LINKS THE DNA DAMAGE RESPONSE TO TISSUE DEGENERATION

Judith Campisi; Buck Institute for Research on Aging and Lawrence Berkeley National Laboratory, USA

E-mail: jcampisi@lbl.gov

Cellular senescence is a multi-faceted stress response that had two main features. The first feature is an essentially irreversible cell cycle arrest that suppresses the development of cancer by preventing stressed or damaged cells from proliferating. The second feature is a robust secretory phenotype, which we term the senescence-associated secretory phenotype (SASP). The SASP entails the secretion of numerous bioactive molecules, including pro-inflammatory cytokines, chemokines, growth factors and proteases. Among the pathways that activate and regulate the SASP is the DNA damage response (DDR) pathway. We found that DNA damage is one of several potent inducers of the SASP.

Morevover, DDR components upstream of p53, including the ATM, CHK2 and NBS1 proteins, are required for both the initiation and establishment of the SASP. The DDR signaling that sustains the SASP emanates from persistent DNA damage foci termed DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence), which contain activated (phosphorylated) ATM, CHK2 and p53 and are particularly important for the pro-inflammatory arm of the SASP. What are the consequences of cellular senescence resulting from DNA damage? Senescent cells, including those resulting from DNA damage, accumulate with age and are now known to drive several phenotypes and pathologies associated with aging. We find that DNA damage induces a senescence response and pro-inflammatory SASP in human and mouse astrocytes. Further, senescent astrocytes can drive symptoms associated with Parkinson's disease (PD), and possibly other neurodegenerative pathologies. Using a transgenic mouse that permits the selective elimination of senescent cells, we show that the senescent astrocytes are major contributors to the loss of motor neuron function associated with PD symptoms.

Our data support the idea that the senescence response to DNA damage is a double-edged sword – protecting organisms from cancer, while driving age-associated pathology, including neurodegeneration.

LINKING ATM FUNCTIONS TO THE CEREBELLAR DEGENERATION IN ATAXIA-TELANGIECTASIA

Yosef Shiloh; Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

E-mail: yossih@post.tau.ac.il

The nervous system is extremely vulnerable to inherited defects in maintenance of genome instability, as evidenced by a variety of human and mouse mutant phenotypes. Ataxia-telangiectasia (A-T) is a prototype human genomic instability syndrome whose cardinal and most devastating symptom is relentless neurodegeneration affecting primarily the cerebellum. A-T is also characterized by immunodeficiency, cancer predisposition, radiation sensitivity, endocrine abnormalities, and segmental premature aging. Our work is guided primarily by our attempt to understand the complex A-T phenotype.

The responsible gene, *ATM*, encodes the homeostatic, powerful ATM protein kinase, which is involved in many cellular circuitries. Despite the extensive investigation of ATM functions, the molecular basis of the major A-T symptom – the cerebellar atrophy – is still in question, implying that a cardinal function of ATM is still not understood. ATM is vigorously activated by double-strand breaks (DSBs) in the DNA, and subsequently sets in motion a broad signaling network – the DNA damage response (DDR) – by phosphorylating numerous targets in its various branches. Recently we concluded that in parallel to its striking activation by DSBs, ATM is also involved in repair pathways directed at other DNA lesions and is thus a major player in maintenance of genome stability in a broader context. We attribute great importance to this general role of ATM in maintaining genome integrity, in cellular and tissue homeostasis and aging. The unique combination of cell types of the cerebellar tissue, the physiology of these cells and their individual and interactive modes of action render the cerebellum particularly vulnerable to ATM loss long before it affects many other tissues. We are establishing genetically manipulated mouse strains to examine these conjectures. Examples will be shown.

COMPUTATIONAL MODELLING THE CELLULAR RESPONSE TO DNA DAMAGE: SHORT TERM DYNAMICS AND LONG TERM CONSEQUENCES

(Poster number I-2)

Daryl Shanley; Newcastle University Institute for Ageing, Newcastle Upon Tyne, UK

E-mail: daryl.shanley@newcastle.ac.uk

Cellular health for neurons and glia as for any other cell depends on internal state and the capacity to respond to challenge. DNA damage presents a serious challenge and a network of signalling systems respond to regulate repair and changes in cellular metabolism. As organisms age the level of challenge is raised through factors such as increased levels oxidative stress. Computational modelling provides a means to study the complex relationships between these different processes and the impact of oxidative stress. I will present recent work with a particular focus on cellular senescence. Our modelling demonstrates the importance of short term dynamics in ageing: 1) progressive change with age such as unrepaired DNA damage and mitochondrial dysfunction affect the short term response (e.g. Dalle Pezze et al. 2014); 2) conversely, signalling dynamics change with age and affect whether or not DNA damage is repaired effectively (Dolan et al. 2015); and 3) potential treatment strategies are affected by these changes which I will illustrate with irradiation therapy (e.g. low dose irradiation delivered at specific time intervals), use of antioxidants and metabolic drugs such as rapamycin and metformin.

References:

1) Dalle Pezze P**,** Nelson G, Otten EG, Korolchuk VI, Kirkwood TBL, von Zglinicki T, Shanley DP (2014) Dynamic modelling of pathways to cellular senescence reveals strategies for targeted interventions. PLoS Comp Biol 10(8): e1003728

2) Dolan DWP, Zupanic A, Nelson G, Hall P, Miwa S, Kirkwood TBL, Shanley DP (2015) Integrated Stochastic Model of DNA damage repair by non-homologous end joining and p53/p21 mediated early senescence signalling. PLoS Comp Biol 11(5): e1004246

MITOCHONDRIAL FUNCTION REGULATES NUCLEOTIDE METABOLISM AND AFFECTS GENOMIC STABILITY: MECHANISMS AND BIOMARKER FOR COGNITIVE FUNCTION

Lene Juel Rasmussen[1,2]
[1]Center for Healthy Aging; [2]Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark

E-mail: lenera@sund.ku.dk

Mitochondria are the powerhouse of the cell and where cellular energy supplies in the form of ATP are generated. Because of this pivotal role, mitochondrial dysfunction is very damaging for the cell and can lead to numerous pathological conditions in humans. Most notably, mitochondrial dysfunction is associated with cognitive decline, neurological abnormalities and aging.

Balanced levels of dNTP are important for genomic stability. Accordingly, imbalance of the cytosolic dNTP pool has been demonstrated to decrease the genetic stability. We show that depletion of mtDNA of human cell lines results in an imbalance of the cytosolic dNTP pools and a decrease of chromosomal stability. MtDNA primarily encodes peptides essential for the activity of the mitochondrial electron transport chain and, therefore, also ATP produced by oxidative phosphorylation. The ETC is also linked to the *de novo* synthesis of pyrimidines through the enzyme dihydroorotate dehydrogenase (DHODHase) located in the inner membrane of the mitochondria. Our findings support a model for the initiation of genome instability through a mitochondrial dysfunction and resulting imbalance of the cytosolic dNTP levels. This places fitness of mitochondria as an important determinant of genomic instability.

Cognitive impairment in adults may be an early indicator of later life dementia. Therefore, it is important to search for early biomarkers of cognitive decline. Our data promote investigation into mitochondrial activities, dNTP levels, and DNA damage as potential correlates or predictors of cognitive decline, which may lead to early treatment initiatives in order to delay or prevent later life dementia.

SPECIFIC ACCUMULATION OF P73 PROTEIN IN IRRADIATION-INDUCED MICRONUCLEI

(Poster number I-3)

Victoria Meltser, Israel Ben-Dor, Nina Reuven, Yosef Shaul; Department of Molecular Genetics, Weizmann Institute of Science, Israel

E-mail: victoria.meltser@weizmann.ac.il

Micronuclei (MNi) are small sub-nuclear compartments that form due to the missegregation of whole chromosomes or chromosome parts during mitosis. MNi can form either from lagging chromosomes or from acentric chromosomal fragments caused by DNA damage-induced double stranded breaks. In both cases the missegregated chromosomes recruit their own nuclear envelope to form MNi. Although MNi structurally resemble the primary nuclei, a large proportion of them were shown to have reduced nuclear functioning and to undergo nuclear envelope collapse.

Such dysfunctional MNi have also been recently suggested to be instrumental for the mutagenic process of chromotripsis, in which MNi-enclosed chromosomes undergo massive rearrangements and can lead to severe genomic instability upon re-integration into the primary nucleus. p73, a member of the p53 family of genes, is an important player in both apoptotic cell death and neuronal cell survival.

We found that MNi induced by DNA damage in cultured cells contain high levels of the p73 protein compared to its levels in the primary nucleus. High p73 protein accumulation within MNi was observed in both cancer and normal cells and occurred in simian, human as well as murine cell lines. Morphologically, the protein-rich MNi manifest normal nuclear lamina and nuclear membrane but have an uneven nuclear pore complex distribution, which may compromise their nuclear import-export capacity.

We demonstrate that p73 accumulation in MNi is a regulated process since it is rapidly diminished by the nuclear export inhibitor leptomycin B. Interestingly, p73 accumulation was more pronounced in MNi that contained less DNA, ruling out DNA association as a mechanism of p73 accumulation.

These results suggest that the nuclear content of the p73 protein in the cell is regulated by a novel mechanism of MNi mediated exclusion, which not only diminishes p73 nuclear accumulation but also constrains p73 cytoplasmic accessibility.

References:

1) Agami, R., Blandino, G., Oren, M., and Shaul, Y. (1999). Nature 399, 809-813.
2) Ben-Yehoyada, M., Ben-Dor, I., and Shaul, Y. (2003). The Journal of biological chemistry 278, 34475-34482.

IMAGING GENETIC ASSOCIATION OF COGNITIVE FUNCTION IN AN AGEING COHORT

Kiyana Zarnani [a, b], Jayachandra Mitta Raghava [a], Naja Liv Hansen [a, b, d], Erik Lykke Mortensen [b, e], Merete Osler [b, e, g], Martin Lauritzen [b, c], Egill Rostrup [a, b]

a) Functional Imaging Unit, Dept. of Clinical Physiology, Nuclear Medicine and PET, Copenhagen University Hospital Rigshospitalet, Glostrup, Denmark
b) Center for Healthy Aging, University of Copenhagen, Denmark
c) Department of Clinical Physiology, Nuclear Medicine and PET, Copenhagen University Hospital
Rigshospitalet Blegdamsvej, Copenhagen, Denmark
d) Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital Bispebjerg, Copenhagen, Denmark
e) Department of Public Health, University of Copenhagen, Denmark
f) Department of Radiology, Copenhagen University Hospital Rigshospitalet Glostrup, Glostrup, Denmark
g) Research Center for Prevention and Health, Copenhagen University Hospital Rigshospitalet Glostrup, Glostrup, Denmark

E-mail: kiyana.zarnani@regionh.dk

Background: The silent epidemic of 'population ageing' has inflated the number of people afflicted with neural disorders, creating a combined social and economical burden. With increased age, we observe alterations in brain homeostasis, a decline in cognitive function, and an increased risk factor for nervous system dysfunction[1]. Common genetic variations

identified by genome-wide association studies (GWAS) have shown to be liable for much of the observed variance in both cognitive function[2], and brain microstructure of ageing individuals. Presently, we investigate whether brain microstructural changes mediate cognitive performance, and which genetic variations are important contributors of the brain microstructural variation seen among non-demented elderly[3].

Method: We implement a multimodal approach using a combination of neuroimaging and genotyping to explore the relationship between genes, brain structure, and cognition in a cohort of healthy males, all born in 1953. Individuals selected based on there estimated change in cognitive function acquired at age 20 completed further extensive cognitive examinations, alongside MR Diffusion Tensor Imaging (DTI). Genome mapping was performed on 200 subjects using a Illumina array (HumanOmniExpress-12), that provided 719665 SNPs. SNPs previously associated with cognitive function or decline, and with DNA repair were identified, and their association with cognition in the present sample were estimated.

Results: We found significant associations between risk-associated genotypes COMT, BDNF, NRG1 IGF-1, DRD-2, NOS, DISC1, DAPK1, SIRT1, FOXO3 and cognitive performance. Furthermore, significant associations were also observed between fractional anisotropy (FA) and genotypes FOXO3 and COMT, mean diffusivity (MD) and FOXO3, axial diffusivity (L1) and FOXO3 and NRG1, and mode (MO) and COMT, Neil2 and SIRT1.

Discussion: Our results present statistically significant associations between common risk alleles, WM integrity and lower cognitive functioning, pointing strongly to a biological background for the differential development in cognitive function.

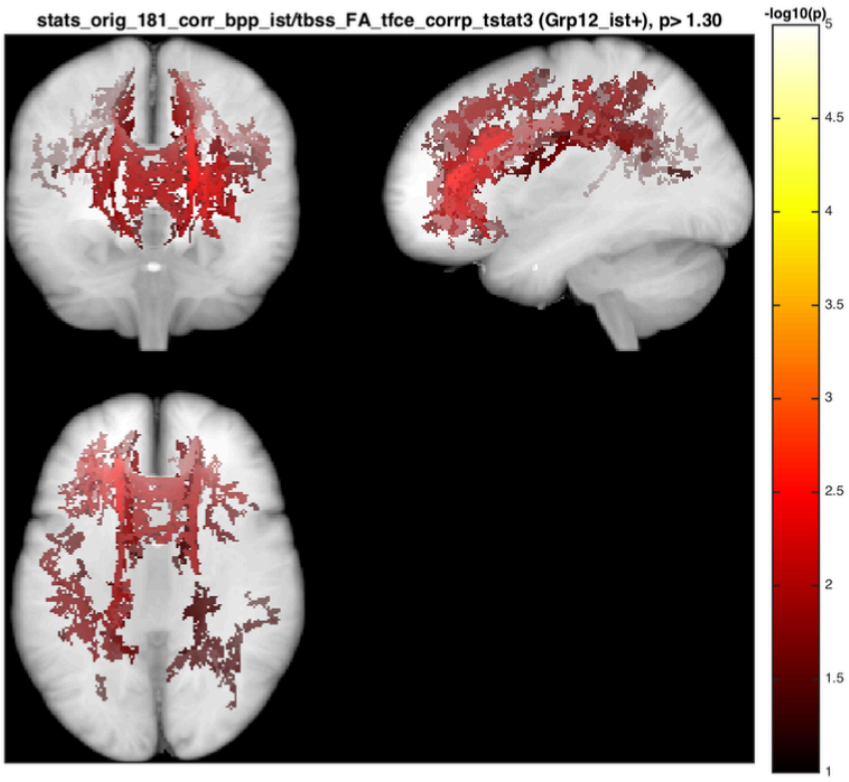


Figure 1: TBSS analyses show significant association between cognitive performance in IST examination and fractional anisotropy (FA) value in n = 181 Metropolit cohort members.

References:
1) Salthouse, T. A. (2010). Selective review of cognitive aging. Journal of the International Neuropsychological Society, 16(05), 754–760. http://doi.org/10.1017/S1355617710000706
2) Petrella, J. R., Mattay, V. S., & Doraiswamy, P. M. (2008). Imaging genetics of brain longevity and mental wellness: the next frontier? Radiology, 246(1), 20–32. http://doi.org/10.1148/radiol.2461061994
3) Harris, S. E., Fox, H., Wright, A. F., Hayward, C., Starr, J. M., Whalley, L. J., & Deary, I. J. (2007). A genetic association analysis of cognitive ability and cognitive ageing using 325 markers for 109 genes associated with oxidative stress or cognition. BMC Genetics, 8(1), 43. http://doi.org/10.1186/1471-2156-8-43

INVESTIGATIONS OF CHROMOSOMAL FRAGILE SITES IN THE AVIAN CELL LINE DT40

Vibe H. Oestergaard(1), Constanze Pentzold(1), Shiraz A. Shah(1), Benoit Le Tallec(2), Andaine Seguin Orlando(3), Maria Avila-Arcos(4), K.J. Patel(5), Michelle Debatisse(6) and Michael Lisby(1)

1) Department of Biology, University of Copenhagen, Denmark
2) Institut Curie, Paris, France
3) National High-throughput DNA Sequencing Centre, University of Copenhagen, Denmark
4) Natural History Museum of Denmark, University of Copenhagen, Denmark
5) MRC Laboratory of Molecular Biology, Cambridge, UK

E-mail: vibe@bio.ku.dk

Chromosomal Fragile Sites (CFSs) are specific regions on the chromosomes, which have a high propensity to break upon inhibition of DNA replication. Moreover CFSs are mutational hotspots in cancer genomes. Various cellular processes seem to affect CFSs including DNA replication, transcription, checkpoints and DNA repair pathways, but detailed mechanistic insight is still sparse. Orthologs of human CFSs have been found in a number of other mammalian species, but the extent of conservation of CFSs beyond the mammalian lineage is unclear. We have taken advantage of the finding that FANCD2 binds CFS in mitosis [1, 2].

Thus we have tagged both alleles of FANCD2 in the avian cell line DT40 and we have performed ChIPseq to identify genomic regions where FANCD2 are enriched in response to replication stress by treatment with aphidicolin (APH). FISH analyses confirm that identified sites for FANCD2 enrichment are bona fide fragile sites. Interestingly many of these regions overlap with the largest genes in the chicken genome, some of which have human orthologs in CFSs. We predict that the obtained map of CFSs in combination with the tools developed for the DT40 system will enable a systematic dissection of fragility in an isogenic genetically tractable system.

To this end we have inserted a counter selectable marker at the CFS PARK2 allowing us to study the mutational processes acting at CFSs. DT40 clones carrying the counter selectable marker at the PARK2 locus display high but variable mutation rates at the locus and large deletions are the predominant mutation events.

References:
1) Chan KL, Palmai-Pallag T, Ying S, Hickson ID. Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. Nat Cell Biol 2009; 11:753-60.
2) Naim V, Rosselli F. The FANC pathway and BLM collaborate during mitosis to prevent micro-nucleation and chromosome abnormalities. Nat Cell Biol 2009; 11:761-8.

HOW MITOCHONDRIAL FITNESS IMPACT TELOMERE HOMEOSTASIS

(Poster number I-6)

Thomas Lau Hansen, Claus Desler Madsen, Lene Juel Rasmussen

Center for Healthy Aging, Institute for Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

E-mail: tlhansen@sund.ku.dk

Telomeres are patches of repeated sequences flanking the ends of each chromosome in the eukaryotic genome. Because of incomplete replication and particularly DNA damage, the telomeres shorten for every cell division while ensuring genomic integrity. To counteract this regression, stem cells, germline cells and many cancer cells apply the telomere extending capabilities of the specialized reverse transcriptase – telomerase[1]. The activity of telomerase is dependent on multiple factors including substrate levels[2]. We therefore propose that mitochondria bioenergetics play a major role in the extension and regulation of telomere length.

Mitochondrial fitness decrease with age through slow but constant accumulation of damage to the mitochondrial DNA (mtDNA)[3,4,5]. We have previously shown that dysfunctional mitochondria are able to affect DNA replication and repair, and we therefore hypothesize that cellular aging is able to influence telomere homeostasis indirectly through its negative impact on mitochondrial fitness, connecting the mitochondrial and telomere theories of aging.

We make use of the high throughput Flow-FISH technique to assay average telomere lengths. By inhibition of mitochondrial respiration or simulation of maximal respiration, the mitochondrial functions are pertubated to record the effects on telomere homeostasis.

All of this is initially done in a human osteosarcoma cell line, to see the overall effects in immortalized cells.

The hTERT-positive cell line is treated with telomerase inhibitor in a comparative assay to record the specific mitochondrial effects on replication, shortening and elongation of telomeres. This effect will be confirmed in an inducible telomerase positive cell line, which further allows for calculation of the direct mitochondrial influence on telomerase activity.

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THE DNA NUCLEOTIDE EXCISION REPAIR PROFILE IN BLOOD AND BRAIN TISSUE FROM PATIENTS WITH ALZHEIMER'S DISEASE

Helge Leander B. Jensen[1], Alberto Rabano[3], Ingun Dina Ulstein[4], Tone Tønjum[1,2] and Meryl S. Lillenes[1,2]

1) Department of Microbiology, University of Oslo, Oslo, Norway
2) Department of Microbiology, Oslo University Hospital, Oslo, Norway
3) Fundación Centro Investigación Enfermedades Neurológicas (CIEN), Spain
4) Department of Old Age Psychiatry, Oslo University Hospital, Oslo, Norway

E-mail: helge.leander@gmail.com

Alzheimer's disease (AD) is a progressive, multifactorial neurodegenerative disorder that is the main cause of dementia globally. AD is clinically well characterized, however, little is known about the early etiology. Aging is the greatest risk factor for the development of dementia including AD, and age-related changes are augmented in patients with AD. Increased oxidative stress, resulting from imbalance in production and clearance of reactive oxygen species (ROS), can damage DNA and other macromolecules, leading to genome instability and disrupted cellular functions.

Oxidative DNA damage is primarily repaired by the base excision repair (BER) pathway, however, the nucleotide excision repair (NER) pathway repairing helix distorting damages also seems to be involved (1, 2). NER is known to be associated with neurodegenerative disorders like Cockayne syndrome and some subtypes of Xeroderma pigmentosum, and may play a role in the early stages of AD (3). To this end, we addressed the role of the NER pathway in the development of AD by comparing the expression of the DNA repair components RAD23B, RPA1, ERCC1, LIG3, PCNA and MPG in blood and post-mortem brain tissue from patients with AD, mild cognitive impairment (MCI) and healthy controls (HC). mRNA levels of the DNA repair components selected were significantly higher in the brain compared to blood. Notably, the expression of LIG3 (frontal cortex) and RPA1 (cerebellum) was higher in the AD brain than in healthy controls. This suggests an important role of NER in the brain relevant for the etiology of AD.

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IMPACT OF DEREGULATION OF dNTP POOLS ON EXPRESSION OF LARGE GENES

Vasileios Voutsinos, Michael Lisby and Vibe H. Oestergaard; Department of Biology, University of Copenhagen, Copenhagen, Denmark

E-mail: billvoutsinos@gmail.com

Deoxyribonucleotides (dNTPs) are synthesized by the ribonucleotide reductase enzyme (RNR), which catalyzes the rate limiting step in dNTP production by reducing the ribonucleoside diphosphates to their corresponding deoxy forms. By inhibiting the activity of RNR we can decrease the dNTP levels in the cell and therefore inhibit replication (1).

Replication inhibition has been shown to increase instability at common fragile sites (CFS), which harbor transcriptionally active large genes (2). It has been shown that transcriptionally active large genes, including several CFS genes, exhibit high rates of double strand breaks (DSBs) and genomic rearrangements. Such genes are very commonly expressed in brain cells and have been associated with neurodevelopmental and neuropsychiatric disorders. Nevertheless, large gene structure at CFSs is evolutionarily conserved leading to the speculation that their size and fragility might have a physiological function in the cell and or the organism (3).

In this study we deregulate RNR by using various drugs (HU, COH29) and genetic manipulations resulting in deregulated dNTP pools. We want to assess the impact that changed dNTP levels have on the stability of CFSs. By fluorescent tagging of genes located within the CFSs, we want to investigate changes in their expression levels in response to replication stress. Then we will assess potential epigenetic changes at these sites, due to the RNR deregulation.

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REV1 DEFICIENCY LEADS TO PARP1 ACTIVATION AND MITOCHONDRIAL DYSFUNCTION

Nima B. Fakouri[1], Christine Regnell[1,2], Maria Angleys[1], Jon Durhuus[1], Claus Desler[1], Anastasia Tsaalbi-Shtylik[3], Mahdi Hasan[2], Niels de Wind[3], Linda Bergersen[1,2], Lene Juel Rasmussen[1]

1) Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark
2) Department of Oral Biology, University of Oslo, Norway
3) Leiden University Medical Center, Leiden, Netherlands

E-mail: fakouri@sund.ku.dk

Endogenous and exogenous agents that damage the DNA constantly challenge genomes of every organism. Unrepaired lesions within the genome act as obstacles for DNA replication and transcription machineries, which can cause replication fork arrest. The stalled replication can generate ssDNA breaks and gaps as well as DSBs, which left unrepaired, lead to genomic instability (Zeman & Cimprich 2014). Translesion synthesis (TLS) is activated in response to

replication stress and fork arrest and carried out by the Y family of DNA polymerases that can replicate the damaged DNA templates. Rev1 is one of the key enzymes in this process that mediate the assembly of TLS machinery on PCNA and has deoxycytidyl transferase activity (Sale et al. 2012). Mice lacking Rev1 protein show premature aging phenotypes, which resemble physiological disorders related to aging such as liver degeneration, type 2 diabetes, and obesity (Tsaalbi-Shtylik A. et al., unpublished). Our data show that mitochondrial functions are impaired in liver and MEF cells derived from $Rev1^{-/-}$. The expression and activity of PARP1 in cells from $Rev1^{-/-}$ mice is increased.

Our results suggest that activation of PARP1 in $Rev1^{-/-}$ cells disrupt mitochondrial homeostasis, which could be mediated through increased consumption of NAD^{+}.

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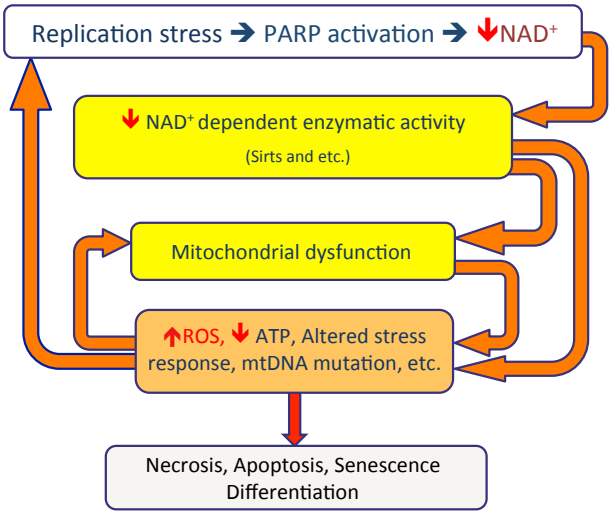

TUESDAY, AUGUST 23, 2016

MOLECULAR BASIS OF CHROMOSOME FRAGILITY

Ian D. Hickson; Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark

E-mail: iandh@sund.ku.dk

Fragile sites in the human genome are defined by their propensity to appear as gaps or breaks on otherwise hyper-condensed metaphase chromosomes. This is termed fragile site 'expression'. The phenomenon of chromosome fragility has been known for decades, but only recently has there been renewed interest into the mechanism by which fragility arises. Common fragile sites (CFSs) are present in all individuals, whilst rare fragile sites (RFSs) are restricted in the population to those individuals who inherit a genetic defect at the affected locus. CFSs and RFSs are associated with human disease – with CFSs being hotspots for genome rearrangements in cancer, and RFSs with neurological and developmental defects. RFSs are generally defined by their repeated sequence features, such as an expansion in a CGG triplet repeat at the FRAXA locus associated with Fragile X syndrome. In contrast, CFSs rarely have a diagnostic sequence motif.

We are analyzing the molecular mechanism by which fragile site expression occurs. Our working model is that defective DNA replication underlies all cases of fragility. Through exacerbating replication difficulties at fragile sites, we have shown that DNA synthesis can still be occurring during mitosis and that it utilizes a mechanism based on homologous recombination. Fragile sites also fail to be evenly or accurately segregated in mitosis, which might promote locus-specific DNA rearrangements. Our latest studies in this area will be discussed.

DNA SINGLE-STRAND BREAK REPAIR AND HUMAN NEUROLOGICAL DISEASE

Keith W. Caldecott; Genome Damage and Stability Centre, School for Life SDciences, University of Sussex, Falmer, Brighton, UK

E-mail: k.w.caldecott@sussex.ac.uk

DNA single-strand breaks (SSBs) are amongst the most frequent DNA lesions arising in cells and if not repaired correctly can threaten both genetic stability and cell survival. The repair of SSBs is regulated and facilitated by protein ADP-ribosylation via the activation of one or more of the ADP-ribosyl transferases PARP1, PARP2, and PARP3. PARP enzymes promote SSB repair by promoting the recruitment of the scaffold protein XRCC1 at sites of SSBs and thereby enabling the assembly of enzyme complexes that process these lesions. Indeed, mutations in XRCC1 protein partners are associated with hereditary neurodegenerative disease in humans, as illustrated by the genetic diseases *ataxia oculomotor apraxia-1* (AOA1; mutated in *APTX*), *spinocerebellar ataxia with axonal neuropathy-1* (SCAN1; mutated in *TDP1*), and *microcephaly with early onset seizures* (MCSZ; mutated in *PNKP*). Here, I will present new data highlighting the mechanism of SSB repair and neuroprotective impact of XRCC1 protein complexes in humans, and I will describe a molecular mechanism by which un-repaired SSBs trigger neuropathology.

DNA DAMAGE RESPONSES IN AGEING AND DISEASE: AN ORGANISMAL PERSPECTIVE

Björn Schumacher; Institute for Genome Stability in Aging and Disease, Medical Faculty, Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD) Research Centre and Centre for Molecular Medicine (CMMC), University of Cologne, Cologne, Germany

E-mail: bjoern.schumacher@uni-koeln.de

The causal contribution of DNA damage in driving the aging process has become evident in a variety of progeroid syndromes that are caused by defects in DNA repair systems. Congenital defects in genome maintenance mechanisms cause complex disease phenotypes characterized by developmental growth failure, cancer susceptibility, and premature aging. The distinct human disease outcomes of DNA repair defects become particularly apparent in syndromes caused by mutations in nucleotide excision repair (NER). While transcription-coupled (TC-) NER defects lead to growth and mental retardation and premature ageing in Cockayne syndrome (CS) patients, global-genome (GG-) NER mutations lead to highly skin cancer prone Xeroderma pigmentosum (XP). Intriguingly, the distinct outcomes of NER deficiencies are conserved in the simple metazoan *C. elegans*. TC-NER deficiency renders worms highly susceptible to DNA damage during developmental growth and with aging, while GG-NER defects give rise to genome instability in proliferating germ cells.

We employed the nematode model to investigate distinct response mechanisms to genome instability in (postmitotic) somatic tissues and in the germline. DNA damage that persists in somatic tissues leads to activation of the insulin-like growth factor signalling (IIS) effector DAF-16. The FoxO transcription factor DAF-16 is efficiently activated in response to DNA damage during development while its DNA damage responsiveness declines with aging. We demonstrated that DAF-16 alleviates growth arrest and enhances DNA damage resistance in somatic tissues even in the absence of DNA repair. We propose that IIS mediates DNA damage responses in somatic tissues and that DAF-16 activity enables developmental growth amid persistent DNA lesions and promotes maintenance of differentiated tissues through enhanced tolerance of DNA damage that accumulates with aging.

DNA damage that persists in germ cells leads to enhanced stress resistance of somatic tissues. The "Germline DNA damage-induced systemic stress resistance" (GDISR) is mediated by the innate immune system that is triggered by genomically compromised germ cells and executed through elevated activity of the ubiquitin proteasome system (UPS) in somatic tissues. We propose that GDISR elevates somatic endurance to extend reproductive lifespan when germ cells require time to reinstate genome stability before resuming offspring generation.

Our findings suggest that somatic tissues adapt to distinct constraints of genome instability in the germline and the soma: Developmental growth can be sustained despite genome instability by DAF-16-mediated DNA damage tolerance, while adult tissues adapt to the requirements of genomically compromised in germ cells through GDISR. Together the DNA damage tolerance mechanism and GDISR systemically promote functional maintenance of differentiated cell types and might therefore open important new avenues for preserving functional integrity of the neuronal system during aging.

DEFECTIVE CHROMOSOMAL BREAK REPAIR IN SPINAL MUSCULAR ATROPHY

(Poster number II-1)

Evangelia Karyka, Mimoun Azzouz, and Sherif El-Khamisy; Krebs and Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK

E-mail: s.el-khamisy@sheffield.ac.uk

Spinal Muscular Atrophy (SMA) is a devastating motor neuron disease representing one of the most common genetic diseases leading to death in childhood. SMA is an autosomal recessive neurodegenerative disorder characterized by progressive muscular weakness and hypotonia as a consequence of the loss of lower motor neurons. On the basis of the age of onset and the severity of the neuromuscular symptoms, four clinical phenotypes have been described. The most severe form, type 1 SMA, is a devastating childhood condition also known as Werdnig-Hoffmann disease. The disease begins by 6 months after birth and is fatal by 2 years of age. It is caused by mutations or deletion of the telomeric copy (*SMN1*) of the *survival motor neuron* (*SMN*) gene, leading to depletion in SMN protein levels. The disease is currently incurable and no effective treatments exist. Using patient fibroblasts, cortical neurons and spinal cord sections derived from SMA mutant mouse models, we will present and discuss our data linking gene transcription to chromosomal instability in SMA.

FOLIC ACID DEFICIENCY INDUCES ANAPHASE DNA BRIDGES AT THE FRAGILE X LOCUS

(Poster number II-2)

Victoria A. Bjerregaard [1*], Lorenza Garribba [1], Wei Wu [1], Javier Pena Diaz [2], Cynthia McMurray [3], Ian D. Hickson [1&2*], and Ying Liu [1*]

1) Center for Chromosome Stability, Department of Cellular and Molecular Medicine, University of Copenhagen, Panum Institute, Copenhagen, Denmark
2) Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Panum Institute, Copenhagen, Denmark
3) Lawrence Berkeley National Laboratory, Berkeley, CA, USA

*: Authors who will attend this symposium

E-mail: viab@sund.ku.dk

Tri-nucleotide repeat sequences (TNRs) can be problematic when they exceed a crucial threshold length. For example, if the TNR lies within a crucial gene, it can lead to gene silencing that then causes severe diseases, including neurodegeneration (reviewed in [1]). Here, we present our current research on how TNR regions are replicated in S phase and separate in mitosis using Fragile X syndrome (FXS) cells as a model. FXS is caused by a CGG repeat in the FRAXA region on chromosome X. The repeat lies in the non-coding (5' UTR) region of the Mental Retardation Gene 1 (FMR1). This TNR expands from the pre-mutation allele length (55-200 units) to a full mutation (200-4000 units) almost exclusively through maternal transmission [2, 3]. Interestingly, the fully mutated FRAXA region is prone to break when the cells are exposed to folic acid deficient growth medium, which is a phenomenon akin to that seen with common fragile sites (CFSs) in response to DNA replication stress.

Our results indicated that: 1) FdU treatment (that mimics folic acid deficiency), but not APH treatment (that causes CFS breakage), in human cells can cause the manifestation of breakage

at the FRAXA locus on chromosome X when the CGG repeats at that locus exceeds 200 units; 2) following the FdU treatment, the FRAXA locus is found as 'lagging' DNA between the two separated DNA masses in anaphase or telophase cells in a significant portion of cells with CGG repeats over 900. This phenomenon is found only associated with FdU treatment and only at the FRAXA locus (not at CFSs).

We conclude that the fully mutated FRAXA locus is very unstable when the cells are starved of folic acid. Future studies will be aimed at deciphering the mechanism underlying the instability of the long CGG repeats under folic acid deficiency conditions.

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ATM AND ATR IN NEURONS -- FUNCTIONS BEYOND THE DNA DAMAGE RESPONSE

Karl Herrup; Division of Life Science, Hong Kong University of Science and Technology, Kowloon, Hong Kong

E-mail: herrup@ust.hk

In a wide range of cell types, including neurons, ATM and ATR are central contributors to the DNA damage response. While both kinases are activated by DNA breakage, ATM tends to be involved in the response to double strand breaks while ATR responds to single strand breaks. Yet focusing solely on these complementary roles in maintaining genomic integrity oversimplifies the full range of ATM and ATR functions. For example, ATM is involved in the regulation of insulin secretion and mitochondrial dynamics. In neurons the association of ATM with vesicles of various descriptions has oft been noted and our lab has reported that both ATM and ATR are associated with synaptic vesicles where they are necessary for normal vesicle trafficking, including spontaneous release.

We now report the use of stochastic optical reconstruction microscopy (STORM) to directly demonstrate the association of both ATM and ATR with synaptic vesicles in mouse cortical neurons both in vivo and in vitro. Electrophysiological measurements in ATM neurons predicts a presynaptic function for ATM and this prediction is validated by STORM where the ATM-associated vesicles are found in closer proximity to pre-synaptic than post-synaptic proteins. STORM has been used to localize ATR to the surface of 40 nm VAMP2 synaptic vesicles. Unlike ATM, however, ATR is found in both pre- and post-synaptic compartments as well as on the surface of larger dense core vesicles. Most intriguingly, in a reprise of their complementary role in the nucleus, we find that ATM and ATR are found in two distinct types of vesicles. ATM is located on VGLUT1-containing excitatory vesicles while ATR is located exclusively on VGAT-containing inhibitory vesicles. We further show that under conditions of ATM deficiency, ATR levels rise leading to a marked change in the excitatory/inhibitory balance. This provides a mechanistic understanding of the changed

inhibitory 'tone' of the ATM-deficient brain. In the aggregate, these new observations emphasize strongly that the complementarity between ATM and ATR extends well beyond their roles in the nucleus and offers potential new insights into the complex neurological phenotypes of individuals with ataxia-telangiectasia.

GENOME INSTABILITY: A CONSERVED MECHANISM OF AGING?

Jan Vijg; Department of Genetics, Albert Einstein College of Medicine, New York

E-mail: Jan.Vijg@einstein.yu.edu

Genome instability has been implicated as a main causal factor in age-related cellular degeneration and death since the 1950s when the first evidence emerged that low doses of ionizing radiation can accelerate aging. Genome instability as a driver of aging is an attractive hypothesis for two main reasons. First, since DNA is the primary informational macromolecule of the genome, loss or alteration of its sequence is essentially irreversible, which is generally not true for changes in other macromolecules, such as proteins. Second, heritable mutations in multiple genes involved in genome maintenance in both humans and mice have been found associated with segmental progeria; there is little evidence that the same is true for other gene families thought to be involved in longevity, such as antioxidant defense or autophagy.

However, due to the random nature of genome instability, alterations in individual cells are obscured when analyzing bulk cells or tissues. This means that we do not know the severity of the genomic mutation load of aging cells and tissues, which has essentially constrained the establishment of reliable cause and effect relationships.

I will describe several approaches to comprehensively characterize the landscape of somatic genome alterations as a function of age and in relation to maximum species life span.

AGING, DAMAGE AND REPAIR: HOW COMPLICATED CAN IT BE?

(Poster number II-3)

Tom Kirkwood; Newcastle University Institute for Ageing, and Center for Healthy Aging, University of Copenhagen

E-mail: tom.kirkwood@newcastle.ac.uk

Evolutionary logic makes clear that the primary cause of intrinsic aging is to be found in the limited priority that is placed by natural selection in preventing the ultimate deterioration of cells and organs that occurs through accumulation of molecular damage, of which genome instability forms an important part. A direct consequence of this logic is that multiple forms of damage are expected to contribute to the underlying pathobiology of age-related chronic diseases and frailty, including neurodegenerative conditions. Recognising the complexity of how diverse forms of damage interact one with another, and how the responses to damage may themselves contribute to downstream deleterious effects, has enabled significant progress. Energetics and metabolism play central roles because (i) some of the damage arises through by-products of essential metabolic functions, (ii) energy is necessary to support proofreading and turnover processes such as autophagy, which when compromised by genome instability may fail to maintain homeostasis, and (iii) the allocation of resources to maintenance processes, as influenced by signalling pathways (e.g. IGF, TOR), may both cause and be caused by changes in internal and external state.

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INTEGRATED BRAINWIDE STRUCTURAL AND FUNCTIONAL ANALYSIS

Karl Deisseroth; Stanford University, James H. Clark Center, Stanford, USA

E-mail: deissero@stanford.edu

The last decade has seen a rapid rise in the adoption of optogenetics in biology – facilitated not only by discovery and development of essential features of modern optogenetics itself (microbial opsin gene variants, opsin-targeting strategies, and light-targeting devices), but also through the integration of optogenetics with complementary technologies and data streams based in chemistry and biochemistry, including CLARITY and crystal structure-guided opsin design. These discoveries allow optogenetics to be used to identify causal underpinnings of physiology and behavior on acute or chronic time scales, and across synaptic, cellular, circuit-level, or brain-wide spatial scales. This talk will focus on discoveries from the molecular level that have reshaped the study of intact biological systems, and illuminated fields of biology ranging from systems neuroscience to psychiatry.

REGULATION OF BASE EXCISION REPAIR IN THE AGING HUMAN BRAIN

(Poster number II-4)

Sofie Lautrup[1,2], Anne Mayntzhusen[1], Kaare Christensen[2,3], Vilhelm A. Bohr[2,4], Christian B. Vægter[5], Tinna Stevnsner[1,2]

1) Danish Center for Molecular Gerontology, Aarhus University, Aarhus, Denmark
2) Danish Aging Research Center
3) University of Southern Denmark, Odense, Denmark,
4) Laboratory of Molecular Gerontology, National Institute on Aging, Baltimore, USA
5) Department of Biomedicine, Aarhus University, Aarhus, Denmark

E-mail: tvs@mbg.au.dk

During the aging process the amount of reactive oxygen species in the brain increases and the DNA repair efficiency seems to decrease. This results in increased oxidative DNA damage, which primarily is repaired by the Base Excision Repair (BER) pathway. One of the key enzymes in BER is DNA Polymerase Beta (PolB). Several studies suggest that PolB plays a role in aging of the brain, especially in cognitive decline as seen in the age-associated neurodegeneration and diseases like Alzheimer Disease. A major contributor to the maintenance of cognitive function is Brain Derived Neurotrophic Factor (BDNF). BDNF stimulates a number of cellular processes in the nervous system ranging from neuronal migration, differentiation, and plasticity. The level of BDNF in the brain decreases with aging and is associated with cognitive loss and neurodegeneration. BDNF acts via the receptor Tyrosine-kinase B (TrkB). The binding of BDNF to TrkB activates various intracellular pathways. One of the pathways activated is the PI3K-Akt pathway, leading to activation of the transcription factor CREB, which binds to specific recognition sites in the promoters of

certain genes. Several of the proteins involved in the BER pathway are predicted to have CREB-recognition sites in their promoter region. Notably, the activity of the PolB promoter in neurons has been shown to be dependent on this recognition site.

Here, we investigate whether BDNF treatment of cultured primary hippocampal neurons results in increased activity of PolB. Furthermore, we examine whether the transcription and protein level of PolB is reduced in hippocampus of BDNF +/- compared to wild type mice. Our preliminary results suggest that PolB expression and activity is positively regulated by BDNF via CREB. This could implicate that reduction in BDNF in the brain affects BER capacity, which may affect brain function including cognitive capacity at old age.

INCREASED DEOXYTHYMIDINE TRIPHOSPHATE LEVELS IS A FEATURE OF RELATIVE COGNITIVE DECLINE

(Poster number II-5)

Claus Desler1,2, Jon Durhuus1,2, Jane H. Frederiksen1,2*, Maria Angleys1,2, Scott Maynard1,2, Guido Keijzers1,2, Birgitte Fagerlund1,3, Erik Lykke Mortensen1,4, Merete Osler4,5, Martin Lauritzen1,6,7, Vilhelm A. Bohr1,8, Lene Juel Rasmussen1′[2]

1) Center for Healthy Aging, Copenhagen, Denmark
2) Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark
3) Lundbeck Foundation Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS), and Center for Neuropsychiatric Schizophrenia Research (CNSR), Mental Health Centre Glostrup, Denmark
4) Department of Public Health, University of Copenhagen, Denmark
5) Research Center for Prevention and Health, Glostrup Hospital, Glostrup, Denmark
6) Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark
7) Department of Clinical Neurophysiology, Glostrup Hospital, Glostrup, Denmark
8) Laboratory of Molecular Gerontology, National Institutes on Aging, NIH, USA

E-mail: cdesler@sund.ku.dk

Purpose: Alzheimer's disease (AD) is a condition involving serious loss of global cognitive function. Changes of life style and therapeutics are known to delay the etiology of AD, but no cure exists for already inflicted neurological damage caused by the condition. This highlights the necessity of the development of early or very early indicators for increased AD risk.

Results: We designated relative cognitive decline as statistically extreme decline in cognitive function between young adulthood and middle age and we defined the term as characteristic of unhealthy brain aging, and as an early indicator of increased risk of dementia and AD. From a cohort of 1985 otherwise healthy individuals, we have demonstrated a 20% increase of deoxythymidine triphosphate (dTTP) from peripheral blood mononuclear cells (PBMCs) of 103 individuals displaying severe unhealthy brain aging. This trend was also apparent in PBMCs from 53 individuals suffering AD, strongly suggesting this factor to be usable as early or very early indicators of AD. We believe that the altered levels of dTTP is correlated with impairments of mitochondrial function, and we have in cultured cells shown, that affecting the mitochondrial enzyme dihydroorotate dehydrogenase involved the *de novo* synthesis of dTTP results in increase of genomic instability and impediment of autophagy which are factors now being recognized important in the etiology of AD

Conclusion: Levels of dTTP in PBMCs are indicators of relative cognitive change suggesting a role of deoxyribonucleotides in the etiology of AD.

THE EFFECTS OF TAU IN DNA REPAIR AND DNA PROTECTION IN AD MODELS

Jin Zheng; Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

E-mail: zhj821107@yahoo.com

Tau is a highly soluble microtubule protein abundantly expressed in neurons of the central nervous system. In Alzheimer's disease brain tau becomes hyperphophorylated and forms intracellular neurofibrillary tangels. But the functions of tau in AD development still largely unexplored. Previous studies have shown that tau is present in the nucleus and may have a DNA protection effect. The expression of polb, encoding DNA polymerases beta, which is a key DNA repair polymers involved in the repair of oxidative DNA damage, was regulated by and interacted with Tau. Our studies in a common AD mouse model (3xTgAD) showed reduction of DNA polymerase beta causes neurodegeneration and aggravates the AD features, which demonstrated that decrement in DNA repair in neutrons can render the brain more vulnerable to AD-related alterations. In this study we further investigate the role of tau in the regulation of DNA repair proteins in both cell and animal models. In addition, the functional link between mtDNA damage and tauopathy will be also explored.

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EFFECT OF IONIZING RADIATION ON NEURAL STEM CELLS DURING DIFFERENTIATION

Bodo Laube, Katja Häupl, Kerstin Rau and Bastian Roth

Neurophysiology and Neurosensory Systems, TU Darmstadt, Germany

E-mail: laube@bio.tu-darmstadt.de

In the developing and adult brain the exact cellular proliferation, migration, and differentiation mechanisms of neural stem cells (NSCs) are of prime importance. Ionizing radiation (IR) affects neurogenesis in the dentate gyrus of the hippocampus. NSCs are characterized by the ability of self-renewal and the ability to differentiate to neurons, astrocytes and oligodendrocytes. Increasing evidence suggests that the activity of receptors and ion channels is intimately related to the control of proliferation and differentiation into defined cell types upon IR.

Here, we have studied the cellular reaction towards radiation induced DNA-damage and the physiology of early differentiation and network formation in neuronal cultures derived from the murine neural stem cell line J1 in a 2D differentiation protocol. Upon differentiation, the J1 cells show a loss in the stem cell marker nestin and a specific expression of differentiation markers and synaptic proteins like GFAP, MAP 2 and PSD95, synaptophysin, respectively. Based on this differentiation protocol, we selectively irradiated (up to 1 Gy) individual differentiation stages and investigated the effects of IR on DNA damage response and on differentiation events like synaptogenesis, migration or neuronal functionality. Since potassium channels regulate cell behaviors such as proliferation and migration, we initially assessed the properties and composition of potassium channels expressed in different developmental stages upon radiation. By using whole-cell patch-clamp recording and

immonohistochemistry, we measured the current responses of irradiated and unirradiated J1 NSCs in selfrenewal state and determined the potassium conductance of undifferentiated and differentiated J1 cells.

We can show that even low dose radiation of neural stem cells leads to significant variations in its electrophysiological homeostasis and the biophysical properties (like potassium conductance) of NSCs. In conclusion, NSCs could be successfully differentiated into functioning neural networks with differential developmental patterns upon radiation that may lead to subtle deficits in neuronal function.

RECURRENT SOMATIC VARIATIONS IN NORMAL AGEING BRAIN

Yumi Kim[1,2], Hong Gil Nam[2,3], and Dario Riccardo Valenzano[1,4]

1) Max Planck Institute for Biology of Ageing, Cologne, Germany
2) Department of New Biology, DGIST, Daegu, Republic of Korea
3) Center for Plant Aging Research, Institute for Basic Science, Republic of Korea
4) CECAD, University of Cologne, Germany

E-mail: ykim@age.mpg.de

Accumulation of mutations in somatic cells has been proposed as a major cause for ageing. Specific somatic alterations are associated with cancer or various diseases, including neurodegeneration and epilepsy. To identify the recurrent somatic alterations associated with normal ageing and test their phenotypic effects, we developed a method that combines transcriptome sequencing in brain and muscle. We used the short-lived turquoise killifish (*Nothobranchius furzeri*) as model organism, due to its short lifespan in captivity (16 weeks of median lifespan) and its recently sequenced and assembled genome. We tested a number of genetic markers associated with DNA damage response and genome instability and found them to be significantly increased in brain and muscle in aged fish. We further tested expression levels of the genes involved in DNA repair and observed decreased expression both in brain and muscle. Taken together, our results strongly suggest that DNA lesions are expected to increase in aged fish.

We next set out to identify somatic variants in total transcripts during normal ageing. Numerous somatic variants were enriched in aged fish compared to young fish. About a half of the total variants were assigned to gene bodies, while the rest occurred in non-protein-coding regions of the genome. A significant number (~1%) of the total variants was recurrently identified in independent biological samples. Furthermore, to test the biological effect of DNA damage and repair on age-related phenotypes, including disease and individual longevity, we generated 6 transgenic fish lines with expected enhanced DNA repair capacity. Altogether, our work will help to understand the importance of recurrent somatic variants on ageing-associated brain dysfunctions and will help to answer the question of whether improving DNA-repair mechanisms can beneficially impact the ageing process and lifespan.

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NUCLEOLAR REGULATION OF THE WRN HELICASE

Dorthe Helena Larsen[1], Vilhelm A. Bohr[2], Lene Juel Rasmussen[3] and Jiri Bartek [1]

1) Unit Genome Integrity, Danish Cancer Society, Denmark
2) Laboratory of Molecular Gerontology, National Institutes on Aging, NIH Center for Healthy Aging, USA
3) Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark

E-mail: dhl@cancer.dk

Average life expectancy is increasing in today's aging populations and new approaches to promote healthy aging are required to maintain the quality of life for individuals and to minimize the economic burden for our healthcare systems. Improved biological understanding of the aging process and age-associated diseases is expected to be essential for the design of effective treatments in the future.

While the molecular mechanisms of aging are slowly being revealed, our understanding is still limited. Premature aging syndromes, such as Werner Syndrome (WS), can be used as model systems to study key processes as they resemble normal aging, albeit accelerated, both at the molecular and phenotypic level(*1*). Patients suffering from WS have mutations in the gene encoding the helicase WRN and the involvement of WRN in aging is conserved through evolution(*2*). During unperturbed growth WRN accumulates in the nucleolus, a nuclear organelle housing the ribosomal genes, where it promotes ribosomal gene transcription(*3*, *4*). Upon exposure to cellular stresses WRN translocates to the nucleoplasm where it is important for proper DNA repair(*2*, *5*). Accurate regulation of WRN is essential to maintain genome stability and cellular homeostasis, but the underlying mechanisms are poorly understood. Our research focuses on the functional interplay between the nucleolus and WRN and our most recent results will be presented.

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MITOCHONDRIA IN OLIGODENDROCYTES AND THEIR MYELIN SHEATHS

Johanne E. Rinholm,[1,2,3] Koen Vervaeke,[3,4] Michael R. Tadross,[3] Ariana N. Tkachuk,[3] Benjamin G. Kopek,[3*] Timothy A. Brown,[3] Linda H. Bergersen,[2] and David A. Clayton[3]

1) Department of Anatomy, The Brain and Muscle Energy Group, University of Oslo, Oslo, Norway
2) Department of Oral Biology, The Brain and Muscle Energy Group, Electron Microscopic Laboratory, University of Oslo, Oslo, Norway
3) Howard Hughes Medical Institute, Ashburn, Virginia
4) Department of Physiology, Laboratory of Neural Computation, University of Oslo, Oslo, Norway

*Benjamin G. Kopek is currently at Department of Biology, Hope College, Holland, MI, USA

E-mail: j.e.rinholm@medisin.uio.no

Mitochondria play several crucial roles in the life of oligodendrocytes. During development of the myelin sheath they are essential providers of carbon skeletons and energy for lipid synthesis. During normal brain function their consumption of pyruvate will be a key determinant of how much lactate is available for oligodendrocytes to export to power axonal function. Finally, during calcium-overload induced pathology, as occurs in ischemia, mitochondria may buffer calcium or induce apoptosis. Despite their important functions, very little is known of the properties of oligodendrocyte mitochondria, and mitochondria have never been observed in the myelin sheaths. We have now used targeted expression of fluorescent mitochondrial markers to characterize the location and movement of mitochondria within oligodendrocytes.

We show for the first time that mitochondria are able to enter and move within the myelin sheath. Within the myelin sheath the highest number of mitochondria was in the cytoplasmic ridges along the sheath. Mitochondria moved more slowly than in neurons and, in contrast to their behavior in neurons and astrocytes, their movement was increased rather than inhibited by glutamate activating NMDA receptors. By electron microscopy we show that myelin sheath mitochondria have a low surface area of cristae, which suggests a low ATP production. These data specify fundamental properties of the oxidative phosphorylation system in oligodendrocytes, the glial cells that enhance cognition by speeding action potential propagation and provide metabolic support to axons.

NEW ROLES FOR 14-3-3 PROTEINS IN RNA METABOLISM AND CELLULAR STRESS RESPONSES

Melanie Blasius and Jiri Bartek; Genome Integrity, Danish Cancer Society Research Center, Denmark

E-mail: blasius@cancer.dk

Cells have evolved elaborate signaling networks to contain the detrimental effects of DNA damage and maintain a stable genome. The DNA damage response (DDR) is particularly important, because un- as well as misrepaired DNA damage can lead to genomic instability and cancer. Several protein kinases are involved in coordinating the DDR, causing a halt in cell division, the activation of DNA repair or programmed cell death. Apart from such primary interventions in cellular homeostasis, it has in recent years become apparent that transcription and RNA processing are also tightly regulated in response to DNA damage, but our understanding of the molecular mechanisms and the exact pathways that govern these

processes remain ill defined (1). Recent research by my collaborators and I, as well as a number of other research groups, hint at an important role for the p38-MK2-14-3-3 pathway in controlling RNA-related events after DNA damage and other cellular stress inducers (2,3).

We currently address novel aspects of how the p38-MK2-14-3-3 pathway controls different aspects of RNA processing and transcription under such conditions. Misregulation of these processes can cause cancer and diseases such as neurodegeneration, inflammatory diseases and fertility disorders. Furthermore, chemical inhibitors for MK2 as well as 14-3-3 are currently being developed as anti-cancer and anti-inflammatory drugs, and thus a better understanding of the full scope of cellular roles for these proteins will be paramount for the advancement of these drugs.

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BASE EXCISION REPAIR AND CELLULAR RESPIRATION ARE COMPROMISED IN LAMIN A/C-DEPLETED CELLS

Scott Maynard[1], Guido Keijzers[1], Arnaldur Hall[2], Susana Gonzalo[3], Jiri Bartek[2], and Vilhelm A. Bohr[4]

[1]Department of Cellular and Molecular Medicine, Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark
[2]Danish Cancer Society Research Center, Copenhagen, Denmark
[3]Department of Biochemistry and Molecular Biology, Saint Louis University, School of Medicine, Saint Louis, MO, USA
[4]Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

E-mail: scottm@cancer.dk

The A-type lamins (lamin A and C encoded by the *LMNA* gene) are important structural components of the nuclear lamina. *LMNA* mutations lead to degenerative disorders termed laminopathies, including the premature aging disease Hutchinson-Gilford progeria syndrome (HGPS). Altered expression levels of lamins are found in various cancers. Reports indicate the lamins play a role in DNA double strand break repair, but a role for lamins in base excision repair (BER) has not be described. In this report, we provide evidence for compromised BER in lamin A/C-depleted cells (MEF LMNA knockout and siRNA knockdown in human cells). We observed altered oxidative stress response indicated by microarray analysis, increased cell sensitivity to oxidative and alkylation stress, less efficient DNA repair of oxidative lesions, and impaired DNA incision activity of two BER enzymes (OGG1 and APE1). Moreover, in *LMNA* null MEF there was a significant reduction in the level of several BER enzymes. The lamin A/C-depleted cells also displayed impaired glycolytic and mitochondrial bioenergetic fluxes, and higher levels of mitochondrial ROS production; the enhanced ROS levels may be a source of genotoxic stress and make it more difficult for the already compromised BER enzymes to repair DNA quickly.

Collectively, these findings indicate the lamin A/C promotes BER and cellular respiration.
This study gives new insight into the role of A-type lamins in the DNA damage response, mitochondrial function and cancer.

BASE EXCISION REPAIR AND CELLULAR RESPIRATION ARE COMPROMISED IN LAMIN A/C-DEPLETED CELLS

Scott Maynard[1], Guido Keijzers[1], Arnaldur Hall[2], Susana Gonzalo[3], Jiri Bartek[2], and Vilhelm A. Bohr[4]

1) Department of Cellular and Molecular Medicine, Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark
2) Danish Cancer Society Research Center, Copenhagen, Denmark
3) Department of Biochemistry and Molecular Biology, Saint Louis University, School of Medicine, Saint Louis, MO, USA
4) Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

E-mail: jb@cancer.dk

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Moreover, in *LMNA* null MEF there was a significant reduction in the level of several BER enzymes. The lamin A/C-depleted cells also displayed impaired glycolytic and mitochondrial bioenergetic fluxes, and higher levels of mitochondrial ROS production; the enhanced ROS levels may be a source of genotoxic stress and make it more difficult for the already compromised BER enzymes to repair DNA quickly. Collectively, these findings indicate the lamin A/C promotes BER and cellular respiration. This study gives new insight into the role of A-type lamins in the DNA damage response, mitochondrial function and cancer.

ROLE OF APTX DEFICIENCY IN MITOCHONDRIAL DYSFUNCTION AND AOA1 PATHOLOGY

Mansour Akbari[1], Peter Sykora[2], Vilhelm A. Bohr[1,2]

1) Center for Healthy Aging, SUND, University of Copenhagen, Denmark
2) Laboratory of Molecular Gerontology, National Institute on Aging, Baltimore, USA

E-mail: akbari@sund.ku.dk

DNA ligation is critical in DNA replication and repair. Aborted DNA ligation can lead to the formation of 5′-AMP termini, which if not repaired cause genome instability. Aprataxin (APTX) is the enzyme that removes 5′-AMP from DNA. Mutations in *APTX* cause an inherited human disease syndrome characterized by early-onset progressive ataxia with ocular motor apraxia (AOA1). APTX resides in the nucleus and in mitochondria. Depletion of APTX causes mitochondrial dysfunction and renders the mitochondrial genome susceptible to damage[1]. To determine the biochemical processes that link APTX deficiency to mitochondrial

dysfunction, we monitored the repair of 5´-AMP DNA damage in nuclear and mitochondrial extracts from human *APTX*$^{+/+}$ and *APTX*$^{-/-}$ cells. We found that the efficiency of repair of 5´-AMP DNA was much lower in mitochondrial than in nuclear protein extracts, and resulted in persistent DNA repair intermediates in APTX deficient cells.

Moreover, the removal of 5´-AMP from DNA was significantly slower in the mitochondrial extracts from human cell lines and mouse tissues compared with their corresponding nuclear extracts[2]. Thus, contrary to nuclear DNA repair, mitochondrial DNA repair is not able to compensate for APTX deficiency resulting in the accumulation of mitochondrial DNA damage. To further elucidate the role of APTX in mitochondrial and nuclear DNA metabolism and mitochondrial bioenergetics and how these processes are related to AOA1, we have knocked-out *APTX* in human cell lines using CRISPR, followed by specific expression of APTX in mitochondria or the nucleus in these cells. Our results suggest that APTX plays a key role in mitochondrial DNA repair and show a possible connection between mitochondrial dysfunction and AOA1 pathology.

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CATALYTICALLY INACTIVE ATM ABROGATES DSB REPAIR MORE THAN ATM ABSENCE, WITHOUT CAUSING A MARKED NEUROLOGICAL PHENOTYPE

Efrat Tal[1], Marina Alfo[1], Rotem Granit[1], Shan Zha[2], Chris I. De Zeeuw[3], Yael Ziv[1], and Yosef Shiloh[1]

1) Department of Human Genetics, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
2) Institute for Cancer Genetics, Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA
3) Department of Neuroscience, Erasmus MC, Rotterdam, and the Royal Netherlands Academy of Art & Science, Amsterdam, Netherlands

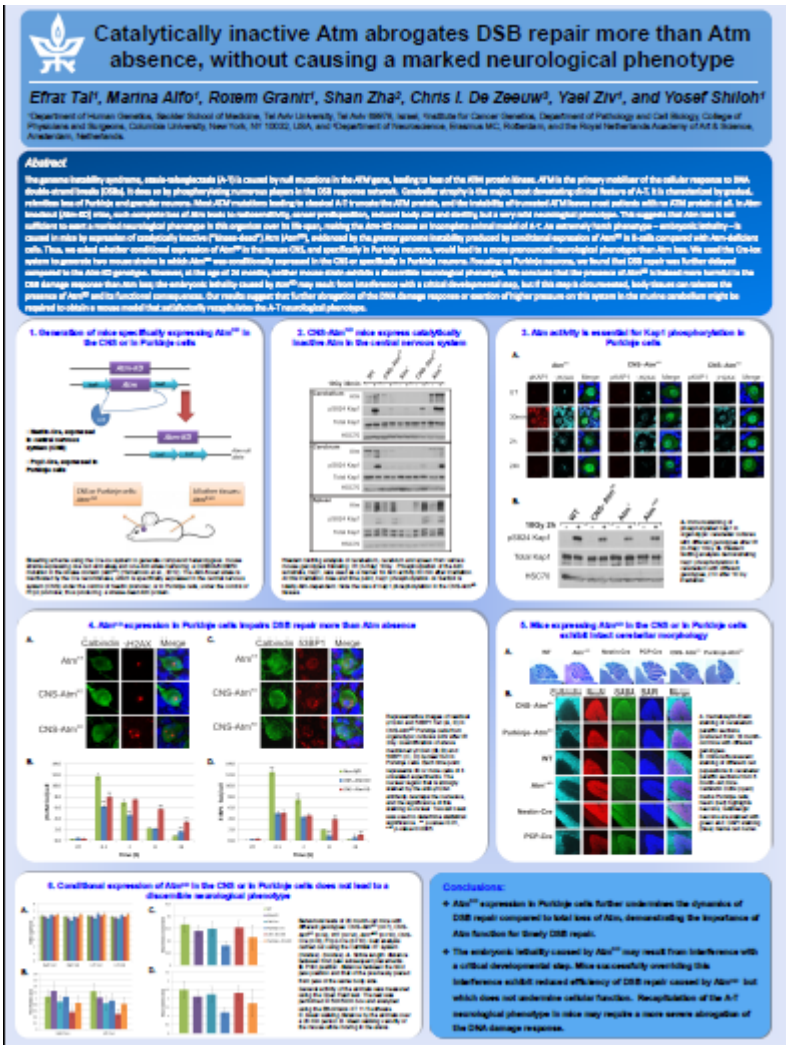
E-mail: mrina9.k@gmail.com

The genome instability syndrome, ataxia-telangiectasia (A-T) is caused by null mutations in the *ATM* gene, leading to loss of the ATM protein kinase. ATM is the primary mobilizer of the cellular response to DNA double-strand breaks (DSBs). It does so by phosphorylating numerous players in the DSB response network. Cerebellar atrophy is the major, most devastating clinical feature of A-T. It is characterized by gradual, relentless loss of Purkinje and granular neurons. Most *ATM* mutations leading to classical A-T truncate the ATM protein, and the instability of truncated ATM leaves most patients with no ATM protein at all. In *Atm*-knockout (*Atm*-KO) mice, such complete loss of Atm leads to radiosensitivity, cancer predisposition, reduced body size and sterility, but a very mild neurological phenotype. This suggests that Atm loss is not sufficient to exert a marked neurological phenotype in this organism over its life span, making the *Atm*-KO mouse an incomplete animal model of A-T. An extremely harsh phenotype – embryonic lethality – is caused in mice by expression of catalytically inactive ("kinase-dead") Atm (Atm^{KD}), evidenced by the greater genome instability produced by conditional expression of Atm^{KD} in B-cells compared with Atm-

deficient cells[1,2]. Thus, we asked whether conditional expression of Atm^{KD} in the mouse CNS, and specifically in Purkinje neurons, would lead to a more pronounced neurological phenotype than Atm loss. We used the Cre-lox system to generate two mouse strains in which Atm^{KD} was conditionally expressed in the CNS or specifically in Purkinje neurons. Focusing on Purkinje neurons, we found that DSB repair was further delayed compared to the *Atm*-KO genotype.

However, at the age of 24 months, neither mouse strain exhibits a discernible neurological phenotype. We conclude that the presence of Atm^{KD} is indeed more harmful to the DSB damage response than Atm loss; the embryonic lethality caused by Atm^{KD} may result from interference with a critical developmental step, but if this step is circumvented, body tissues can tolerate the presence of Atm^{KD} and its functional consequences.

Our results suggest that further abrogation of the DNA damage response or exertion of higher pressure on this system in the murine cerebellum might be required to obtain a mouse model that satisfactorily recapitulates the A-T neurological phenotype.



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REDUCED CYTOSOLIC CALCIUM CAUSED BY SERCA ACTIVATION IS AN EARLY AND PATHOGENIC EVENT IN THE CELLULAR STRESS CAUSED BY ALPHA-SYNUCLEIN OLIGOMERS

Cristine Betzer[1], Louise Berkhoudt Lassen[1], Jens Peter Andersen[1], Anders Olsen[2], Marisa Brini[3], Tito Cali[3], Wei-Ping Gai[4], Poul Henning Jensen[1]

1) Department of Biomedicine & Dandrite, Arhus University, Denmark
2) Department of Molecular Biology and Genetics, Aarhus University, Denmark
3) Department.of Comparative Biomedicine and Food Science, University of Padova, Italy
4) Department of Human Physiology, Flinders University School of Medicine, Australia

E-mail: phj@biomed.au.dk

Epidemiological studies demonstrate protective effect of brain penetrating calcium channel antagonists against development of sporadic PD in hypertensive patients, suggesting aggregated AS alters the calcium homeostasis. We analyzed the cellular calcium levels in cell based models of AS aggregation dependent degeneration in live cells by use of Fura-2 and fluorescence microscopy. Measurement of cytosolic calcium in cell lines, and primary mouse hippocampal neurons revealed an early decrease and a later increase in cellular calcium when degeneration occurs. Endoplasmic calcium ATPase, SERCA is critical for maintaining normal cytosolic calcium by pumping cytosolic calcium into the endoplasmic-reticulum. Soluble AS oligomers, in contrast to monomers, bind SERCA and activate the pump as measured in vitro by increased ATPase activity and transport of calcium. We hypothesize that the increased ATPase activity causes the early decrease in cellular calcium level, therefore we treated cells with low doses of a SERCA-inhibitor, *cyclopiazonic acid* (CPA). CPA-treatment normalized both the initial reduction in cellular calcium, but also the later increase, suggesting that there is a direct link between the two phenomena.

Furthermore, we found that CPA treatment improved the viability in the cell based models, and *C. elegans* overexpressing AS in dopaminergic neurons, strengthening our hypothesis that the early decreased cellular calcium is harmful and untreated will lead to increased cellular calcium and cellular degeneration. In conclusion, decreased cytosolic calcium is an early disease-propagating event in AS oligomer cytotoxicity that can be pharmacologically targeted by SERCA-inhibitors and represents a novel therapeutic strategy to be tested in synucleinopathies.

WEDNESDAY, AUGUST 24, 2016

NUCLEAR TO MITOCHONDRIAL DNA DAMAGE SIGNALING IN NEURODEGENERATION

Vilhelm A. Bohr; Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, USA

E-mail: BohrV@grc.nia.nih.gov

We find that some DNA repair defective diseases with severe neurodegeneration have mitochondrial defects. Our studies involve cell lines, the worm (c.elegans), and mouse models and include the conditions Xeroderma pigmentosum group A, Cockaynes syndrome and Ataxia telangiectasia. We find a pattern of hyperparylation, deficiency in the $NAD^+$and Sirtuin signaling and mitochondrial stress. We are pursuing mechanistic studies of this signaling and interventions at different steps to improve mitochondrial health and the neurodegeneration. I will discuss intervention studies in these diseases models including a new Alzheimer mouse model with NAD supplementation.

THE ROLE OF MITOCHONDRIA IN PARKINSON´S DISEASE

Nils-Göran Larsson; Max Planck Institute for Biology of Ageing, Cologne, Germany

E-mail: larsson@age.mpg.de

Parkinson's disease (PD) is a common neurodegenerative disorder caused by progressive degeneration of dopamine (DA)-producing neurons in the midbrain. The pathophysiological events that lead to the degeneration of DA neurons are not well established, but may involve mitochondrial dysfunction. Mitochondria are highly dynamic organelles and continuous fission and fusion of mitochondria is necessary for maintaining mtDNA and mitochondrial function. Unbalanced control of mitochondrial dynamics can contribute to increase of dysfunctional mitochondria in the cells.

We have previously addressed the consequences of mitochondrial dysfunction in DA neurons by disruption of Mitofusin 1 (Mfn1) and Mfn2, two homologous GTPases that involved in mitochondrial fusion. We reported that disruption of Mfn2 in DA neurons caused early postnatal onset of a Parkinsonian phenotype, whereas loss of Mfn1 in DA neurons had no apparent phenotype. However, the exact cause of early death remained to be established. Here, we generated conditional knockout mice in which Mfn2 is disrupted in adult midbrain DA neurons by using mice expressing an inducible Cre recombinase under the control of the DA transporter promoter (DATCreERT2). We found that loss of Mfn2 in mature DA neurons resulted in progressive degeneration of nigrostriatal pathway and death by 12 weeks after induction of Cre recombinase expression. The conditional knockout mice showed impaired motor behaviors and reduction of body weight before death. We also found disrupted mitochondrial network in Mfn2 depleted DA neurons and fragmented mitochondria became enlarged with impaired respiratory chain function. Further elucidation of the underlying molecular mechanism may provide a role of mitochondrial dynamics in PD with degeneration of striatal DA nerve terminals.

SYSTEMIC REGULATION OF BRAIN AGING AND PLASTICITY

Tony Wyss-Coray; VA Palo Alto and Stanford University School of Medicine

E-mail: twc@stanford.edu

Age is the main risk factor for sporadic forms of neurodegenerative diseases, and aging of peripheral organs may affect brain function. How the systemic environment affects brain health is largely unknown and while some of these interactions may involve cells entering the nervous tissue it is likely that others are mediated by soluble factors. We use a combination of physiological methods to manipulate systemic aging and proteomic methods to try to identify factors that age or potentially rejuvenate the brain. Our findings point to systemic changes in immune responses and cellular signaling factors with aging and may be relevant for the understanding of age-related neurodegeneration.

RESOLVING RNA-DNA DAMAGE-INDUCED GENOMIC INSTABILITY: WHERE THERE IS SETX, THERE IS A WAY

(Poster number III-1)

Kanagaraj Radhakrishnan, & Stephen C. West; The Francis Crick Institute, Clare Hall Laboratory, South Mimms, UK

E-mail: Kanagaraj.Radhakrishnan@crick.ac.uk

Defects in DNA repair have been linked to multiple human genetic disorders. The neurodegenerative disease Ataxia with Oculomotor Apraxia 2 is caused by defects in Senataxin (SETX) {1}. SETX, a putative RNA/DNA helicase, is considered to be an important player in the resolution of RNA/DNA hybrids (R-loops) formed either during transcription termination or the RNA-DNA damage response (RDDR) {2}. Previous studies have shown that SETX localizes to sites of collision between components of the replisome and the transcription apparatus and that it is targeted to R-loops, where it plays an important role at the interface of transcription and RDDR {2,3}. We find that loss of SETX in both human and mouse cells causes hypersensitivity to treatment with agents that cause either replication stress or induce the formation of R-loops.

Furthermore, SETX deficiency promotes the formation of replication stress-induced genomic instability and chromosomal rearrangements. Using genomic approaches, we find that loss of SETX results in altered gene expression, differential methylation patterns and copy number alterations. A combination of chromatin and DNA/RNA immunoprecipitation experiments revealed that SETX deficiency promotes the accumulation of both DNA damage markers and R-loops simultaneously across many replication stress hotspots, such as regions of the genome that contain transcribing genes, fragile sites and repetitive DNA sequences. The mechanistic links between SETX, replication stress and genomic instability will be discussed.

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XRCC1 MUTATIONS IN HUMAN NEUROLOGICAL DISEASE

(Poster number III-2)

Hana Hanzlikova[1], Nicolas Hoch[1], Grace Yoon[2] and Keith W. Caldecott[1]

1) Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Falmer, Brighton, United Kingdom
2) Division of Clinical and Metabolic Genetics and Division of Neurology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

E-mail: H.Hanzlikova@sussex.ac.uk

The DNA repair protein XRCC1 also known as X-ray repair cross-complementing protein 1 is required for the rapid repair of chromosomal DNA single-strand breaks (SSBs). XRCC1 does not have enzymatic activity, but acts as a scaffold protein that interacts with multiple enzymes (e.g., PARP1, PNKP, Polβ, APTX, Lig3) and promotes their stability and/or function. SSBs are amongst the most frequent DNA lesions arising in cells and if not repaired correctly can threaten both genetic stability and cell survival. Moreover, SSB repair defects are associated with hereditary neurodegeneration in humans, as illustrated by the genetic diseases *ataxia oculomotor apraxia-1* (AOA1; mutated in *APTX*), *spinocerebellar ataxia with axonal neuropathy-1* (SCAN1; mutated in *TDP1*), and *microcephaly with early onset seizures* (MCSZ; mutated in *PNKP*).

Here, we describe for the first time a human patient with bi-allelic mutations in XRCC1. We define the cellular and pathological consequences of XRCC1 loss in human and mouse brain and identify a molecular mechanism by which unrepaired SSBs trigger neuropathology. Collectively, these data establish the importance of XRCC1 protein complexes for normal neurological function and identify a possible therapeutic approach for treating DNA strand break repair-defective neurodegenerative diseases.

DYNAMIC DNA DEMETHYLATION VIA BASE-EXCISION REPAIR REGULATES NEURONAL FLEXIBILITY

Hongjun Song; Institute for Cell Engineering, Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA

E-mail: shongju1@jhmi.edu

Epigenetic modifications of chromatin, including the genomic DNA and histone proteins, play critical roles in orchestrating transcriptomes of all cell types. We found that neuronal stimulation induces region-specific active DNA demethylation in a Gadd45b- and TET1-dependent fashion in the adult mouse dentate granule neurons in vivo (Ma et al. *Science* 2009; Guo et al. *Cell* 2011). Our genome-wide analysis further revealed that 1.4% of all CpGs measured exhibit rapid activity-induced demethylation or de novo methylation (Guo et al. *Nat. Neurosci.* 2011). These activity-modified CpGs exhibit a broad genomic distribution with significant enrichment in low-CpG density regions, and are associated with brain-specific genes related to neuronal plasticity. Our single-base methylome analysis discovered significant levels of nonCpG methylation in these neurons and we identified DNMT3A and MeCP2 as a writer and a reader for nonCpG DNA modification (Guo et al. *Nat. Neurosci.* 2013).

More recently, our studies suggest that active DNA demethylation and DNA repair serve as a synaptic activity sensor to epigenetically regulate fundamental properties and meta-plasticity of neurons and animal behavior (Yu et al. *Nat. Neurosci.* 2015 and unpublished). Together, our studies implicate novel modifications of the neuronal DNA methylome and DNA repair as a previously under-appreciated mechanism for activity-dependent epigenetic regulation in the adult nervous system under both physiological and pathological conditions.

THE ROLE OF DNA BREAKS IN NEURONAL GENE EXPRESSION DETERMINED BY END-SEQ

Andre Nussenzweig; National Institutes of Health; NCI/CCR/Laboratory of Genome Integrity, Bethesda, MD, USA

E-mail: nussenza@exchange.nih.gov

DNA double strand breaks (DSBs) arise during physiological transcription, DNA replication, and antigen receptor diversification. Mis-targeting or mis-processing of DSBs can result in pathological structural variation and mutation. Here we describe a sensitive method (END-seq) to monitor DNA end resection and DSBs genome-wide at base-pair resolution in vivo.

We utilized END-seq to determine the frequency and spectrum of restriction enzyme-, zinc-finger nuclease-, and neuronal activity-induced DSBs. END-seq can detect at least 1 DSB per cell amongst 10,000 cells not harboring DSBs. In addition to site-specific cleavage, we detect DSBs distributed over extended regions during immunoglobulin class switch recombination.

Thus END-seq provides a snapshot of DNA ends genome-wide, which can be utilized for understanding genome-editing specificities and the influence of chromatin on DSB pathway choice. In this seminar, we focus on how neuronal activity-induced DSBs are related to gene transcription and mutation.

ROLE OF TUMOR SUPPRESSOR WWOX, GENE PRODUCT OF A COMMON FRAGILE SITE, IN BRAIN DEVELOPMENT AND NEURODEGENERATION

(Poster number III-3)

Srinivasarao Repudi[1], Oded Behar[2] and Rami I. Aqeilan[1]

1) Lautenberg Center for Immunology and Cancer Research, Hebrew University-Hadassah Medical School, Jerusalem, Israel
2) Developmental Biology and Cancer Research, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem, Israel

E-mail: ramiaq@mail.huji.ac.il

WW domain-containing oxidoreductase (WWOX) is well known in tumor suppression. Surprisingly, germline mutations in *WWOX* causes a recessive form of early onset neurodegenerative and neurodevelopmental symptoms in man including epilepsy, mental retardation and cerebellar ataxia[1]. Other symptoms noted in some cases were prominent spasticity, or prominent upper motor neuron disease. Somewhat reminiscent phenotype was also found in *Wwox*-null mice and *Wwox*-deficient rats. WWOX is also shown to play a crucial role in the pathogenesis of Alzheimer's disease and neuronal injury. Thus it is apparent that WWOX plays a surprising and critical role in brain development.

Our efforts aim to study the underlined mechanism by which WWOX regulates the development of the brain and how its loss leads to neuropathy. We have recently shown that

WWOX directly plays a key role in DNA damage response (DDR) through regulating ATM function. In this aspect, WWOX loss is associated with impaired DDR and increased genomic instability[2,3]. Interestingly, we observed that the brains of WWOX mutant mice display increased number of γH2AX foci, a marker of DNA double strand breaks, and increased percentage of apoptosis. Using high-throughput deep sequencing, we are mapping the breakome in *Wwox*-deficient neural stem cells and exploring the relevance of these breaks on biology of the brain.

Our studies shed light on the underlining mechanisms by which WWOX influence brain development and hopefully will extend our understanding of how to benefit patients suffering from neurodegeneration through implementation of regenerative medicine approaches.

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AGE–DEPENDENT EXPANSION IN HUMAN HUNTINGTON DISEASE: FROM GENETICS TO METABOLISM

Yanhao Lai[1], Helen Budworth[4], Jill M. Beaver[1,2], Nelson L. S. Chan[4], Zunzhen Zhang[5], Cynthia T. McMurray[4]*, Yuan Liu[1,2,3]*

Lawrence Berkeley Laboratory, Berkeley, USA

E-mail: ctmcmurray@lbl.gov

Both *in vivo* and *in vitro* results support a toxic oxidation model in which 7,8-dihydro-8-oxo-guanine-DNA glycosylase (OGG1) causes age-dependent somatic mutation associated with Huntington's Disease (HD) occurs in the process of removing oxidized base lesions, and is remarkably dependent on the base excision repair enzyme, OGG1 initiates an escalating "oxidation-excision-expansion" cycle that leads to progressive age-dependent expansion. After removal of the oxidized bases, strand displacement during gap filling synthesis causes TNR expansion by creating a TNR flap, which is stabilized by MSH2-MSH3. We have recently discovered that MSH2-MSH3 adopts an entirely new function when it operates wthin BER, and opens up new insight into how MSH2-MSh3 might operate in cells. Our results provide direct evidence that MMR and BER, operating together, create a novel hybrid pathway to protect the genome against DNA loss or breaks, at the expense of DNA instability and mutation. Cells appear to implement crosstalk strategies and share machinery when a single canonical pathway is ineffective in removing blocks to DNA replication.

NEURODEGENERATION IN ACCELERATED AGING

(Poster number III-4)

Morten Scheibye-Knudsen; Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

E-mail: mscheibye@sund.ku.dk

Accelerated aging disorders represent a phenotypically diverse group of diseases all with defects in DNA maintenance. In a subset of these disorders, neurodegeneration is prominent. Notably, the neurological phenotype is strikingly similar to what is seen in primary mitochondrial disorders, an observation that we have investigated and corroborated using *in silico*, *in vitro* and *in vivo* methods. The mitochondrial dysfunction in these neurodegenerative diseases may be caused by hyperactivation of a nuclear DNA damage response involving the enzyme poly-ADP-ribose-polymerase 1 (PARP1) leading to loss of NAD and alterations in cellular and organismal metabolism. Interventions at steps in this pathway lead to normalization of the mitochondrial phenotypes suggesting that new treatments may be possible for these, previously, untreatable disorders.

ALTERED DNA BASE EXCISION REPAIR PROFILE IN BRAIN TISSUE AND BLOOD IN ALZHEIMER'S DISEASE

(Poster number III-5)

Meryl S. Lillenes[1,2], Tahira Riaz[1,2], Alberto Rabano[3], Tormod Fladby[4] and Tone Tønjum[1,2]

1) Department of Microbiology, Oslo University Hospital, Oslo, Norway
2) Department of Microbiology, University of Oslo, Oslo, Norway
3) Fundación Centro Investigación Enfermedades Neurológicas (CIEN), Spain
4) Department of Neurology, Faculty Division, Akershus University Hospital, Norway

E-mail: m.s.lillenes@medisin.uio.no

Alzheimer's disease (AD) is the major contributor to cognitive decline and dementia worldwide. Considering the expanding numbers of elderly in our society, studies of health and disease in the aging population is increasingly important. In order to understand AD, one must understand normal aging. AD is preceded by mild cognitive impairment (MCI), and once MCI occurs, early diagnostics and therapies are urgently needed. MCI/AD is associated with increased oxidative stress, resulting from imbalance in production and clearance of reactive oxygen species (ROS). ROS can damage DNA and other macromolecules, leading to genome instability and disrupted cellular functions. To counteract the harmful effects of oxidative DNA damage, cells use the base excision repair (BER) pathway.

We have monitored the expression of the DNA repair profile in human blood and post-mortem brain biopsies from AD and MCI patients and healthy aged individuals by transcriptional profiling and mass spectrometry. In this context, we have a particular a focus on selected BER components, to define if the expression profile varies between AD patients as compared to healthy controls. Notably, BER expression was significantly higher in brain tissue compared to blood. BER mRNA levels were correlated to clinical signs and cerebrospinal fluid biomarkers for AD. Blood mRNA levels of *OGG1* was low and *PARP1* high in MCI and AD. These findings suggest that alteration in BER gene expression is an event preceding AD and reflect the oxidative stress-generating energy-consumption in the brain and the importance of BER in repairing these damage events.

Collectively, these studies provide new keys to understanding early events in the progression of AD and also expand the pool of potential biomarkers for pre-clinical AD.

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TARGETING OF PROTEINS INVOLVED IN REPAIR OF OXIDATIVE DNA DAMAGE TO THE MITOCHONDRIA

Debora Lia[1], Tristan Piolot[2], Juan-Pablo Radicella[1], Anna Campalans[1]

1) Institute of Cellular and Molecular Radiobiology, UMR967 INSERM, Commisariat A L'Energie Atomique (IRCM, CEA), Fontenay aux Roses, France
2) Institute Curie, Paris, France

E-mail: debora.lia@hotmail.com

Mitochondria are involved in a plethora of essential functions in the cells. Mitochondria have their own genome that encodes for 13 proteins involved in the electron transport chain and ATP synthesis. Maintenance of mtDNA is mandatory for the health of the cell, and accumulation of mitochondrial DNA damage plays an important role during aging and has also been involved in the development of neurodegenerative disorders such as Parkinson and Alzheimer diseases. The mitochondrial genome is highly exposed to reactive oxygen species (ROS), and the base excision repair (BER) seems to be the main DNA repair pathway inside these organelles. 8-oxoG is one of the most abundant lesions induced by ROS on DNA and the DNA glycosylase activity of OGG1 is essential for its removal. Almost all BER proteins have been detected in mitochondria and they are all encoded by the nuclear genome.

However, the molecular mechanisms involved in the targeting of BER proteins to mitochondria are poorly understood. OGG1 presents a mitochondrial targeting sequence (MTS) in its N-terminus and a Nuclear Localisation Signal (NLS) in its C-terminus. Both signals are functional and required for the proper subcellular localisation of the protein. OGG1 is imported in mitochondria where it colocalizes with mtDNA in the nucleoids. We are actually characterising the role has OGG1 in the maintenance of mtDNA and mitochondrial function.

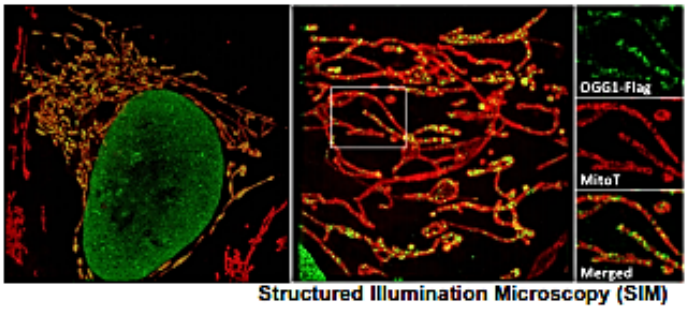

Figure 1: αOGG1 is present in both mitochondrial and nuclear compartments.
In red Mitotracker RED that stain mitochondrial matrix, in green αOGG1-FLAG.

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AGE-RELATED CHANGES IN APP AND AMYLOID BETA DRIVING ALZHEIMER'S PATHOLOGY

Maria Luisa Moro[1]*, Andrew Phillips[1], Katie Gaimster[1], James Nicoll[1], and Delphine Boche[1]

1) Faculty of Medicine, Clinical and Experimental Sciences, University of Southampton, Southampton General Hospital, Southampton, UK

Email: m.l.moro@soton.ac.uk

***Introduction**:* Abundant extracellular deposits of Amyloid beta (Aβ) characterize the brain of patients affected by Alzheimer's Disease (AD). This may derive from changes in the cellular localization of the amyloid precursor protein (APP), from which Ab is released[1], but also by age-related post-translational modifications of Aβ, e.g. aspartate isomerization and pyroglutamate formation, which can influence protein folding and accumulation[2].

In this study, we investigated if ageing can act as risk factor in AD, inducing specific changes in APP and Aβ in human brain.

***Material and methods**:* Parietal cortex from 70 cases: 11 young controls (YC), 32 old controls (OC) and 27 AD cases (table 1) were immunostained for: a) APP and Aβ (clone 4G8), b) specifically Aβ (clone 82E1), c) specifically APP (clone 22C11), d) isoaspartate modified Aβ (IsoAsp-Aβ, clone 22C8) and pyroglutamate modified Aβ (pE-Aβ, clone 337.48).

***Results**:* We observed that APP is uniformly distributed in the cytoplasm of YC neurons. This characteristic was lost in OC and AD, in correlation with the increase of extracellular Aβ load, which became significantly higher in AD cases (82E1: YC vs AD and OC vs AD, $p<0.001$). Deposits of pE-Aβ and IsoAsp-Aβ were present in the brain of OC and AD cases, but significantly increased only in AD cases (IsoAsp-Aβ: YC vs AD $p=0.001$; OC vs AD, $p<0.001$; pE-Aβ: YC vs AD $p<0.001$ and OC vs AD, $p=0.001$)

Conclusions: These data suggest that with ageing the cytoplasmic intracellular pool of APP declines and this is concomitant with increased of extracellular Aβ and of IsoAsp-Aβ and pE-Aβ. Our findings suggest that ageing drives AD pathology by reducing cytoplasmic APP, and inducing age-related post-translational modifications of Aβ.

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TABLE 1

GROUP	Number of cases	Age range	Sex	Post-mortem brain analysis	Source
YC	11	26-62 years old	54% Female 46% Male	Normal Brain	BRAIN UK
OC	32	Over 63 years old	50% Female 50% Male	Normal Brain	Brains for Dementia Research
AD	27	Over 63 years old	52% Female 48% Male	Presence of AD neuropathological hallmarks	Brains for Dementia Research

CROSSTALK BETWEEN NOTCH SIGNALING AND DNA DAMAGE RESPONSE PATHWAYS

Murat Kirtay[1], Zhong-Wei Zhou[1], Zhao-Qi Wang[1, 2]

1) Leibniz Institute on Aging, Fritz Lipmann Institute (FLI), Jena, Germany
2) Faculty of Biology and Pharmacy, Friedrich-Schiller-University Jena, Germany

E-mail: Murat.Kirtay@leibniz-fli.de

The Mre11-Rad50-Nbs1 (MRN) complex functions to activate ATM and also ATR under certain cellular physiology conditions, in response to DNA double-strand breaks (DSBs). Mutations of ATM, ATR, NBS1 and MRE11 cause Ataxia-Telangiectasia (A-T), Seckel Syndrome, Nijmegen Breakage Syndrome (NBS) and A-T Like Disorder (A-TLD), respectively. Neurological defects are in common within these disorders. Whereas A-T and A-TLD are associated with cerebellar degeneration, NBS and ATR-Seckel Syndrome show microcephaly and mental retardation. These suggest that NBS1 and ATR may function in the same pathway to regulate neurogenesis and brain development.

Our previously study showed that while NBS1 is crucial for the proliferation and survival of neural progenitors, ATR are critical for both neural progenitors and neurons [1]. Here in this study, we showed NBS1, unlike other MRN subunits, controls the migration of neurons in cerebral cortex. Moreover, we found that NBS1 directly interacts with Notch1 intracellular domain (NICD) and knock down of Nbs1 increases NICD levels as well as Notch1 activity, independent of the p53 pathway. Furthermore, modulation of Notch activity rescues partially the migration defect in Nbs1 deficient cells. In parallel with this, deletion of ATR in the forebrain neurons (ATR-FBΔ) and in Purkinje cells (ATR-PCΔ) lead to epileptic seizure and defective locomotor functions in mice, respectively, suggesting an important role of ATR in regulating the survival and function of post-mitotic neurons.

We are currently investigating the neuronal activity, neurodegeneration as well as molecular pathways behind these phenomena in ATR-FBΔ and ATR-PCΔ neurons.

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INCREASED HETEROGENEITY OF MITOCHONDRIAL SIZE IS ASSOCIATED WITH DECREASED COMPLEX I-LINKED RESPIRATION IN CORTEX AND HIPPOCAMPUS DURING BRAIN AGING

Kirsten Thomsen, Niloofar Sherazi, Takashi Yokota, Mahdi Hasan-Olive, Christine Regnell Nima Fakouri, Claus Desler, Steen Larsen, Flemming Dela, Linda H. Bergersen, Lene Juel Rasmussen, Martin Lauritzen

Institute of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark
Graduate School of Medicine, Hokkaido University, Hokkaido, Japan
The Brain and Muscle Energy Group, Department of Anatomy, and Department of Oral Biology, University of Oslo, Oslo, Norway
The Brain and Muscle Energy Group, Department of Anatomy and Department of Oral Biology, University of Oslo, Oslo, Norway
Cellular and molecular medicine, University of Copenhagen, Copenhagen, Denmark
Department of Cellular and Molecular Medicine, Center for Healthy aging, Copenhagen, Denmark
Department of Biomedicine, University of Copenhagen, Copenhagen, Denmark
Xlab, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark
Institution of Oral Biology, University of Oslo, Oslo, Norway
Department of Cellular and Molecular Medicine, University of Copenhagen & Center for Healthy Aging, Copenhagen, Denmark
Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark & Department of Clinical Neurophysiology, Glostrup Hospital, Glostrup, Denmark

E-mail: kthomsen@sund.ku.dk

The aging brain is characterized by reduced mitochondrial respiration, which may contribute to impaired cognition and ultimately, to the pathogenesis of neurodegeneration. We hypothesized that the age-related decline in brain mitochondrial respiration would be accompanied by alterations in mitochondrial morphology and dynamics. This was assessed in a segmental progeroid mouse model lacking Cockayne syndrome protein B ($CSB^{m/m}$) and in C57Bl/6 controls (WT) using young (2-5 months) and middle-aged (14 months) mice. Using high-resolution respirometry and NADH-dependent substrates, complex I-linked state 3 (CI) respiration and complex I-linked respiratory control ratio were found to be reduced in middle-aged $CSB^{m/m}$ hippocampus, but not in middle-aged $CSB^{m/m}$ cortex or WT brain. At middle-age, cortical mitochondria were enlarged and hippocampal mitochondria were diminished in size compared to those of young animals using transmission electron microscopy. Importantly, the size of mitochondria in each brain region separately became more heterogeneous with age; this achieved significance in the middle-aged $CSB^{m/m}$ hippocampus.

Thus, an age-dependent inverse linear relation between CI respiration and mitochondrial size heterogeneity was found in both genotypes. Gene expressions of fusion-fission, PINK1-mediated mitophagy, and mitochondrial biogenesis markers and of mitochondrial DNA copy numbers were measured using real-time qPCR. None of these were associated with the age-related alterations in mitochondrial morphology or with the reduction in CI respiration in middle-aged $CSB^{m/m}$ hippocampus. Our findings show that decreased CI respiration and increased mitochondrial size heterogeneity are highly associated and occur early in the brain aging process, indicating accumulation of defective mitochondria with age.

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IDENTIFICATION OF MISMATCH REPAIR ASSOCIATED FACTORS INVOLVED IN THE GENOMIC INSTABILITY OF HUNTINGTON'S DISEASE

Sara Thornby Bak, Despoina Sakellariou and Javier Peña-Diaz

Center for Healthy Aging, Department of Neuroscience and Pharmacology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

E-mail: jdiaz@sund.ku.dk

Trinucleotide repeat (TNR) expansion is the underlying cause of several neurological diseases such as Huntington's disease (HD) in which an autosomal dominant mutation in the Huntingtin gene causes an expanded CAG repeat tract[1,2]. Knowledge of the mechanisms underlying the pathogenic TNR expansion in HD has advanced substantially in recent years; however, many molecular aspects of the mutational processes at TNRs remain enigmatic[3,4]. Expansion of TNRs is believed to be initiated by DNA damage that is inadequately processed by DNA repair and/or replication machineries such as mismatch repair (MMR), which is a repair mechanism crucial in maintaining the stability of the genome. Surprisingly, certain key factors of the MMR machinery have been shown to contribute to repeat instability in cell cultures and mouse models of HD and other TNR expansion disorders. The role of MMR in repeat expansion is not fully understood as it contradicts its main role as a repair mechanism involved in maintaining stability of the genome[1,4].

The aim of this study is to identify and characterize the components of the MMR machinery, which contributes to TNR instability in HD and investigate the mechanisms underlying the pathogenic expansion. Through a global proteomic comparative analysis we identified a short list of factors involved in the MMR damage response. By use of a genetic assay of TNR expansion we investigate the level of TNR instability obtained in human cells after knockdown of factors found in our experiment. Our studies will provide essential knowledge about the factors contributing to TNR expansion and thereby the initiation of disease in HD patients.

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Sara Thornby Bak, Despoina Sakellariou and Javier Peña-Diaz

Center for Healthy Aging, Department of Neuroscience and Pharmacology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

E-mail: sbak@sund.ku.dk

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OVEREXPRESSION OF TAU IN SH-SY5Y CELLS CAUSE AUTOPHAGY

Marya Morevati, Mansour Akbari, Lene Juel Rasmussen and Vilhelm A. Bohr

Center of Healthy aging, Copenhagen University, Copenhagen, Danmark

E-mail: marya@sund.ku.dk

Background: Tau is a microtubule-associated protein, it promotes assembly and stabilizes microtubules. Tau is encoded by the *MAPT* gene, and is expressed in six isoforms in the adult human brain (1). Recent reports have implicated pathological levels of tau, hyperphosphorylated tau, and mitochondrial dysfunction in the pathogenesis of Alzheimer's disease (AD) (2). In this project we are investigating the effect of overexpression of tau protein on mitochondrial function.

Material and Method: Tau-5 is one of the most abundant tau isoforms in adult brain. We have made human neuroblastoma cells, SH-SY5Y, that stably express isoform 5 and the P301L mutant of isoform 5 (found in patients with frontotemporal dementia), all C-terminally fused with green fluorescent protein (GFP). The purpose of this study is to identify which function of mitochondria can be affected by tau overexpression and to elucidate the underlying molecular mechanisms responsible for those changes. Here we will look at autophagy, since failure to remove damaged mitochondria has been implicated in the pathogenesis of neurodegenerative diseases. Furthermore, we are analyzing the effect of tau overexpression on mitochondrial DNA damage and repair.

The results of this ongoing study have so far shown increased levels of hyperphosphorylated tau following treatment of the tau5 expressing SH-SY5Y cells with rotenone, chloramphenicol andmenadion. The level of TFAM protein is increased in tau5 expressing cells, possibly because of higher level of stress in those cells and the cells also show decreased basal autophagic flux and mitophagy.

Cellular and mitochondrial stress may contribute to the hyperphosphorylation of tau, which leads to tau aggregation. These observations suggest that tau hyperphosphorylation is at least partially mediated by oxidative stress and mitochondrial dysfunction. We are further exploring these relationships in disease models with DNA repair defects and neurodegeneration.

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AGE-RELATED COGNITIVE DECLINE AND THE G-PROTEIN COUPLED LACTATE RECEPTOR

Elisabeth Holm Diget[1,6], Mahdi Hasan-Olive[2], Charu Ramakrishnan[3], Thue W. Schwartz[4,5], Siv Annegrethe Hjorth[5], Lene Juel Rasmussen[6], Albert Gjedde[1], Karl Deisseroth[3], Linda Hildegard Bergersen[1, 2,6]

1) Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark
2) The Brain and Muscle Energy Group, Department of Anatomy, and Department of Oral Biology, University of Oslo, Oslo, Norway
3) Department of Bioengineering, Stanford University, Stanford, CA 94305, USA
4) Department of Neuroscience and Pharmacology University of Copenhagen, Copenhagen, Denmark
5) The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark
6) Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark

E-mail: ediget@sund.ku.dk

In the present graduate program project, we will investigate the physiological effects and molecular mechanisms of the lactate receptor GPR81 (also named HCAR1). GPR81 is present and active in the brain (Lauritzen KH et al. 2014, Cereb Cortex) and a growing body of evidence indicates the receptor's importance in age-related cognitive decline.

To explore the functional role of the endogenous GPR81 in the brain we will investigate expression and activity of the receptor in cell lines as well as primary cells. We have recently shown GPR81 expression in a human Blood Brain Barrier (BBB) cell line (unpublished data). We propose that the involvement of GPR81 in age-related cognitive decline involves a change in expression and/or activation of the receptor in cells at the BBB. GPR81 agonists (and antagonists, when available) will further be used to explore the effects the receptor may have on transcriptomics and proteomics in the BBB cell line. In addition a SeaHorse assay will be used to investigate whether GPR81 signaling causes changes in metabolic flux. Canonical cAMP signaling, alternate signaling pathways and effects on neurotransmitter receptors will further be investigated. Using this range of methods we will explore the effect of GPR81 on age-related cognitive decline.

As an alternative approach, we will investigate the possibility of using optogenetics as an *in vivo* technology for activating GPR81. By incorporating a photosensitive molecule with the receptor, we are constructing a chimeric receptor construct, OptoHCAR1, which will be transfected *in vitro* and *in vivo*. (Airan RD et al. 2009, Nature). The optogenetic method will allow the function of the receptor to be studied with greater spatiotemporal resolution than possible with classical pharmacological methods.

References:

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SSB SIGNALLING AND NEURODEGENERATION IN ATAXIA TELANGIECTASIA

Stéphanie Rosciglione and Svetlana Khoronenkova; Department of Biochemistry, University of Cambridge, Cambridge, UK

E-mail: sk870@cam.ac.uk

ATM protein kinase plays a fundamental role in the cellular response to various types of stress, including DNA single- (SSBs) and double-strand breaks. ATM is mutated in individuals with the rare genetic disorder Ataxia Telangiectasia (A-T) characterised by progressive neurodegeneration, genomic instability and immunodeficiency. The genomic instability and immunodeficiency phenotypes characteristic of A-T are relatively well studied, whereas the reasons for neurodegeneration are not yet fully understood. To date, the cytoplasmic functions of ATM that are not associated with DNA repair, global epigenetic silencing and cell cycle re-entry in the absence of ATM, have all been implicated in the neurodegenerative phenotype observed in A-T. Importantly, however, the progressive cerebellar degeneration that underlies the neurological phenotype of A-T is mainly thought to relate to the high levels of DNA damage that occur in neural cells. Indeed, ATM-dependent signalling is important for the efficient repair of DNA base damage and SSBs that represent the most abundant endogenous DNA lesions[1-3]. Moreover, ATM-deficient cells are sensitive to a range of DNA damaging agents that cause SSBs.

Here, I will present our recent findings on the links between deficiencies in the signalling of SSBs and the molecular nature of neurodegeneration in A-T.

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DNA DAMAGE AND DNA DAMAGE RESPONSE IN ALZHEIMER'S DISEASE

Sara Sepe[1] and Fabrizio d'Adda di Fagagna[1-2]

1) IFOM; Milan, Italy
2) IGM-CNR, Pavia, Italy

E-mail: sara.sepe@ifom.eu

Alzheimer's disease (AD) is the most common neurodegenerative disorder. The pathogenesis of AD is complex and involves several cellular pathways including redox balance, mitochondrial dysfunction and damage to macromolecules. Emerging evidences of the involvement of DNA damage in AD has recently been gathered in human and mouse model studies. Noteworthy, patients and mouse model carrying mutations in genes involved in DNA repair and genome integrity show neurological symptoms. Consistently, markers of activated DNA damage response (DDR) have been observed in the brains of AD patients and in AD mouse models. These observations support the essential role of DDR for the homeostasis of neurons, even though the molecular mechanisms of correlations between pathogenesis and DDR remain unclear. Interestingly enough, the administration of Abeta oligomers, the major component of amyloid plaques - hallmark of AD-, to mouse primary neurons, has been

observed to induce DNA damage [1]. We plan now to investigate the DDR activation in mouse primary neurons upon administration of Abeta oligomers in great depth. Thus we will analyze the molecular players of the DDR cascade such as activation of gammaH2AX which is required to recruit mediator of DNA damage checkpoint MDC1, 53BP1 to amplify DDR signaling dependent on ATM. Further downstream factors such as CHK2 and p53 will be studied too. In parallel, we plan to study markers of DNA replication stress to explore the link between Abeta oligomers and potential cell-cycle re-entry of neurons as observed by some laboratories [2]. Thus we will carefully study DNA replication patterns by molecular DNA combing. Here we show our preliminary results strongly supporting a link between Abeta oligomers and the activation of specific DDR markers in neurons.

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THE USE OF MEMBRANE TETHERED Ca_V CHANNEL TOXINS FOR LONG-TERM SILENCING OF CIRCUITS CONTROLLING SOCIAL AND EMOTIONAL BEHAVIORS

Ines Ibanez-Tallon; Laboratory of Molecular Biology, The Rockefeller University, New York, NY, USA

E-mail: iibanez@rockefeller.edu

We discuss the design of genetically encoded peptide tethered toxins (t-toxins) that are anchored to the cell-membrane via a glycolipid or transmembrane domain and their application for cell-specific and long-term manipulation of ion channel-mediated activities in vivo. For instance, Cre–dependent t-toxins against Cav2.1 and Cav2.2 channels were employed for silencing a newly identified oxytocin receptor interneuron population and determine its contribution to sociosexual behavior in female mice (Nakajima et al., 2014). Similarly Ca_v t-toxins were employed for chronic silencing cholinergic interneurons in the nucleus accumbens to demonstrate the contribution of this population to the regulation of mood and motivation (Warner-Schmidt et al., 2012). We also validated the toxin-based strategy in non transgenic mouse models using a dual viral injection system where retrogradely transported AAV2/5-Cre was injected into the striatum and Cre-dependent AAV expressing t-toxins, where injected in either the thalamus or the prefrontal cortex to compare the contribution of thalamo-striatal and cortico-striatal projections to social stress behavioral responses (Christoffel et al., 2015). Taken together, these studies show that the Cav t-toxin strategy can be implemented to interrogate complex circuits in the responses to social stress, depression and sociosexual behaviors.

THURSDAY, AUGUST 25, 2016

EXPLORING THE MOLECULAR LANDSCAPES OF CNS CELL TYPES: 5HMC, MECP2 AND STABILIZATION OF NEURONAL PHENOTYPES

Nathaniel Heintz; The Rockefeller University, New York, NY, USA

E-mail: heintz@rockefeller.edu

As demonstrated over a century ago, the mammalian brain contains over 500 distinct post-mitotic cell types. Each of these cell types is fine-tuned to provide critical functions within brain circuitry, and to respond to physiological factors that modulate behavior. Despite the molecular flexibility neurons display in response to a variety of stimuli, the molecular ground state of each cell type is remarkably stable. This discussion will focus on recent studies of the detailed properties of CNS cell types, the role of 5-hydroxymethylcytosine and MeCP2 in the neuronal genome, and the stability of neuronal phenotypes over the lifetime of the organism.

ROLE OF OXIDATIVE STRESS IN THE ATAXIA-TELANGIECTASIA PHENOTYPE

Martin Lavin[1],Mark Graham [2], Ashley Waardenberg [2] and Sergei Kozlov [1]

1)University of Queensland Centre for Clinical Research, Brisbane, Australia
2) Children's Medical Research Institute, Westmead, Sydney, Australia

E-mail: m.lavin@uq.edu.au

Ataxia-telangiectasia (A-T) is a human autosomal recessive disorder characterized by cancer predisposition and neurodegeneration. ATM (ataxia-telangiectasia, mutated) protein plays a central role in phosphorylating a network of proteins in response to DNA damage. These proteins function in signalling pathways designed to maintain the stability of the genome and minimize the risk of disease by controlling cell cycle checkpoints, initiating DNA repair and regulating gene expression. We have shown that ATM is activated by redox stress and over the years demonstrated that several aspects of the A-T phenotype including survival of neurons, neurobehavioural deficits in mice and onset of tumours can be corrected/delayed by antioxidants. We recently confirmed activation of cytoplasmic ATM by autophosphorylation at multiple sites. In that approach we employed global quantitative phosphoproteomics to identify cytoplasmic proteins altered in their phosphorylation state in control and A-T cells in response to oxidative damage.

We demonstrated that ATM was activated by oxidative damage in the cytoplasm as well as in the nucleus and identified a total of 9,833 phosphorylation sites including 6,686 high confidence sites mapping to 2,536 unique proteins. A total of 62 differentially phosphorylated peptides were identified; of these 43 were phosphorylated in control but not in A-T cells and 19 varied in their level of phosphorylation. Motif enrichment analysis of phosphopeptides revealed that consensus ATM SQ sites were overrepresented. When considering phosphorylation events only observed in control cells (not observed in A-T cells), with predicted ATM sites p(S/T)Q, we narrowed this list to 11 candidate ATM-dependent cytoplasmic proteins. Two of these 11 were previously described as ATM substrates (HMGA1 and RAP80/ UIMCI), another five were identified in a whole cell extract phosphoproteomic screens and the remaining four proteins had not been identified previously

in DNA damage response screens. We validated the phosphorylation of three of these proteins (OSR1, HDGF and ccdc82) as ATM-dependent after H2O2 exposure and another protein (S100A11) demonstrated ATM-dependence for translocation from the cytoplasm to the nucleus. These data provide new insights into the activation of ATM by oxidative stress through identification of novel substrates for ATM in the cytoplasm.

ON THE BRAIN-GUT AXIS: DIFFERENTIAL EXPRESSION OF DNA REPAIR PATHWAYS IN HUMAN BRAIN AND MUCOSAL GUT TISSUE

(Poster number III-6)

Tahira Riaz [1,2], Meryl S. Lillenes[2], Trond Espen Detlie[3] , Alfredo Rabano[4] and Tone Tønjum[1,2]

1) Department of Microbiology, Oslo University Hospital, Norway
2) Department of Microbiology, University of Oslo, Norway
3) Department of Gastroenterology, Akershus University Hospital, Lørenskog and Campus Ahus, Institute of Clinical Medicine, University of Oslo
4) Fundación Centro Investigación Enfermedades Neurológicas (CIEN), Spain

E-mail: tone.tonjum@medisin.uio.no

The biology of the neurological and gastrointestinal systems are tightly interconnected, interacting with and influencing each other through multiple bidirectional signaling pathways. The human microbiome strongly influences the physiology of all organs including the central nervous system (CNS), and the CNS in turn modulates gut function, beyond the effects of the vagal nerve. Therefore, imbalance in the brain-gut axis could correlate with incipient pathology in the CNS and/or the gastrointestinal tract. We asked if and how the gut microbiome modulates susceptibility to progressive neurodegenerative Alzheimer's disease (AD). The primary hypothesis we are testing is that imbalance in the brain-gut axis promotes neurodegeneration or gastrointestinal dysfunction or both. ‘

The proteomic expression profile was tested in post-mortem human brain biopsies and gut mucosal biopsies. In terms of DNA repair, predominant base excision repair (BER) was expressed in brain tissue, while nucleotide excision repair (NER) was more highly expressed in the gut mucosa. This differential expression pattern reflects local stress and organ environments, both the nature of non-replicating versus replicating cells as well as the state of a sterile organ versus that of an organ with a rich microbiome. Different DNA repair responses were evident in the prodromal versus late stages of AD and in IBD. We have previously shown that signature reactions in BER patterns in brain appear before AD pathology is evident and may represent a response to increased oxidative stress. These studies extend our findings on DNA repair and bioenergetics in AD and IBD, and will also address the contribution of the gut microbiome.

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A NOVEL MECHANISM FOR EXERCISE-INDUCED ANGIOGENESIS IN THE BRAIN

(Poster number III-7)

Cecilie Morland*[1,2], Krister A. Andersson*[1,3], Alena Hadzic[1,2,3], Øyvind P. Haugen[1], Andreas Gille[4], Johanne Egge Rinholm[1,3], Lauritz H. Kennedy[1,3], Liv Kleppa[1,3], Tomas Stølen[5], Yiqing Cai[1], Maja Puchades[3], Stefan Offermanns[6], Ulrik Wisløff[5], Jon Storm-Mathisen[3], and Linda H. Bergersen[1].

1) The Brain and Muscle Energy Group, and Electron Microscopy Laboratory, Department of Oral Biology, University of Oslo, Oslo, Norway
2) Institute for Behavioral Sciences, Faculty of Health Sciences, Oslo and Akershus University Collage, Oslo, Norway
3) The Synaptic Neurochemistry Lab, Division of Anatomy, Department of Molecular Medicine, Institute of Basic Medical Sciences, and Healthy Brain Ageing Centre, University of Oslo, Oslo, Norway
4) Institute for Experimental and Clinical Pharmacology and Toxicology, Mannheim Medical Faculty, Heidelberg University, Mannheim, Germany, and Clinical and Translational Science Strategy Therapy Area Lead – Breakthrough Medicines, CSL Limited, Parkville, Victoria, Australia
5) Department of Neuroscience and Pharmacology, and Center for Healthy Aging, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
6) Department of Circulation and Medical Imaging, K.G. Jebsen Center of Exercise in Medicine, Norwegian University of Science and Technology, Trondheim, Norway
7) Laboratory of Neural Computation, Department of Physiology, University of Oslo, Oslo, Norway
8) Max-Planck-Institute for Heart and Lung Research, Department of Pharmacology, Bad Nauheim, Germany

- These authors contributed equally to the work

E-mail: cecilie.morland@medisin.uio.no

Angiogenesis, the formation of new blood vessels, is a key mechanism through which physical activity enhances brain function. This mechanism might be particularly important to maintain cognitive function in conditions where the metabolic capacity of the brain is reduced, such as in ageing, vascular dementia, Alzheimer's disease, and ischemia. Angiogenesis in the brain is stimulated by increased levels of vascular endothelial growth factor A (VEGFA), and exercise is known to stimulate brain VEGFA expression. However, the signals that initiate cerebral VEGFA expression in response to exercise are yet to be determined. Here we identify a novel mechanism for regulation of VEGFA and angiogenesis in the brain in response to exercise: the activation of a receptor located in the fibroblasts of the pia mater, especially along the pial arteries. Our findings are based on the use of knock-out and wild type mice, which were exposed to high intensity interval exercise five days a week for seven weeks. At the end of the treatment, wild type mice showed a ~40% increase in VEGFA protein level, as measured by Western blotting of hippocampal homogenates, and a ~40% increase in capillary density of the dentate gyrus of the hippocampus compared to controls. These effects were absent in the knock-outs.

The same results were seen in the motor cortex area 1. Further, fibroblast cultures and endothelial cultures were used to pinpoint the detailed inter-communication between these two cell types that links receptor activation to increased VEGF signalling.

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A NOVEL MECHANISM FOR EXERCISE-INDUCED ANGIOGENESIS IN THE BRAIN

Cecilie Morland*[1,2,3], Krister A. Andersson*[1,3], Alena Hadzic[1,2,3], Øyvind P. Haugen[1], Liv Kleppa[1,3], Andreas Gille[4], Johanne Egge Rinholm[1,3], Elisabeth Holm-Diget[1,5], Lauritz H. Kennedy[1,3], Tomas Stølen[6], Eivind Hennestad[7], Yiqing Cai[1], Maja Puchades[3], Sjoerd van den Berg[1,3], Vidar Gundersen[3,8], Stefan Offermanns[9], Koen G.A. Vervaeke[7], Ulrik Wisløff[6], Jon Storm-Mathisen[3], and Linda H. Bergersen[1,3,5]

1) The Brain and Muscle Energy Group, and Electron Microscopy Laboratory, Department of Oral Biology, University of Oslo, Oslo, Norway
2) Institute for Behavioral Sciences, Faculty of Health Sciences, Oslo and Akershus University Collage, Oslo, Norway
3) The Synaptic Neurochemistry Lab, Division of Anatomy, Department of Molecular Medicine, Institute of Basic Medical Sciences, and Healthy Brain Ageing Centre, University of Oslo, Oslo, Norway
4) Institute for Experimental and Clinical Pharmacology and Toxicology, Mannheim Medical Faculty, Heidelberg University, Mannheim, Germany, and Clinical and Translational Science Strategy Therapy Area Lead – Breakthrough Medicines, CSL Limited, Parkville, Victoria, Australia
5) Department of Neuroscience and Pharmacology, and Center for Healthy Aging, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
6) Department of Circulation and Medical Imaging, K.G. Jebsen Center of Exercise in Medicine, Norwegian University of Science and Technology, Trondheim, Norway
7) Laboratory of Neural Computation, Department of Physiology, University of Oslo, Oslo, Norway
8) Max-Planck-Institute for Heart and Lung Research, Department of Pharmacology, Bad Nauheim, Germany

* These authors contributed equally to the work

E-mail: l.h.bergersen@medisin.uio.no

Physical exercise can improve learning and memory and postpone the cognitive decline associated with aging and neurodegenerative disease. Enhanced angiogenesis has been suggested as a key mechanism through which exercise supports brain function. Angiogenesis is stimulated by vascular endothelial growth factor A (VEGFA), but the initial signal that leads to increased cerebral VEGFA in response to exercise has not been determined. Here we identify a novel mechanism for regulation of VEGFA and angiogenesis in the brain in response to exercise. The effects of exercise were reproduced by injecting L-lactate. The novel mechanism for regulation of VEGFA and angiogenesis in the brain were not found in skeletal muscle.

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Lene Juel Rasmussen
Vilhelm Bohr

A NOVEL MECHANISM FOR EXERCISE-INDUCED ANGIOGENESIS IN THE BRAIN

Cecilie Morland*[1,2,3], Krister A. Andersson*[1,3], Alena Hadzic[1,2,3], Øyvind P. Haugen[1], Liv Kleppa[1,3], Andreas Gille[4], Johanne Egge Rinholm[1,3], Elisabeth Holm-Diget[1,5], Lauritz H. Kennedy[1,3], Tomas Stølen[6], Eivind Hennestad[7], Yiqing Cai[1], Maja Puchades[3], Sjoerd van den Berg[1,3], Vidar Gundersen[3,8], Stefan Offermanns[9], Koen G.A. Vervaeke[7], Ulrik Wisløff[6], Jon Storm-Mathisen[3], and Linda H. Bergersen[1,3,5]

1) The Brain and Muscle Energy Group, and Electron Microscopy Laboratory, Department of Oral Biology, University of Oslo, Oslo, Norway
2) Institute for Behavioral Sciences, Faculty of Health Sciences, Oslo and Akershus University Collage, Oslo, Norway
3) The Synaptic Neurochemistry Lab, Division of Anatomy, Department of Molecular Medicine, Institute of Basic Medical Sciences, and Healthy Brain Ageing Centre, University of Oslo, Oslo, Norway
4) Institute for Experimental and Clinical Pharmacology and Toxicology, Mannheim Medical Faculty, Heidelberg University, Mannheim, Germany, and Clinical and Translational Science Strategy Therapy Area Lead – Breakthrough Medicines, CSL Limited, Parkville, Victoria, Australia
5) Department of Neuroscience and Pharmacology, and Center for Healthy Aging, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
6) Department of Circulation and Medical Imaging, K.G. Jebsen Center of Exercise in Medicine, Norwegian University of Science and Technology, Trondheim, Norway
7) Laboratory of Neural Computation, Department of Physiology, University of Oslo, Oslo, Norway
8) Max-Planck-Institute for Heart and Lung Research, Department of Pharmacology, Bad Nauheim, Germany

* These authors contributed equally to the work

E-mail: jon.storm-mathisen@medisin.uio.no

Angiogenesis, the formation of new blood vessels, is a key mechanism through which physical activity enhances brain function. This mechanism might be particularly important to maintain cognitive function in conditions where the metabolic capacity of the brain is reduced, such as in ageing, vascular dementia, Alzheimer's disease, and ischemia. Angiogenesis in the brain is stimulated by increased levels of vascular endothelial growth factor A (VEGFA), and exercise is known to stimulate brain VEGFA expression.

However, the signals that initiate cerebral VEGFA expression in response to exercise are yet to be determined. Here we identify a novel mechanism for regulation of VEGFA and angiogenesis in the brain in response to exercise: the activation of a receptor located in the fibroblasts of the pia mater, especially along the pial arteries. Our findings are based on the use of knock-out and wild type mice, which were exposed to high intensity interval exercise five days a week for seven weeks. At the end of the treatment, wild type mice showed a ~40% increase in VEGFA protein level, as measured by Western blotting of hippocampal homogenates, and a ~40% increase in capillary density of the dentate gyrus of the hippocampus compared to controls. These effects were absent in the knock-outs.

The same results were seen in the motor cortex area 1. Further, fibroblast cultures and endothelial cultures were used to pinpoint the detailed inter-communication between these two cell types that links receptor activation to increased VEGF signalling.

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THE GLYMPHATIC SYSTEM AND ITS IMPORTANCE IN AMYLOID CLEARANCE

Nedergaard, Maiken; University of Rochester Medical Center School of Medicine and Dentistry, Rochester, NY, USA

E-mail: Maiken_Nedergaard@URMC.Rochester.edu

We have recently described a macroscopic pathway in the central nervous system – the glymphatic system that facilitates the clearance of interstitial waste products from neuronal metabolism. Glymphatic clearance of macromolecules is driven by cerebrospinal fluid (CSF) that flows in along para-arterial spaces and through the brain parenchyma via support from astroglial aquaporin-4 water channels. The glymphatic circulation constitutes a complete anatomical pathway; para-arterial CSF exchanges with the interstitial fluid, solutes collect along para-venous spaces, then drain into the vessels of the lymphatic system for ultimate excretion from the kidney or degradation in the liver. As such, the glymphatic system represents a novel and unexplored target for prevention and treatment of neurodegenerative diseases.

CANONICAL AND NON-CANONICAL FUNCTIONS OF DDR MOLECULES IN NEURO(DE)GENERATION

Zhao-Qi Wang[1,2]
1) Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena, Germany
2) Faculty of Biology and Pharmacy, Friedrich-Schiller-University Jena, Jena, Germany

E-mail: zhao-qi.wang@leibniz-fli.de

ATM and ATR govern two key DNA damage response (DDR) pathways, which are activated by DNA double strand breaks (DSBs) and single stranded DNA (SSBs) or replication stress, respectively. ATR-Seckel and Nijmegen Breakage Syndrome (NBS) patients exhibit similar neurological defects, such as microcephaly and mental retardation. NBS1 (mutated in NBS, an activator of ATM) and ATR are essential genes and their absence leads to lethality of cells and mice, presumably due to their essential function in dealing with replication-born DSBs. We studied the impact of the neural specific deletion of either Nbs1 or Atr during mouse development and found that Nbs1 deletion compromised survival of neuroprogenitors, whereas Atr null induced death of both neuroprogenitors and postmitotic neurons. Nbs1 deletion in neuroprogenitors leads to microcephaly by compromising neuroprogenitor proliferation and survival. We recently found that Nbs1 deletion causes premature cell cycle exit of neuroprogenitors, which exhausts progenitor pools and reduces neurogenesis capability to the late born neurons. Although Nbs1 is not essential for the survival of postmitotic cells, inactivation of Nbs1 in neurons affects neurite outgrowth and neuron migration.

Our data suggest that NBS1 regulates brain development not only through its classic DDR function but also a non-canonical function. Currently we are characterizing specific pathways and mechanisms controlled by Nbs1 in neuron maturation and migration.

MAINTAINING GENOME STABILITY IN THE NERVOUS SYSTEM

Peter J. McKinnon; St Jude Children's Research Hospital, Memphis, TN, USA

E-mail: peter.mckinnon@stjude.org

Genome stability is a prerequisite for the development and function of the nervous system. Multiple DNA damage response pathways ensure that DNA lesions resulting from replication stress and other types of damage such as oxidative damage do not impact neural homeostasis. The DNA damage response is especially critical during early neurogenesis when rapid proliferation and progenitor expansion and differentiation generates cellular diversity in the nervous system. For example, numerous congenital human neurologic syndromes are associated with defective DNA damage signaling and compromised genome integrity. These syndromes arise from inactivation of key DNA damage response factors, and can involve diverse neuropathology, including neurodegeneration, neurodevelopmental defects and brain tumors, highlighting the varied tissue-specific needs for neural genome stability.

Data from these syndromes and from genetically engineered mouse models have been critical for understanding the physiologic context for different DNA repair pathways. Recent findings from our work using nervous system-directed genome instability in the mouse as models of neurologic disease will be discussed. These data will emphasis genome stability mechanisms that maintain neural homeostasis to prevent disease.

NELF-E FACILITATES TRANSCRIPTION SILENCING AT DNA DOUBLE-STRAND BREAKS AND PROMOTES DNA REPAIR

(Poster number III-8)

Samah W. Awwad and Nabieh Ayoub[1]; Department of Biology, Technion - Israel Institute of Technology, Haifa, Israel

E-mail: ayoubn@technion.ac.il

DNA damage triggers rapid and transient transcription pause to prevent collisions between repair and transcription machineries at DNA breakage sites (1). DSB-induced transcription silencing is therefore essential for intact DNA repair and genomic stability. Several factors such as ATM, PARP1 and DNA-PK enzymes are implicated in transcription silencing at DNA damage sites, however little is known about the molecular mechanisms for ensuring transcription block after DNA damage. Here we reveal dual functions of the negative elongation factor (NELF) in blocking transcription after damage and double-strand break (DSB) repair. It is known that NELF complex negatively regulates the elongation of transcription by RNA polymerase II (2). Interestingly, it was also shown that NELF complex play a role in the expression of neuronal immediate early genes (3). We show that NELF-E subunit is rapidly recruited to DSBs in a PARP1-dependent manner to shutdown transcription. Remarkably, using *I-Sce-I* endonuclease and CRISPR-Cas9, we demonstrate that NELF-E is preferentially recruited to DSBs induced upstream transcriptionally active genes. Furthermore, we describe a non-canonical function of NELF-E in promoting BRCA1 recruitment to damage sites to foster homology-directed repair of DSBs.

Altogether, our data reveal a hitherto unknown pathway by which PARP1 promotes DSB-induced transcription silencing and identified NELF complex as the first component of the DNA damage response that selectively accumulates at DSBs surrounding transcriptionally active genes.

Highlights

NELF-E is a new component of the DNA damage response.
NELF-E is preferentially recruited to DSBs nearby transcriptionally active genes.
NELF-E is recruited in a PARP1-dependent manner to shutdown transcription near DSB.
NELF-E promotes BRCA1 foci formation and homology-directed repair of DSBs.

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INEFFICIENT DNA REPAIR IS AN AGING-RELATED MODIFIER OF PARKINSON'S DISEASE

(Poster number III-9)

Sara Sepe[1], Chiara Milanese[1,2], Cesar Payan-Gomez[1,3], Kasper W.J. Derks[1], Sandra Moreno[4], Jan H. J. Hoeijmakers[1], Silvia Cerri[5], Fabio Blandini[5], and Pier G. Mastroberardino[1, *]

1) Department of Molecular Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
2) Ri.Med Foundation, Palermo, Italy
3) Universidad del Rosario, Facultad de Ciencias Naturales y Matemáticas, Bogotá, Colombia
4) University of "Roma Tre", Rome, Italy
5) Laboratory of Functional Neurochemistry, Center for Research in Neurodegenerative Diseases, C. Mondino National Neurological Institute, Pavia, Italy

E-mail: p.g.mastroberardino@erasmusmc.nl

The underlying relation between Parkinson's disease (PD) etiopathology and its major risk factor, aging, is largely unknown. Recent evidence established a strong and causative link between genome stability and aging. To investigate a possible nexus between DNA damage accumulation, aging, and PD we examined DNA repair pathways associated with aging in laboratory animal models and human cases. We demonstrate that dermal fibroblasts from PD patients display flawed nucleotide excision repair (NER) capacity and that *Ercc1* mutant mice with mildly compromised NER exhibit typical PD-like pathological alterations, including decreased dopaminergic innervation in the striatum, increased phospho-synuclein levels, and defects in mitochondrial respiration. *Ercc1* mouse mutants are also more sensitive to the prototypical PD toxin MPTP and their transcriptomic landscape shares important similarities with that of PD patients.

Our results demonstrate that specific defects in DNA repair impact the dopaminergic system, are associated with human PD pathology, and might therefore constitute an age-related risk factor for PD.

THE IMPACT OF DNA DAMAGE ON NEURODEGENERATION AND THE POTENTIAL OF NUTRITIONAL INTERVENTIONS

Jan H.J. Hoeijmakers, Department of Genetics, Erasmus MC, Rotterdam, The Netherlands

E-mail: j.hoeijmakers@erasmusmc.nl

The molecular basis underlying ageing and ageing-related diseases is one of the main unsolved questions in biology. Ageing in various model organisms appears remarkably plastic: e.g. suppressing insulin signalling extends lifespan in worms flies and mice. On the other hand, virtually all premature aging syndromes in man provide a link with genome instability. We have generated mouse models, which strikingly mimic human DNA repair deficiency syndromes and display wide-spread accelerated aging. For instance, DNA repair-deficient Ercc1$^{\Delta/-}$ mice defective in 3 or more repair pathways show numerous accelerated aging features in post-mitotic and proliferative organs and tissues (e.g. pronounced neurodegeneration and hematopoietic stem cell exhaustion), limiting lifespan to 4-6 month. Simultaneously they exhibit an anti-aging 'survival response', which suppresses growth and enhances maintenance, resembling the longevity response induced by dietary restriction (DR). Interestingly, subjecting these progeroid, dwarf mutants to actual DR resulted in the largest lifespan increase recorded in mammals. Thirty percent DR tripled median and maximal remaining lifespan, and drastically retarded numerous aspects of accelerated aging, e.g. DR animals retained 50% more neurons and maintained full motoric function, delaying motor decline ~30-fold. Repair-deficient Xpg$^{-/-}$ mice also showing many premature aging symptoms responded similarly to DR, extending this observation beyond Ercc1. The DR response in Ercc1$^{\Delta/-}$ mice resembled DR in wild type animals including (further) reduced insulin signaling. Interestingly, ad libitum Ercc1$^{\Delta/-}$ liver expression profiles showed gradual preferential extinction of expression of long genes, consistent with genome-wide accumulation of stochastic, transcription-blocking lesions, which affect long genes more than short ones. DR largely prevented this decline of transcriptional output, indicating that DR prolongs genome function.

We will present phenotypes of conditional DNA repair models targeting aging to selected organs. Also we report links between DNA damage accumulation and stressed protein homeostasis involved in protein aggregation disorders such as Alzheimer's and Parkinson diseases. Our findings strengthen the link between DNA damage and aging, establish Ercc1$^{\Delta/-}$ mice as powerful model for identifying interventions to promote healthy aging, reveal untapped potential for reducing endogenous damage, provide new venues for understanding the molecular mechanism of DR, and suggest a counterintuitive DR-like therapy for human progeroid genome instability syndromes and DR-like interventions for preventing neurodegenerative diseases.