ERIBA
10th anniversary 2023

fli Leibniz Institute on Aging – Fritz Lipmann Institute

GRONINGEN-JENA AGING MEETING (G-JAM) 2023

University Medical Center Groningen (UMCG), The Netherlands

28 – 30 September 2023

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www.g-jam.eu

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Welcome note

Dear participants,

We are delighted to welcome you for the G-JAM 2023 meeting jointly organized for the second time by the European Research Institute for the Biology Aging (ERIBA) and the Leibniz Institute on Aging – Fritz Lipmann Institute (FLI). The G-JAM meetings were preceded by a history of separate aging meetings by both institutes starting from 2015. Additionally, this year holds special significance for ERIBA as it marks its 10th anniversary of its foundation.

In keeping with the tradition of our previous meetings, we cover a diverse range of topics related to the field of aging research. Our primary goal is to facilitate scientific exchange and interaction. We firmly believe that collaborative efforts are paramount in unraveling the intricate facets of the aging process and in identifying mechanisms of resilience. While our meeting sessions may appear to focus on distinct areas, the very titles of the presentations already hint at the numerous interdisciplinary discoveries.

We wish all of you a great meeting and we sincerely hope our gathering will inspire you to collaborate and lay down the basis for new discoveries in the aging field.

Helen Morrison, Claudia Waskow, Björn von Eyss, Ellen Nollen, Eugene Berezikov and Cornelis Calkhoven

Practical Information

Conference venue
University Medical Center Groningen
Blauwe Zaal (near fonteinstraat)
Hanzeplein 1
9713 GZ Groningen

Reception
Date: 28 September 2023
Time: 20:00
Location: City Hall, Grote markt

Conference dinner & party
Date: 29 September 2023
Time: 18:00
Location: DOT
Vrydemalaan 2
9713 WS Groningen

Free Wifi:
UMCG-guest network

Public transport
The website www.9292.nl very useful for planning your journey in the Netherlands. The planner combines all available public transportation – trains, buses, trams, metro, and boats – to provide an optimal route. Train schedules: Information on travelling by train is available on the NS website (www.ns.nl)

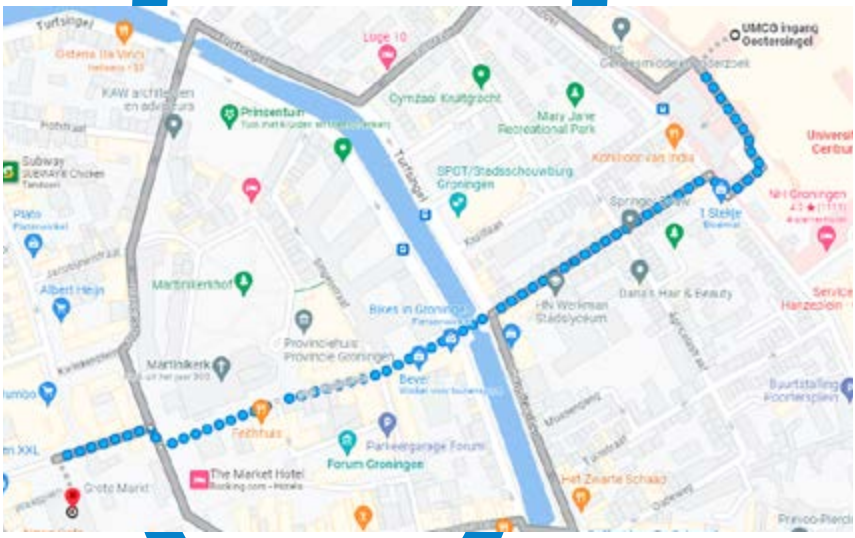
Taxis in Groningen
Taxi Groningen: +31 (0)50 – 549 7676,
www.taxicentralegroningen.nl

Taxi Noord: +31 (0)50 – 549 4940,
www.taxinoord.nl

Schiphol taxi Groningen:
+31 (0)50 – 850 7519,
www.schipholtaxigroningen.nl

Map of the City

Reception Venue



Scientific Programme

Groningen Jena Aging Meeting (G-JAM) 2023

Thursday, September 28	
9:00-10:00	**Registration & Coffee**
10:00-10:15	**Opening remarks - Welcome note**
10:15-10:55	**Session 1. Protein homeostasis 1** *Chair: Ellen Nollen* Speakers
10:15-10:40	Alessandro Ori, Genentech, South San Francisco, USA / Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany *The aging brain proteome of a short-lived vertebrate*
10:40-10:55	Milos Filipovic, Leibniz Institute for Analytical Sciences, ISAS, Berlin, Germany *Age-induced thiol oxidation predisposes brain for neurodegeneration via aberrant phase separation*
10:55-11:30	**Coffee break**
11:30-12:25	**Session 1. Protein homeostasis 2** *Chair: Ellen Nollen* Speakers
11:30-11:55	Malene Hansen, The Buck Institute, Novato, USA *Regulation of autophagy in aging and disease*
11:55-12:10	Paola Gallardo, ERIBA-UMCG, Groningen, The Netherlands *Poor old Pores: Surveillance of intrinsically disordered nucleoporins*
12:10-12:25	Janine Kirstein, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany *The role of chaperones in the phase-separation of Huntingtin*
12:25-13:25	**Lunch**

13:25-14:45	**Session 2: Metabolism** *Chair: Cor Calkhoven*
13:25-13:50	Speakers Dudley Lamming, University of Wisconsin-Madison, USA *When a calorie is not just a calorie: The regulation of health and longevity by dietary macronutrients*
13:50-14:15	Peter Tessarz, Max Planck Institute for Biology of Ageing, Cologne, Germany *Metabolism-epigenetics crosstalk in ageing*
14:15-14:30	Sanne van der Rijt, Amsterdam UMC, The Netherlands Targeting phospholipid metabolism via Pla2g15 results in reduced senescence in murine kidney and *increases longevity in C. elegans*
14:30-14:45	Friedrich Becker, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany *Dietary vitamin A restriction rescues declines in liver fat metabolism and ameliorates sarcopenia in aging mice*
14:45-15:15	**Coffee Break**
15:15-16:10	**Session 3: Gut microbiome** *Chair: Folkert Kuipers*
15:15-15:40	Speakers Sasha Zhernakova, University Medical Center Groningen, The Netherlands *Biological aging markers in population cohorts - microbiome and more.*
15:40-15:55	Aki Minoda, RIMLS, Radboud University Nijmegen, The Netherlands *Mu-kin Mouse Ageing Atlas: How the microbiota affect ageing*
15:55-16:10	Dennis De Bakker, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany *Microbiota transplantation can mitigate age-related brain inflammation and functional decline in a model of spontaneous Alzheimer's-like pathology*
16:10-18:00	**Poster Session**
18:00-18:45	**Dinner at UMCG Fontein Patio**
18:45-19:30	**Keynote Lecture** *Introduction: Cor Calkhoven* Michael Hall, Biozentrum, University of Basel, Switzerland *mTOR signaling in growth and metabolism*
20:00-22:00	**Reception at the City Hall invited by the Mayor of Groningen**

Friday, September 29	
9:00-10:20	**Session 4. Senescence** *Chair: Tamar Tchkonia*
	Speakers
9:00-9:25	Marco Demaria, ERIBA-UMCG, Groningen, The Netherlands *Purinergic signalling reinforces the production of SASP factors and promotes senescence-associated dysfunctions*
9:25-9:50	Raffaella di Micco, I.R.C.C.S. Ospedale San Raffaele, Milano, Italy *Exploiting senescence immunogenicity for leukemia treatment*
9:50-10:05	Akiko Mammoto, Medical College of Wisconsin, USA *Endothelial senescence in hypoxia-induced lung vascular remodeling*
10:05-10:20	Boshi Wang, ERIBA-UMCG, Groningen, The Netherlands *Sex dimorphism in senescent cell turnover is mediated by FABP5*
10:20-10:50	**Coffee break**
10:50-12:10	**Session 5. Immune aging** *Chair: Claudia Waskow*
	Speakers
10:50-11:15	Vishwa Deep Dixit, Yale School of Medicine, New Haven, USA *Metabolic control of inflammaging*
11:15-11:40	Mihai Netea, Radboud MC, Nijmegen, the Netherlands/ Limes, University of Bonn, Germany *Impact of aging on trained immunity*
11:40-11:55	Enric Urena Sala, University College London, UK *Exploring the mechanisms of action of the life-extending drug trametinib in Drosophila*
11:55-12:10	Patrick Schädel, Friedrich Schiller University, Jena, Germany *Oxylipins as novel biomarkers of cellular and organismal inflammaging*
12:10-13:30	**Lunch and meet with the speakers (PhD students and Postdoc career advise)**
13:30-14:50	**Session 6. Stem cells and regeneration** *Chair: Björn von Eyss_*
	Speakers
13:30-13:55	Maximina Yun, Center for Regenerative Therapies TU Dresden, Germany *Tow*imin *uncovering the basis of negligible senescence in vertebrates: enter the salamander*
13:55-14:20	Julia von Maltzahn, B-TU Cottbus-Senftenberg, Germany *Muscle stem cells in age and disease*
14:20-14:35	Alberto Minetti, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany *Proteostasis stress delays regeneration following injury in old small intestine epithelium*
14:35-14:50	Hugo Fernandes, University of Coimbra, Portugal *Unlocking the regenerative potential of extracellular vesicles: Bioactivity enhancement through miRNA modulation*
14:50-15:20	**Coffee break**

15:20-16:40	**Session 7. DNA damage and genome regulation** *Chair: Michael Chang* Speakers
15:20-15:45	George Garinis, University of Crete, Greece *DNA damage and innate immune responses during aging*
15:45-16:10	Björn Schumacher, CECAD, University of Cologne, Germany *Genome Stability in aging and inheritance: new insights from C. elegans*
16:10-16:25	Rouven Arnold, SBP Medical Discovery Institute, La Jolla, USA *Unraveling protective mechanisms of aging: A new role for histone chaperone HIRA*
16:25-16:40	Mihailo Mirkovic, ETH, Zürich, Switzerland *Introns drive asymmetric chromosome inheritance in ageing*
16:40-17:25	**Keynote Lecture** *Introduction: Helen Morrison* Linda Partridge, UCL, London, UK/ MPI, Cologne, Germany *Ageing: a gut feeling*
18:00-00:00	**Dinner and party at the DOT – 10th anniversary ERIBA** **B-flat Carpet Jazz Quintet – DJ – The Blues Cowboys**

Saturday, September 30

9:30-10:10	**Session 8. Organismal aging** *Chair: Eugene Berezikov* Speakers
9:30-9:55	Thomas Bosch, Christian Albrechts University of Kiel, Germany *Longevity, cellular senescence and the microbiome - lessons from the non-senescent model Hydra*
9:55-10:10	Johannes Krug, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany *The transparent klara line as a tool for in vivo analyses*
10:10-10:40	**Socioeconomic aspects of health and lifespan extension therapies** *Introduction: Ellen Nollen* Jochen Mierau, Lifelines, University of Groningen, The Netherlands
10:40-11:00	Coffee break
11:00-11:45	**Keynote Lecture** *Introduction: Folkert Kuipers* James (Jim) Kirkland, Mayo Clinic, Rochester, USA *Clinical studies of agents targeting aging mechanisms: the Translational Geroscience Network*
11:45-12:15	**Closure**
12:15	**Lunch**

Session 1. Protein homeostasis
Thursday, September 28

Alessandro Ori

Genentech, South San Francisco, USA & Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany

The aging brain proteome of a short-lived vertebrate

Proteostasis, the maintenance of protein homeostasis, is disrupted in both aging and neurodegenerative diseases, but how aging impairs proteostasis in the brain is not well understood. Here, we measured and integrated the effects of aging on the transcriptome, translatome and multiple layers of the proteome in the brain of short-lived killifish. We found that aging disrupts the relationship between transcripts and proteins leading to a decrease of basic proteins, including ribosomal and DNA/RNA-binding proteins. In contrast, abundant and long-lived proteins increase or remain stable despite a decrease in their mRNA levels. Chronic proteasome inhibition can induce some aging signatures in vivo, but it does not recapitulate the age-related decoupling between transcripts and proteins. Instead, we find that increased translation pausing reprograms the protein synthesis landscape independently of transcription. These changes in protein biogenesis likely reduce availability of key protein complexes and contribute to remodeling of organelles in older brains. Job title: Principal investigator

Relevant publications: Di Sanzo, S.*, Spengler, K.*, Leheis, A., Kirkpatrick, J.M., Rändler, T.L., Baldensperger, T., Dau, T., Henning, C., Parca, L., Marx, C., Wang, Z.-Q., Glomb, M.A., Ori, A.#, and Heller, R.# (2021). Mapping protein carboxymethylation sites provides insights into their role in proteostasis and cell proliferation. Nat. Commun. 12. Schüler, S.C., Kirkpatrick, J.M.*, Schmidt, M.*, Santinha, D., Koch, P., Di Sanzo, S., Cirri, E., Hemberg, M., Ori, A.#, and von Maltzahn, J.# (2021). Extensive remodeling of the extracellular matrix during aging contributes to age-dependent impairments of muscle stem cell functionality. Cell Reports. 35. Kelmer Sacramento, E.*, Kirkpatrick, J.M.*, Mazzetto, M.*, Baumgart, M., Bartolome, A., Di Sanzo, S., Caterino, C., Sanguanini, M., Papaevgeniou, N., Lefaki, M., Childs, D., Bagnoli, S., Terzibasi Tozzini, E., Di Fraia, D., Romanov, N., Sudmant, P.H., Huber, W., Chondrogianni, N., Vendruscolo, M., Cellerino, A.#, and Ori, A.# (2020). Reduced proteasome activity in the aging brain results in ribosome stoichiometry loss and aggregation. Mol. Syst. Biol. 16, 1–22. Gebert, N., Cheng, C.W., Kirkpatrick, J.M., Di Fraia, D., Yun, J., Schädel, P., Pace, S., Garside, G.B., Werz, O., Rudolph, K.L., Jasper, H., Yilmaz, Ö.H., and Ori, A. (2020).

Region-Specific Proteome Changes of the Intestinal Epithelium during Aging and Dietary Restriction. Cell Reports. 31. Ori, A.*, Toyama, B.H.*, Harris, M.S., Bock, T., Iskar, M., Bork, P., Ingolia, N.T., Hetzer, M.W., and Beck, M. (2015). Integrated Transcriptome and Proteome Analyses Reveal Organ-Specific Proteome Deterioration in Old Rats. Cell Systems 1, 224–237

Milos Filipovic

Leibniz Institute for Analytical Sciences, ISAS, Berlin, Germany

Age-induced thiol oxidation predisposes brain for neurodegeneration via aberrant phase separation

Liquid-liquid phase separation (LLPS) has been shown to be a mechanism through which components of the cytoplasm can assemble into distinct compartments not delimited by a membrane. Although the links between LLPS and aging have been subject of numerous speculations, the actual evidence linking those two processes is lacking. One possible way aging could influence LPPS is through posttranslational modifications (PTM). We recently proposed that protein persulfidation, a cysteine PTM, could have protective and "anti-aging" effects. Using chemoproteomics we now addressed the changes of different cysteine PTMs in aging brain of mice and observed that cysteine oxidation to sulfenic acids linearly increases with aging, while protein persulfidation shows opposite trend. Among identified targets is the main inducer of stress granule formation, G3BP2, and we observed that cysteine oxidation is essential inducer of LLPS during stress. Cells lacking ability to produce H2S and therefore form persulfides uncontrollably form stress granules. Conversely, addition of H2S to cells results in "melting" of the stress granules. To our surprise, GAPDH, the central glycolytic enzyme, also showed propensity to LLPS and colocalization with G3BP2. We observed that once C245 of GAPDH is hyperoxidized, the protein enters phase separation more readily than when the cysteine is reduced. Persulfides of GAPDH did not enter phase separation at all. Hyperoxidation of GAPDH in brain samples of aged mice increased several-fold and was particularly found in aggregated fraction. Indeed, phase separated hyperoxidized GAPDH started aggregating under in vitro conditions. Finally, protein aggregates were observed throughout the whole brain sections of mice lacking main H2S producing enzyme, CSE, which correlated well with their memory decline. Taken together these data show that age-induced changes in cysteine oxidation status control LLPS forcing proteins to spend more time in condensates and to eventually aggregate leading to the perfect setting for aging-induced neurodegeneration.

Session 1. Protein homeostasis 2
Thursday, September 28

Malene Hansen

The Buck Institute, Novato, USA

Regulation of autophagy in aging and disease

The cytosolic recycling process of autophagy plays an important role in many age-related diseases and has been directly linked to aging, including in the nematode C. elegans where autophagy appears beneficially induced in many conserved longevity models. As a critical process to ensure cellular homeostasis, autophagy is regulated at multiple levels, yet it remains a challenge in the field to understand how the regulation of autophagy is integrated at the cellular and molecular level to ensure health- and lifespan benefits. I will here discuss our progress on understanding the different molecular mechanisms employed by cells and organisms to regulate canonical as well as non-canonical functions of autophagy in aging and disease. Job title: Chief Scientific Officer & Professor

Paola Gallardo

European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

Poor old Pores: Surveillance of intrinsically disordered nucleoporins

The Nuclear Pore Complex (NPC) is the macromolecular conduit embedded at the nuclear envelope, responsible for the selective trafficking of macromolecules between the nucleus and cytoplasm. It is composed of more than 500 proteins, called nucleoporins (Nups), which makes the NPC an exceptionally large structure. On the one hand, some Nups are extremely long-lived, which make them prone to accumulate damage during ageing. Age-related deterioration of NPCs is associated with imbalances in the stoichiometry of nucleoporins, reduced nucleoporin turnover, and diminished quality control systems. On the other hand, approximately one-third of all Nups are intrinsically disordered proteins (IDPs), which are particularly vulnerable during the ageing process. They have the ability phase separate from soluble into gel-like and amyloid-like particles, and mislocalisation and aggregation of certain nucleoporins has been described in several age-related and neurodegenerative diseases. I will present data demonstrating how chaperones directly protect the intrinsically disordered nucleoporins, preventing unfavorable phase transitions. This quality control mechanism plays a crucial role in NPC biogenesis, with significant implications for NPC function and maintenance throughout the ageing process.

Janine Kirstein

Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany

The role of chaperones in the phase-separation of Huntingtin

The aggregation of mutant Huntingtin (HTT) bearing an expanded polyQ stretch in Exon1 can be suppressed by a trimeric chaperone complex consisting of Hsc70, DNAJB1 and Apg2. The same chaperone complex is able to disaggregate pre-formed HTT fibrils. Thus, the chaperones interact with soluble HTT and with mature fibrils. It has recently been established that HTT can phase separate and undergo a liquid to solid phase transition with subsequent fibril formation. It was unknown however if and how the condensation process of HTT is regulated by the polyQ-flanking domains and by chaperones. We have now established the phase separation of HTTExon1Q23 in our lab and observed that the polyQ-flanking domains that control the aggregation of HTT also modulate the condensation of HTT. I will present data on how Hsc70, DNAJB1 and Apg2 can regulate the phase separation of HTT and HTT variants lacking the polyQ flanking domains.

Session 2. Metabolism
Thursday, September 28

Dudley Lamming

Univ. of Wisconsin-Madison, USA

When a calorie is not just a calorie: The regulation of health and longevity by dietary macronutrients

Dietary protein is a key regulator of metabolic health and aging. Low protein (LP) diets promote health and longevity in diverse species. Reducing dietary levels of the three branched-chain amino acids (BCAAs) leucine, isoleucine and valine recapitulates many of the benefits of a LP diet in young C57BL/6J mice. While usually considered as a group, we find that that each individual BCAA has unique metabolic effects, and that restriction of isoleucine is both sufficient to promote metabolic health and required for the metabolic benefits of an LP diet in C57BL/6J males. Further, isoleucine restriction (IleR) improves the metabolic health of both young and old HET3 mice, promoting leanness and glycemic control in both sexes, and reprograms hepatic metabolism, blunting age-related molecular changes in males and, to a lesser extent, in females. IleR reduces frailty and extends the lifespan of both male and female HET3 mice, but to a much greater degree in males. Our results demonstrate that restricting dietary isoleucine can increase healthspan and longevity in a genetically diverse population of mice and suggests that reducing dietary levels of isoleucine, or pharmaceuticals that mimic this effect, may have great potential as a geroprotective intervention.

Richardson NE, Konon EN, Schuster HS, Mitchell AT, Boyle C, Rodgers AC, Finke M, Haider LR, Yu D, Flores V, Pak HH, Ahmad S, Ahmed S, Radcliff A, Wu J, Williams EM, Abdi L, Sherman DS, Hacker T, Lamming DW. Lifelong restriction of dietary branched-chain amino acids has sex-specific benefits for frailty and lifespan in mice, Nature Aging. 1, 73–86 (2021). doi: 10.1038/s43587-020-00006-2. PMID: 33796866 PMCID: PMC8009080 Yu D*, Richardson NE*, Green CL, Spicer AB, Murphy ME, Flores V, Jang C, Kasza I, Nikodemova M, Wakai MH, Tomasiewicz JL, Yang SE, Miller BR, Pak HH, Brinkman JA, Rojas JM, Quinn WJ 3rd, Cheng EP, Konon EN, Haider LR, Finke M, Sonsalla M, Alexander CM, Rabinowitz JD, Baur JA, Malecki KC, Lamming DW. The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine, Cell Metabolism. 2021 May 4; 33(5):905-922.e6. doi: 10.1016/j.cmet.2021.03.025. Epub 2021 Apr 21. PMID: 33887198 PMCID: PMC8102360

Peter Tessarz

Max Planck Institute for Biology of Ageing, Cologne, Germany

Metabolism-epigenetics crosstalk in ageing

Cellular metabolism and chromatin architecture are heavily intertwined as many metabolites serve as essential cofactors and substrates for chromatin-modifying enzymes. Importantly, their availability can strongly affect the activity of enzymes catalyzing histone and DNA modifications. Many of these metabolites are generated in mitochondria and this establishes a tight mitochondrial–nuclear connection. Given the observed age-related mitochondrial dysfunction, it is not surprising that downstream epigenetic processes will be impacted as well. We recently showed how mild, but persistent mitochondrial stress impacts the export of acetyl-CoA from mitochondria, which subsequently is no longer available for histone acetylation and renders chromatin less plastic in ageing mesenchymal stem cells. Here, I will present new data on the role of the mito-nuclear axis during ageing and under stress conditions and will highlight how changes in tissue architecture upon ageing impact the complex interplay between mitochondrial function and the epigenome.

Sanne van der Rijt
Amsterdam UMC, The Netherlands

Targeting phospholipid metabolism via Pla2g15 results in reduced senescence in murine kidney and increases longevity in C. elegans

Aging was long considered an irreversible and passive process. However, genetic and pharmacological interventions have shown that aging can be actively regulated and influenced to reduce the burden of age-related diseases, such as chronic kidney disease. Dysregulated lipid metabolism plays a vital role in aging and age-related kidney diseases, however, the mechanisms are still not well understood. We previously found an accumulation of phospholipid bis(monoacylglycero) phosphates (BMP) in both aged mice kidney and human muscles, compared to young controls. BMP is a class of lipids primarily found in lysosomes and endosomes, synthesized from phospholipid PG, a reaction that is reported to involve Pla2g15. We tested whether targeting pla2g15 could ameliorate aging and features of age-related kidney disease.

We found that the expression of Pla2g15 is increased in human and murine aged mice and in doxorubicin-induced senescence in the kidney epithelial cells. Global deletion of Pla2g15 via CRISPR/Cas9 expression showed reduced Pla2g15 enzyme activity,and reduces senescent features such as decreased p21 expression and G2/M arrest.

To further study the role of Pla2g15 in aging, we turned to a model of C. elegans. The predicted worm orthologue of Pla2g15 is M05B5.4. RNAi of M05B5.4 results in lifespan extension and increased healthspan in worms. Transcriptional profiling identified upregulation of defense response, transmembrane transport and proteolysis. We found that lysosome regulating genes hlh-30, elt-3 and pmp-5 were required for longevity induced by M05B5.4 RNAi.

To conclude, Pla2g15 expression is increased in aged human and murine kidney. Deleting Pla2g15 ameliorates kidney epithelial cells senescence in vitro. We confirmed that the Pla2g15 ortholog M05B5.4 is an aging regulator. The lifespan extension of M05B5.4 is dependent on lysosomal functions, mediated by hlh-30, elt-3 and pmp-5. Altogether these data suggest that targeting phospholipid metabolism might be a potential therapeutic target for healthy aging.

Friedrich Becker
Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany

Dietary vitamin A restriction rescues declines in liver fat metabolism and ameliorates sarcopenia in aging mice

Aging associates with an impaired liver fat metabolism and an increased risk for fatty liver disease, such as non-alcoholic fatty liver disease (NAFLD). Loss of muscle maintenance and strength (sarcopenia) is a major health problem in the elderly. NAFLD associates with an increased risk of sarcopenia in an unexplained way. Changes in Vitamin A (VitA) metabolism occur in NAFLD but its functional role in aging and liver disease remains unknown. Here we show that a VitA fasting diet (VAFD) rescues aging-related declines in liver fat metabolism in laboratory mice. This metabolic rescue associates with the reversion of aging-associated decline of proteins that are known to be regulated by PPARa - a master regulator of lipid metabolism. Single cell ATACseq confirms a significant increase of chromatin accessibility at PPARa binding sites specifically in hepatocytes. Retinoic acid signaling, - a known competitor for RXR-mediated PPARα signalling is reduced in livers of VAFD treated mice. As expected, the study shows a mobilization of hepatic VitA storages in response to the VAFD, while systemic levels of VitA remain stable. In the ophthalmic retina - the most sensitive organ affected by VitA-deficiency - the VAFD does not lead to pathological declines but to an alleviation of age-related accumulation retinoids. Also, in skeletal muscle, retinoid levels and retinoic acid signaling remain unaffected by the VAFD. Despite the absence of systemic changes in retinoids, VAFD strongly reverts aging-related activation of protein catabolism in skeletal muscle associating with improved maintenance of overall mass and functionality.

Together, this study identifies aging-associated impairments in liver fat metabolism as a causal factor for sarcopenia. While the signalling components of this liver-muscle cross talk remain currently unknown, the study shows that VAFD rescues sarcopenia by direct, liver-specific effects on alleviating aging-associated impairments in fat metabolism.

Session 3. Gut microbiome
Thursday, September 28

Sasha Zhernakova

University Medical Center Groningen, The Netherlands

Biological aging markers in population cohorts - microbiome and more.

Aki Minoda

RIMLS, Radboud University Nijmegen, The Netherlands

Mu-kin Mouse Ageing Atlas: How the microbiota affect ageing

Old age is one of the biggest risk factors for many diseases, suggesting cellular ageing contributes to the development of a variety of diseases. How much the host microbiota plays a role in this aspect is currently unknown. To gain insights into this question, we have constructed the MU-KIN (germ-free in Japanese) Mouse Ageing Atlas. The atlas is composed of single cell 5' RNA-seq and single cell ATAC-seq data of ten different tissues from young and old mice that have microbiota (SPF; specific pathogen-free) as well as mice that are born and raised free of microbiota (germ-free (GF) mice). Our analysis enabled demarcation of age-associated changes that are microbiota-dependent and independent, showcasing that the MU-KIN Mouse Ageing Atlas will serve to be a great resource for the community of both ageing and microbiota research fields. Our results show that the microbiota plays a causative role in some of the age-associated changes, many of which are observed across multiple cell types in multiple tissues.

Dennis de Bakker
Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany

Microbiota transplantation can mitigate age-related brain inflammation and functional decline in a model of spontaneous Alzheimer's-like pathology

Abstract
Neurodegenerative diseases such as Alzheimer's disease are correlated with an imbalance of the microbial community of the gut. However, whether the gut microbiota plays a causal role in the etiology of neurodegenerative diseases remains to be elucidated. Here, we investigate whether microbiota transfer can mitigate age-related phenotypes of Alzheimers-like pathology. To address the aim of this study, we employ the turquoise killifish (Nothobranchius furzeri). Turquoise killifish are naturally short-lived vertebrates which spontaneously develop age-related changes in their gut microbiota which shares characteristics with those observed in Alzheimer's patients. In addition, turquoise killifish have been reported to spontaneously develop key phenotypes described in human neurodegenerative diseases, such as neuronal degeneration, protein aggregation, microgliosis, astrogliosis and learning capacity.

First, we successfully validated the spontaneous and age-related onset of neuronal degeneration, protein aggregation, microgliosis, astrogliosis and learning capacity. Furthermore, we investigated the accumulation of amyloid beta (Aβ), a key hallmark of Alzheimer's disease. We found that pyroglutamated-Aβ, a highly toxic and aggregation-prone form of Aβ, accumulated throughout the killifish brain which directly correlated with poor learning performance.

Heterochronic microbiome transplant in killifish extends lifespan. However, we have no insights as to whether it reduces organ-specific inflammation. Our hypothesis is that reducing brain-specific inflammation by targeting the gut microbiota could mitigate Alzheimer's-like pathology. To test our hypothesis, we transplanted gut microbiota from young to aged turquoise killifish and observed that the fish which received young microbiota display significantly less microgliosis and astrogliosis, indicating reduced inflammation. Furthermore, although no differences were detected in the number of actively degenerating neurons at 6 months of age, we did observe a mitigation of the age-related decline in learning performance. Together, these findings indicate that microbiota transplantation can reduce age-related brain inflammation and mitigates functional decline in a model of spontaneous Alzheimer's-like pathology.

Keynote Lecture
Thursday, September 28

Michael Hall

Biozentrum, University of Basel, Switzerland

mTOR signaling in growth and metabolism

TOR (target of rapamycin) is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, and cellular energy. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. TOR is found in two structurally and functionally distinct multiprotein complexes termed TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth. As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.

While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in disease and in coordinating and integrating overall body growth and metabolism in multicellular organisms. This will require elucidating the role of TOR signaling in individual tissues. Data on the role of mammalian TORC1 (mTORC1) and mTORC2 in controlling cellular processes and in specific tissues will be presented.

Session 4. Senescence
Friday, September 29

Marco Demaria

European Institute for the Biology of Ageing – University Medical Center Groningen, The Netherlands.

Purinergic signalling reinforces the production of SASP factors and promotes senescence-associated dysfunctions

Aging is characterized by chronic, low-grade, systemic inflammation (inflammaging), a significant risk factor for morbidity and mortality in older individuals. A significant contributor to inflammaging is the accumulation of senescent cells and their senescence-associated secretory phenotype (SASP), which is enriched in pro-inflammatory mediators[1]. Here, we demonstrate that senescent cells release significant amounts of ATP through connexin43 channels. Increased ATP enhances purinergic signalling in senescent cells, exacerbating the production and secretion of pro-inflammatory molecules in a calcium- and NF-kB-dependent mechanism. Strikingly, pharmacological inhibition of the purinergic receptor P2X1 reduces NF-kB activation and expression of pro-inflammatory factors in mouse models of chemotoxicity and osteoarthritis, eventually leading to improved physical performance and reduced inflammation. Taken together, we uncover a new mechanism regulating the inflammatory phenotype of senescent cells, and a potential new therapeutic target against inflammaging and age-related diseases (ARD).

Raffaella di Micco

I.R.C.C.S. Ospedale San Raffaele, Milano, Italy

Exploiting senescence immunogenicity for leukemia treatment

AML is traditionally treated by intensive chemotherapy. Still, early treatment failure is frequent. Therapy-induced senescence is a stress-triggered mechanism that may elicit immune-mediated responses. By combining transcriptional and cell-based evaluation of senescence markers in AML patient samples, we found upregulation of senescence-associated and interferon gene categories concomitantly with induction of HLA class I and class II, revealing a link between TIS and blasts immunogenicity. Consistently, senescent AML samples activated autologous CD4+ and CD8+ T cells, leading to improved recognition of leukemic blasts. We identified a novel epigenetic regulator of senescence-triggered immune-mediated responses and found that its inhibition recovers HLA expression and T cell-mediated recognition of non-senescent blasts. Lastly, the anti-leukemic activity of ICB was enhanced upon senescence, indicating state-dependent utilization of agents otherwise not seen effective in AML. Altogether, our results identify a potent senescence-based immune-related anti-leukemic mechanism, that may rapidly translate into innovative strategies to prevent AML relapse.

Gambacorta et al Cancer Discovery 2022 PMID: 35255120 Gilioli et al 2022 on Biorxiv Schiroli et al 2019 Cell Stem Cell PMID: 30905619 Biavasco et al. 2021 Nature Communications PMID: 34315896

Akiko Mammoto

Medical College of Wisconsin, USA

Endothelial senescence in hypoxia-induced lung vascular remodeling

Abstract

Pulmonary hypertension (PH) is a fatal pulmonary vascular disease characterized by a sustained elevation of pulmonary arterial (PA) pressure. Remodeling of distal PAs is a key feature of PH and involves accumulation of PA smooth muscle cells (PASMCs) to PAs, increasing PA pressure. Disrupted PA endothelial cell (EC) signaling stimulates PASMC proliferation and accumulation to distal PAs. Cellular senescence is characterized by irreversible cell cycle arrest and contributes to age-related pathologies, including chronic lung diseases associated with PH. The mechanistic role of EC senescence in pathogenesis of PH has not been fully understood. The Hippo pathway signaling transducer, Yes-associated protein (YAP1) stimulates angiogenesis and controls cell proliferation, survival and differentiation. YAP1 activity is upregulated in idiopathic pulmonary arterial hypertension (IPAH) patient-derived PAECs. Expression of a major senescence marker p16INK4A is higher in PAECs isolated from IPAH patients compared to healthy controls, as is platelet-derived growth factor (PDGFB), a known stimulator of PASMC proliferation and migration. p16INK4A knockdown decreases YAP1 expression, and loss of p16INK4A or YAP1 also decreases PDGFB in IPAH patient PAECs. Accumulation of alpha-smooth muscle actin (alpha-SMA)-positive cells to the PAs in a hypoxia-induced mouse PH model is attenuated in p16INK4afl/fl-Cdh5(PAC)-CreERT2 (p16INK4aiΔEC) mice, in which p16INK4a expression is knocked down in ECs after tamoxifen induction. These results suggest that increases in EC senescence mediate vascular remodeling in PH through endothelial YAP1-PDGFB signaling.

Boshi Wang

European Research Institute for the Biology of Ageing, University medical Center Groningen, UMCG

Sex dimorphism in senescent cell turnover is mediated by FABP5

Sex is still often considered as confounding variation rather than biological variation in aging studies, which is limiting our understandings of basic mechanisms in aging biology. Sex chromosome linked genes and sex-specific hormones are supposed to play crucial roles for sex dimorphism in many agingrelated phenotypes, but numerous data suggest that the influence of sex-associated genes/hormones is insufficient to fully explain the disparity, thus implying the involvement of other indirect cellular and molecular mechanisms. Senescent cells play crucial roles in aging and related dysfunctions and they are generally characterized by the upregulation of Cyclin-Dependent Kinase (CDK) inhibitor p16. Using this feature, our lab has developed the p16-3MR mouse model to selectively isolate, visualize and kill senescent cells. Using a chemotherapy-induced premature senescence/aging model and xenograft transplantation mouse models, we have shown that the temporal dynamics of senescent cell turnover were distinct between females and males. Our data clearly indicated that females have advantages in both cell intrinsic features and extrinsic microenvironment for the immune clearance of senescent cells. With a proteomics approach, we have identified a profile of differentially expressed proteins to distinguish senescent female and male mouse dermal fibroblasts (MDFs). Based on the fold change and p-value in the volcano plot, we picked the fatty acid binding protein FABP5 to further study its role in mediating the sex dimorphism in senescent cell turnover. Interestingly, knock-down (KD) of Fabp5 in male senescent MDFs made their immune clearance more efficient and these KD cells gained a gene expression profile that is more comparable to the female senescent cells. In summary, our study has clearly shown that there is sex dimorphism in the dynamics of senescent cell turnover, which provide biomarkers and insights of underlying mechanisms to decipher sex disparity in aging-related dysfunctions and diseases.

Session 5. Immune aging
Friday, September 29

Viswa Deep Dixit

Yale School of Medicine, New Haven, USA

Metabolic control of inflammaging

Mihai Netea

Radboud MC, Nijmegen, The Netherlands/ Limes University of Bonn, Germany

Impact of aging on trained immunity

The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Long-term reprogramming of innate immunity, that induces adaptive traits and has been termed trained immunity characterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfection in a T/B-cell-independent manner. In contrast, trained immunity has been shown to be able to induce protection against reinfection in a lymphocyte-independent manner. Non-specific protective effects dependent on trained immunity have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce trained immunity, through induction of histone modifications (methylation, acetylation) and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to a long-term epigenetic reprogramming of its function. Moreover aging impacts trained immunity and influences the responses to vaccination. The concept of trained immunity represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines in individuals of older age.

Enric Urena Sala

University College London, UK

Exploring the mechanisms of action of the life-extending drug trametinib in Drosophila

Pharmacological therapies are promising interventions to slow down ageing and reduce multimorbidity in the elderly. Studies in animal models are the first step towards translation of candidate molecules into human therapies, as they aim to elucidate the molecular pathways, cellular mechanisms and tissue pathologies involved in the anti-ageing effects. Trametinib, an allosteric inhibitor of MEK within the Ras/MAPK pathway and currently used as an anticancer treatment, emerged as a geroprotector candidate since it extended lifespan in the fruit fly Drosophila melanogaster. Our research shows a sexually dimorphic effect of trametinib on fly ageing, as it robustly extends lifespan in females, while it has weaker and inconsistent effects in males. We find that at least two tissues mediate the pro-longevity effect of trametinib in fly females, the gut and the fat body, the fly equivalent of adipose tissue and the liver. In the gut, trametinib increases autophagy and reduces intestinal stem cell proliferation, tumour formation, tissue dysplasia and barrier disruption, improving intestinal homeostasis at old ages. In the fat body, it reduces innate immune ageing, slowing down the chronic transcriptional up-regulation of antimicrobial peptides (AMPs) observed at old ages. Altogether, our findings advance the understanding of the anti-ageing properties of trametinib and pave the way for further studies in higher animals and in humans.

Patrick Schädel

Friedrich Schiller University Jena, Germany

Oxylipins as novel biomarkers of cellular and organismal inflammaging

Unresolved, chronic inflammation is a central hallmark of cellular and organismal aging driven by a complex network of immunomodulatory mediators. Among others, oxylipins derived from omega-3 and omega-6 fatty acids orchestrate the initiation, perpetuation, and resolution of inflammation. The biosynthesis of those mediators is based on lipoxygenase and cyclooxygenase activity of resident innate immune cells and their surrounding tissue. Balance between pro-inflammatory and pro-resolving oxylipins is crucial to maintain tissue homeostasis – an equilibrium majorly impacted by aging. To which extent oxylipin signaling drives inflammaging and impacts the immunological competence of the host is vastly understudied. Our mass spectrometry-based approach reveals a preserved niche-specificity and age-dependency in oxylipin dynamics in line with alterations to the inflammatory microenvironment alongside aberrant plasticity and functionality of tissue-resident macrophages (TRM). Mechanistically, aging establishes a niche-specific phenotype for TRM that affects their ability to initiate oxylipin biosynthesis after bacterial infection with pathogenic Escherichia coli. This is exacerbated by decreased plasticity to adapt distinct pro-inflammatory (M1) or pro-resolving (M2a) phenotypes and therefore limits their ability to perpetuate or resolve the oxylipin-driven inflammatory response. Even though macrophages constitute just one part of the innate immune cell population within the respective niches, we find striking similarities in the oxylipin profile of the niche and the corresponding TRM. Commonly, cyclooxygenase-derived prostanoids are depleted as consequence of aging, while 12/15-lipoxygenation of omega-3 and omega-6 fatty acids rises. Nevertheless, a unifying concept for age-associated alterations in oxylipin dynamics cannot be proposed, as niche-specificity persists during aging. To determine whether a pro-inflammatory or pro-resolving oxylipin signaling advances with age, an approach considering the respective inflammatory microenvironment is required. In conclusion, mass spectrometry-based oxylipin profiling is a useful tool to identify age-related phenotypes on both cellular and organismal level, while being applicable across well-established aging models ranging from killifish over mice to humans.

Session 6. Stem cells and regeneration
Friday, September 29

Maximina Yun

Center for Regenerative Therapies TU Dresden, Germany

Towards uncovering the basis of negligible senescence in vertebrates: enter the salamander

Negligible senescence, a remarkable phenomenon defined by lack of evident biological ageing, is found in a few exceptional vertebrates including salamanders such as axolotls and newts. Renowned for their exceptional abilities to regenerate organs and complex structures, salamanders also display additional noteworthy traits, namely extraordinary longevity, indefinite regenerative potential, lack of traditional signs of age-related decay and defiance of Gompertz's law of mortality. As such, they constitute valuable models for addressing the nature of organismal senescence and the interplay between regeneration and ageing. Here, I will present our lab's efforts towards understanding how salamanders regulate key hallmarks of ageing through regeneration and lifespan, and discuss the potential of salamander models to illuminate the nature of complex regeneration and the basis of negligible senescence.

Julia von Maltzahn

Brandenburg Technische Universität Cottbus-Senftenberg, Germany

Muscle stem cells in age and disease

Skeletal muscle has diverse functions in the organism and a remarkable ability to adapt to physiological demands such as growth, training and injury. Furthermore, it is one of the organs with the highest ability to regenerate, a process depending on muscle stem cells. During aging muscle stem cell numbers are reduced, but most importantly their functionality decreases resulting in impaired regeneration of skeletal muscle. The reduced regenerative capacity of skeletal muscle can be attributed to intrinsic changes in muscle stem cells, changes in their niche as well as systemic changes and changes in supporting cells.

Changes in different signaling in muscle stem cells, changes in systemic factors as well as changes in the extracellular matrix are examples for age-related changes affecting muscle stem cell functionality and thereby regeneration of skeletal muscle in the aged. However, inhibition of aberrantly active signaling pathways or replenishing missing systemically delivered factors or factors in the immediate muscle stem cell niche allow the improvement of regeneration in the aged.

Alberto Minetti
Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany

Proteostasis stress delays regeneration following injury in old small intestine epithelium

Aging has been associated with reduced regenerative capacity of intestinal epithelium in different species including humans. However, how aging influences the molecular dynamics of intestinal epithelium regeneration remains still unclear.

Here, we used proteome profiling and functional assays of intestinal tissues to characterize the dynamics of epithelium regeneration following injury induced by a single injection of 5-fluorouracyl (5-FU), an anti-metabolite drug causing intestinal mucositis.

We show that 5-FU induces a decrease of body weight, which is more pronounced and prolonged in old mice. Multiple histological parameters including crypts number and villi length confirmed age-related differences in intestinal regeneration. Next, we used quantitative mass spectrometry to analyze proteome changes in response to injury and found a precise temporal dynamic in the abundance of ribosomal proteins, reflecting the different phases of the regeneration process. Comparison of regeneration dynamics in mice of different ages revealed the emergence of proteotoxic stress signatures following injury exclusively in old epithelia. Mechanistically, we show that delayed regeneration is a cell-intrinsic feature of old epithelial cells that display reduced protein synthesis. We then established an organoid based platform that recapitulate the delayed regeneration observed in old mice, and we are planning to use this system to screen for interventions aimed to restore a proper regenerative capacity.

Altogether our data define different phases of the regeneration process in the small intestine and the emergence of a cell-intrinsic proteotoxic stress in old animals following the activation of protein synthesis during the recovery phase after injury.

Hugo Fernandes

MIA – University of Coimbra, Portugal

Unlocking the Regenerative Potential of Extracellular Vesicles: Bioactivity Enhancement through miRNA Modulation

Introduction: Extracellular vesicles (EVs) are secreted by virtually all cells and are key mediators of intercellular communication. EV cargo can be used as a fingerprint of the cell proteome, metabolome, and transcriptome. EVs can be used as therapeutic agents; however, in many cases, the bioactivity of native EVs is insufficient to elicit therapeutic effects. To overcome this, we have used bioengineering strategies to modulate the bioactivity of extracellular vesicles using pre-selected miRNAs.

Methods:
miRNAs: We screened a library of 2080 miRNAs and identified prosurvival miRNAs, i.e., miRNAs capable of enhancing the survival of endothelial cells (ECs) exposed to ischaemic conditions (growth factor depletion and 0.1% O2).

Modulation of EVs: prosurvival miRNAs were transfected into EVs using Exo-Fect™ and qPCR was used to confirm transfection. The internalization and intracellular trafficking of miRNA-modulated EVs were analyzed in cell types known to be involved in wound healing and neurogenesis. In vivo studies: we used two in vivo models to study the effect of miRNA-modulated EVs: wound healing on STZ-induced diabetic mice and neuronal regeneration of a 6-hydroxydopamine-lesioned striatum mouse model.

Results: after transfection of EVs, we showed a >30 000-fold increase in miRNA expression compared to native EVs. miRNA-modulated EVs were efficiently internalized by all the cells involved in skin wound healing and neurogenesis. Colocalization of miRNA-modulated EVs with the endo-lysosomal compartment differed from native EVs suggesting improved trafficking and escape. Topical administration of miRNA-modulated EVs into the wound of diabetic mice significantly accelerated the healing kinetics whereas intracerebro-ventricularly administered EVs protected dopaminergic neurons and fully restored motor behavior symptoms.

Conclusions: Our findings demonstrate that transfection of EVs with specific miRNAs can substantially enhance their bioactivity, thereby enhancing their regenerative potential.

Acknowledgements: This work was supported by the EU Project RESETAgeing (Ref:952266) and BIOMED (CENTRO-01-0145-FEDER-181228).

Session 7. DNA damage and genome regulation
Friday, September 29

George Garinis

University of Crete, Greece

DNA damage and Innate immunes responses during aging.

DNA damage and innate immune responses are intimately linked, leading to chronic inflammation and tissue degenerative changes as individuals age. Here, we will present evidence regarding the functional role of DNA damage signalling in immune activation, as well as the impact of DNA damage-driven chronic inflammation on health and disease.

Björn Schumacher

CECAD, University of Cologne, Germany

Genome Stability in aging and inheritance: new insights from C. elegans.

While the soma ages over the course of an individual's lifespan, germ cells indefinitely perpetuate the genetic information. The stability of germline genomes is prerequisite for the inheritance, germline immortality, and species preservation. We will here discuss new concepts of genome maintenance mechanisms in the germline. First, we focus on the regulation of the genome quality control in the female germline. We uncovered how somatic stress signaling impacts the apoptotic response during oogenesis and impacts the occurrence of aneuploidy. Our results established that somatic stress can impact genetic inheritance. Second, we investigate the genome quality control in the male germline. Here, we uncovered unexpected transgenerational consequences of DNA damage in paternal genomes. We show that DNA damage in mature sperm leads to structural variants that are generated by maternal Theta-mediated endjoining (TMEJ), which consequently results in genome instability in the progeny. The progeny is incapable to repair the damage due to heterochromatization leading to transgenerational embryonic lethality. Alleviation of the heterochromatization, in contrast, allows access for homologous recombination repair (HRR) thus resolving the genome instability and reversing the transgenerational lethality. We thus uncover a novel mechanism of the specific consequences of paternal DNA damage and the restrictive repair types fueling genome instability in the consequent generation.

Rouven Arnold

SBP Medical Discovery Institute, La Jolla, USA

Unraveling protective mechanisms of aging: A new role for histone chaperone HIRA

Influences on the integrity of chromatin affect aging, health, and disease during adulthood. The aging process is accompanied by a progressive alteration of the chromatin landscape, but how these changes affect the aging process is not well defined. We have previously proposed that chromatin homeostasis (chromostasis) is important to maintain chromatin integrity and hence cell identity and function during aging. However, the mechanisms underlying chromostasis are unknown. Replacements of histones by specific variants, such as H3.3, are among the epigenetic changes associated with aging. The histone chaperone HIRA deposits H3.3 into chromatin, mostly at actively transcribed gene bodies, enhancers, and sites of DNA damage repair. H3.3 accumulates with time in several post-mitotic organs and has profound consequences on cellular fate. We show that deposition of H3.3 is necessary for maintenance of tissue function during aging. Transcriptomic data suggest that targeted ablation of HIRA in hepatocytes leads to a loss of cell “identity”, impaired metabolism, and age-associated pathology, such as liver fibrosis. We suspect that incorporation of H3.3 by HIRA during aging is a protective mechanism contributing to chromostasis and hence cell identity, phenotype, and function. Interestingly, HIRA seems to particularly maintain the transcription of long and highly expressed genes. We propose that HIRA maintains the integrity and function of cells during aging by sustaining nucleosome turnover and histone modifications. A better understanding of the mechanism that links chromatin integrity to healthy adult aging and disease can yield new approaches to improve quality of life and prevent diseases in later life.

Mihailo Mirkovic
ETH, Zurich, Switzerland

Introns drive asymmetric chromosome inheritance in ageing

Accumulation of extrachromosomal DNA circles is a major cause of replicative aging in budding yeast but we know little about how DNA circles limit cellular longevity. Here we show that circle accumulation drives chromosomes to asymmetrically segregate to the bud as the mother cell undergoes its last division. Chromosomes mis-segregated with the old spindle pole body (SPB, functionally equivalent to the centrosome of animal cells), phenocopying the effect of Aurora B inactivation. Strikingly, this ageing phenotype correlated with the intron-dependent alteration of the expression of three regulators of Aurora B, Nbl1/Borealin, the kinetochore protein Mcm21/CENP-O and the phosphatase Glc7/PP1. Removing their unique intron from all three genes abrogated chromosome drive. Together our data establish that the remodelling of nuclear pore with age, as caused by circle accumulation, promotes aging phenotypes such as aneuploidy by relaxing the nuclear retention of pre-mRNAs.

Keynote Lecture
Friday, September 29

Linda Partridge

University College London, UK/ MPI, Cologne, Germany

Ageing: a gut feeling

Ageing is characterized by evolutionarily conserved hallmarks that are conserved in different organisms. Targeting these hallmarks genetically can therefore delay or prevent ageing-related impairment and disease in animals, with increasing evidence of their importance in human ageing. Dysregulation of the nutrient-sensing insulin/insulin/like growth factor/mTOR/Ras signaling network is one of these ageing hallmarks. Activity of this network is well tuned for youth, where it controls key processes such as cell growth and division, metabolism and reproduction, but later in life it drives the ageing process. Inhibiting its activity can therefore increase lifespan and numerous aspects of health during ageing in diverse laboratory model organisms. Reduced activity can also combat pathology in animal models of human age-related diseases. To translate these findings into health benefits for humans, drugs that target ageing hallmarks will be needed. The nutrient-sensing network plays key roles in age-related diseases, including cancer and metabolic disease. Many drugs have therefore already been developed to inhibit targets within it. These are potential candidates for repurposing for geroprotection. Based on genetic evidence, we have been focusing on drugs that inhibit different nodes in the signaling network, to understand the mechanisms by which they combat age-related pathology and to identify dosing-regimes that maximise the ratio of benefits to side-effects. This talk will focus on one of these drugs, rapamycin.

Session 8. Organismal aging
Saturday, September 30

Thomas Bosch

Christian Albrechts University of Kiel, Germany

Longevity, cellular senescence and the microbiome - lessons from the non-senescent model Hydra

Aging results from a complex interplay between genetic endowment and environmental exposures during lifetime. As our understanding of the aging process progresses, so does the need for experimental animal models that allow a mechanistic understanding of the genetic and environmental factors involved. One such well-studied animal model is the freshwater polyp Hydra. Hydra are remarkable because they are non-senescent. Much of this nonsenescence can be ascribed to a tissue consisting of stem cells with continuous self-renewal capacity. Another important fact is that Hydra's ectodermal epithelial surface is densely colonized by a stable multispecies bacterial community. The symbiotic partnership is driven by interactions among the microbiota and the host. Here, we review key advances over the last decade that are deepening our understanding of the genetic and environmental factors contributing to Hydra's non-senescent lifestyle. We conclude that the microbiome prevents pathobiont invasion (colonization resistance) and stabilizes the patterning mechanisms, and that microbiome malfunction negatively affects Hydra's continuous self-renewal capacity.

Johannes Krug
Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany

The transparent klara line as a tool for in vivo analyses

Body pigmentation is a limitation for in vivo imaging and thus for the performance of longitudinal studies in biomedicine. A possibility to circumvent this obstacle is the employment of pigmentation mutants, which have already been used in fish species like zebrafish and medaka. Recently, we generated a stable transparent line of the short-lived African turquoise killifish Nothobranchius furzeri, called klara. Via CRISPR/Cas9-mediated simultaneous inactivation of three genes (mitfa, ltk and csf1ra), a loss of body pigmentation was achieved.

Fish of the klara line were analyzed concerning general health and physiology as well as lifespan, showing no differences to wild type animals. Moreover, the lack of pigmentation did not affect the breeding behavior and egg quality. However, while breeding behavior was normal among klara fish, both wild-type and klara fish preferred pigmented mating partners in competitive breeding situations.

One of the hallmarks of aging is the accumulation of senescent cells, which are characterized among others by the expression of cdkn1a (p21). To elucidate the role of senescent cells, we generated an in vivo senescence reporter line by HDR-mediated integration of a fluorophore (GFP) into the cdkn1a locus of klara. Using light sheet microscopy, we performed in vivo imaging of living juvenile fish and were able to detect GFP-positive cells in an age-dependent manner. With this reporter line we aim to further characterize cellular senescence to elucidate the role of the respective cell population in aging and regeneration.

Socioeconomic aspects of health and lifespan extension therapies

Jochen Mierau
Lifelines, University of Groningen, The Netherlands

Keynote Lecture
Friday, September 30

James (Jim) Kirkland

Mayo Clinic, Rochester, USA

Clinical Studies of Agents Targeting Aging Mechanisms: The Translational Geroscience Network

The Hypothesis of the National Institutes of Health-funded Translational Geroscience Network is that clinical interventions targeting fundamental mechanisms of aging can delay, prevent, or treat multiple diseases and disabilities linked to fundamental aging mechaisms. The TGN provides advice/support for over 80 clinical trials of agents and lifestyle interventions targeting aging processes across the lifespan that are underway or about to begin, with more planned. These studies include testing interventions targeting aging processes (senolytics, senomorphics [including metformin, rapamycin], NAD precursors, anti-inflammatories, MitoQ, sodium nitrite, dietary and lifestyle interventions [including ketone ester, caloric restriction, exercise], and others) for such conditions as the accelerated aging-like state that can occur in survivors of childhood or adult cancers, Alzheimer's disease, mild cognitive impairment, acute complications of COVID-19, Longhauler and chronic HIV syndromes, frailty in the elderly, osteoarthritis, are-related osteoporosis, eye diseases, diabetes/ obesity, enhancing function of organs from older donors to allow use in transplantation, complications of space travel, and others as well as observational studies. The TGH is developing Gerodiagnostics (composites of "biomarkers" of fundamental aging processes for predicting or diagnosing conditions contributed to by these processes that can predict which intervention or combinations of interventions to use and when, monitor responses to these interventions that may eventually be translatable to clinical practice), discover further mechanisms that can be targeted to prevent or treat multiple disorders and diseases, and speed translation of interventions from bench to bedside.

Poster session

Thursday, September 28

Poster# 1

Abdullah Altulea
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Identifying CDKN1C/p57 as a unique marker for wound-associated cellular senescence in mice.

Cellular senescence is a state of a stable cell cycle arrest that cells undergo in response to various stressors, such as DNA damage. Senescent cells accumulate in the aging organism and contribute to various age-related diseases, such as skeletomuscular, cardiovascular, and neurodegenerative diseases. Research has demonstrated that the targeted removal of senescent cells alleviates the burden of these diseases and results in positive outcomes. At the molecular level, senescent cells display elevated expression of cell cycle inhibitors, such as p16 (CDKN2A) and p21 (CDKN1A), as well as diverse secreted molecules recognized as the senescence-associated secretory phenotype (SASP). Notably, our lab discovered that the removal of CDKN2A-positive cells leads to delayed wound healing in mice. Here, we present a novel observation of the presence of the cell cycle inhibitor p57 (CDKN1C) in damaged skin following wounding. Using single-cell RNA sequencing, we identified a unique, transient, wound-associated cluster of fibroblasts that express CDKN1C, as well an array of SASP factors throughout the wound healing period. The expression of CDKN1C is significantly increased in the transient cluster compared to the resident cluster on day 4 post injury and gradually declines by day 7. We observed that the protein expression of p57 follows the same pattern as the transcriptomic pattern, as confirmed by Western Blotting. Importantly, we found that aged mice have delayed onset of CDKN1C transcription at the transcriptome level, and generally lower levels of p57 at the protein level compared to younger mice.

We hypothesize that this delay could potentially underlie the slowed wound healing phenotype that is observed in aged mice. Furthermore, a subset of CDKN1C-positive cells was also found to be CDKN2A-positive, which might partially explain the delayed wound healing upon the selective removal of CDKN2A-positive cells. Together, these findings illuminate the role of other cell cycle inhibitors in cellular senescence and substantiate the potential advantageous role of cellular senescence in tissue remodeling and organismal development.

Poster# 2 Alessio Vagnoni
MIA Portugal

Age-specific and compartment-dependent changes in mitochondrial homeostasis and cytoplasmic viscosity in mouse peripheral neurons

“Ensuring precise distribution and functionality of mitochondria is essential for maintaining cellular homeostasis. The remarkably long processes of neurons means that these cells are particularly dependent on mechanisms for long-range cytoskeletal transport of mitochondria and on continuous mitochondrial functionality along an extended cellular architecture. This is a particularly critical challenge for neuronal cells that can live throughout the adult life of an organism. Combined evidence from Drosophila, mammalian and nematode neurons suggests that decline in the axonal transport of mitochondria during ageing plays a crucial role in reducing neuronal healthspan.

We have previously shown that axonal transport of mitochondria decreases in the peripheral nervous system of ageing Drosophila melanogaster (PMID: 26598558) and that neuronal ageing phenotypes can be manipulated by modulation of mitochondrial transport (PMID: 26598558; 29606421). However, a study focused on mitochondrial trafficking and mitochondrial functionality in young and old neurons within the same experimental system has been lacking. We have now expanded our Drosophila studies by using dorsal root ganglion neurons (DRGs) cultured from young and old mice, combined with work in the sciatic nerve of the ageing MitoMouse in vivo.

I will present data describing important aspects of mitochondrial biology in this experimental system, including mobility in the axons of neurons, mitochondrial membrane potential, calcium uptake and intra-mitochondrial viscosity. We describe age and compartment-dependent alterations of these properties and we expand on these data by quantifying intracellular viscosity and diffusiveness of the neuronal cytoplasm. Finally, we propose a model that correlates mitochondrial functionality and the viscoelastic properties of the neuronal cytoplasm during ageing"

Poster# 3

Andrea C. Postmus
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Failure to repair endogenous DNA damage in β-cells causes adult-onset diabetes in mice

Matthew J. Yousefzadeh1,2*, Ana P. Huerta Guevara3*, Andrea C. Postmus3, Rafael R. Flores1,2, Tokio Sano1, Angelika Jurdzinski3, Luise Angelini1,2, Sara J. McGowan1,2, Ryan D. O'Kelly1,2,Erin A. Wade1, Lisa V. Gonzalez-Espada1, Danielle Henessy-Wack1, Shannon Howard1, Tania A. Rozgaja1, Christy E. Trussoni4, Nicholas F. LaRusso4, Bart J.L. Eggen7, Johan W. Jonker3, Paul D. Robbins1,2, Laura J. Niedernhofer1,2 and Janine K. Kruit3

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*contributed equally

Objective: Age is a major risk factor for type 2 diabetes mellitus (T2DM), yet the underlying reason for this remains unclear. Age-related decline in tissue homeostasis is attributed to the accumulation of stochastic damage. Here, we asked if spontaneous, endogenous DNA damage in β-cells is sufficient to drive loss of β-cell function and consequently, diabetes.

Methods: ERCC1-XPF is an endonuclease required for multiple DNA repair pathways and reduced expression of ERCC1 causes accelerated accumulation of unrepaired endogenous DNA damage. We generated mice with an Ercc1 deletion in pancreatic β-cells and studied the effect on glucose homeostasis, β-cell function and the development of diabetes.

Results: Mice harboring Ercc1-deficient β-cells developed adult-onset diabetes as demonstrated by increased random and fasted blood glucose levels, impaired glucose tolerance, and reduced insulin secretion. The inability to repair endogenous DNA damage led to an increase in oxidative DNA damage and apoptosis in β-cells and a significant loss of β-cell mass. Using electron microscopy, we identified β-cells in clear distress characterized by an increased cell size, enlarged nuclear size, reduced number of mature insulin granules, and decreased number of mitochondria. Some β-cells were more affected than others consistent with the stochastic nature of spontaneous DNA damage. Ercc1-deficiency in β-cells also resulted in loss of β-cell function as glucose-stimulated insulin secretion and mitochondrial function were impaired in islets isolated from mice harboring Ercc1-deficient β-cells.

Conclusion: These data demonstrate that unrepaired endogenous DNA damage is sufficient to drive β-cell dysfunction and T2DM features and provide a mechanism by which age increases the risk of T2DM.

Poster# 4

Anna Ainslie
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Investigating kynurenine-pathway mediated neuroprotection in ageing

With a progressively ageing population, the need to understand mechanisms of neuroprotection is increasing. It is well established that depletion of the Kynurenine Pathway (KP) enzyme tryptophan 2,3-dioxygenase (TDO-2) rescues motility and lifespan in ageing worms and in multiple neurodegenerative disease models. However, our understanding of the precise mechanisms and tissue-specificity of neuroprotection by TDO-2 depletion is still limited. In our research, we use the C. elegans model to elucidate the dynamic local and systemic changes that are induced by TDO-2 depletion. We have optimized a novel technique (split-wrmScarlet) for tissue-specific visualization of TDO-2 and other KP enzymes, in order to analyze the precise tissue-localization and dynamics in ageing. Preliminary analysis suggests that TDO-2 is primarily expressed in the hypodermis throughout adulthood, an organ that transcriptomically mimics the human liver in C. elegans. Additionally, we have established a pipeline to perform tissue-specific depletion (using auxin-inducible degradation) of TDO-2 and other KP enzymes, and subsequently analyze dynamic changes in KP metabolite levels and motility over time. Overall, this data will help us understand the tissue-specific role of KP enzymes and metabolites in neuroprotection, and thus elucidate potential mechanisms to help fight neurodegeneration in ageing.

Poster# 5 Antonio Marino
The Leibniz Institute on Aging – FLI

Extensive remodeling of the protein ubiquitination landscape in the vertebrate aging brain

Many pathways are involved in maintaining proteostasis like the ubiquitin-proteasome system (UPS), autophagy, and chaperones collectively defined as the quality control system. These processes are regulated by protein posttranslational modifications (PTMs). However, it is unclear how aging affects the PTMs landscape and the functional consequences of such alterations. Here, we quantified proteome-wide changes of protein ubiquitination, acetylation, and phosphorylation in the aging brain of mice and the short-lived killifish Nothobranchius furzeri. We found that aging has the strongest impact on the ubiquitylome, with a conserved signature between species. Notably, 42% of protein ubiquitination changes could not be explained by changes in total protein abundance, suggesting altered stoichiometry of protein modification. We have also identified age-related alterations in the abundance of enzymes involved in the ubiquitin cycle that might be, at least in part, responsible for the observed changes in the ubiquitylome landscape.

Finally, we inhibited proteasome activity and lysosomal acidification in human iPSCs-derived neurons (iNeurons). Changes in ubiquitinations during aging could be partially recapitulated in proteasome-inhibited iNeurons. Remarkably, neurodegeneration-associated proteins were more susceptible to proteostasis impairment both in vivo and in vitro leading to increasing ubiquitination, showing that these proteins regulation is highly dependent on the UPS.

Poster# 6 Baixue Yu
Maastricht university

DPEP1 regulates age-related increases in diastolic blood pressure in humans and mice

"Aim: Our previous single cell sequencing data indicated that dipeptidase 1 (Dpep1) demarks an abundant adventitial fibroblast population. GWAS and mendelian randomization indicated an inverse correlation of DPEP1 with blood pressure in subjects from the UK biobank and the International Consortium for Blood Pressure. As hypertension is a consequence of vascular fibrosis and ageing, and DPEP1 marks profibrotic cells, we hypothesized DPEP1 knockout to amplify fibroblast density or activation, and increase blood pressure (BP) and age-related vascular stiffening.

Methods and results
In vivo blood pressure, and vascular stiffness (pulse wave velocity), and ex vivo myographic contractility/relaxation were measured in 3- and 18-month-old wildtype and whole body DPEP1-/- mice (n=15-20 per group). Diastolic blood pressure and adventitial (myo) fibroblast density were increased with age and in aged DPEP1-/- versus wildtype mice, while phenotypes of young mice were similar. Unexpectedly, vascular stiffness, collagen content, elastin breaks and local inflammation were unchanged by DPEP1-/-. However, ex vivo carotid arteries of aged DPEP1-/- (n=6-10) showed blunted contraction to U46619 (-36%) and relaxation to Acetylcholine and Nitroprusside of up to -62% (p=0.0047) and -46% (p=0.0006) respectively, explaining increased blood pressure. Short-term ex vivo DPEP1 inhibition with cilastatin did not affect myography in young or aged arteries, indicating chronic alterations of smooth muscle cell function. Indeed, medial expression of SMC contractility markers TAGLN and MYH11 was reduced by ~30% (p=0.02), without changes in SMC density or media thickness. In vitro, conditioned medium of primary fibroblasts derived from aged wildtype and DPEP1-/- aorta to wildtype aged aorta SMCs indeed reduced MYH11 mRNA expression.

Conclusion:
DPEP1 inversely correlates to diastolic blood pressure in human and mice, which may be partly explained by fibroblast-dependent changes in SMC phenotype and function. Future studies will reveal paracrine mediators of fibroblast-SMC communication regulated by DPEP1."

Poster# 7 Cagla Donmez
Leibniz Institute on Aging-Fritz Lipmann Institute

Elucidation of Biological Hematopoietic Stem Cell Aging on the Epigenomic Level

"The Hippo signalling pathway, which includes transcription co-activators YAP and TAZ, is a crucial regulator of organ growth and development, tissue homeostasis, tumourigenesis (Piccolo et al., 2014). Additionally, YAP and TAZ have been reported to control stem cell fate decisions, including self-renewal and differentiation (Moya & Halder, 2019). Previous studies have shown that epigenetic profiling of young and old hematopoietic stem cells (HSCs) has shown alterations in the levels of the histone modification H3K4me3 across self-renewal genes and discovered an increase in DNA methylation at genes associated with differentiation, resulting in impairments in differentiation during HSC aging (Sun et al., 2014).

In my study, I aim to elucidate the biological aging of hematopoietic stem cells (HSCs) at the epigenomic level. I am investigating how histone modifications at the chromatin level and methylation signatures at the DNA level affect aging in hematopoietic stem cells obtained using a mouse model. Recently, our group discovered that the Taz coactivator particularly induces the surface marker Clca3a1 gene in aged HSCs, which is absent in young HSCs (Kim et al., 2022). We can distinguish and isolate ""young-like"" HSCs from an aged mouse using this marker. With the help of this marker, I am isolating Clca3a1high/low HSC populations from aged mice to analyze global chromatin changes and changes in DNA methlylation signatures by using Clca3a1high/low HSCs. Taken together, my study enables me to determine the contribution of DNA methylation vs. chromatin modifications in "young-like" vs. old HSCs from the same mouse. Hereby, it will allow me to produce epigenomic profiles from HSCs, and contribute to a better understanding of biological aging, as opposed to chronological aging, on the molecular level.

My experiments are still ongoing, so my data analysis will be completed in the coming weeks."

Poster# 8 Chiara Giannuzzi
Scuola Normale Superiore

Epigenetic clock and methylation studies in the short-lived killifish Nothobranchius furzeri

"The concept of "biological age" was coined to define the relative fitness of an individual within an aging cohort. Therefore, to understand aging, a method of measuring "biological age" is needed.

Since epigenetic alterations are known to be a hallmark of aging, in the last years the methylation levels in specific CpG positions in the genome have been used to develop predictors of biological age, called 'epigenetic clocks'. Initially built to monitor human aging (Horvath, 2013), epigenetic clocks have since been used in several mammalian species and methylation of some key CpGs seems to be preserved in these. However, no epigenetic clock has yet been developed for the Nothobranchius furzeri, an interesting model for aging studies.

First, we generated a small dataset with 12 fish, 2 tissues (fin and brain) and 3 ages (corresponding to sexual maturity, young adults and old age) to study the N. furzeri methylome. Then we increased the number of samples (up to 70 fin biopsies) and time points (up to 17 different ages, spanning between 3 weeks and 44 weeks) to create our epigenetic clock, able to predict with high precision the chronological age. In a third step, we collected fin DNA at 10 and 20 weeks of age from 165 fish longitudinally and recorded the age of death for each, with the aim to build a lifespan predictor based on epigenetic age.

The method we have established can now be used as a biomarker to assess the effects of interventions on aging in killifish."

Poster# 9

Christine Müller
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Tweaking the C/EBPα isoform ratio to slow down ageing in the African turquoise killifish

Christine Müller1, Joscha S. Muck1, Gertrud Kortman1, Eugene Berezikov1 and Cornelis F. Calkhoven1

C/EBPα and -β transcription factors regulate an overlapping set of target genes involved in metabolism, innate immunity, cell proliferation and differentiation. The C/EBPα and -β mRNAs are both translated into long and short isoforms with opposing functions in target gene regulation. Expression of the short inhibitory-acting isoforms depends on a small upstream open reading frame (uORF) located in the 5'- untranslated region (5'UTR) of the CEBPA- and CEBPB-mRNAs and is stimulated by mTORC1 signaling. Mutation of the uORFs result in loss of inhibitory isoform expression and increased C/EBPα and -β transactivation function. We have shown earlier that mice with an increased C/EBPβ function resulting from uORF mutation (C/EBPβ super-mice) are metabolically healthier than their wild-type littermates and have an extended healthspan, reduced spontaneous cancer incidence, and show a female-specific lifespan extension. To examine whether increased C/EBPα function similarly results in increased health- and lifespan we choose the short-lived African turquoise killifish as a new model for studying the effects on ageing and lifespan determination. We generated fish with a C/EBPα uORF mutation (C/EBPα super-fish) using CRISPR/Cas9 genome editing and performed a lifespan experiment comparing uORF-mutant and wild-type fish derived from heterozygeous breeding pairs using separate cohorts for males and females. Survival curves show that male C/EBPα super-fish are longer-lived with a median lifespan extension of 8% and a maximum lifespan extension of 10%, while no lifespan extension was observed in females. Since the lifespan experiment with males is not fully completed these results are preliminary, still they indicate that C/EBPα might be a similarly promising anti-ageing target as C/EBPβ."

Poster# 10

Daniele Novarina
ERIBA (UMCG, University of Groningen)

Systematic investigation of disease-related short tandem DNA repeat instability

Daniele Novarina, Sandra Ollivaud, Liesbeth Veenhoff and Michael Chang
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Short tandem DNA repeats, consisting of stretches of repeating tracts of 2-12 bp DNA units, are intrinsically unstable due to the formation of non-B DNA structures, which can interfere with DNA replication and repair. To date, the expansion of 13 such repeats has been linked to over 50 neurodegenerative diseases in humans, including Huntington's disease, Friedrich ataxia, fragile X syndrome and several spinocerebellar ataxias. These diseases are collectively referred to as "repeat expansion diseases" (REDs). Importantly, longer repeats correlate with a more severe disease phenotype and an earlier age of onset. Furthermore, somatic and intergenerational instability of human short tandem DNA repeats increases with age. Even though the pathogenetic mechanisms might be different among these diseases, in each case the initial event is the expansion of the corresponding repeated sequence at the DNA level. Therefore, elucidating the mechanisms leading to DNA repeat instability and the cellular pathways counteracting it is essential for understanding (and ultimately curing) these diseases.

We take advantage of the Saccharomyces cerevisiae genetic model to systematically investigate the instability of the 13 RED-linked human short tandem repeats by inserting repeat tracts of varying length into the yeast genome. We measure their length- and orientation-dependent fragility with the gross chromosomal rearrangement (GCR) assay, and we assess their potential localization at the nuclear pore complex (NPC) during S-phase via live-cell microscopy, since CAG relocation to the NPC has been shown to be important for their stability. Furthermore, we perform genome-wide screens using the GCR assay and our recently-developed high-throughput replica-pinning technique to identify genes and pathways that affect the fragility of these sequences.

Poster# 11

Domenico Di Fraia
Leibniz-Institut für Alternsforschung - Fritz-Lipmann-Institut e.V. (FLI)

Post-transcriptional characterization of the vertebrate aging brain sheds light on the origin of protein-transcript decoupling

Proteostasis, the maintenance of protein homeostasis, is disrupted in both aging and neurodegenerative diseases, but how aging impairs proteostasis in the brain is not well understood. Here, we measured and integrated the effects of aging on the transcriptome, translatome and multiple layers of the proteome in the brain of short-lived killifish. We found that aging disrupts the relationship between transcripts and proteins leading to a decrease of basic proteins, including ribosomal and DNA/RNA-binding proteins. In contrast, abundant and long-lived proteins increase or remain stable despite a decrease in their mRNA levels. Chronic proteasome inhibition can induce some aging signatures in vivo, but it does not recapitulate the age-related decoupling between transcripts and proteins. Instead, we find that increased translation pausing reprograms the protein synthesis landscape independent of transcription. These changes in protein biogenesis likely reduce availability of key protein complexes and contribute to remodeling of organelles in older brains.

Poster# 12

Elizabeth C. Riquelme Barrientos
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Quality control of the Nuclear Pore Complex: tools to permeabilize and block pores

The nuclear pore complex (NPC) is the sole gateway to the nuclear interior, and its function is essential to all eukaryotic life. NPC malfunction occurs in ageing, both in dividing and non-dividing cells, and is emerging as a feature shared by several neurodegenerative diseases. Given the importance of the NPC and its vulnerability in ageing, it is important to understand how cells recognize and deal with malfunctional NPCs. We use Saccharomyces cerevisiae as a model to develop methods to inflict specific damage to mature NPCs in live cells and assess if cells can detect and distinguish permeabilized, obstructed and bona vide NPCs.

In a first essay we use 1,6-hexanediol, an alcohol that has previously been shown to be able to perturb the permeability barrier of NPCs1. We optimized the conditions and surveyed all aspects of cell physiology that we deemed relevant and could assess and find no changes that could explain the increase in permeability. This increase in passive permeability is also not related to dissociation or degradation of nucleoporins. Instead, multiple NTRs are displaced from NPCs. We then showed that when mature NPCs become more permeable by the treatment with 1,6-hexanediol, the recruitment of Chm7, a protein known to also detect misassembled NPCs, is triggered.

In the second strategy, we aimed to obstruct the central channel by inducibly anchoring a 40 nm sized nanoparticle adapted from2, in the similarly sized NPC central channel. We developed and optimized two tools to obstruct the pore. The first tool is anchored to the central channel of the NPC using the anchor away system. The second tool encodes nuclear localization signals where the particles are recruited to the central channel of the NPC by interacting with the nuclear transport factors Kap60 and Kap95. In both setups, the anchoring of GEMs in the NPCs is confirmed through biochemical and imaging-based methods. Interestingly, our data suggests that the cellular response to anchoring both types of particles is distinct. The assays developed will enable to further explore how cells respond to malfunctional NPCs.

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Poster# 13 Georgia Chatzinikolaou
IMBB-FORTH

R-loop-mediated CTCF DNA looping by XPF/TOP2B complex during transcription

Co-transcriptional RNA-DNA hybrids cause DNA damage threatening genome integrity but the mechanism remains unclear. Here, we show that the NER factor XPF interacts with TOP2B, the insulator binding protein CTCF and the Cohesin subunits SMC1A and SMC3 on active promoters to facilitate R-loop processing. We show that transcription stimulation leads to DSB accumulation, the activation of a DNA damage response and to the recruitment of XPF to active gene promoters. Upon UV irradiation, XPF is released from all regulatory and gene body elements. Abrogation of TOP2B or inhibition of TOP2-mediated DNA cleavage leads to the diminished recruitment of XPF, CTCF and the Cohesin subunits to promoters of actively transcribed genes and R-loops and the concurrent impairment of CTCF-mediated DNA looping. Taken together, our findings disclose an essential role for XPF with TOP2B and the CTCF/Cohesin complex in R-loop processing with important ramifications for DNA repair-deficient syndromes associated with transcription-associated DNA damage.

Poster# 14 Joanne Heida
DIfE

Analysis of age-related metabolic changes in thermogenic brown adipocytes

“Brown adipose tissue (BAT) is responsible for energy expenditure in a process known as thermogenesis, where energy is used for maintenance of body temperature upon cold-induced, adrenergic stimulation. In the process, triglycerides and glucose are cleared from the circulation, enhancing glucose tolerance and insulin resistance. Age-related metabolic changes cause a decrease in BAT activity, resulting in a deterioration of thermogenic efficiency, excessive accumulation of white adipose tissue and insulin insensitivity. The regulatory mechanisms mediating the age-related loss of function of BAT are not fully understood.

In transcriptomic and proteomic analyses comparing brown and white adipose tissue of young and aged mice, the enzyme L-asparaginase, encoded by the gene Aspg, was identified as a potential biomarker of aging in BAT. Specifically, the protein was found to be downregulated in aged BAT and induced by cold exposure.

In order to assess the tissue-specific role of Aspg in age-related loss of metabolic flexibility, mouse models with conditional Aspg-inactivation specifically in brown adipocytes, or in brown and white adipocytes combined were generated using the Cre-loxP system. Mice with BAT-specific Aspg-inactivation were cold intolerant upon acute cold exposure and showed a reduction in plasma glycerol and free fatty acids. Histological analysis of the white adipose tissue revealed larger white adipocyte size in the knock-out animals. In addition, indirect calorimetry data showed a shift from carbohydrate oxidation towards fat oxidation. These results indicate an impaired lipid mobilization in mice with BAT-specific Aspg-inactivation, which mirrors the impact of aging on BAT. A similar effect was seen in mice with Aspg-inactivation in all adipose tissue depots.

In summary, our results show impaired lipid mobilization and cold intolerance in both mouse models of Aspg-inactivation, suggesting that the loss of Aspg in brown and white adipocytes impairs metabolic flexibility in mice.”

Poster# 15 Kalliopi Stratigi
IMBB, FORTH

Transcription stress-associated telomere dysfunction and cellular senescence

"Gene regulation is inextricably linked to proper cell function and proliferation, tissue development and organismal health. However, the genome is continuously challenged by damaging agents that interfere with the process of mRNA synthesis. Although most endogenous lesions can be bypassed by the transcription machinery, bulkier or helix-distorting lesions obstruct the progression of RNA Polymerase II causing transient pauses or more detrimental arrests. As a result of such persistent lesions, transcription stress leads to cellular malfunction and ultimately to premature cell death or senescence, resulting in DNA damage-induced, accelerated aging and disease. Transcription elongation factor S-II (TFIIS), also known as TCEA, plays a key role in stimulating RNAPII to bypass sites of oxidative damage or resume transcription following elongation arrest. Whereas correlations between transcription stress and genome instability have been established, the mechanisms underlying their impact on cell homeostasis and function remain poorly understood.

To elucidate such mechanisms, we used a Tcea1fl/fl ;CMV.Cre mouse model to isolate Tcea1-/- Mouse Embryonic Fibroblasts (MEFs) and found that complete ablation of TFIIS results in intrinsic transcription stress, as evidenced by impaired transcription, RNAPII stalling and R-loop formation. We also show that the TFIIS defect leads to telomere crisis and cellular senescence. We further propose to investigate how a transcription defect can lead to this telomere-associated phenotype, describing the exact mechanism by which these events are happening and if there is a physiological connection to the telomere attrition we observe during ageing."

Poster# 16 Liesbeth Veenhoff
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Poor old Pores: Surveillance of the intrinsically disordered FG-nucleoporins in ageing

Nuclear pore complexes (NPCs) are the sole gateways to the interior of the nucleus and their function is essential to all eukaryotic life. The NPC's function is intimately connected to the primary hallmarks of ageing of protein homeostasis and genome stability, and several processes underlying these hallmarks are orchestrated at NPCs. As aging progresses, the NPC's performance becomes compromised, motivating our objective to elucidate the mechanisms responsible for maintaining NPC quality control. In this talk I will discuss how the intrinsically disordered proteins of the NPC are surveilled and how this fails in ageing.

Poster# 17 Mareike Wichmann-Costaganna
Friedrich Schiller University Jena, Dept. of Pharm./Med. Chemistry

Metabolic modulation of lipid mediator-driven inflammaging via exercise and caloric restriction

"Lipid mediators (LM), a class of endogenous signaling molecules that are derived from polyunsaturated omega-6 and omega-3 fatty acids, are key regulators of complex inflammatory processes. Aging is associated with a dysregulated LM synthesis, resulting in a reduced ability to resolve inflammation and restore tissue homeostasis. This ultimately leads to a chronic, inflamed state, coined inflammaging. Nutritional and exercise interventions have been used as a promising strategy to reduce age-associated phenotypes and improve both life- and healthspan. However, the effect of these interventions on the metabololipidome is barely described in literature and warrants further research.

Here, we show the implications of two major life- and healthspan-extending strategies - caloric restriction (CR) and exercise - onto the inflammatory LM signatures in healthy, aged (18-20 months) C57BL/6 mice and evaluate their rejuvenating potential.

Firstly, we investigated how four weeks of CR by 30% alter the hepatic metabololipidome and discovered a shift from bioactive LM to their corresponding monohydroxylated precursors. Surprisingly, a 2-day period of ad libitum re-feeding further aggravated the depletion of the metabololipidome. Both interventions failed to re-establish a LM profile as found in young mice (3 months). Secondly, we have screened eight organs from group-housed mice following 8 weeks of voluntary wheel running (VWR) against the ones of aged, sedentary mice. Each mouse was implanted with a transponder which allows to correlate their individual running performance with the levels of the evaluated LM. In addition to screening whole organ lysates, we analyzed the metabololipidome within serum as well as resident peritoneal macrophages in response to an ex vivo infection with pathogenic Staphylococcus aureus. In all test systems we discovered that VWR leads to an overall reduction of pro- and anti-inflammatory LM.

These findings offer new insights into the lifestyle-driven modulation of the metabololipidome in the elderly and their rejuvenating potential."

Poster# 18 Marian Breuer
Maastricht University

Computational metabolic network modeling in aging and chronic diseases

“Many age-related and chronic diseases, as well as aging processes overall, involve changes in metabolism on a cellular level. The complexity of cellular metabolism calls for a systems approach to study these changes. In particular, while omics technologies provide extensive data on the state of a cell, interpretation is enhanced by combining the vast data with knowledge-based mechanistic computational modeling methods.

Here we show applications at the Maastricht Centre for Systems Biology (MaCSBio) to study the role of cellular metabolism in chronic diseases and aging processes through cell-scale metabolic network modeling. On the one hand, samples can be analyzed by mapping gene expression data onto metabolic networks to calculate sample-specific metabolic task/functionality scores. On the other hand, condition-specific metabolic network models can be constructed from omics data, and analyzed for their functional states beyond the structure of the network.

In this way, computational modeling of cellular metabolic networks has the potential to complement experimental research into the role of metabolism in aging and chronic diseases.”

Poster# 19

Paola Gallardo
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Poor old Pores: Surveillance of intrinsically disordered nucleoporins

E. F. Elsiena Kuiper1,4, Paola Gallardo 2,7, Tessa Bergsma 2,7, Muriel Mari 1,5,7, Maiara Kolbe Musskopf 1, Jeroen Kuipers 1,3, Ben N. G. Giepmans 1, Anton Steen 2, Harm H. Kampinga 1, Liesbeth M. Veenhoff 2 and Steven Bergink 1,6.

The Nuclear Pore Complex (NPC) is the macromolecular conduit embedded at the nuclear envelope, responsible for the selective trafficking of macromolecules between the nucleus and cytoplasm. It is composed of more than 500 proteins, called nucleoporins (Nups), which makes the NPC an exceptionally large structure. On the one hand, some Nups are extremely long-lived, which make them prone to accumulate damage during ageing. Age-related deterioration of NPCs is associated with imbalances in the stoichiometry of nucleoporins, reduced nucleoporin turnover, and diminished quality control systems. On the other hand, approximately one-third of all Nups are intrinsically disordered proteins (IDPs), which are particularly vulnerable during the ageing process. They have the ability phase separate from soluble into gel-like and amyloid-like particles, and mislocalisation and aggregation of certain nucleoporins has been described in several age-related and neurodegenerative diseases. I will present data demonstrating how chaperones directly protect the intrinsically disordered nucleoporins, preventing unfavorable phase transitions. This quality control mechanism plays a crucial role in NPC biogenesis, with significant implications for NPC function and maintenance throughout the ageing process.

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Poster# 20

Sara Mouton
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

A physicochemical perspective on cellular ageing

Cellular ageing is a multifactorial process that is characterized by a decline in homeostatic capacity, best described at the molecular level. Physicochemical properties such as pH and macromolecular crowding are essential to all molecular processes in cells and require maintenance. Whether a drift in physicochemical properties contributes to the overall decline of homeostasis in ageing is not known. Here, we present a framework of the physicochemical parameters of replicatively aged yeast cells where we combine the use of ageing-tailored fluorescent biosensors and microfluidics.

Poster# 21

Stijn Mouton
European Research Institute for the Biology of Aging (ERIBA), University Medical Center Groningen, the Netherlands

Slowing down ageing: from immortal planarians to short-lived yeast

"Animals age at different rates. Some extreme cases, such as asexual planarians, even seem to be able to avoid ageing. This is possible thanks to a high regenerative capacity, high cellular turnover, and a very active population of stem cells, called neoblasts. There are indications that especially these neoblast evolved efficient and/or novel molecular mechanisms to counteract primary causes of ageing.

We will present a cross-species approach to identify planarian anti-ageing genes by testing their potential to improve stress-resistance and slow down ageing in yeast. In these studies, we focus on non-conserved genes which encode molecular functions which are normally not present in yeast, and can have an added beneficial impact on yeast health and ageing. Currently, we are focusing on developing assays to screen for planarian genes which can improve proteostasis and limit protein aggregation, a known cause of ageing and multiple diseases. The long-term aim of this project is to characterize novel molecular mechanisms which can be used to improve healthy ageing in diverse organisms."

Poster# 22 Vincent Gyande Kangah
University Medical Center Groningen

Fisetin does not alleviate cholangiopathy in Cyp2c70-deficient mice with a hydrophobic bile acid pool despite modulation of cellular senescence

“Cyp2c70-deficient mice have a hydrophobic bile acid (BA) pool and display cholangiopathic features. In livers of patients with cholangiopathies, cellular senescence has been observed and has been suggested to contribute to disease development. Therefore, the role of cellular senescence in the cholangiopathy in cyp2c70-deficient mice was examined and the impact of senolytic treatment was assessed.

Fisetin, a natural senolytic, was given to female cyp2c70-deficient mice and their wild type littermates once a week for 8 weeks by oral gavage. Hepatic transcriptome analysis was performed to evaluate gene expression patterns associated with cellular senescence. In addition, BA concentration and composition as well as liver pathophysiology, including ductular reactions, inflammation and fibrosis were assessed.

Gene set enrichment analysis (GSEA) revealed the up-regulation of senescence-associated gene expression patterns in livers of female cyp2c70-deficient mice. Ursodeoxycholic acid (UDCA), a hydrophilic BA, that has been reported to restore cholangiopathy in female cyp2c70-deficient mice, normalized these gene expression patterns in the livers, supporting a potential role for BA-induced senescence in cholangiopathy in female cyp2c70-deficient mice. Fisetin treatment had limited effects on senescence gene sets and senescence-associated secretory phenotype (SASP) genes. However, despite down-regulation of the pro-fibrogenic marker Tgfβ1 and the hepatic stellate cell activation marker αSMA, fibrosis was not significantly impacted by fisetin treatment. Furthermore, fisetin did not alleviate the ductular reactions despite reducing proliferation-associated gene expression patterns as revealed by GSEA. Finally, BA levels and composition remained unaffected by fisetin treatment.

BA-induced senescence indeed appears to play a role in the development of cholangiopathy in mice with a hydrophobic, human-like BA profile. Fisetin reduces the expression of SASP factors and of genes involved in proliferation-associated pathways. However, fisetin does not considerably ameliorate cholangiopathy in female cyp2c70-deficient mice."

Poster# 23

Wenming Huang
Max Planck Institute for Biology of Ageing

Decreased spliceosome fidelity inhibits mTOR signalling and promotes longevity

Wenming Huang1, Chun Kew1, Stephanie A. Fernandes1, Anna Loerhke1, Lynn Han1, Constantinos Demetriades1,2, Adam Antebi1,2*

Changes in splicing fidelity are associated with loss of homeostasis and ageing, yet only a handful of splicing factors have been shown to be causally required to promote longevity, and the underlying mechanisms and downstream targets in these paradigms remain elusive. Surprisingly, we found a hypomorphic mutation within ribonucleoprotein RNP-6/poly(U)-binding factor 60 kDa (PUF60), a spliceosome component promoting weak 3' splice site recognition, which causes aberrant splicing, elevates stress responses, and enhances longevity in Caenorhabditis elegans. Through genetic suppressor screens, we identify a gain-of-function mutation within rbm-39, an RNP-6 interacting splicing factor, which increases nuclear speckle formation, alleviates splicing defects and curtails longevity caused by rnp-6 mutation. By leveraging the splicing changes induced by RNP-6/RBM-39 activities, we uncover intron retention in egl-8/phospholipase C B4 (PLCB4) as a key splicing target prolonging life. Genetic and biochemical evidence show that neuronal RNP-6/EGL-8 downregulate mTORC1 signaling to control organismal lifespan. In mammalian cells, PUF60 downregulation also potently and specifically inhibits mTORC1 signaling. Altogether, our results reveal that splicing fidelity modulates lifespan through mTOR signaling.

Poster# 24 Yuliia Haluza
TU Dresden – CRTD

The Axolotl Epigenetic Clock Offers Insights Into the Nature of Negligible Senescence

Salamanders such as axolotls have extraordinary regenerative capacities, including the ability to regenerate complex structures such as limbs and spinal cord throughout the lifespan. Furthermore, they exhibit strong resistance to age-related diseases, defiance of the Gompertz law of mortality and notably long lifespans, constituting species of negligible senescence. To date, little is known about the molecular changes that accompany salamander ageing. Recently, it has emerged that the methylation level of particular genomic CpG sites is predictably altered with ageing. By profiling these changes, it is possible to computationally build 'epigenetic clocks', able to predict chronological ageing with impressive accuracy across vertebrate species, including amphibians. Here, we present the first pan-tissue DNA methylation (DNAm) clock for the axolotl. Both at specific or pan-tissue levels, the clock is characterised by a bimodal nature, accurately predicting age in animals up to 4,5 years old, while not capturing evident DNAm changes for the rest of its lifespan. The trajectory of DNAm age deceleration beyond animal maturation suggests the axolotl deviates from the conventional notion of epigenetic ageing. This study offers a first glimpse into the epigenetic profile across axolotl lifespan, supporting the idea that axolotls exhibit negligible senescence. Further exploration of the unique epigenetic traits in this species could offer valuable insights into the interplay between ageing and regeneration.

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