European Research Institute for the Biology of Ageing

ERIBA
Annual Report
2020

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Annual Report 2020

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Foreword
by the Director

It is a great pleasure to present to you the 2020 Annual Report of the European Research Institute for the Biology of Ageing. This report provides you with an overview of all our activities and achievements, in science, education, business development and outreach. We value all these domains equally, and are proud to share with you all that has been accomplished in 2020.

Last year I ended this foreword with the following lines: "*The upcoming year, 2020, will be an odd year, which all of us will remember vividly. It is uncertain how much longer Covid-19 will affect the research and education agenda. However, whatever the future will bring, our mission and ambition are more viable and relevant than ever before*."

Although these words were written in the early days of the pandemic, they happened to very well forecast what lay ahead of us. The entire year of 2020, and in fact also most of 2021, we have been facing the consequences of a virus that has affected all of us in one way or another. Fortunately, in ERIBA only very few of our people became infected, and the precautions that we undertook to work safely turned out to be effective. As everywhere in the academic world, we worked in shifts and complicated schemes, which allowed us to continue our research as much as possible. For those of who did not need to be in the lab, working from home was enforced.

While the conditions of our research and educational efforts were severely affected, we can also proudly state that we managed to keep teaching efforts at a high quality, that we kept accepting intern students, that many research papers were published, and that grants were successfully obtained. You will find an overview of all these achievements in this Annual report.

This report will be the last one that I oversee. After 23 years in the UMCG, of which 10 years were committed to establish and lead ERIBA, I will step down as Director to become Director of Research at Sanquin in Amsterdam. My move comes with some sadness, but most importantly with great satisfaction. Together with many colleagues (who became friends), an exceptional management team led by Henk Heidekamp, innumerable PhD students, postdocs and technicians, it is fair to say that ERIBA in 2021 is where we had hoped it to be when we started in 2010. ERIBA is a great academic Institute, well-known in the national and international field of aging research. We have recruit excellent group leaders from anywhere in the world. We have recruited excellent PhD students and postdocs, who, when graduated from ERIBA, easily find new positions in prestigious academic or industrial environments. We are collaborating with a rapidly increasing number of biotech companies.

It has been truly a pleasure to be part of the ERIBA founding team, and I wish the team and my future successor all the best. I leave you with the same words that I wrote for the 2020 Annual Report: It is uncertain how much longer Covid-19 will affect the research and education agenda. However, whatever the future will bring, our mission and ambition are more viable and relevant than ever before.

Gerald de Haan
Scientific Director
March 2021

Our mission and ambition are more viable and relevant than ever before

Ageing Research at ERIBA

ERIBA is an internationally recognized research institute. The institute is located in the picturesque city of Groningen, The Netherlands. Since its inception, the institute has grown and established itself as one of the leading institutes, focusing on fundamental biology to understand the causes of ageing. The core research focuses on the mechanisms that result in the loss of cells with age, and decline in cellular functioning.

Research and discoveries at ERIBA aims to address ageing phenomena such as loss of protein homeostasis, metabolic decline, stem cell loss-of-function, genome instability. These phenomena are at the root of age-related frailty, and have been implicated in various age-related diseases such as cancer, neurodegeneration, and diabetes.

ERIBA aims to develop new strategies to prevent or combat age-related diseases with the goal of increasing the healthy life span of individuals. The approach is to discover new drug targets, specialized disease models, and assays for the purpose of adding more healthy years to human life.

Mitochondria - microbiology

ERIBA hosts various research groups that cover a wide range of age-related research.

Stem Cell Regulation and Mechanisms of Regeneration

Eugene Berezikov

Resilience is the capacity of a complex system to recover from perturbations. In essence, ageing and age-related diseases are manifestations of failing resilience of a living organism in the face of various intrinsic and extrinsic stresses. Some animal species evolved better resilience mechanisms than others, and investigation of these mechanisms will broaden our understanding of the underlying fundamental biology, and can eventually contribute to the development of novel therapies in human.

Towards this, we study the model organism *Macrostomum lignano* - a flatworm that can regenerate its body, is long-lived and highly resistant to various stresses, including ionizing and UV radiation. To translate our findings in flatworms to other model organisms, we also utilize the nematode C. elegans and one of the shortest living vertebrate models, the killifish *Nothobranchius furzerii*.

Eugene Berezikov
Stem cells and resilience mechanisms

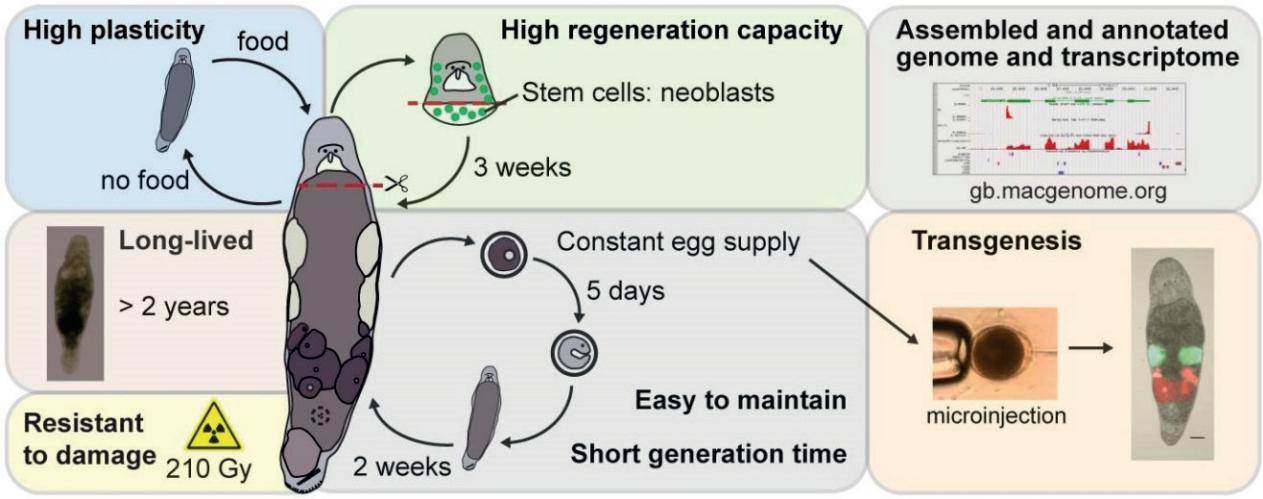


Figure. The flatworm **Macrostomum lignano** is a versatile model organism to study stem cells, regeneration, ageing and resilience mechanisms. Adapted from Wudarski et al., EvoDevo 11:5 (2020).

RESEARCH FOCUS

The flatworm *Macrostomum lignano* has an impressively advanced resilience, far beyond other animals (Fig. 1). Besides regeneration, it can also de-grow in the absence of food and survive long periods of starvation, and grow back when food becomes available again. It can live several years, and its mortality hazard does not increase with age. It sustains very high doses of ionizing radiation (120 Gy), as well as sterilization-level doses of ultraviolet C (100 mJ/cm2). We think that all these remarkable resilience properties of *M. lignano* are conferred primarily at the level of the stem cells (neoblasts), because as long as the neoblasts are functional, the damaged cells can be continuously replaced.

In order to start understanding the remarkable biology of this animal, in recent years we focused on developing a genetic toolbox for *M. lignano*. We identified stem cell and germline transcriptional signatures[1], sequenced, assembled and annotated the genome[2], and establishing a robust transgenesis method[2]. Importantly, *M. lignano* is the only flatworm species in which transgenesis is available, and it allowed us to generate the first-ever stem-cell-specific *M. lignano* transgenic lines, which opens up tremendous research opportunities. Furthermore, we demonstrated that for its size the animal is remarkably long-lived (more than 2 years), and appears resilient to aging via active regulation of the stem cells[3]. To translate these findings from flatworms into vertebrates, we initiated and coordinated the establishment of the killifish (*Nothobranchius furzeri*) facility in the UMCG. We are currently the only facility in the Netherlands where research on this remarkable short-lived vertebrate model organism is possible.

THE FUTURE

We envision three interconnected research directions in the future:

1. **Mechanisms of regeneration in *M. lignano***
   Regeneration is an efficient organismal resilience strategy to injury but understanding its mechanisms is still incomplete. Using the power of transgenesis in *M. lignano*, combined with single cell sequencing, we plan to characterize regulatory programs that drive cell fate specification during regeneration.

2. **Mechanisms of DNA damage control in *M. lignano***
   Damage to DNA is a major factor of ageing and cancer. Hence, preventing and repairing DNA damage is an important resilience strategy. We will investigate how *M. lignano* survives high doses of gamma- and UVC radiation by combining transgenesis, genomics and proteomics approaches.

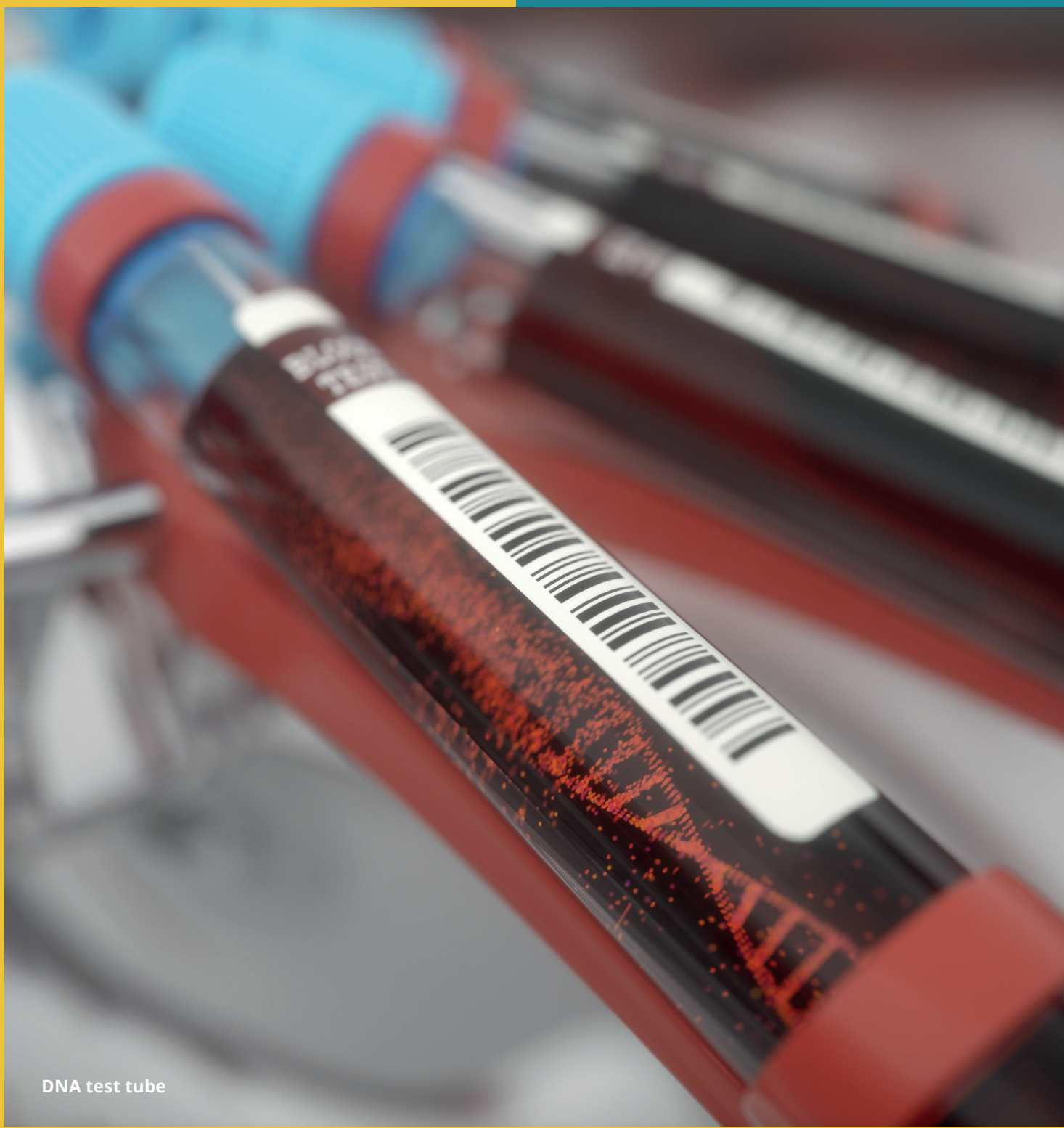
3. **Engineering healthspan extension**
   We propose that *M. lignano* is a rich source of genetic information for molecular engineering of healthspan extension in other animals[3]. We will test this hypothesis to identify pro-longevity genes using *C. elegans*, killifish and mouse models.

TOP 3 PUBLICATIONS

1. Grudniewska, M., Mouton, S., Simanov, D., Beltman, F., Grelling, M., de Mulder, K., Arindrarto, W., Weissert, P.M., van der Elst, S., **Berezikov, E.** (2016). Transcriptional signatures of somatic neoblasts and germline cells in *Macrostomum lignano*. *eLife* 5:e20607.

2. Wudarski, J., Simanov, D., Ustyantsev, K., de Mulder, K., Grelling, M., Grudniewska, M., Beltman, F., Glazenburg, L., Demircan, T., Wunderer, J., Qi, W., Vizoso, D.B., Weissert, P.M., Olivieri, D., Mouton, S., Guryev, V., Aboobaker, A., Schärer, L., Ladurner, P., **Berezikov E.** (2017). Efficient transgenesis and annotated genome sequence of the regenerative flatworm model *Macrostomum lignano*. *Nat. Commun.* 8:2120.

3. Mouton, S., Grudniewska, M., Glazenburg, L., Guryev, V., **Berezikov, E.** (2018). Resilience to aging in the regeneration-capable flatworm *Macrostomum lignano*. *Aging Cell* 17:e12739.



DNA test tube

Gene Regulation In Ageing and Age-Related Diseases

Cor Calkhoven

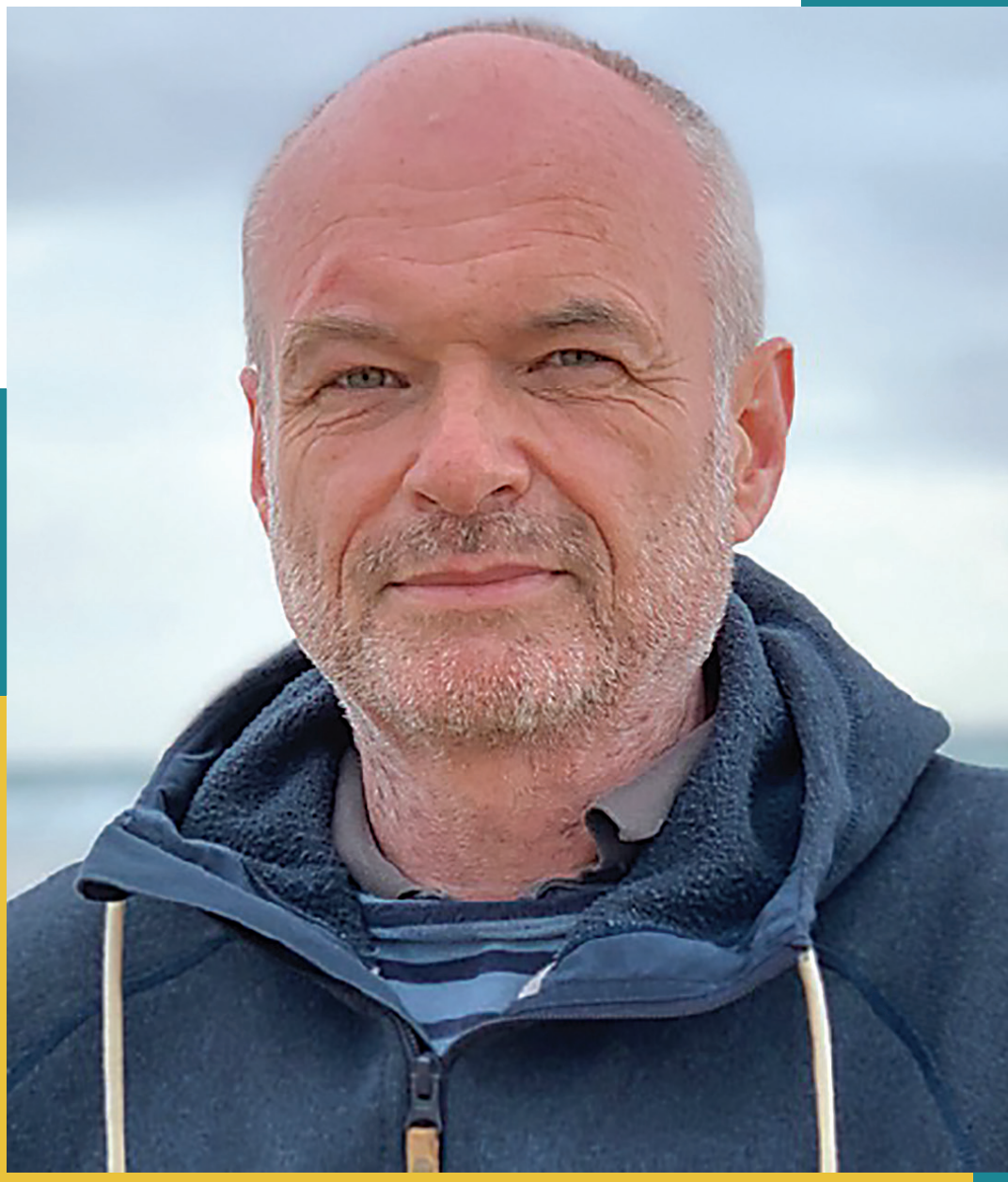
Our research aim is to identify and understand the role of regulatory networks that control the function of C/EBPα and C/EBPβ transcription factors in ageing and age-related diseases. We showed that mRNA-translational regulation of C/EBPβ expression through the mTORC1 nutrient and energy signaling pathway are linked to ageing and health- and lifespan determination. Others showed that deficiency of DNA-demethylation factors that regulate access of C/EBPβ to its genome binding sites result in premature ageing. In addition, the NAD+-SIRT1 pathway controls the function in mitochondrial biogenesis and respiration through regulation of C/EBPα protein-deacetylation. Apart from physiologic metabolic functions, C/EBPβ functions as an oncogene by inducing cancer type metabolism. In another line of research, we aim to understand the pro-tumorigenic role of TSC-mTORC1 regulation in lung cancer.

RESEARCH FOCUS

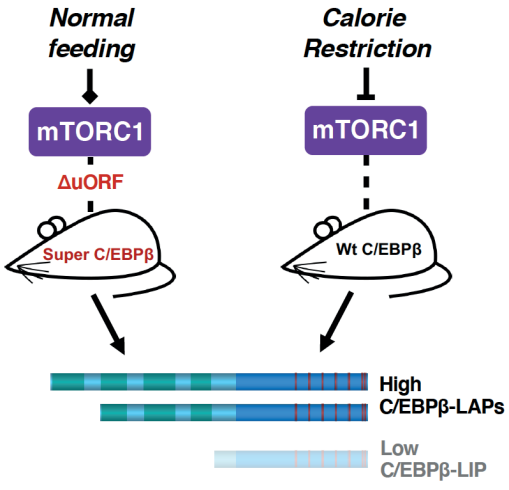
CEBPA- and CEBPB-mRNAs are translated into complete and active transcription factors, called C/EBPβ-LAP and C/EBPα-p42 as well as into shorter inhibitory factors, called C/EBPβ-LIP and C/EBPα-p30. A single uORF in the mRNAs acts as a *cis*-regulatory element required for translation into LIP and p30 and confers sensitivity to specific translational regulation pathways, in particular to mTORC1 nutrient signaling. We have shown that obstructing mTORC1 from regulating LIP by removal of the uORF results in a wide range of delays in age-related conditions in mice, akin those observed by calorie restriction or other mTOIRC1 inhibitory measures (Figure). This *Cebpb*$^{\Delta uORF}$ mutation is characterized by C/EBPβ super-function since only the transactivating LAP is expressed, unrestrained by expression of the inhibitory LIP. We know that the C/EBPα-p30 expression is similarly regulated and that removal of the uORF results in C/EBPα super-function in cell culture. Physiological relevance of p30 regulation awaits examination of *Cebpa*$^{\Delta uORF}$ mice that just have been generated.

Another prominent regulation of C/EBPα function is through lysine-acetylation. The acetylation status of C/EBPα is controlled through deacetylation by SIRT1 in response to changes in NAD^+ homeostasis. Hypoacetylated C/EBPα stimulates the transcription of mitochondrial genes and results in increased mitochondrial function, identifying C/EBPα as a key mediator of SIRT1-controlled adaption of energy homeostasis. Mouse models mimicking hypo- and hyperacetylation C/EBPα of are currently being generated to investigate the related physiology.

In another line of research, we discovered that oncogenic MYC restrains mTORC1 signaling in Burkitt's lymphoma by safeguarding the expression of the tuberous sclerosis complex (TSC). Interference with MYC-TSC1-mTORC1 regulation results in enhanced mitochondrial respiration, accumulation of toxic reactive oxygen species and cell death. Since TSC expression is high in small cell lung cancer cells we currently are investigating TSC's potential tumor promoting role in lung cancer and the involved regulatory and pathological mechanisms.



Cor Calkhoven
Gene regulation in ageing and age-related diseases



Metabolic trait	C/EBPβ −ΔuORF	Calorie restricted
Body weight	↓	↓
Fat content	↓	↓
Steatosis	↓	↓
Adiponectin	↑	↑
Glucose	↓	↓
Insulin	↓	↓
Glucose tol.	↑	↑
Ins. sensitivity	↑	↑
GH/IGF-1	↓	↓

Ageing phenotypes	C/EBPβ −ΔuORF	Calorie restricted
Rotarod	↑	↑
Beam walking	↑	↑
Wire hang	↑	↑
naïve/memory T-cells	↑	↑
Better maintained during ageing (4 vs. 20 months)		
Cancer	↓	↓

Figure. The table shows a compilation of phenotypes induced by the Cebpb$^{\Delta uORF}$ mutation resulting in C/EBPβ super-function through loss of LIP expression. Simmilar metabolic phenotypes and delay in age-related conditions can be achieved by calorie restriction.

THE FUTURE

Future studies will address the role of mTORC1- and SIRT1-C/EBPα regulation in health and lifespan determination using genetic engineered mouse models. In addition, we have identified RNA-methylation as a new regulatory stage of C/EBPs as well as other regulatory factors in metabolism and cancer that will require further investigations. As part of both the C/EBP and TSC projects we aim to develop drug screening strategies in order to develop new therapies for metabolic disorders and cancer.

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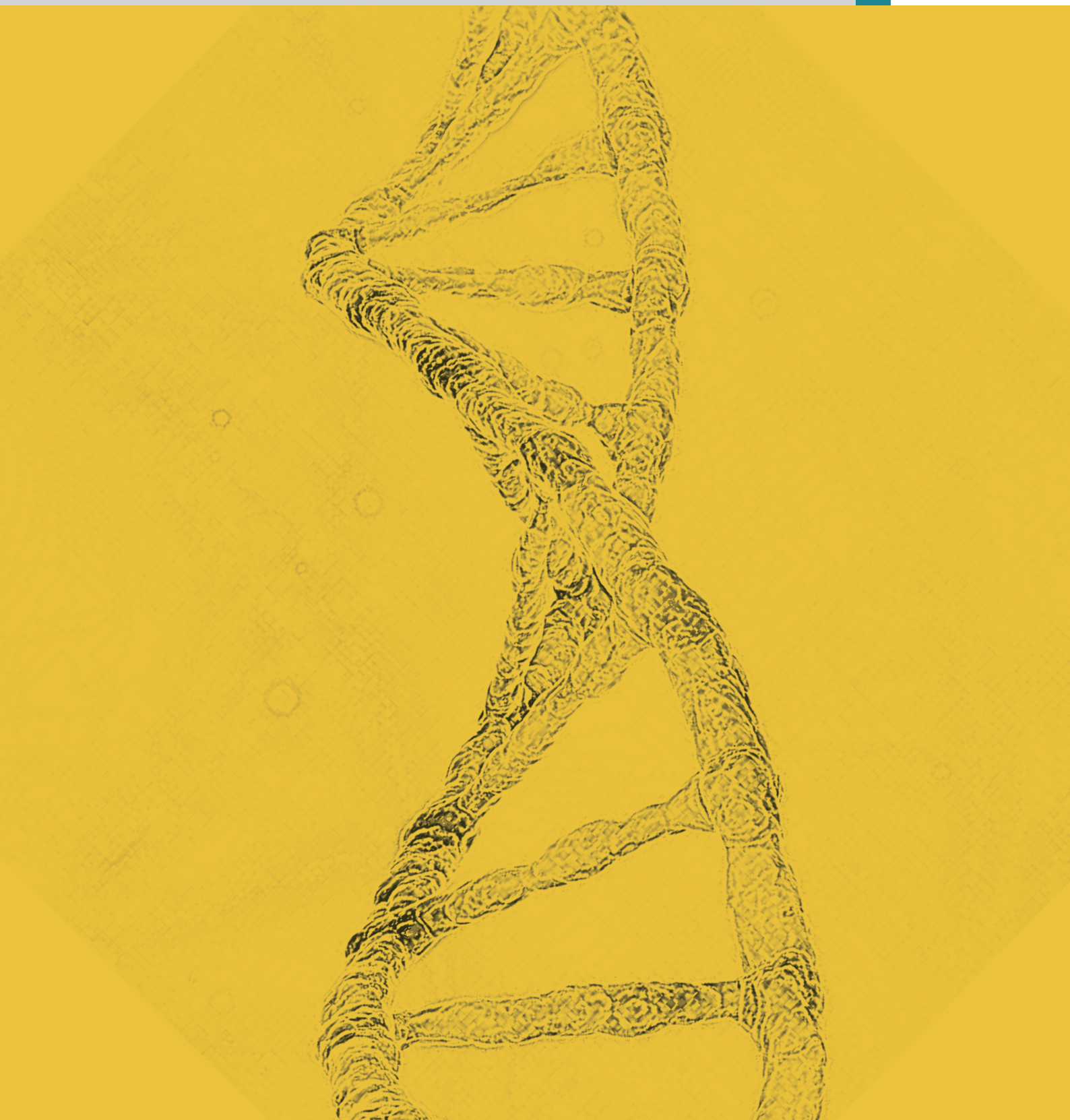
1. Müller, C., Zidek, L.M., Ackermann, T., de Jong, T. Liu, P., Kliche, V., Zaini, M.A., Kortman, G., Harkema, L., Verbeek, D.S., Tuckermann, J.P., von Maltzahn, J., de Bruin, A., Guryev, V., Wang, Z-Q and **Calkhoven, C.F.** (2018). Reduced expression of C/EBPβ-LIP extends health- and lifespan in mice. eLife 2018;7:e34985 DOI: 10.7554/eLife.34985

2. Zaini, M.A., Müller, C., de Jong, T., Ackermann, T., Hartleben, G., Kortman, G., Gührs, K-H., Fusetti, F., Krämer, O.H., Guryev, V. and **Calkhoven, C.F.** (2018) A p300 and SIRT1 regulated acetylation switch of C/EBPα controls mitochondrial function. Cell Reports 22, 497-511. DOI: 10.1016/j.celrep.2017.12.061

3. Hartleben, H., Müller, C, Krämer, A., Schimmel, H., Zidek L.M., Dornblut, C., Winkler, R., Eichwald, S., Kortman, G., Kosan, C., Kluiver, J., Petersen, I., van den Berg, A., Wang, Z-Q and **Calkhoven, C.F.** (2018). Tuberous sclerosis complex is required for tumor maintenance in MYC-driven Burkitt's lymphoma. *EMBO J*, 37, e98589 DOI: 10.15252/embj.201798589



Telomeres and Genome Integrity

Michael Chang

INTRODUCTION

The overall goal of our lab is to characterize the mechanisms used by a cell to protect its genome from becoming mutated or inappropriately altered or rearranged. The genome is duplicated in a process called DNA replication. If DNA becomes damaged, either as a consequence of normal cellular processes or due to exposure to DNA damaging agents, DNA repair pathways are employed to fix the damage. Defective DNA replication or DNA repair results in genome instability, which is a hallmark of both cancer and ageing.

RESEARCH FOCUS

The main focus of the lab is to understand how the ends of our chromosomes, the telomeres, are properly maintained. Telomeres help cells distinguish natural chromosome ends from double-strand DNA breaks in need of repair. Recent work in our lab has found that multiple proteins work together to protect telomeres from getting degraded.

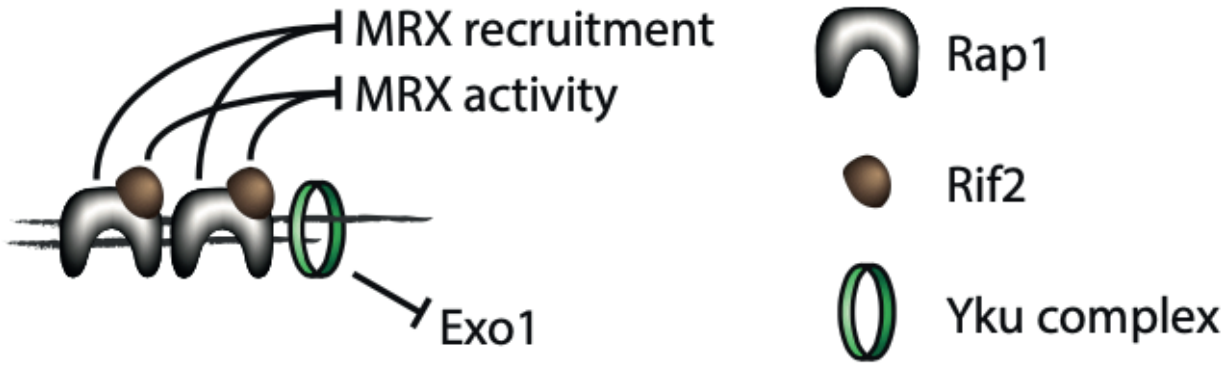


Figure. A model to illustrate how Rap1, Rif2, and the Yku complex work in concert to prevent MRX- and Exo1-mediated telomere degradation (from Rosas Bringas et al., 2020).

THE FUTURE

Our lab will continue to study how cells protect their genome from the accumulation of mutations, chromosomal rearrangements, and telomere dysfunction. In particular, we are examining how telomere length affects different aspects of telomere biology. We will also examine how repetitive DNA sequences such as telomeres, which pose unique obstacles for the DNA replication machinery, are dealt with by genome maintenance mechanisms.

TOP 3 PUBLICATIONS

1. Rosas Bringas, F.R., Stinus, S., Wanders, L., de Zoeten, P., Cohn, M., and **Chang M.** (2020) Rap1, Rif2, and the Ku complex work in concert to cap chromosome ends. *bioRxiv*. doi: 10.1101/2020.12.30.424824.

2. Novarina, D., Janssens, G.E., Bokern, K., Schut, T., van Oerle, N.C., Kazemier, H.G., Veenhoff, L.M., and **Chang, M.** (2020) A genome-wide screen identifies genes that suppress the accumulation of spontaneous mutations in young and aged yeast cells. *Aging Cell*, 19(2): e13084.

3. Novarina, D., Desai, R., Vaisica, J.A., Ou, J., Bellaoui, M., Brown, G.W., and **Chang. M.** A genome-wide screen for genes affecting spontaneous direct-repeat recombination in *Saccharomyces cerevisiae*. *G3*, 10(6): 1853-1867.

Michael Chang
The goal of our research group is to characterize the mechanisms that maintain the integrity of the genome. Genome instability of a hallmark of ageing and cancer.

Ageing Biology and Stem Cells

Gerald de Haan

INTRODUCTION

Our research is aimed to understand the molecular mechanisms that contribute to hematopoietic stem cell (HSC) aging. A small population of selfrenewing HSCs ensures that all the various types of blood cells are produced during the lifetime of an organism. Nevertheless, during aging blood cell production is impaired, resulting in either a deficit of blood cells (leading to anemia, thromocytopenia, or susceptibility to infections) or an excess of blood cells (myeloproliferative diseases or leukemias). The identification of the molecular machinery that is associated with aberrant selfrenewal of aged HSCs should allow the development of interventions that improve or restore selfrenewal of normal aged stem cells, and that target exessive selfrenewal of leukemic stem cells.

Gerald de Haan
Hematopoietic stem cell ageing

RESEARCH FOCUS

Transplanting aged HSCs into young recipients does not rejuvenate these cells. Thus, it appears as if aging of HSCs is at least partially the result of cell intrinsic perturbations. In our lab we have therefore focused on how epigenetic mechanisms, controlled by the activity of the Polycomb Repressive Complex proteins, regulate selfrenewal of HSCs. We have a particular interest into the role of the CBX proteins. These proteins bind the H3K27me3 marks, and in HSCs one of the core CBX proteins, CBX7, represses genes that are important for selfrenewal. Enhancing or inhibiting the activity of CBX7 results in enhanced or repressed HSC selfrenewal. Thus, targeting CBX7 in leukemic cells may be a viable strategy to eridate leukemic cells.

We speculate that potentially random, stochastic, epigenetic changes occur in HSCs as they divide, and that the ensueing epigenetic drift results in an altered transcriptome that impairs functioning of aged HSCs. To assess whether a defined 'aging signature' exists in aged HSCs, we performed a meta-analysis of multiple HSC transcriptome datasets (Figure). Although we observed

very substantial heterogeneity, we were able to identify a core set of some 150 genes that are robustly differentially expressed in young vs. aged murine HSCs. Interestingly, these HSC aging genes are strongly enriched for cell membrane-encoding proteins. This suggests that HSC aging results from altered communication of HSCs with their immediate environment. Many of the genes that we identified have no known role in HSC biology. We have provided functional data on the 'top' HSC aging gene, P-selectin. These data show that differential P-selectin expression levels among aged HSCs defines populations of HSCs that are functionally distinct. Collectively, our results have made it possible to predict, based on the expression levels of a limited number of genes, whether HSCs are molecularly aged or young.

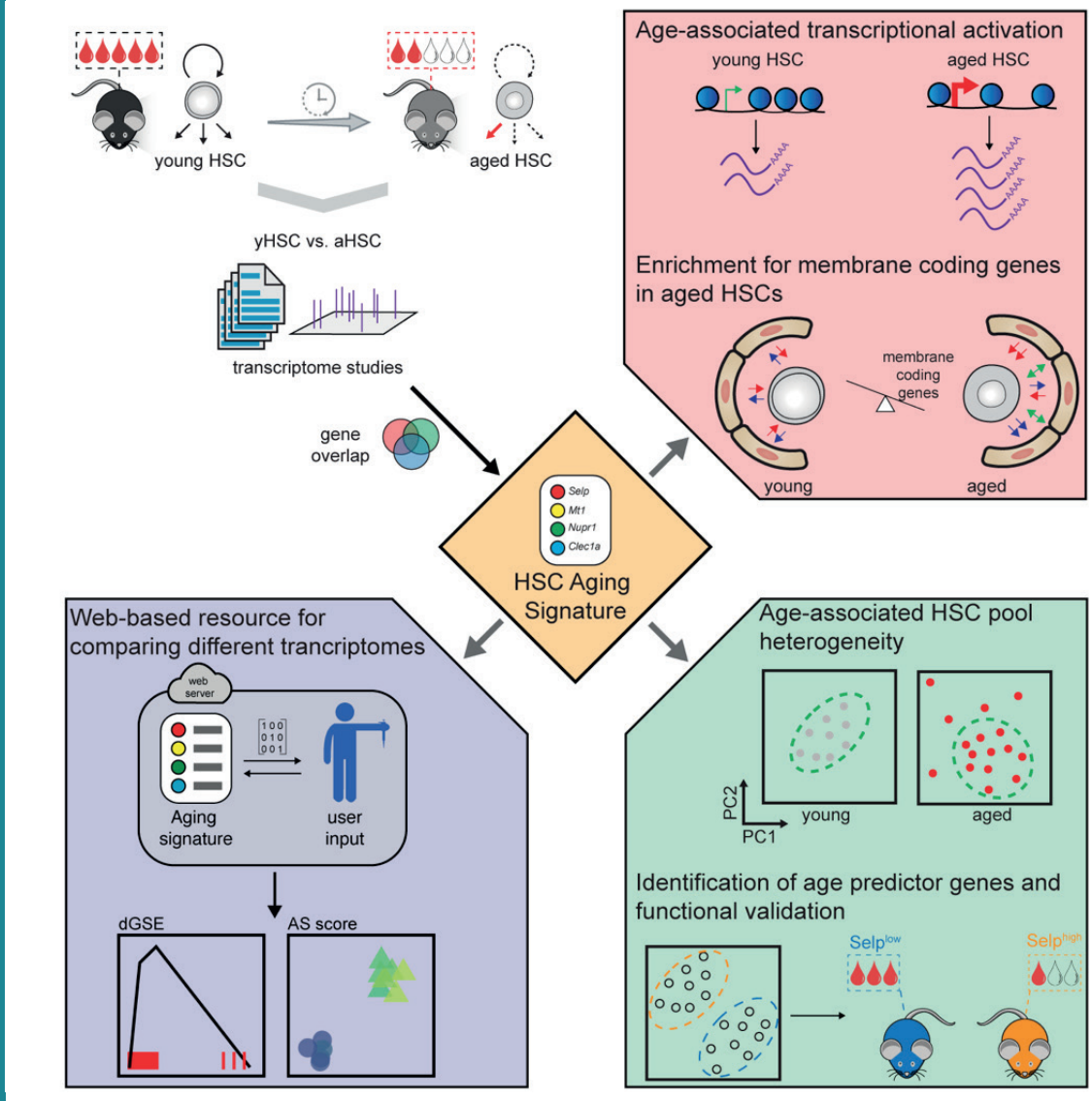
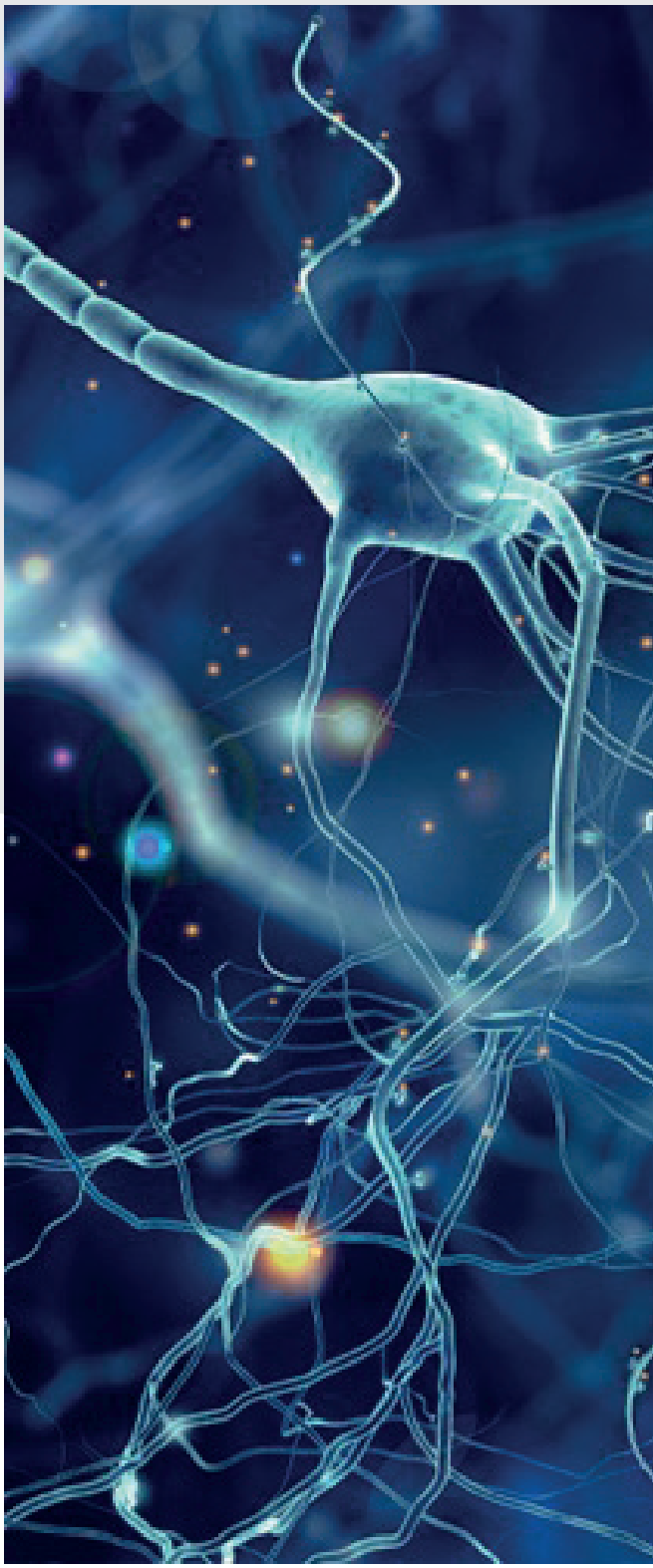


Figure. Meta-analysis of multiple HSC transcriptome datasets

THE FUTURE

In the future we aim to use newly identified cell surface markers that are aberrantly expressed on aged HSCs to prospectively purify and functionally test distinct HSC subpopulations. We predict that these subpopulations are differentially susceptible to leukemic derailment, and will test this using various experimental approaches. In addition, we will aim to improve the functioning of aged HSCs by genetic or pharmacologic targeting of novel pathways, controlled by candidate aging genes.



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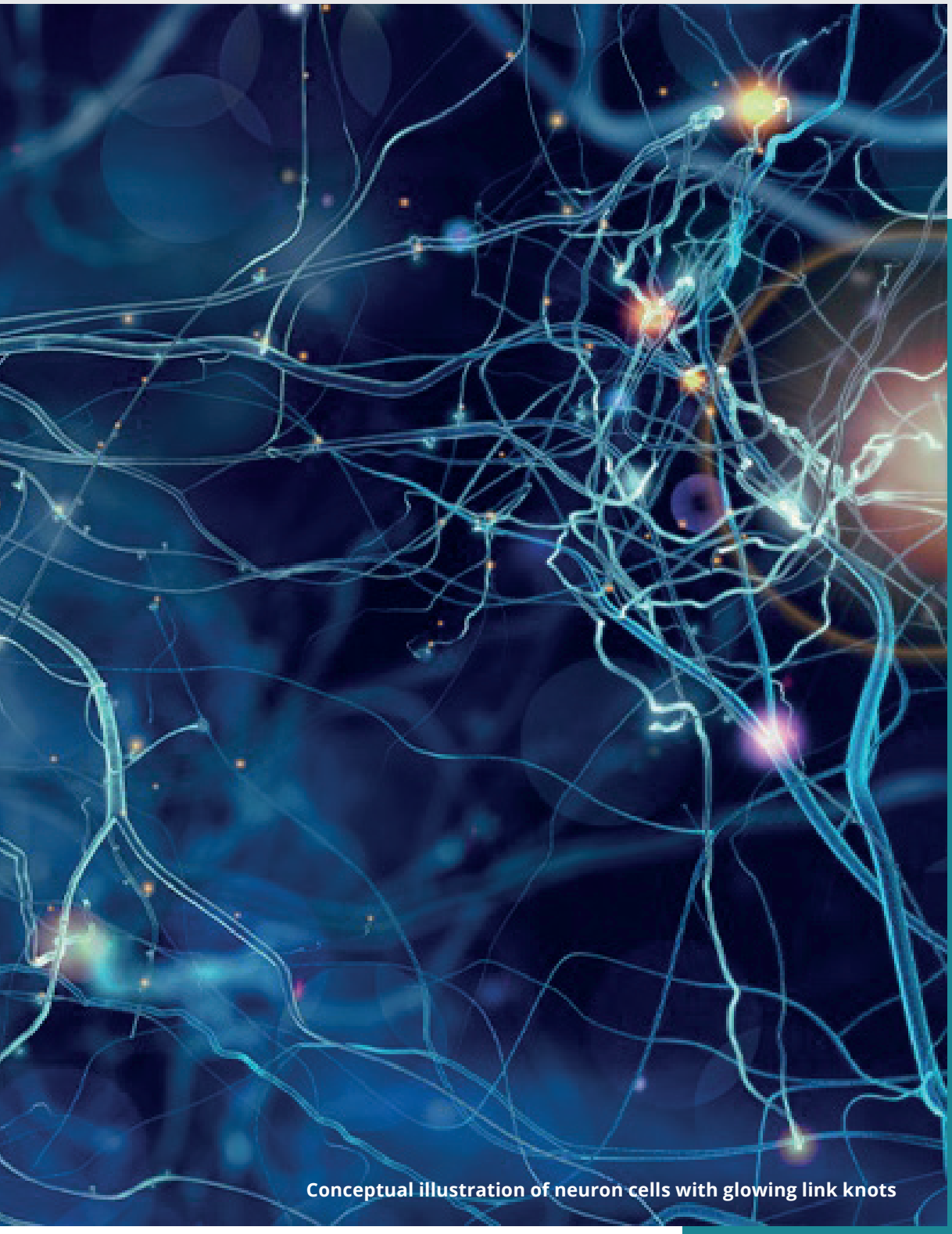
1. Simon Renders, Arthur Flohr Svendsen, ... Leonid Bystrykh, Paul S. Frenette, Patrick Mehlen, **Gerald de Haan**, Nina Cabezas-Wallscheid, Andreas Trumpp. Niche Derived Netrin-1 Regulates Hematopoietic Stem Cell Dormancy via its Receptor Neogenin-1. Niche derived netrin-1 regulates hematopoietic stem cell dormancy via its receptor neogenin-1. Nat Commun 12, 608 (2021). https://doi.org/10.1038/s41467-020-20801-0.

2. Arthur Flohr Svendsen, Daozheng Yang, KyungMok Kim, Seka Lazare, Natalia Skinder, Erik Zwart, Anna Mura-Meszaros, Albertina Ausema, Björn von Eyss, **Gerald de Haan**, Leonid Bystrykh. A comprehensive transcriptome signature of murine hematopoietic stem cell aging. Blood, in revision. bioRxiv 2020.08.10.244434; doi: https://doi.org/10.1101/2020.08.10.244434.

3. Jung J, Buisman SC, Weersing E, Dethmers-Ausema B[1], Zwart E, Schepers H, Dekker MR, Lazare SS, Hammerl F, Skokova J, Kooistra SM, Klauke K, Poot RA, Bystrykh LV and **de Haan G**. CBX7 induces self-renewal of human normal and malignant hematopoietic stem and progenitor cells by canonical and non-canonical interactions. Cell Rep. 2019 Feb 12;26(7):1906-1918.



Conceptual illustration of neuron cells with glowing link knots

Cellular Senescence and Age-related Pathologies

Marco Demaria

INTRODUCTION

Our research is aimed to understand the mechanisms that regulate induction and biological functions of senescent cells. Increasing evidence indicates that a common mark of older organisms is the accumulation of senescent cells: cells that enter a state of irreversible growth arrest in response to diverse damages. Senescence-associated growth arrest represents a well-established tumor suppressive mechanism. However, gradual accumulation of growth-arrested senescent cells during a lifetime can cause inability to maintain important cell pools with age. Remarkably, most senescent cells activate the transcription of various cytokines, growth factors and matrix metalloproteases (MMPs), developing a complex but highly heterogeneous secretory phenotype known as SASP (senescence-associated secretory phenotype). The SASP plays an essential role in tissue remodeling and repair during embryogenesis and adulthood and guarantees effective clearance of senescent cells. In contrast, when senescent cells aberrantly accumulate, such as during aging or under excessive stress, they cause chronic low-level inflammation and aberrant tissue growth and remodeling via SASP factors. The identification of deleterious pro-disease senescent cells prompted the development of senolytic drugs able to induce selective death of senescent cells.

Marco Demaria
Heterogeneity of cellular senescence

To determine the heterogeneity of senescent cells, we have recently performed a comprehensive transcriptomic study to compare different senescence subsets. We have analyzed the effect of various factors including cell and tissue of origin, senescence inducer and time point after senescence induction. We have identified a number of subset-specific differentially expressed genes and a 'core' senescence signature which we are further characterizing and validating for their impact on functions and their potential as new biomarkers. Moreover, we have predicted a number of novel broad and subset-specific senolytic compounds which we are currently screening and validating.

A major limitation for the understanding of basic aging mechanisms and the effect of anti-aging interventions is the lack of reliable models. Our laboratory has pioneered the use of senescence-reporter mice to study aging and diseases associated to senescent cells. One such model is the p16-3MR mouse, which carries a reporter gene called 3MR, under the regulation of the $p16^{INK4A}$ promoter. 3MR contains Renilla luciferase (LUC), monomeric red fluorescence protein (mRFP), herpes simplex virus (HSV), and thymidine kinase (Tk), a suicide gene activated by ganciclovir (GCV) (Figure 2). With this model we are now interrogating the senescence heterogeneity *in vivo* by tracking, isolating and eliminating different senescence subsets.

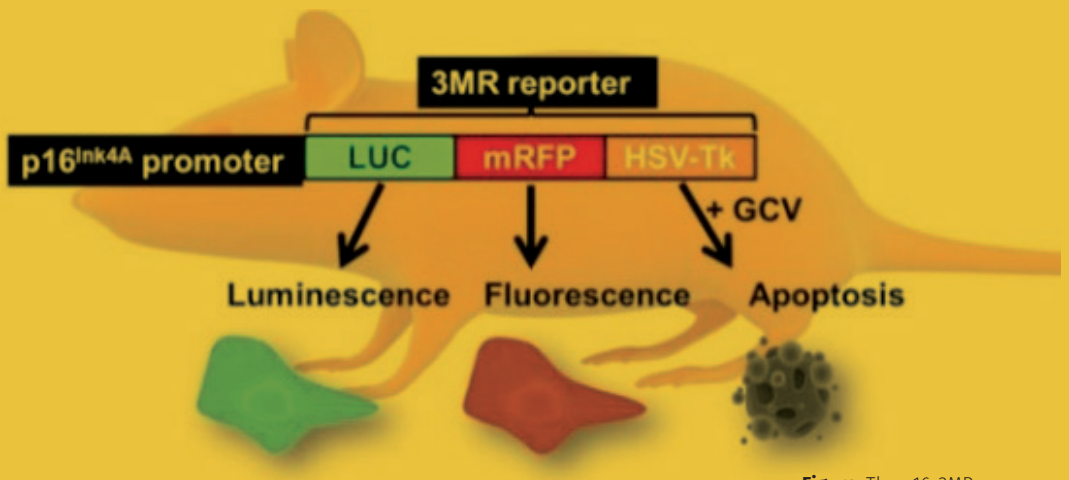


Figure. The p16-3MR mouse.

THE FUTURE

In the future we aim to expand our understanding on senescence heterogeneity by analyzing and phenotyping senescence subsets associated to specific physiological and pathological conditions. Our goal is to contribute to the understanding of the multifaceted role of senescence and to the development of potent anti-aging and disease interventions.

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1. **Demaria M**[#], O'Leary M, Chang J, Shao L, Liu S, Alimirah F, Koenig K, Le C, Mitin N, Deal AM, Alston S, Academia E, Kilmarx S, Valdovinos A, Wang B, de Bruin A, Kennedy BK, Melov S, Zhou D, Sharpless NE, Muss H and Campisi J. 2017. *Cellular senescence promotes adverse effects to chemotherapy and cancer relapse*. Cancer Discovery. 7:165-176. [#] corresponding author

2. Hernandez-Segura A, de Jong TV, Guryev V, Campisi J, **Demaria M**. 2017. *Unravelling transcriptional heterogeneity of senescent cells*. Current Biology. 27:2652-2660

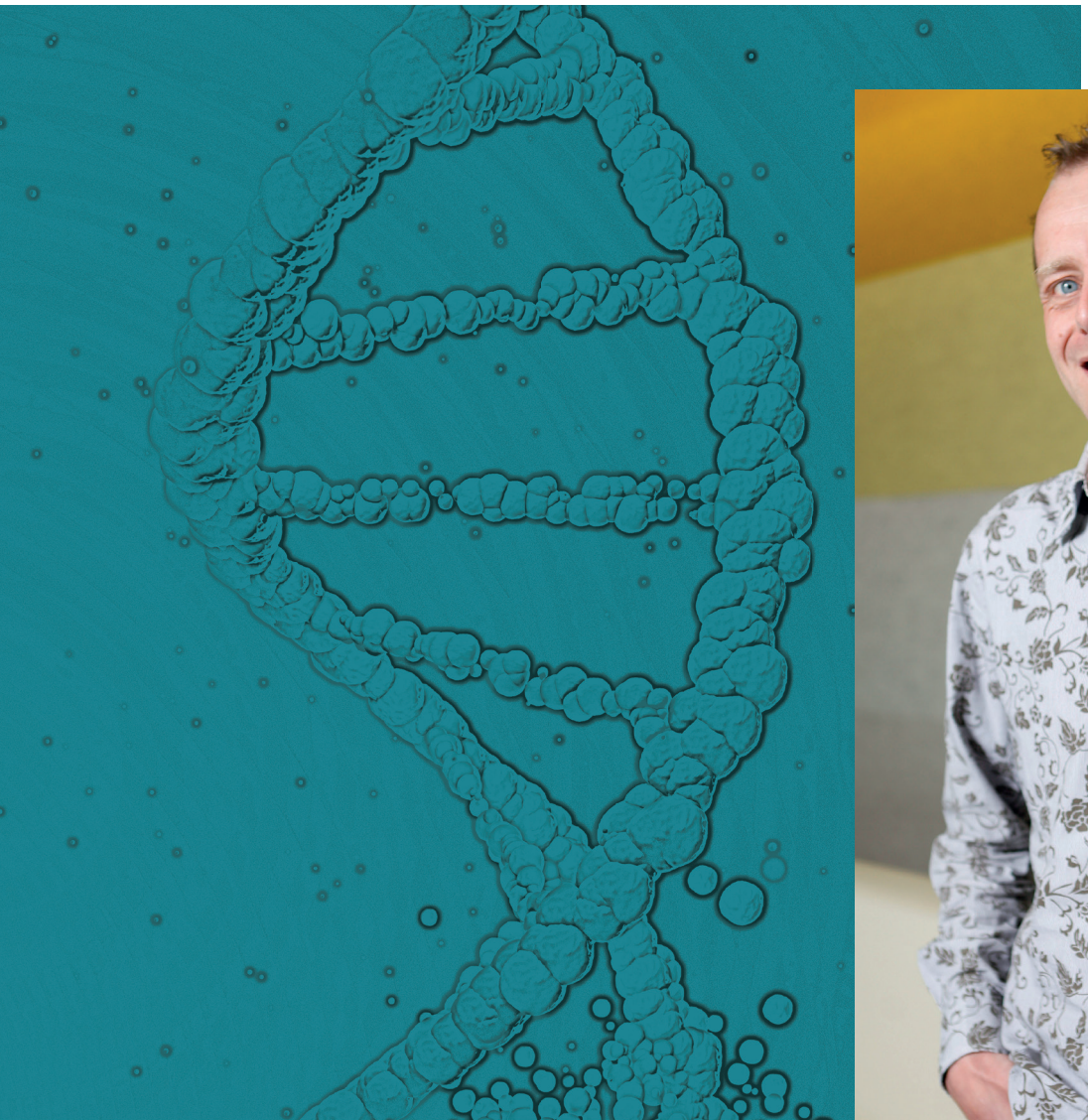
3. Gorgoulis V**,** Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, Campisi J, Collado M, Evangelou K, Ferbeyre G, Gil J, Hara E, Krizhanovsky V, Jurk D, Maier AB, Narita M, Niedernhofer N, Passos JF, Robbins PD, Schmitt C, Sedivy J, Vougas K, von Zglinicki T, Zhou D, Serrano M, **Demaria M.** 2019. *Cellular senescence: defining a path forward*. Cell. 179:813-827

Genomic Instability in Development and Disease

Floris Foijer

INTRODUCTION

Chromosomal instability (CIN) is a hallmark feature of cancer. CIN leads to cells with an abnormal DNA content, a state known as aneuploidy affecting >80% of all cancers. Paradoxically, in untransformed cells, CIN and aneuploidy decrease cellular fitness and lead to activation of stress pathways. This suggests that cancer cells have found ways to cope with the downsides of CIN. A better understanding of these coping strategies can lead to new therapies that target these mechanisms, and thus selectively kill the aneuploid cancer cells with fewer side effects on healthy cells. We study the how cells deal with chromosomal instability and aneuploidy, in vitro as well as in vivo. For this we 1) develop and exploit models and technology to faithfully measure chromosomal instability and aneuploidy in cultured cells as well as in living mice, 2) we develop mouse and cultured cell models to study CIN, which we 3) use to better understand the mechanisms that trigger the responses to CIN and 4) we exploit these mechanistic findings to design therapies that selectively kill cells with a CIN phenotype.



Floris Foijer Chromosomal instability in ageing and cancer

RESEARCH FOCUS

Ongoing CIN leads to cells with variable karyotypes and thus to intratumour karyotype heterogeneity. CIN is therefore a strong driver of cancer cell evolution and associated with poor prognosis. Together with the research sequencing facility, we heavily invested in single cell DNA sequencing as a tool to quantify karyotype heterogeneity (ref 1). We for instance used this tool to map karyotype evolution over time in two distinct mouse models for acute T-cell lymphoma (T-ALL), one model in which CIN was chronic and another in which CIN was transient. Interestingly, we found that both models yielded cancers with identical average karyotypes. However, the tumors exhibiting chronic CIN displayed higher intratumor heterogeneity (ref 2). While these experiments indicate that both transient and chronic CIN lead to comparable tumours, the obvious next step is to compare therapy response between both models.

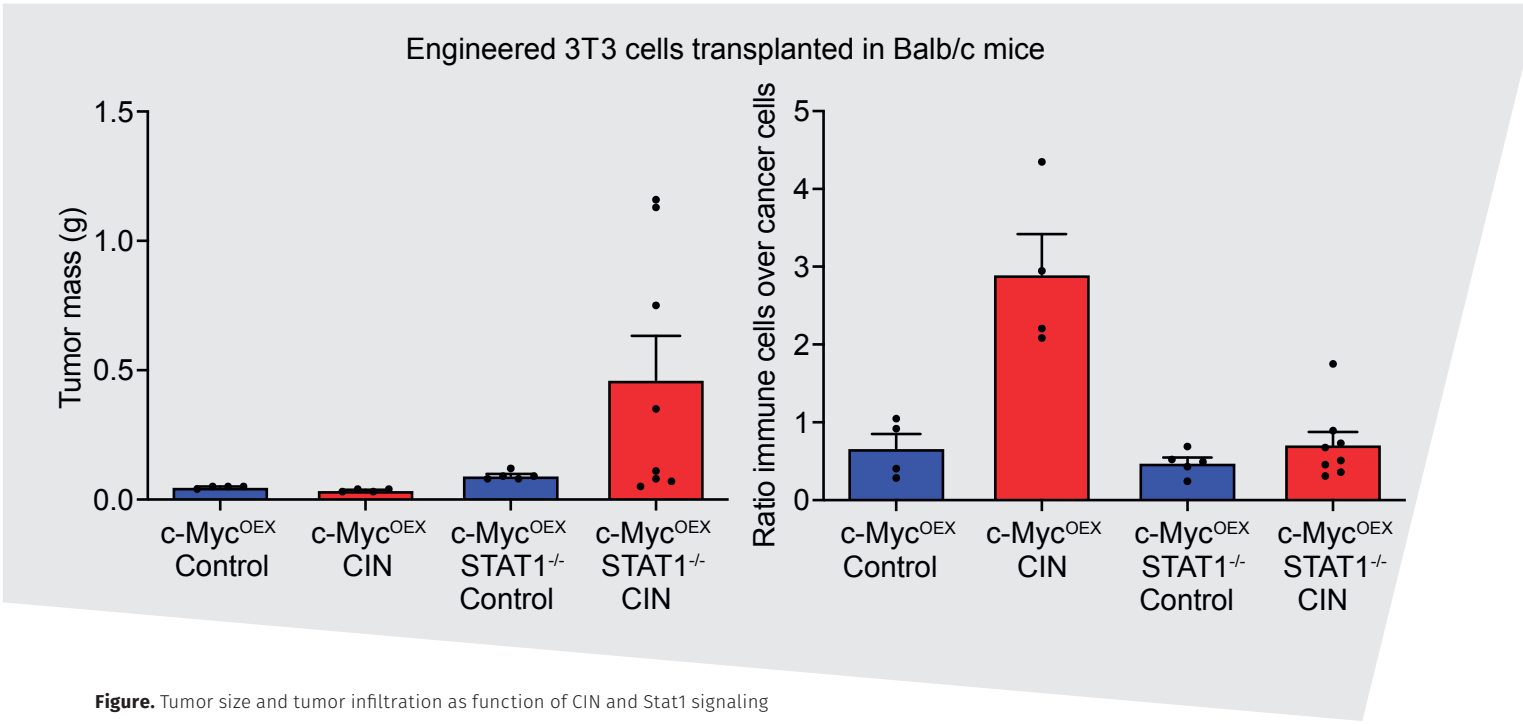


Figure. Tumor size and tumor infiltration as function of CIN and Stat1 signaling

In another effort, we compared the cancer drivers between isogenic tumours with or without a CIN phenotype. For this purpose, we provoked hematopoietic malignancies in two cohorts of mice, one solely driven by random transposon mutagenesis and another in which transposon mutagenesis and ongoing CIN were combined. We found that the key difference between both groups is an impaired inflammation response in the aneuploid malignancies as a result of inactivation of the Stat1 signaling pathway. Indeed, when we allograft cells with a CIN phenotype into immune-proficient mice either or not combined with Stat1 inactivation, we find that CIN triggers massive immune infiltration of the tumor, but that inflammation can be prevented by concomitant Stat1 inactivation, which coincides with increased tumor size. We conclude that tumours with a CIN phenotype, in order to thrive, need to circumvent immunosurveillance, for instance through inactivation of Stat1 signaling. The next important step will be to find ways to reinstate immunosurveillance in aneuploid cancers, which could provide a powerful means to selectively kill aneuploid cancer cells.

Our findings indicate that CIN triggers an inflammation response in cells that relies on Stat1 signaling. Intriguingly, while Stat1 inactivation promoted tumorigenesis in combination with CIN, we also found that alleviating the inflammatory response upstream of Stat1 is very toxic to chromosomal instable cells. We also found that the inflammatory response triggered by CIN critically relies on Il6 activity upstream of Stat1. Indeed, blocking Il6 signaling by means of the clinically approved Il6R inhibitor Tocilizumab is toxic to CIN tumor cells in vitro and in vivo, but well-tolerated by chromosomal stable cancers, potentially revealing an Achilles heel of aneuploid cancers, which we plan to validate in a clinical trial soon.

THE FUTURE

Now that we identified that lack of immunosurveillance is an essential feature of tumours displaying a CIN phenotype, we next want to understand which immune cells clear aneuploid cells and which interactions between immune cells and cancer cells trigger clearance. Furthermore, we want to map the molecular mechanisms that cancers exploit to inactivate immune signaling and translate this knowledge into therapeutic interventions that selectively target aneuploid cells. In addition to mapping how immune cells clear aneuploid cells, we will also further investigate the molecular mechanisms that trigger the initial inflammation response, including CRISPR genome-wide screens.

Furthermore, we want to better understand how karyotype dynamics drive tumor evolution. For this, we will investigate how chromosome copy number changes change cellular fitness in cell models, but also in vivo, including intravital imaging models to visualize aneuploidy in vivo. For this, we will develop fitness reporters as well as new mouse models in which we can determine cellular fitness in cultured cells including genome-wide screens as well as *in vivo*.

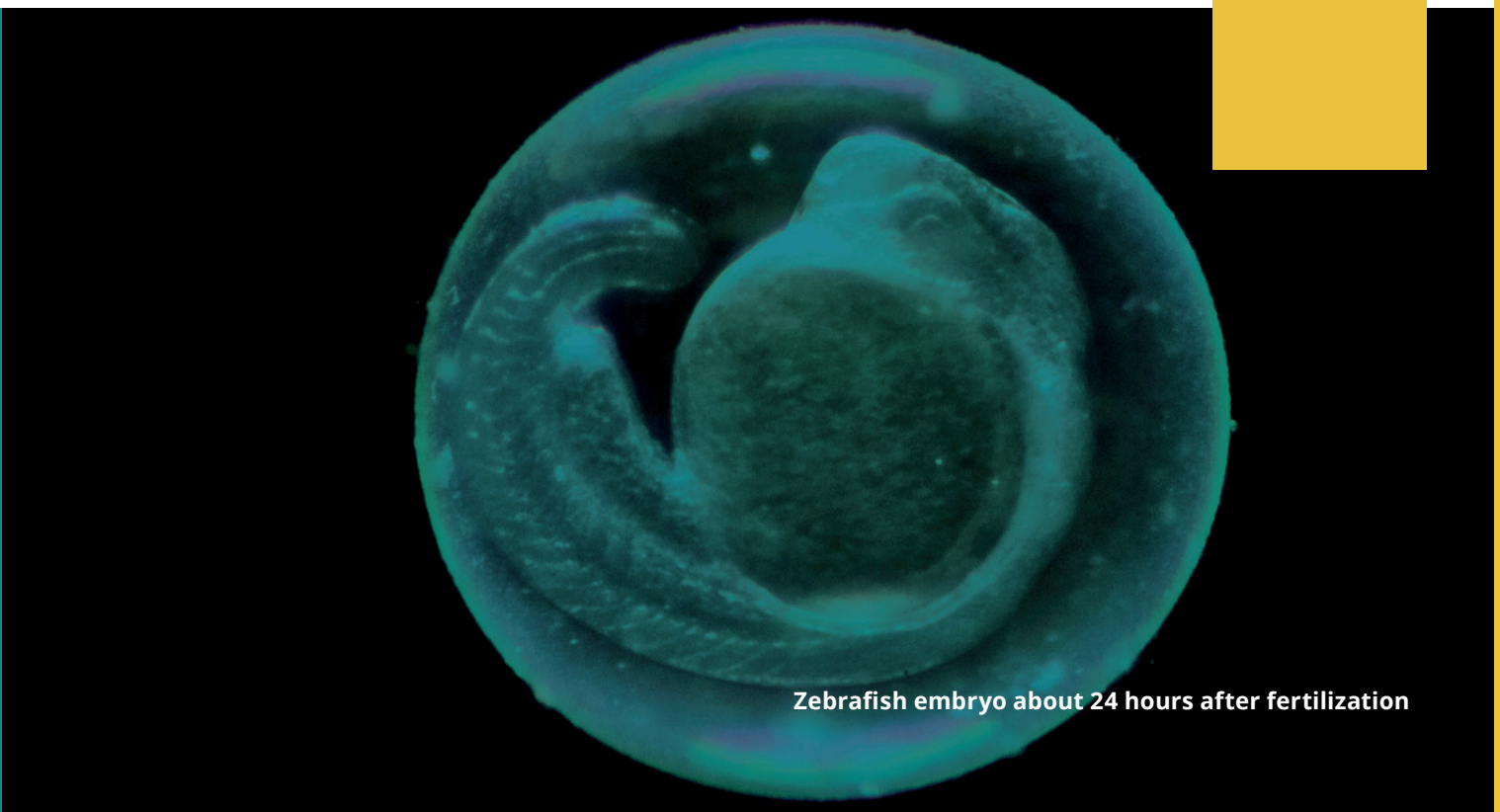
TOP 3 PUBLICATIONS

1. Shoshani O, Bakker B, WangY, Kim DH, Maldonado M, Demarest MA, Artates J, Zhengyu O, Mark A, Wardenaar R, Sasik R, Spierings DCJ, Vitre B, Fisch K, **Foijer F#**, and Cleveland DW#. Transient genomic instability drives tumorigenesis through accelerated clonal evolution. bioRxiv 2020.11.17.387753. Under revision (Genes & Development)

2. Bakker B*, Taudt A*, Belderbos ME, Porubsky D, Spierings DC, de Jong TV, Halsema N, Kazemier HG, Hoekstra-Wakker K, Bradley A, de Bont ES, van den Berg A, Guryev V, Lansdorp PM, Colomé-Tatché M#, **Foijer F#.** Single-cell sequencing reveals karyotype heterogeneity in murine and human malignancies. Genome Biol. 2016 May 31;17(1):115. doi: 10.1186/s13059-016-0971-7.

3. Schukken KM, Lin YC*, Bakker PL*, Schubert M, Preuss SF, Simon JE, van den Bos H, Storchova Z, Colomé-Tatché M, Bastians H, Spierings DC, **Foijer F#.** Altering microtubule dynamics is synergistically toxic with spindle assembly checkpoint inhibition. Life Sci Alliance. 2020 Jan 24;3(2):e201900499. doi: 10.26508/lsa.201900499.

*Equal contributions, #corresponding author.



Zebrafish embryo about 24 hours after fertilization

Genome structure and Ageing

Victor Guryev

INTRODUCTION

Even though the completion of the human genome project was announced over 20 years ago, our knowledge of genome variants and their effects on the onset of ageing-related diseases is still far from being complete. Under-investigated large and complex alterations in our genomes affect more DNA bases than single-nucleotide changes. Some of these structural genome changes can be predicted using routine analysis procedure of DNA data, others, like large inversions or non-reference insertions, deserve further investigation.

Our research aims to identify a wide spectrum of DNA alterations, fine-map them to corresponding genomic locations and characterize their effects on molecular function. Our group combines analysis of genome, transcriptome, and proteome profiling (functional genomics and proteogenomics approaches) to distinguish deleterious genomic variants from benign ones. These results should contribute to a better understanding of the content and function of variable segments in our genomes.

RESEARCH FOCUS

Our research is focused on several approaches for investigating ageing-related molecular changes:

A Investigation of genome alterations potentially associated with ageing-related diseases (Fig 1A). We are studying the distribution and role of large variants in our genomes. My team applies expertise developed in the Dutch genome project to characterize SVs in patients suffering from early-onset severe COPD, cancer, and other diseases.

B Transcriptome regulation in ageing and onset of diseases (Fig 1B). Previous studies already identified several trends (e.g. more retained introns) in transcriptome processing that happen as we get older. Our group analyses transcriptomes of several patient cohorts to identify sources of these changes and their potential roles in disease etiology.

C Combining differential expression and differential variability analysis (Fig 1C). Since many human diseases are very heterogeneous in their molecular and clinical manifestations, molecular subtyping and analysis differential variability provide orthogonal approaches to classical disease association methods. We successfully employed methods for quantifying biological variability to get insight into cellular processes affected by ageing, lung diseases, sepsis, and COVID-19.

D Multi-level data integration for personalized diagnostics and treatment (Fig 1D). Combining DNA variation data with other omics layers, such as gene expression, proteomics, metabolomics, and phenotypic data, is key for the discovery of function for DNA polymorphisms. Previously, we used a rat model of hypertension to demonstrate that such analysis of DNA, RNA, and proteins, where information 'flows' across omics-layers, is an efficient way to study disease (PMID:24290761). This observation supports the validity of our approach and suggests that it can be useful for studying relations between structural genome variants, molecular phenotypes that manifest themselves at RNA and protein levels and potentially play roles in human diseases.

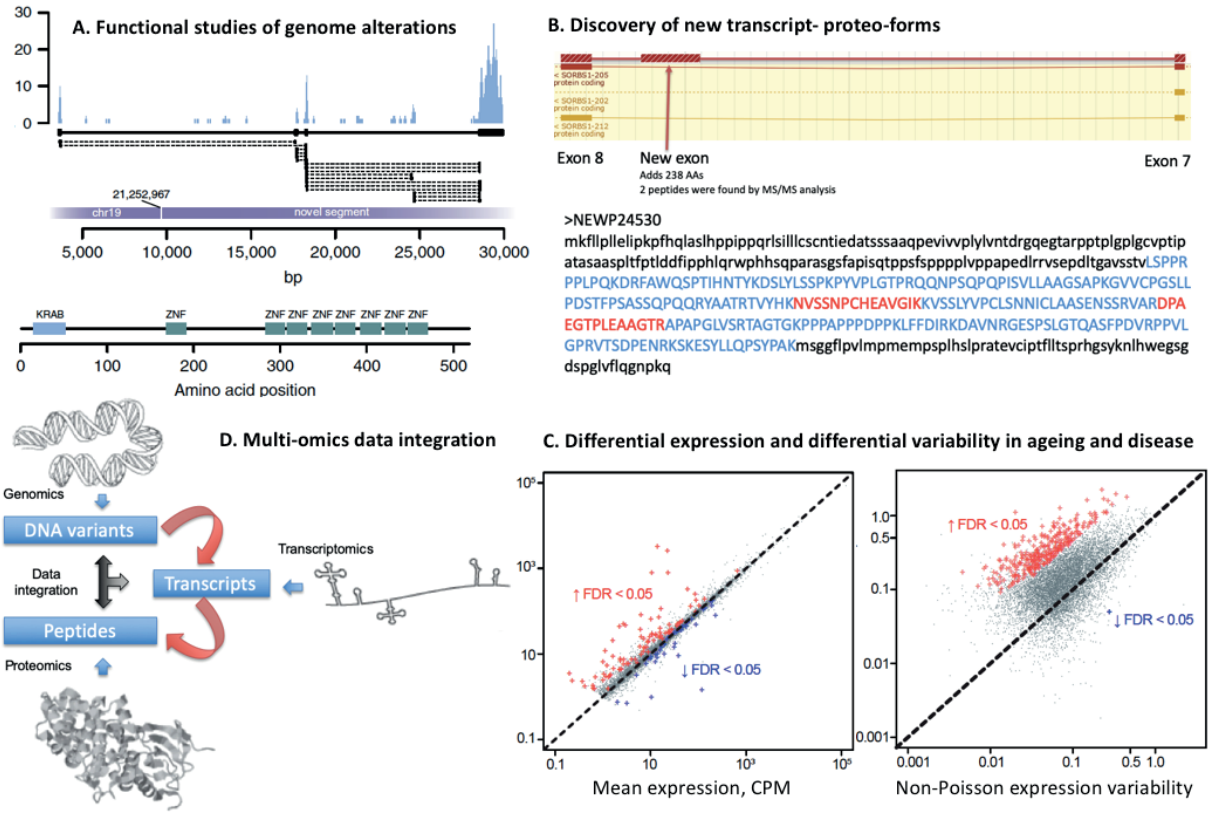


Figure. Major research directions. (A). Investigation and functional characterization of large genome alterations and their role in the onset of age-related diseases. An example of a long non-reference insert on chr19, that encodes for a new zinc finger gene. Transcript coverage by RNA-seq reads (top), reads supporting splicing events (middle) and domain structure of resulting protein product (bottom) are displayed. (B) Transcriptome analysis identifies new disease-associated protein-coding exons. An example of an exon in SORBS1 gene that is differentially present in transcripts of COPD patients. The exon adds 238 amino acids to the protein product and was confirmed by 2 corresponding peptides (in red) with LC-MS/MS data. (C) Differential expression and differential variability analysis show age-specific changes in gene expression. Left panel: more genes show upregulation of expression level in old individuals (y-axis) compared to young (x-axis). Right panel: many more genes show an increase in inter-individual variability in old individuals (y-axis) than in young individuals (x-axis). (D) Our multi-omics data integration approach. A common analysis strategy is to perform separate analyses for each omics level using public reference (black arrows). In our studies, we perform sequential integration where each omics layer informs the analysis of the next levels by providing data on DNA variants (genomics), splice variants, and new transcript units (transcriptome) for better interpretation of ageing- and disease-related molecular changes.

THE FUTURE

In the future, we aim to improve the prediction of functional consequences for large genome alterations in relation to human diseases. We plan to identify new transcriptional units and novel isoforms for known genes and link them to genome variation and dysregulated splicing factors (age- and disease-specific). Our short-term goal is to employ pathway and biochemical complex-centered analysis of gene expression variability. This will allow us to identify disruptions in particular gene ensembles on disease and better understand its underlying mechanism. Finally, we will continue to develop our computational framework for personalized multi-omics data integration and will utilize it for the analysis of omics data from other ageing-related diseases.

TOP 3 PUBLICATIONS

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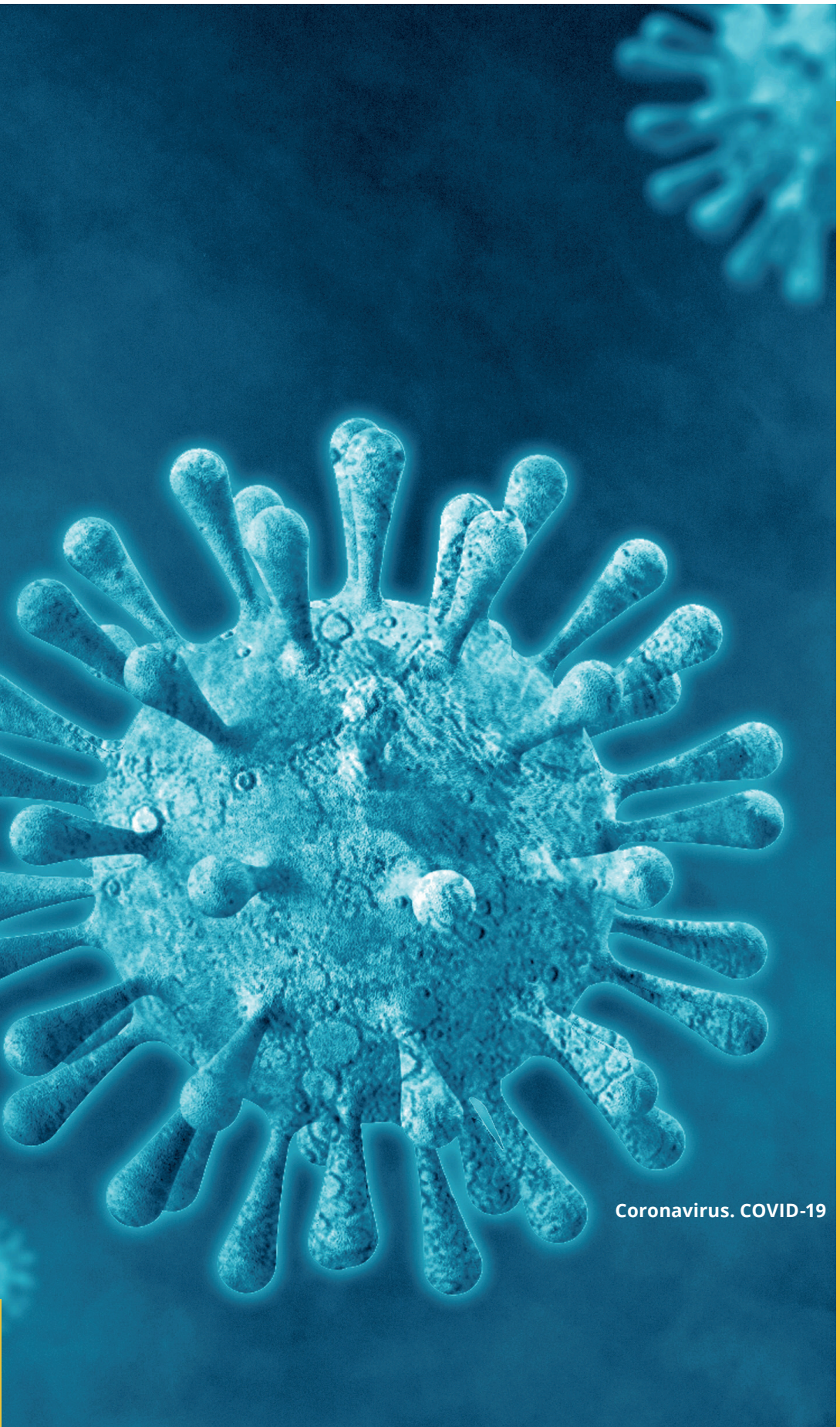
3. Kloosterman WP, Francioli LC, [..], Ye K, **Guryev V**. 2015. Characteristics of de novo structural changes in the human genome. *Genome Res*. 25:792-801.



Coronavirus. COVID-19

Genetic Instability and Ageing

Peter Lansdorp

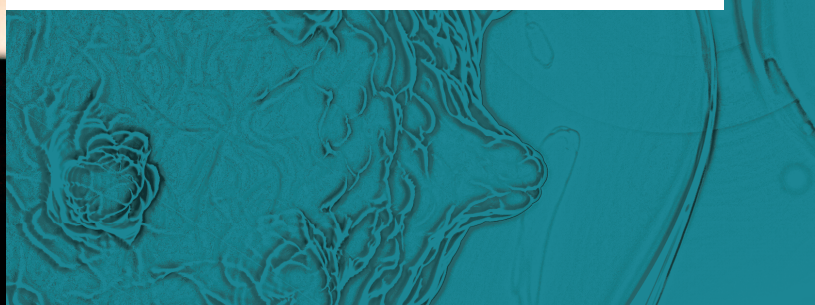
INTRODUCTION

The Lansdorp lab's main research interests include stem cell biology, genome instability and understanding genotype-phenotype relations in ageing and cancer. For these studies we have developed novel techniques including single cell DNA (Strand) sequencing that allow accurate identification of numerical chromosomal abnormalities, generation of haplotypes along entire chromosomes and mapping of structural genomic variations such as polymorphic inversions. The focus of our group has mostly been on further development of the Strand-seq technique and, more generally, on techniques that can be used to sequence the genome of single cells. This work will be continued in the UMCG Research Sequencing Facility, a core facility spearheaded by Diana Spierings as Peter Lansdorp officially retired from his UMCG position in 2020.

RESEARCH FOCUS

Peter Lansdorp
Single cell Strand-seq: the future of genome analysis

Work by former ERIBA student David Porubsky published in the prestigious Nature Biotechnology journal highlights the power of the single cell Strand-seq method developed by the Lansdorp lab for genome analysis. Human genomes are typically assembled as consensus sequences that lack information about parental haplotypes. In this paper David et al., describe a reference-free workflow for diploid de novo genome assembly that combines the chromosome-wide phasing and scaffolding capabilities of single-cell strand sequencing with continuous long-read or high-fidelity sequencing data. Employing this strategy, a completely phased de novo genome assembly for both parental haplotypes was established in the absence of any parental data. The haplotype assemblies are accurate and provide fully phased single-nucleotide variants, indels and structural variants. The results are illustrated in this figure:



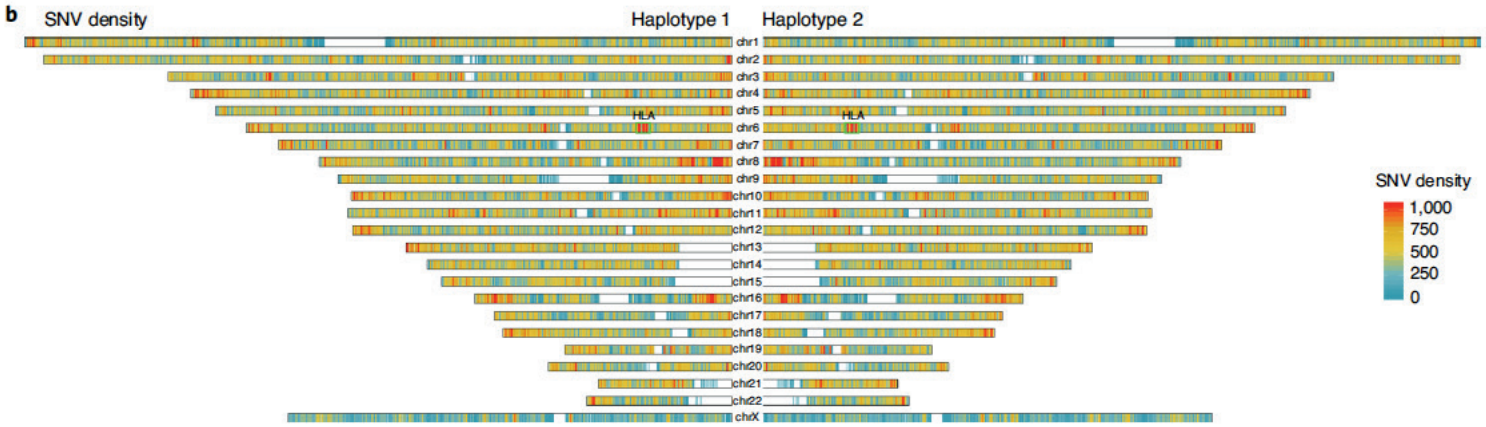


Figure. By combining single cell Strand-seq with results of long-read sequencing technologies a complete phased assembly of a human genome was put together without analysis of parental DNA. Note the high density of SNP's in the HLA region on Chromosome 6 and, more generally, at subtelomeric locations.

THE FUTURE

Work in the Lansdorp lab will from 2021 onwards be performed in the Research Sequencing Facility under supervision of Diana Spierings.

TOP 3 PUBLICATIONS

1. Vollger MR, Logsdon GA, Audano PA, Sulovari A, Porubsky D, Peluso P, Wenger AM, Concepcion GT, Kronenberg ZN, Munson KM, Baker C, Sanders AD, Spierings DCJ, **Lansdorp PM**, Surti U, Hunkapiller MW, Eichler EE. Improved assembly and variant detection of a haploid human genome using single-molecule, high-fidelity long reads. Ann Hum Genet. 2020 Mar;84(2):125-140. doi: 10.1111/ahg.12364. Epub 2019 Nov 11. PMID: 31711268; PMCID: PMC7015760.

2. Hamadeh Z, **Lansdorp P**. RECQL5 at the intersection of replication and transcription. Front Cell Dev Biol. 2020 May 25;8:324. doi: 10.3389/fcell.2020.00324. PMID: 32523948; PMCID: PMC7262407.

3. Porubsky D, Ebert P, Audano PA, Vollger MR, Harvey WT, Marijon P, Ebler J, Munson KM, Sorensen M, Sulovari A, Haukness M, Ghareghani M; Human Genome Structural Variation Consortium, **Lansdorp PM**, Paten B, Devine SE, Sanders AD, Lee C, Chaisson MJP, Korbel JO, Eichler EE, Marschall T. Fully phased human genome assembly without parental data using single-cell strand sequencing and long reads. Nat Biotechnol. 2021 Mar;39(3):302-308. doi:10.1038/s41587-020-0719-5. Epub 2020 Dec 7. PMID: 33288906; PMCID: PMC7954704.

Molecular Neurobiology of Ageing

Ellen Nollen

INTRODUCTION

Loss of protein homeostasis during ageing is thought to accelerate ageing and contribute to age-related neurodegenerative diseases, such as Parkinson's disease and amyotrophic lateral sclerosis (ALS). As a consequence, disease-related proteins become aggregation-prone and self-assemble to form aggregates. The biological mechanisms that drive these structural changes and their toxicity are poorly understood. Our research group aims to identify and understand the genes and molecular pathways involved, which may include targets for therapeutic inhibition to prevent or delay protein toxicity in age-related diseases.

RESEARCH FOCUS

Using genetic screens in *C.elegans* worm models for disease protein aggregation, we have previously identified several drivers of protein toxicity, including the modifiers of aggregation MOAG-4/SERF, MOAG-2/LIR-3 and the tryptophan metabolizing enzyme TDO. Our recent research has focused on identifying mechanisms by which these modifiers act. For example, using a peptide-binding screen on a panel of disease-related aggregation-prone proteins and functional studies in human cell and worm models, we have found that MOAG-4/SERF drives amyloid aggregation via charge interactions[1] (see figure x). In addition, to translate our findings in worms to mammalian brain, we have developed knockout mice for SERF and combined these with mouse models for disease protein aggregation. We find that deletion of SERF alters aggregation and shifts the structure of the aggregates being formed. Together, our studies have identified an evolutionary conserved mechanisms the drives protein aggregation and pin point the inhibition of charge interactions as a strategy to explore in order to prevent or delay protein toxicity in disease.

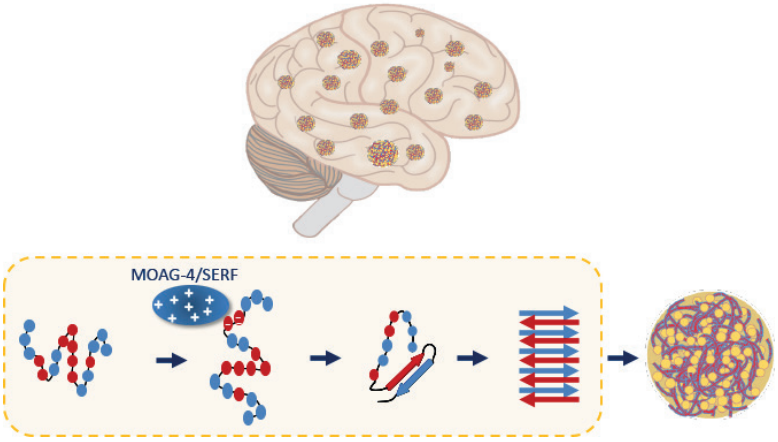


Figure. Charge interactions drive aggregation of neurodegenerative disease-related proteins. Protein aggregates of toxic disease-related proteins are hallmarks of several age-related diseases. In a C.elegans model for disease, we find that removing the charge of a single endogenous factor can suppress aggregation, providing the prevention of such charge interactions as a new directions to explore for interventions.

THE FUTURE

To further exploit the power of C.elegans to identify genes and pathways that regulate protein toxicity, we are developing tools and methods to automate and accelerate screens for ageing related phenotypes, such as motility decline[3]. We use these methods to identified mechanisms involved in motor impairment in ALS and in the toxicity of alpha-synuclein and to explore possible genetic and pharmacological interventions.

Ellen Nollen
Preventing protein toxicity in ageing and age-related diseases.

TOP 3 PUBLICATIONS

1. The cellular modifier MOAG-4/SERF drives amyloid formation through charge complementation. A.Pras, B. Houben, F. A. Aprile, R. Seinstra, R.o Gallardo, L. Janssen, W. Hogewerf, M. De Vleeschouwer, A. Mata-Cabana, M. Koopman, E. Stroo, M. de Vries, S. L. Edwards, M. Vendruscolo, S. F. Falsone, F.Rousseau, J. Schymkowitz, **Nollen EAA**, bioRxiv 2020.12.09.417709; doi: https://doi.org/10.1101/2020.12.09.417709 (in revision at EMBO J.)

2. The Effect of Tryptophan 2,3-Dioxygenase Inhibition on Kynurenine Metabolism and Cognitive Function in the APP23 Mouse Model of Alzheimer's Disease. Sorgdrager F, van Der Ley CP, van Faassen M, Calus E, **Nollen EA**, Kema IP, van Dam D, De Deyn PP.Sorgdrager F, et al. Int J Tryptophan Res. 2020 Dec 28;13:1178646920972657. doi: 10.1177/1178646920972657. PMID: 33447045

3. Assessing motor-related phenotypes of Caenorhabditis elegans with the wide field-of-view nematode tracking platform. Koopman M, Peter Q, Seinstra RI, Perni M, Vendruscolo M, Dobson CM, Knowles TPJ, **Nollen EAA**. Nat Protoc. 2020 Jun;15(6):2071-2106. doi: 10.1038/s41596-020-0321-9. Epub 2020 May 20.Nat Protoc. 2020. PMID: 32433626

Asymmetric Cell Division and Ageing

Judith Paridaen

INTRODUCTION

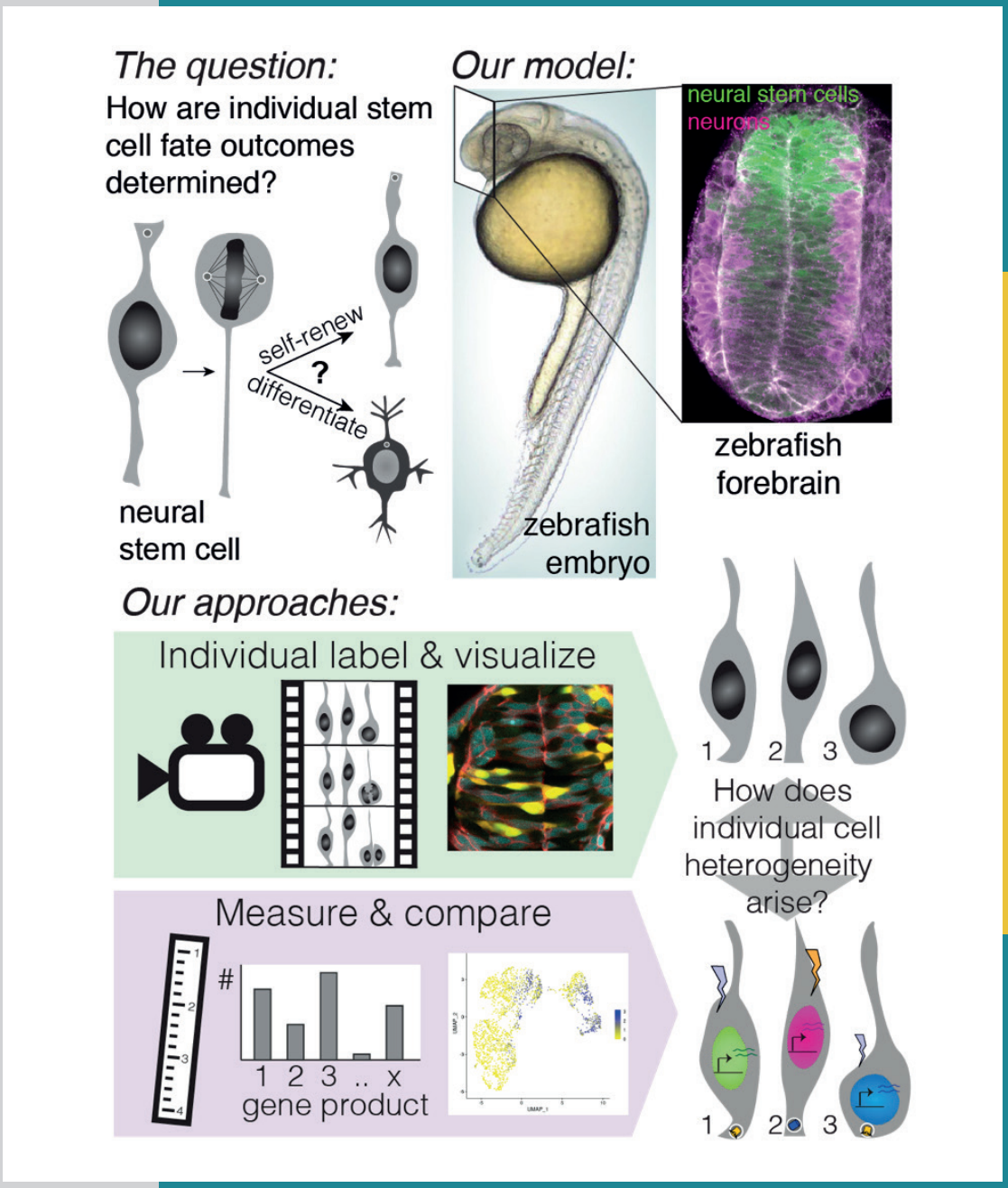
In order to advance our understanding of what goes wrong in disease and during ageing, it is key to understand the mechanisms underlying how individual stem cells develop and function normally. With our research, we aim to contribute to the understanding of which mechanisms are crucial for proper functioning of neural stem cells, and how they can help growing and maintaining a healthy brain.

In our group, we focus on the molecular and cell biology of individual neural stem cells in the developing, adult and ageing brain. These neural stem cells produce most of the cells in the brain including neurons. Interestingly, individual stem cells within a population or tissue can show considerable differences in their individual function, behavior and gene expression profiles. In our research, we aim to find whether and how molecular and cell biological neural stem cell heterogeneity contributes to functional differences in individual neural stem cell output.

RESEARCH FOCUS

Despite individual stem cell heterogeneity, their functional output in normal development is very robust. However, it is unclear how this heterogeneity comes about, and what the contributions of truly noisy or truly random (stochastic) processes is. We hypothesize that hidden features of neural stem cells may explain some of the heterogeneity in cell production/output. We study these questions in the zebrafish developing forebrain (Figure). Here, we investigate how neural stem and progenitors differ in certain cellular properties, such as fate determinant inheritance, signaling state and gene expression profile.

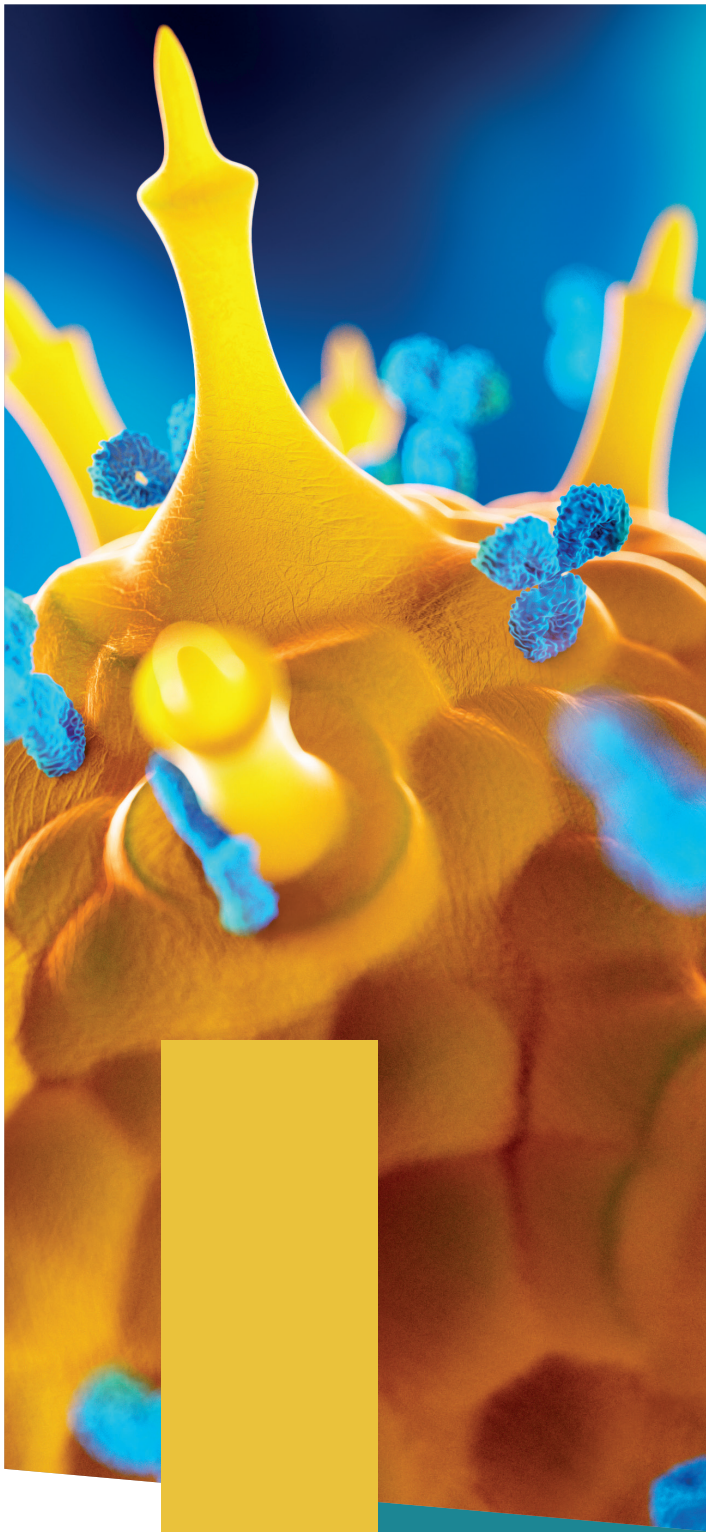
Judith Paridaen
Neural stem cell biology at the single-cell level



One aspect of neural stem cells that we investigate is the regulation of the onset of neurogenic division, which occurs concomitantly to induction of asymmetric cell division. This seems to be related to cell biological changes in junctional complexes and intracellular trafficking. Here, we are currently performing genomic editing to identify candidate regulators of the cell biological changes underlying onset of neurogenesis. Differential segregation of organelles is a mechanism involved in determining the symmetry of stem cell division outcomes. In this context, centrioles are interesting candidates as they are inherently asymmetrical because of their semi-conservative generation as the cell undergoes division. Using microscopy, signaling reporters, genetic markers and genomic editing, we are currently investigating whether centriole inheritance plays a direct role in mediating division asymmetry through inferring asymmetrical signaling states.

THE FUTURE

We aim to generate a comprehensive view of how cell biological and molecular heterogeneities influence individual neural stem cell division outcomes. In the future, we would like to extend our analyses of these heterogeneities to later life stages. For example, we will investigate the selection mechanism of specification of adult neural stem cells. Moreover, closer study of the similarities and differences of the cell biological and molecular mechanisms underlying neural stem cell division outcomes between development and adult stages will aid in unravelling how stem cell function is affected in neurodegenerative disease and ageing.

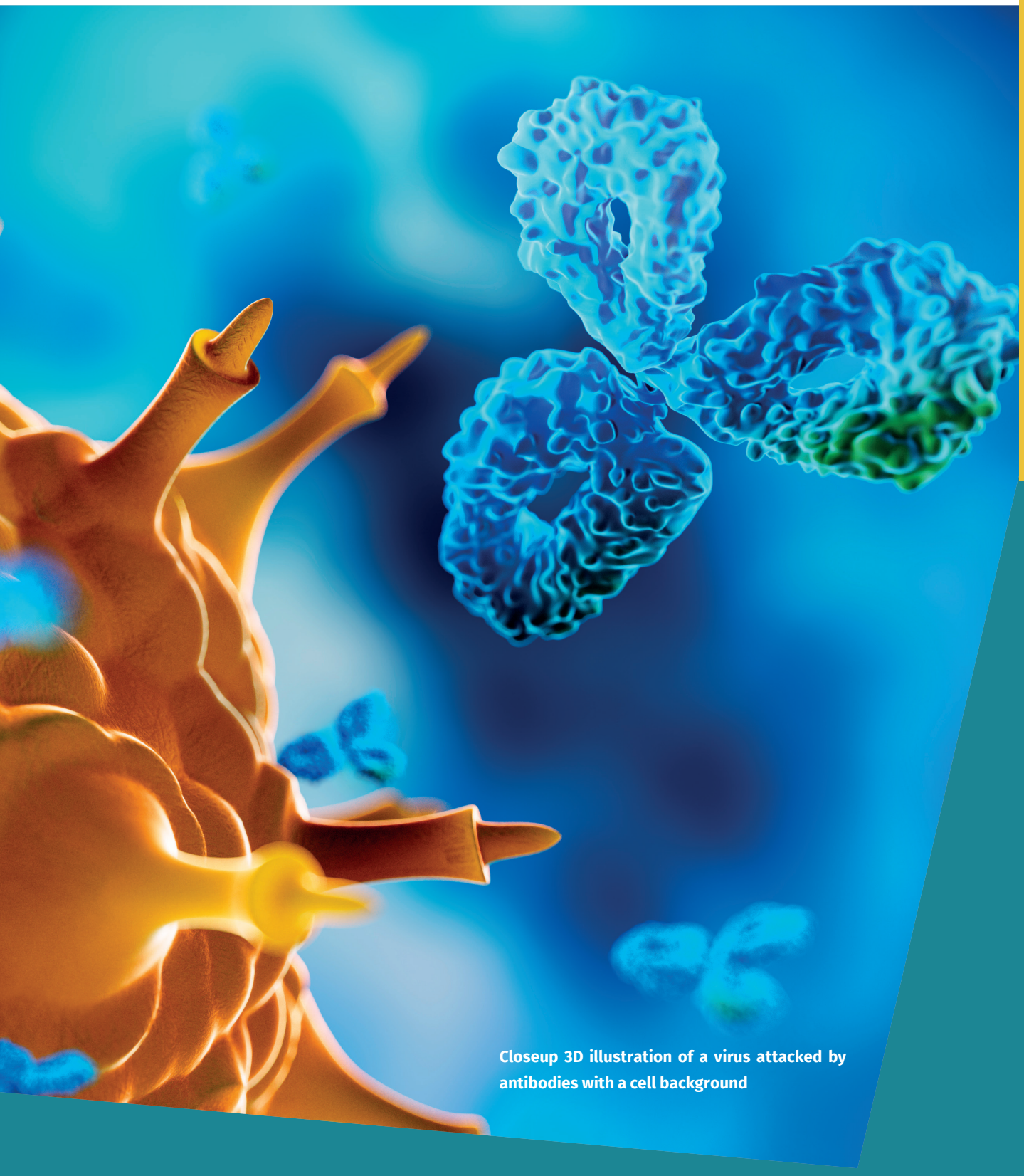


TOP 3 PUBLICATIONS

1. The developmental stage of the medulloblastoma cell-of-origin restricts Hedgehog pathway usage and drug sensitivity. MJ Smit, I Armandari, I Bockaj, TEI Martini, WW Zomerman, Z Siragna, TGJ Meeuwsen-de Boer, FJG Scherpen, MH Schoots, M Ritsema, WFA den Dunnen, EH Hoving, **JTML Paridaen**, G de Haan, V Guryev, S Bruggeman (2021). bioRxiv.

2. YAP activity is necessary and sufficient for basal progenitor abundance and proliferation in the developing neocortex. M Kostic, **JTML Paridaen**, KR Long, N Kalebic, B Langen, N Grübling, P Wimberger, H Kawasaki, T Namba, WB Huttner (2019). Cell reports 27 (4), 1103-1118. e6.

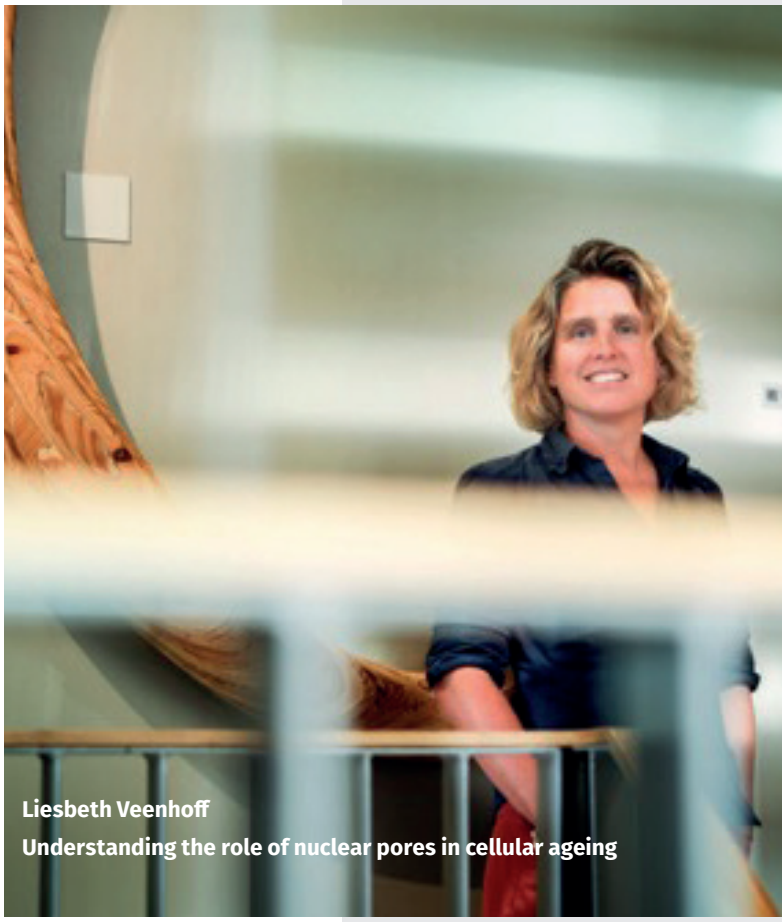
3. Insm1 induces neural progenitor delamination in developing neocortex via downregulation of the adherens junction belt-specific protein Plekha7. S Tavano, E Taverna, N Kalebic, C Haffner, T Namba, A Dahl, M Wilsch-Bräuninger, **JTML Paridaen**, WB Huttner (2018). Neuron 97 (6), 1299-1314. e8.



Closeup 3D illustration of a virus attacked by antibodies with a cell background

Cellular Biochemistry

Liesbeth Veenhoff



Liesbeth Veenhoff
Understanding the role of nuclear pores in cellular ageing

INTRODUCTION

The main research line in the group is to understand the role of the nuclear pore complex (NPC) in ageing. The nuclear pores 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. The NPC's function is compromised in ageing, and we aim to uncover the mechanisms responsible for NPC quality control. Complementing these NPC-centered studies, we aim to contribute to a better understanding of the cellular ageing process in general. Here, our strategy is to 'simply' observe live ageing cells, and quantify new molecular and physicochemical aspects of ageing.

RESEARCH FOCUS

The NPC's function has been related to ageing and several age-related human diseases, most prominently aggregation pathologies. We contributed to this field by showing that in ageing dividing yeast cells, it is the assembly of new NPCs that is compromised. The age-related vulnerabilities of NPCs may well be conserved in humans as they relate, at least in part, to the conserved and unique biochemical characteristics of NPCs, as illustrated in figure 1. First, NPCs are composed of hundreds of proteins, the assembly of which is complex and occasionally goes awry. This is particularly troublesome in ageing dividing cells. Second, once formed, several proteins of the NPC exist for extremely long periods of time; as a result, NPCs are liable to accumulate damage, especially in ageing postmitotic cells. Last, a significant part of the NPC is composed of intrinsically disordered proteins that are prone to aggregation; this may be a problem in the assembly of NPCs as well as the long-term stability of NPCs.

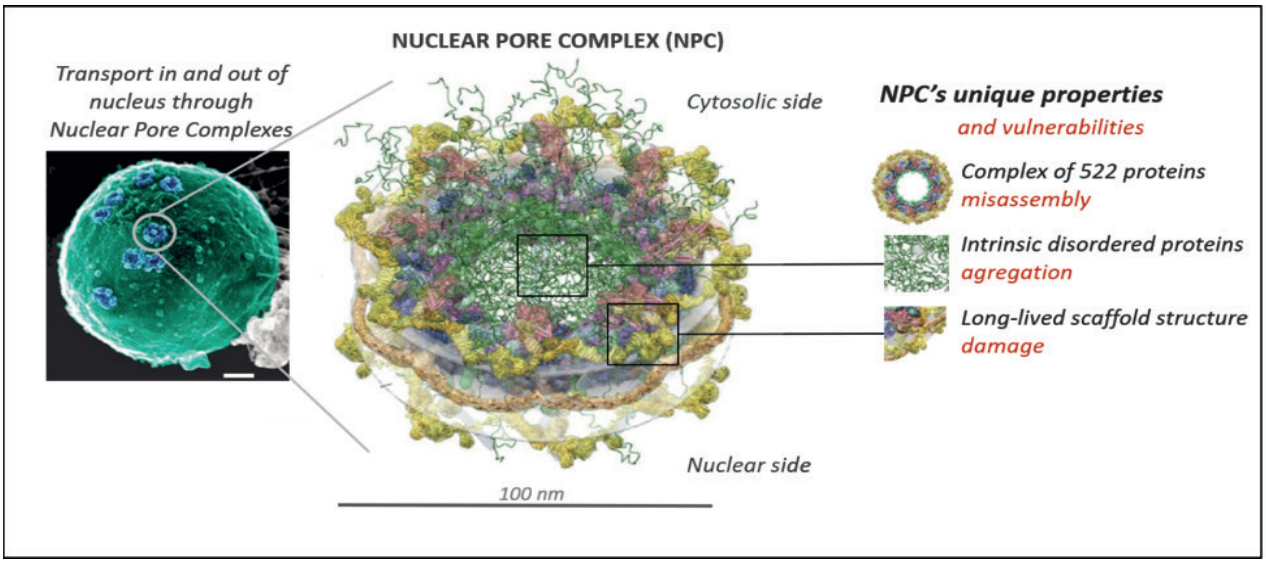


Figure. Left: field emission scanning electron micrograph of yeast nucleus with blue-colored NPCs (adapted from E. Kiseleva, Nature Cell Biology, 2004). Middle: structure of yeast NPC (adapted from Kim et al., Nature, 2018), highlighting its size and complexity and the presence of intrinsically disordered proteins (in green) and long-lived proteins in the scaffold (other colors).

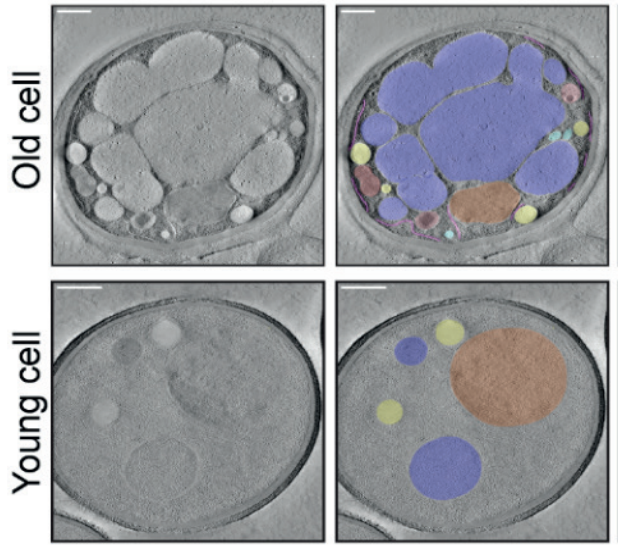


Figure. Single slices of electron microscopic tomograms of a young and old cell; right panes show an overlay to emphasize organelles where nuclei are orange, vacuoles blue and lipid droplets yellow. Scale bars are 500 nm.

Contributing to a more comprehensive understanding of the ageing process in general, we mapped physicochemical aspects of the cytosol of ageing cells. The rational being that aspects like the pH, ionic strength, redox state, or crowding impact the functionality of all biomolecules, and a failure to maintain homeostasis of these physicochemical aspects would broadly impact all hallmarks of ageing. In a collaborative effort, we provided an initial framework and showed how pH and crowding, on the scale of proteins and on the scale of organelles, changes in the cytosol of mitotically ageing yeast cells. The image illustrates that major changes occur in organellar crowding.

THE FUTURE

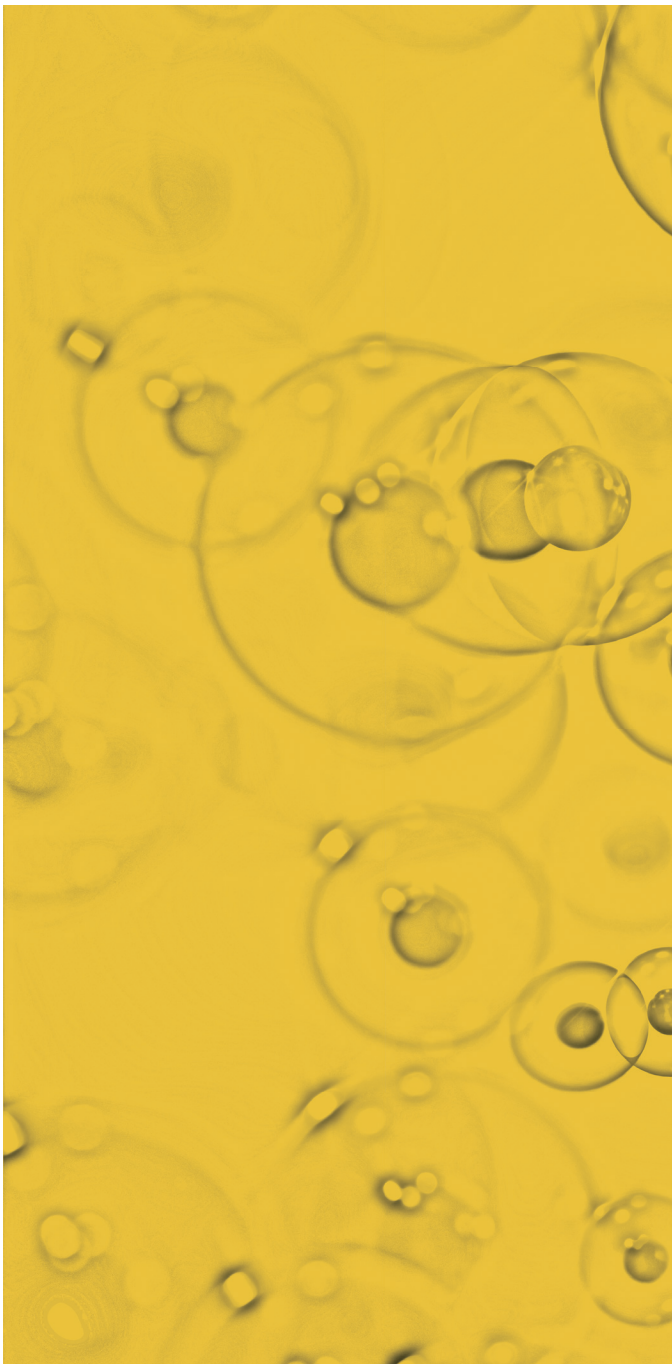
We will continue to design our research from the viewpoint that studying "biology in time" is an unbiased way to reveal fundamental knowledge; knowledge that is needed to combat age-related diseases. Specifically, we aim to identify the proteins that detect damaged NPCs, to know the destiny of damaged NPCs, and to uncover the mechanisms that prevent damage to NPCs. Particularly the intrinsically disordered inside of the NPC is interesting to study, as it appears that mechanisms that guard it's structural state, may also guard other intrinsically disordered protein, such as those related to aggregation pathologies. Together, the planned research aims to uncover how the quality control of NPCs and intrinsically disordered proteins can be better safeguarded in ageing.

TOP 3 PUBLICATIONS

1. Age-dependent deterioration of nuclear pore assembly in mitotic cells decreases transport dynamics. Rempel IL, Crane MM, Thaller DJ, Mishra A, Jansen DP, Janssens G, Popken P, Akşit A, Kaeberlein M, van der Giessen E, Steen A, Onck PR, Lusk CP, **Veenhoff LM**. Elife. 2019 Jun 3;8:e48186.

2. A physicochemical perspective of aging from single-cell analysis of pH, macromolecular and organellar crowding in yeast. Mouton SN, Thaller DJ, Crane MM, Rempel IL, Terpstra OT, Steen A, Kaeberlein M, Lusk CP, Boersma AJ, **Veenhoff LM**. Elife. 2020 Sep 29;9:e54707.

3. Flexible and Extended Linker Domains Support Efficient Targeting of Heh2 to the Inner Nuclear Membrane. Rempel IL, Popken P, Ghavami A, Mishra A, Hapsari RA, Wolters AHG, Veldsink AC, Klaassens M, Meinema AC, Poolman B, Giepmans BNG, Onck PR, Steen A, **Veenhoff LM**. Structure. 2020 Feb 4;28(2):185-195.e5.



Macromolecules and Interactomes

John LaCava

John LaCava
We study the contributions of dysregulated macromolecular interactions to human diseases.

INTRODUCTION

Our group has a specific technology focus: improving methods for interactome analyses. We specialize in the development of affinity proteomic approaches. Presently, we aim to translate our research tools, which explore and characterize protein interactions within multi-component macromolecular complexes, towards the clinic: for example, identifying differences in protein complexes between healthy and diseased patient tissues. In addition to our technology focus, we explore different biological foci - primary among them, the characterization of human LINE-1 retrotransposons. Over evolutionary time, LINE-1 sequences have come to compose a large proportion of the human genome and the latest studies suggest clinical implications for LINE-1 expression in autoimmunity and cancer. Presently, we are exploring the roles of LINE-1 in systemic lupus erythematosus and in colorectal cancers. We are also exploring emerging connections between LINE-1 and aging-related diseases, in particular Alzheimer's disease.

RESEARCH FOCUS

Proteins and the multi-component macromolecular complexes they form are the effectors of cell biology. Studying cell biology therefore requires the ability to isolate distinct proteins along with other constituents of their associated macromolecules. Affinity capture techniques have greatly facilitated the discovery, purification, and characterization of endogenous protein complexes. These techniques leverage reagents able to target and capture proteins of interest assembled with physiological binding partners, from cell extracts. Although affinity capture has matured steadily as an approach, many technical shortcomings still limit its efficacy in the retrieval of intact, endogenous macromolecules. We address these challenges and develop mass spectrometry-based, affinity proteomic techniques for interactome mapping. We place special emphasis on approaches that also enable downstream structural and biochemical studies of purified macromolecules. In the context of this technology focus, we are agnostic to the specific disease or underlying biology and collaborate widely with e.g., surgeons and clinicians on diverse projects.

Long Interspersed Element 1 (LINE-1, L1) is a retrotransposon. As a result of its "copy and paste" method of proliferation, L1 activity has contributed a large proportion of DNA to the human genome (including those sequences mobilized by L1, such as Alus). Since the insertion of new DNA sequences into the genome is inherently mutagenic, understanding the lifecycle of L1 is crucial to understanding human genome dynamics and cell biology. L1 DNA proliferates through an RNA intermediate whose protein products bind the L1 RNA to form a ribonucleoprotein (RNP) complex. L1 RNPs also co-opt and contend with a variety of host factors that facilitate or repress L1's ability to reach the chromatin and reintegrate into the genome. Thus, different subpopulations of L1s consist of different assortments of constituents, depending both on the subcellular

compartment and on the pathway being traversed (proliferation or repression). Our goal is to expand our breadth of knowledge concerning the L1 interactome, and we study the structural and biochemical properties of L1 RNPs. In doing so, we also explore LINE-1 contributions to pathobiology.

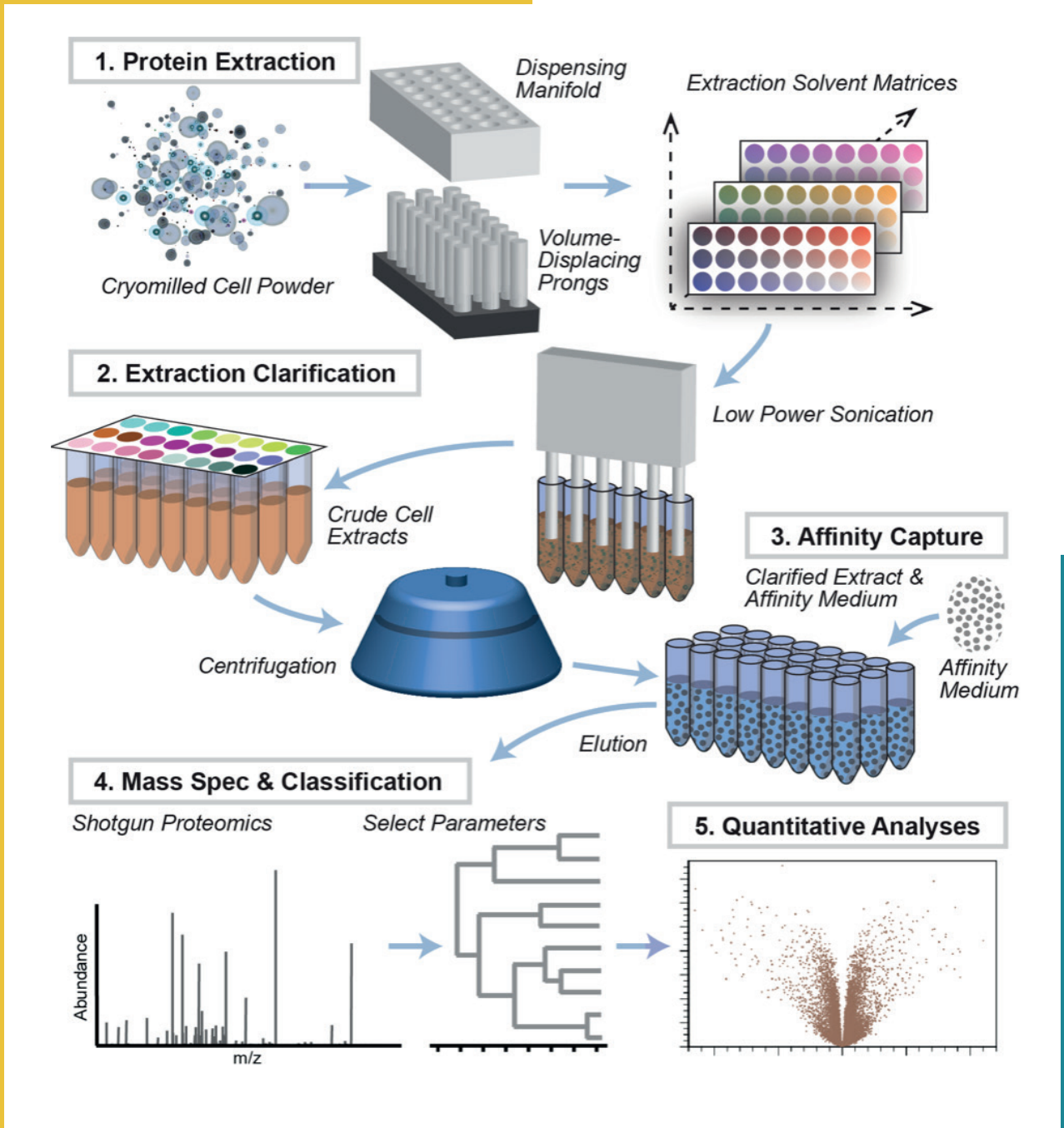


Figure. Methodological approach. Cryomilled cell powders are distributed with a dispensing manifold and macromolecules are extracted with different extraction solutions (1). Brief sonication is applied to disperse and homogenize the extracts (2). After clarifying the extracts by centrifugation, affinity capture is performed (3) and protein eluates are subjected to MS analysis (4) and data processing

THE FUTURE

We are expanding our interactome charting approaches to include *in situ* proximity labeling. We will cross-reference macromolecular compositions defined by immunoprecipitation, which transfers macromolecules out of cells prior to identification, with those obtained by 'marking' the associating proteins while they still reside within cellular milieux. Taken together, the combination of these technique will provide complementary data to inform more comprehensive studies of protein complexes. We anticipate that proximity labeling may also allow us to make judicious use of FFPE tissue banks, whereas immunoprecipitation is carried out on fresh-frozen tissue, which is comparatively rare.

Our work with LINE-1 is growing in numerous directions, chief among them, our development of a sensitive, quantitative biomarker assay for the detection of LINE-1 ORF proteins in e.g., serum and cerebrospinal fluid. This assay is enabling us to explore diagnostic implications of LINE-1 expression in cancers, autoimmunity, and neurodegeneration, which we are pursuing in on-going research.

TOP 3 PUBLICATIONS

1. Dou, Y. *et al.* Affinity proteomic dissection of the human nuclear cap-binding complex interactome. *Nucleic Acids Res* gkaa743 (2020)

2. Ardeljan, D. *et al.* LINE-1 ORF2p expression is nearly imperceptible in human cancers. *Mobile DNA* **11**, 1–19 (2020).

3. Taylor, M. S. *et al.* Dissection of affinity captured LINE-1 macromolecular complexes. *eLife* **7**, e30094 (2018).

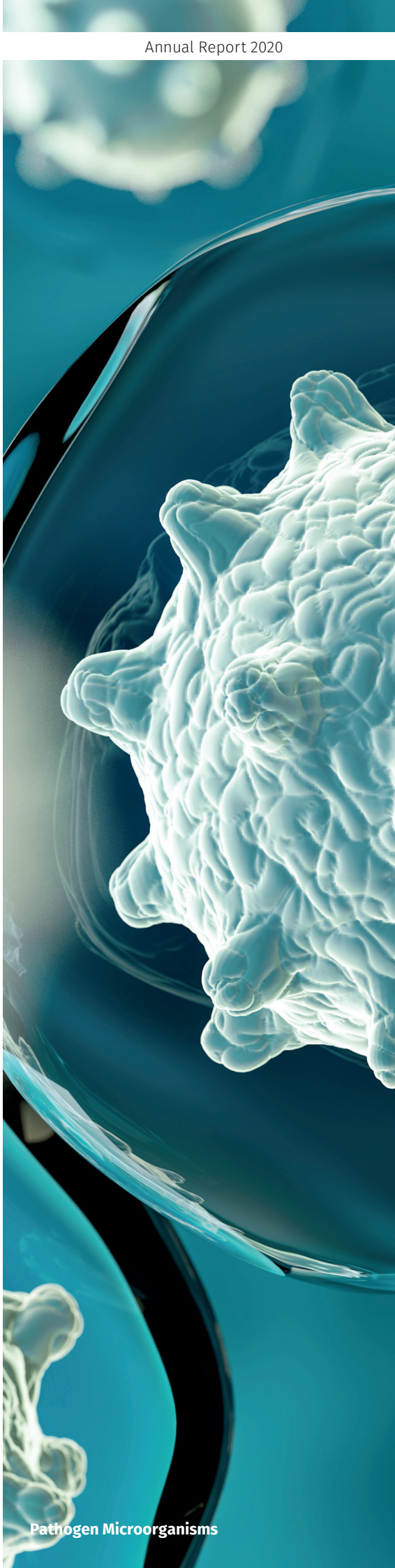
Highlights

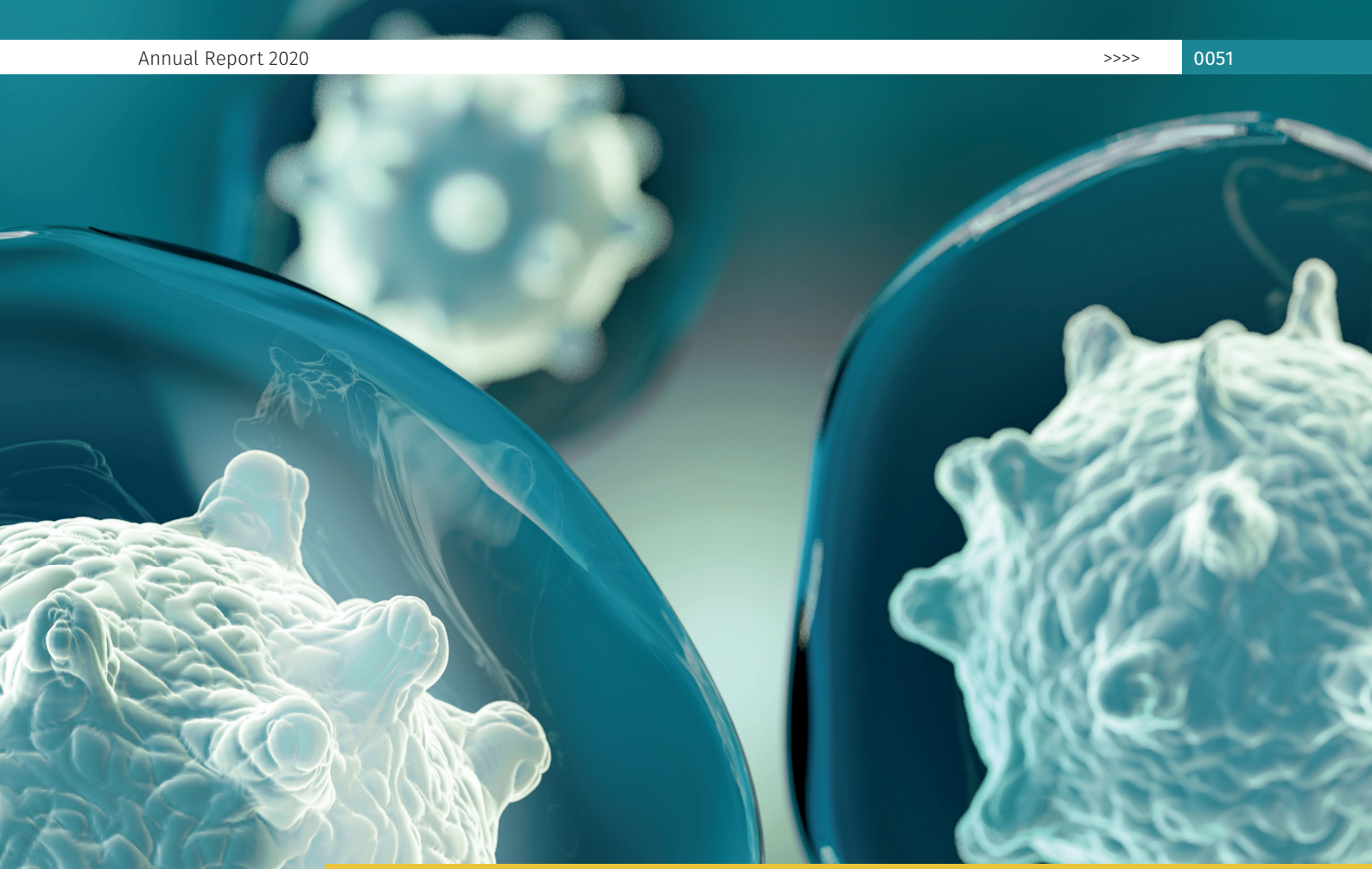
2020: Highlights

This section reports a selected number of achievements that have been accomplished by ERIBA staff in 2020.

Scientific Publications

In 2020, ERIBA scientists published a record number of 71 papers in reputed scientific journals. Many of the published papers were the result of fruitful national and international collaborations employing a multidisciplinary approach. Numerous joint projects were initiated within ERIBA, between scientists in ERIBA and research groups at the University Medical Center Groningen and the Faculty of Science and Engineering of the University of Groningen, and with international partners. A snapshot of ERIBA's team scientific achievements are highlighted on page no. 52.


Pathogen Microorganisms



2020 Highlights

Cor Calkhoven's group

C. Niehrs, **C. F. Calkhoven** (2020) Emerging Role of C/EBPβ and Epigenetic DNA Methylation in Ageing. Trends in genetics.

Changes in epigenetic DNA methylation are the most promising predictor of biological age and lifespan in humans, but whether methylation changes affect ageing is unresolved. Here, we discuss converging data, which indicate that one mode by which aberrant DNA methylation can affect ageing is via CCAAT/enhancer binding protein beta (C/EBPb). This basic leucine-zipper (bZIP) transcription factor is controlled by the lifespan regulator mechanistic/mammalian target of rapamycin complex 1 (mTORC1) and plays an important role in energy homeostasis and adipose tissue differentiation. Emerging evidence indicates that access of C/EBPb proteins to cognate binding sites is regulated by DNA demethylation via ten-eleven translocation (TET) methylcytosine dioxygenases and their adaptor proteins growth arrest and DNA damage-inducible protein 45 alpha (GADD45a) and inhibitor of growth 1 (ING1). We discuss the emerging causal nexus between C/EBPb, energy metabolism, and DNA demethylation in organismal ageing.

Michael Chang's group

Novarina D, Desai R, Vaisica JA, Ou J, Bellaoui M, Brown GW, **Chang M** (2020) A Genome-Wide Screen for Genes Affecting Spontaneous Direct-Repeat Recombination in Saccharomyces cerevisiae. G3 (Bethesda).

Homologous recombination is an important mechanism for genome integrity maintenance, and several homologous recombination genes are mutated in various cancers and cancer-prone syndromes. However, since in some cases homologous recombination can lead to mutagenic outcomes, this pathway must be tightly regulated, and mitotic hyper-recombination is a hallmark of genomic instability. We performed two screens in Saccharomyces cerevisiae for genes that, when deleted, cause hyper-recombination between direct repeats. One was performed with the classical patch and replica-plating method. The other was performed with a high-throughput replica-pinning technique that was designed to detect low-frequency events. This approach allowed us to validate the high-throughput replica-pinning methodology independently of the replicative aging context in which it was developed. Furthermore, by combining the two approaches, we were able to identify and validate 35 genes whose deletion causes elevated spontaneous direct-repeat recombination. Among these are mismatch repair genes, the Sgs1-Top3-Rmi1 complex, the RNase H2 complex, genes involved in the oxidative stress response, and a number of other DNA replication, repair and recombination genes. Since several of our hits are evolutionarily conserved, and repeated elements constitute a significant fraction of mammalian genomes, our work might be relevant for understanding genome integrity maintenance in humans.

Floris Foijer's group

Schukken KM , Lin YC, Bakker PL , Schubert M, Preuss SF, Simon JE , van den Bos H, Storchova Z, Colomé-Tatché M, Bastians H, Spierings DCJ, **Foijer F** (2020) Altering Microtubule Dynamics Is Synergistically Toxic With Spindle Assembly Checkpoint Inhibition. Life Science Alliance.

Chromosome instability (CIN) and aneuploidy are hallmarks of cancer. As the majority of cancers are aneuploid, targeting aneuploidy or CIN may be an effective way to target a broad spectrum of cancers. In this study, we performed two small molecule compound screens to identify drugs that selectively target cells that are aneuploid or exhibit a CIN phenotype. We found that aneuploid cells are much more sensitive to the energy metabolism regulation drug ZLN 005 than their euploid counterparts. Furthermore, cells with an ongoing CIN phenotype, induced by spindle assembly checkpoint (SAC) alleviation, were significantly more sensitive to the Src kinase inhibitor SKI606. We found that inhibiting Src kinase increases microtubule polymerization rates and, more generally, that deregulating microtubule polymerization rates is particularly toxic to cells with a defective spindle assembly checkpoint. Our findings suggest that tumors with a dysfunctional SAC are particularly sensitive to microtubule poisons and, vice versa, that compounds alleviating the SAC provide a powerful means to treat tumors with deregulated microtubule dynamics.

Gerald de Haan's group

Jacobs S, Ausema A, Zwart E, Weersing E, Kingma MJ, El Menshawi YAS, **de Haan G**, Bystrykh LV, Belderbos ME. (2020) Quantitative distribution of patient-derived leukemia clones in murine xenografts revealed by cellular barcodes. Leukemia.

.In this study we developed a DNA barcoding method to label individual leukemic stem cells derived from pediatric patients. These barcoded leukemic stem cells were then transplanted in immune deficient mice to assess where leukemic stem cells and their progeny localize. We show that, at end-stage disease, murine xenografts harbor millions of human leukemia cells, with ~10% localized in blood and pelvis. Leukemia cells are derived from hundreds of leukemia-propagating cells, which are asymmetrically distributed across skeletal sites. We demonstrate that, sampling of a single-site allows for half of the LPC clones to remain undetected. Therefore, multi-site sampling of xenografts will increase the yield of cells for experimental analysis and provide a more in-depth view of the clonal heterogeneity.

Liesbeth Veenhoff's group

Mouton SN, Thaller DJ, Crane MM, Rempel IL, Terpstra OT, Steen A, Kaeberlein M, Lusk CP, Boersma AJ, **Veenhoff LM**. (2020) A physicochemical perspective of aging from single-cell analysis of pH, macromolecular and organellar crowding in yeast. Elife.

To properly function, the molecules in cells need a specific environment. Sara Mouton and colleagues show that the characteristics of this environment changes with ageing. The researchers think this physicochemical aspect of ageing complements the traditional views of ageing that are centered around the molecules themselves.

In short, we show that the cytosol of yeast cells acidifies modestly in early aging and sharply after senescence. Using a macromolecular crowding sensor optimized for long-term FRET measurements, we show that crowding is rather stable. Additionally, in aged cells, we observe drastic changes in organellar volume, leading to crowding on the micrometer scale, which we term organellar crowding. Our measurements provide an initial framework of physicochemical parameters of replicatively aged yeast cells.

For a 10 minute explanation of the paper please see https://www.youtube.com/watch?v=mD0qCPCivzo&t=2823s

Peter Lansdorp's group

Porubsky D, Ebert P, Audano PA, Vollger MR, Harvey WT, Marijon P, Ebler J, Munson KM, Sorensen M, Sulovari A, Haukness M, Ghareghani M; Human Genome Structural Variation Consortium, **Lansdorp PM**, Paten B, Devine SE, Sanders AD, Lee C, Chaisson MJP, Korbel JO, Eichler EE, Marschall T. (2020) Fully phased human genome assembly without parental data using single-cell strand sequencing and long reads. Nat Biotechnol.

Work by former ERIBA student David Porubsky highlights the power of the single cell Strand-seq method developed by the Lansdorp lab for genome analysis. Human genomes are typically assembled as consensus sequences that lack information on parental haplotypes. In this paper David et al., describe a reference-free workflow for diploid de novo genome assembly that combines the chromosome-wide phasing and scaffolding capabilities of single-cell strand sequencing with continuous long-read or high-fidelity sequencing data. Employing this strategy, a completely phased de novo genome assembly for both parental haplotypes was established in the absence of any parental data. The haplotype assemblies are accurate and provide fully phased single-nucleotide variants, indels and structural variants."

Marco Demaria's group

Casciaro F, Borghesan M, Beretti F, Zavatti M, Bertucci E, Follo MY, Maraldi T, **Demaria M.** (2020) Prolonged hypoxia delays aging and preserves functionality of human amniotic fluid stem cells. Mechanisms of ageing and development.

Human amniotic stem cells (hAFSCs) are an emerging tool in regenerative medicine because they have the ability to differentiate into various lineages and efficiently improve tissue regeneration with no risk of

tumorigenesis. A major limitation for the use of hAFSCs in regenerative medicine is their limited replicative potential. In this study, we show that low oxygen (1% O_2) extends stemness and proliferative features, and delays induction of senescence-associated markers. Hypoxic hAFSCs activate a metabolic shift and increase resistance to pro-apoptotic stimuli. Moreover, we observe that cells at low oxygen remain capable of osteogenesis for prolonged periods of time, suggesting a more youthful phenotype. Together, these data demonstrate that low oxygen concentrations might improve the generation of functional hAFSCs for therapeutic use by delaying the onset of cellular aging.

Ellen Nollen's group

Koopman M, Peter Q, Seinstra RI, Perni M, Vendruscolo M, Dobson CM, Knowles TPJ, **Nollen EAA** (2020) Assessing motor-related phenotypes of Caenorhabditis elegans with the wide field-of-view nematode tracking platform. Nature Protocols

How aging causes relatively common diseases such as Alzheimer and Parkinson is still a mystery. Since toxic structural changes in proteins are likely to be responsible, we investigated biological mechanisms that could drive such changes. We made use of a modifying factor called MOAG-4/SERF, which we previously found to accelerate structural changes and aggregation of several disease-related proteins. Through a peptide-binding screen, we found that MOAG-4/SERF acts on negatively charged protein regions. The abundance of such regions in the disease-related proteins may explain why MOAG-4/SERF has its effect. Removing positive charge in MOAG-4/SERF was sufficient to suppresses protein aggregation in models for disease. We propose that blocking charge-interactions with MOAG-4/SERF or similar modifiers deserves exploration as an approach to treat age-related protein toxicity.

Eugene Berezikov's group

Wudarski J, Egger B, Ramm SA, Schärer L, Ladurner P, Zadesenets KS, Rubtsov NB, Mouton S, **Berezikov E**. (2020) The free-living flatworm Macrostomum lignano. EvoDevo.

Macrostomum lignano is a free-living flatworm that is emerging as an attractive experimental animal for research on a broad range of biological questions. This comprehensive review summarizes the current state-of-the art of this model organism.

John LaCava's group

Dou Y, Kalmykova S, Pashkova M, Oghbaie M, Jiang H, Molloy KR, Chait BT, Rout MP, Fenyö D, Jensen TH, Altukhov I, **LaCava J**. (2020) Affinity proteomic dissection of the human nuclear cap-binding complex interactome. Nucleic Acids Research.

Our lab has developed an interactome screening method that improves the coverage of protein-protein interactions discovered by immunoprecipitation / mass spectrometry (an affinity proteomic approach). In an effort to explore missing information and disambiguate protein-protein associations in human mRNA processing, we applied our screen to human nuclear cap binding proteins (NCBP1, 2, and 3). NCBP3 is a newly described protein with unclear roles in mRNA processing. In two papers published together in the same issue of Nucleic Acids Research (one of which is highlighted here) we described the similarities and differences between the protein associations of the canonical "cap-binding" NCBP1 and NCBP2 proteins and also compare them to the newly described NCBP3.

A 5',7-methylguanosine cap is a quintessential feature of RNA polymerase II-transcribed RNAs, and a textbook aspect of co-transcriptional RNA processing. The cap is bound by the cap-binding complex (CBC), canonically consisting of nuclear cap-binding proteins 1 and 2 (NCBP1/2). Interest in the CBC has recently renewed due to its participation in RNA-fate decisions via interactions with RNA productive factors as well as with adapters of the degradative RNA exosome. A novel cap-binding protein, NCBP3, was recently proposed to form an alternative CBC together with NCBP1, and to interact with the canonical CBC along with the protein SRRT. The theme of post-transcriptional RNA fate, and how it relates to co-transcriptional ribonucleoprotein assembly, is abundant with complicated, ambiguous, and likely incomplete models. In an effort to clarify the compositions of NCBP1-, 2- and 3-related macromolecular assemblies, we have applied an affinity capture-based interactome

screen where the experimental design and data processing have been modified to quantitatively identify interactome differences between targets under a range of experimental conditions. This study generated a comprehensive view of NCBP-protein interactions in the ribonucleoprotein context and demonstrates the potential of our approach to benefit the interpretation of complex biological pathways.

Victor Guryev's group

Brandsma, **Guryev** et al, 2020. Integrated proteogenomic approach identifying a protein signature of COPD and a new splice variant of SORBS1. Thorax 75:180-183. doi: 10.1136/thoraxjnl-2019-213200

Chronic obstructive pulmonary disease (COPD) is a progressive ageing-related disease and is about to make its entry to the 'pedestal' as the third leading cause of death with a yearly toll of over 3 million human lives. Despite the roles of some of the genetic (AAT-deficiency) and environmental (smoking, air pollution) factors are well established, our knowledge about all players in disease development and interactions among them is very fragmentary.

In this collaborative study researchers from ERIBA, departments of pathology and medical biology; pulmonary diseases; and analytical biochemistry performed multi-omics characterization of molecular changes in COPD.

Using integrated proteomic and RNA sequencing analysis of COPD and control lung tissues, we identified a protein signature in COPD associated with extracellular matrix changes and a potential regulatory role for SUMO2. Furthermore, we identified 61 differentially expressed novel, non-reference, peptides in COPD compared with control lungs. This included two peptides encoding for a new splice variant of SORBS1, of which the transcript usage was higher in COPD compared with control lungs. These explorative findings and integrative proteogenomic approach open new avenues to further unravel the pathology of COPD.

Grants

In 2020 ERIBA secured € 4.49 million Euros in grants. This included prestigious grants from the Nederlandse Organisatie voor Wetenschappelijk (NWO) and grants secured through public-private partnerships. Detailed information about ERIBA grants and funding can be found on page 70.

Graduations

In 2020, two young scientists graduated from ERIBA as PhD students, and have moved to new positions.

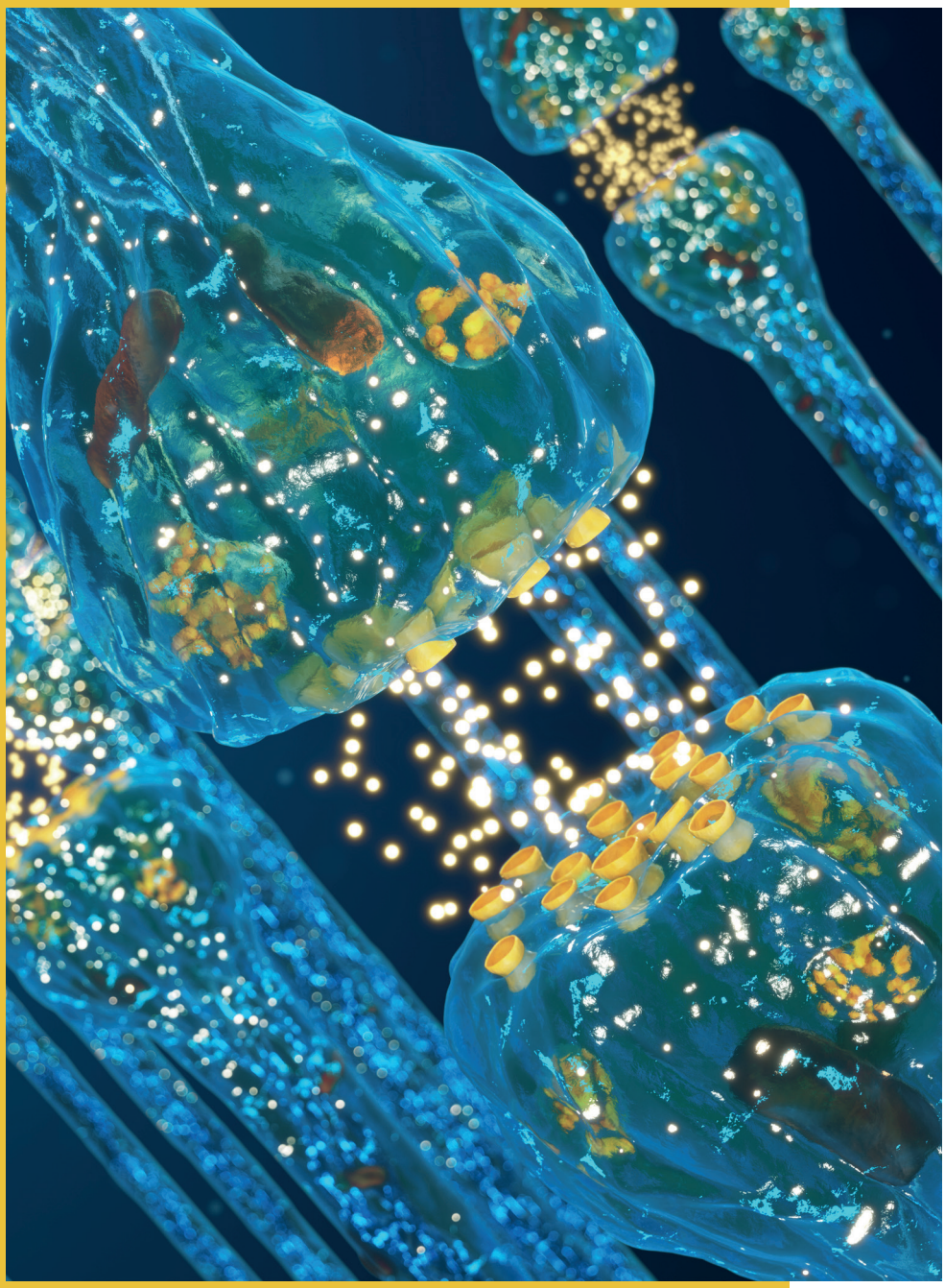
Klaske Schukken (Floris Foijer) successfully defended her PhD thesis. She is currently a post-doctoral fellow at Cold Spring Harbor, New York, USA.

Boshi Wang (Marco Demaria) after defending his PhD thesis is continuing as a post-doctoral fellow at ERIBA.

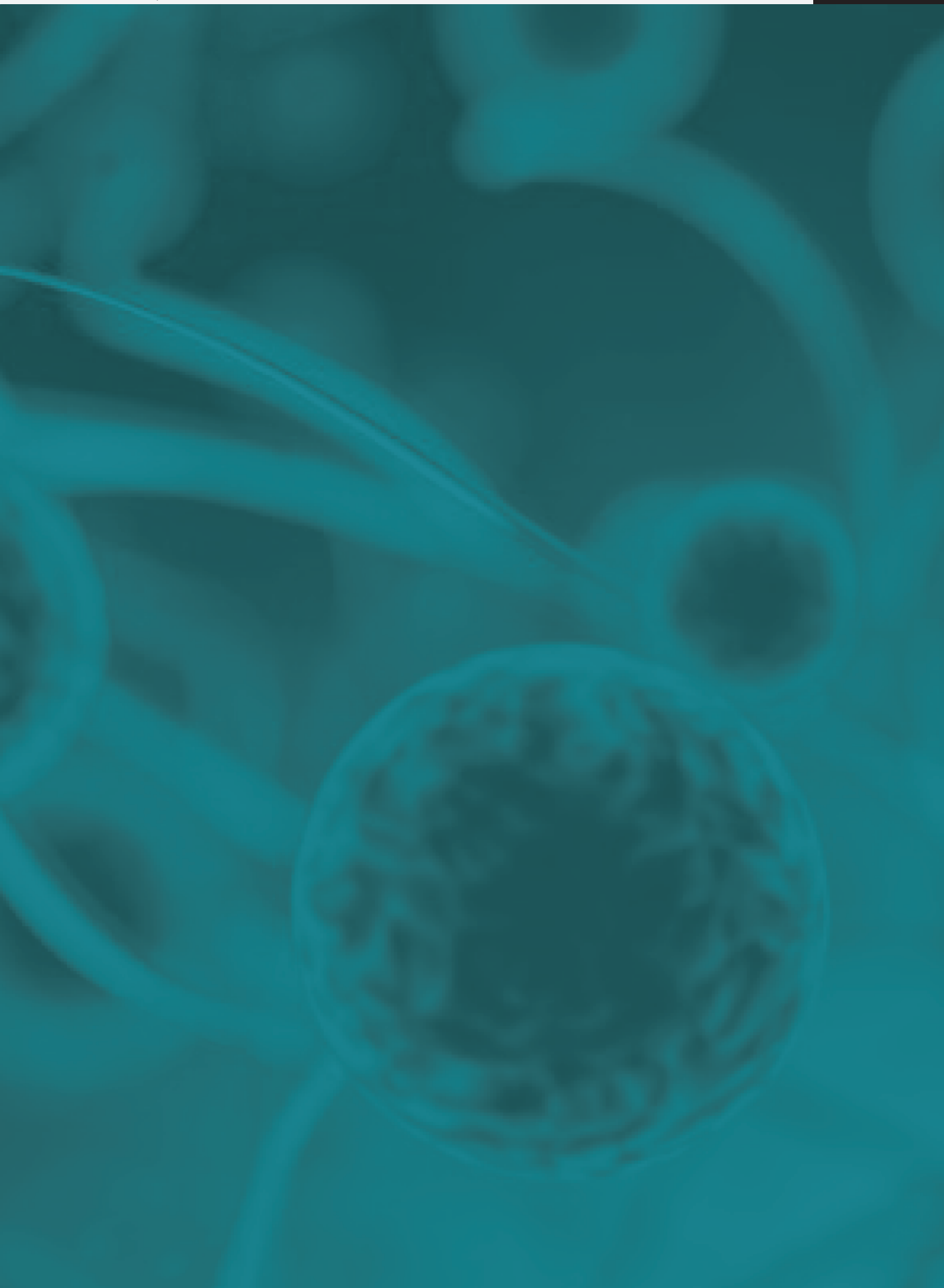
Scientific Dissemination and Outreach

ERIBA has been actively involved in scientific dissemination and public outreach. ERIBA participates in various outreach activities to motivate and create potential future scientists such as high school students, general public, bachelor and master students. Keeping our commitment to the cause, ERIBA scientists participated in the fundraising campaigns, provided leadership courses.

To nurture the young minds, our scientists also spoke at various national and international seminars sharing their experience(s) with the participants. Our scientists also hosted webinars to share scientific knowledge with the young crowd so that the scientists of tomorrow can be motivated. See page 78 for details.



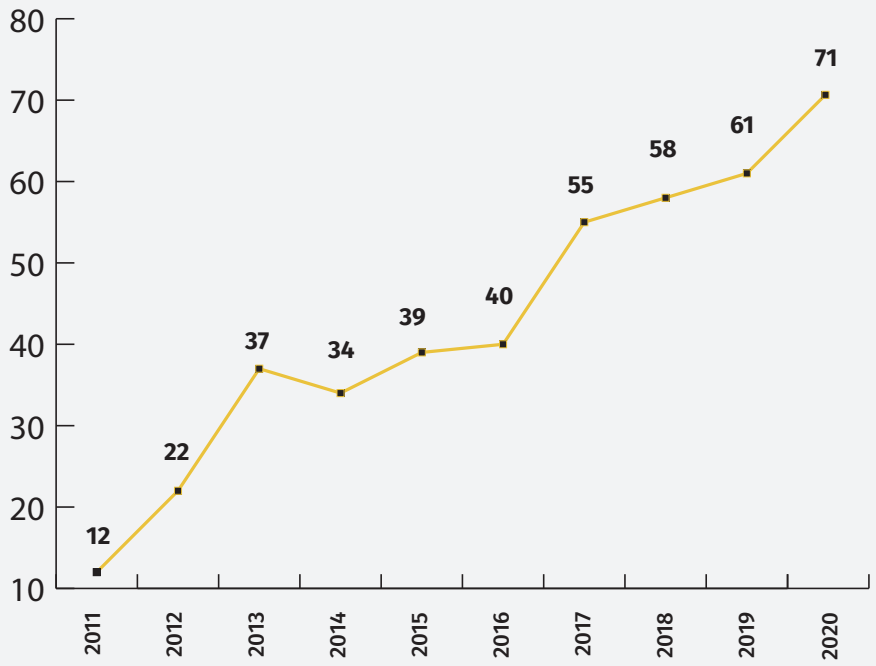
Facts & Analysis



Facts & Figures

NUMBER OF SCIENTIFIC PUBLICATIONS IN 2020

71



Publications:

Publications per Research Group

Laboratory of Gene Regulation in Ageing and Age-related Diseases

Group Leader: Cor Calkhoven

C. Niehrs, **C. F. Calkhoven** (2020) Emerging Role of C/EBPβ and Epigenetic DNA Methylation in Ageing. Trends in genetics.

van der Voort PHJ, Moser J, Zandstra DF, Muller Kobold AC, Knoester M, **Calkhoven C.F.**, Hamming I, van Meurs M. (2020) Leptin levels in SARS-CoV-2 infection related respiratory failure: A cross-sectional study and a pathophysiological framework on the role of fat tissue. Heliyon.

Preprints:

Müller, C.*, Zidek, L.M.*, Eichwald, S. and **Calkhoven, C.F.** (2020) C/EBPβ regulates hypertrophic versus hyperplastic fat tissue growth. *BioRxiv.* DOI: 10.1101/2020.09.02.278911

Ackermann, T., Zuidhof, H.R., Kortman, G., Rutten, M.G.S., Broekhuis, M., Zaini, M.A., Hartleben, G. and **Calkhoven, C.F.** (2020) C/EBPβ-LIP induces the malate aspartate shuttle causing reliance on glycolysis for NADH regeneration and cell survival. *BioRxiv.* DOI: 10.1101/2020.10.09.333104

Laboratory of Molecular Neurobiology of Ageing

Group Leader: Ellen Nollen

Koopman M, Peter Q, Seinstra RI, Perni M, Vendruscolo M, Dobson CM, Knowles TPJ, **Nollen EAA** (2020) Assessing motor-related phenotypes of Caenorhabditis elegans with the wide field-of-view nematode tracking platform. Nature Protocols

Preprints:

Anita Pras, Bert Houben, Francesco A. Aprile, Renée Seinstra, Rodrigo Gallardo, Leen Janssen, Wytse Hogewerf, Matthias De Vleeschouwer, Alejandro Mata-Cabana, Mandy Koopman, Esther Stroo, Minke de Vries, Samantha Louise Edwards, Michele Vendruscolo, S. Fabio Falsone, Frederic Rousseau, Joost Schymkowitz, **Ellen A. A. Nollen** doi: https://doi.org/10.1101/2020.12.09.417709 The cellular modifier MOAG-4/SERF drives amyloid formation through charge complementation

Observation of an α-synuclein liquid droplet state and its maturation into Lewy body-like assemblies Maarten C. Hardenberg, Tessa Sinnige, Sam Casford, Samuel Dada, Chetan Poudel, Lizzy Robinson, Monika Fuxreiter, Clemens Kaminksi, Gabriele S. Kaminski Schierle, **Ellen A. A. Nollen**, Christopher M. Dobson, Michele Vendruscolobio Rxiv 2020.06.08.140798; doi:https://doi.org/10.1101/2020.06.08.140798

Laboratory of Stem cell regulation and mechanisms of regeneration

Group Leader: Eugene Berezikov

Wudarski J, Egger B, Ramm SA, Schärer L, Ladurner P, Zadesenets KS, Rubtsov NB, Mouton S, **Berezikov E.** (2020) The free-living flatworm Macrostomum lignano. EvoDevo.

Yvernogeau L, Klaus A, Maas J, Morin-Poulard I, Weijts B, Schulte-Merker S, **Berezikov E**, Junker JP, Robin C. (2020) Multispecies RNA tomography reveals regulators of hematopoietic stem cell birth in the embryonic aorta. Blood.

Laboratory of Genomic Instability in Development and Disease

Group Leader: Floris Foijer

Sladky VS, Knapp K, Soratroi C , Heppke J, Eichin F, Rocamora-Reverte L, Szabo TG, Bongiovanni L, Westendorp B, Moreno E , van Liere EA, Bakker B, Spierings DCJ, Wardenaar R, Pereyra D, Starlinger P,

Schultze S , Trauner M, Stojakovic T, Scharnagl H, Fava LL , **Foijer F**, de Bruin A, Villunger A (2020) E2F-Family Members Engage the PIDDosome to Limit Hepatocyte Ploidy in Liver Development and Regeneration. Dev. Cel.

Schukken KM , Lin YC, Bakker PL , Schubert M, Preuss SF, Simon JE , van den Bos H, Storchova Z, Colomé-Tatché M, Bastians H, Spierings DCJ**, Foijer F** (2020) Altering Microtubule Dynamics Is Synergistically Toxic With Spindle Assembly Checkpoint Inhibition. Life Science Alliance.

Sieben CJ, Jeganathan KB, Nelson GG, Sturmlechner I, Zhang C, van Deursen WH , Bakker B, **Foijer F**, Li H, Baker DJ , van Deursen JM (2020) BubR1 Allelic Effects Drive Phenotypic Heterogeneity in Mosaic-Variegated Aneuploidy Progeria Syndrome. J. Clinic Invest.

Michael Schubert , Maria Colomé-Tatché, **Floris Foijer** (2020) Gene networks in cancer are biased by aneuploidies and sample impurities. Biochim Biophys Acta Gene Regul Mech.

Nelson L, Tighe A, Golder A, Littler S, Bakker B, Moralli D, Murtuza Baker S, Donaldson IJ, Spierings DCJ, Wardenaar R, Neale B, Burghel GJ, Winter-Roach B, Edmondson R, Clamp AR, Jayson GC, Desai S, Green CM, Hayes A, **Foijer F**, Morgan RD, Taylor SS. (2020) A living biobank of ovarian cancer ex vivo models reveals profound mitotic heterogeneity. Nature Communications

Lin Zhou, Laura J Jilderda, **Floris Foijer** (2020) Exploiting aneuploidy-imposed stresses and coping mechanisms to battle cancer. Open Biology

Saba KH, Cornmark L, Hofvander J, Magnusson L, Nilsson J, van den Bos H, Spierings DC, **Foijer F**, Staaf J, Brosjö O, Sumathi VP, Lam SW, Szuhai K, Bovée JV, Kovac M, Baumhoer D, Styring E, Nord KH. (2020) Loss of NF2 defines a genetic subgroup of non-FOS-rearranged osteoblastoma. Journal of Pathology. Clinical Research.

Andersson N, Bakker B, Karlsson J, Valind A, Holmquist Mengelbier L , Spierings DCJ, **Foijer F**, Gisselsson D (2020) Extensive Clonal Branching Shapes the Evolutionary History of High-Risk Pediatric Cancers. Cancer Research van den Bos H , Spierings DCJ , Westendorp B, Curinha A , Stojakovic T, Scharnagl H , Timelthaler G , Tsuchia K , Pinter M , Semmler G , **Foijer F** , de Bruin A , Reiberger T , Rohr-Udilova N , Villunger A (2020) PIDDosome-induced p53-dependent ploidy restriction facilitates hepatocarcinogenesis. EMBO Rep.

Phan TP, Maryniak AL, Boatwright CA, Lee J, Atkins A, Tijhuis A, Spierings DC, Bazzi H, **Foijer F**, Jordan PW, Stracker TH, Holland AJ.(2020) Centrosome defects cause microcephaly by activating the 53BP1-USP28-TP53 mitotic surveillance pathway. EMBO J.

Sladky VC, Knapp K, Szabo TG, Braun VZ, Bongiovanni L, van den Bos H, Spierings DC, Westendorp B, Curinha A, Stojakovic T, Scharnagl H, Timelthaler G, Tsuchia K, Pinter M, Semmler G, **Foijer F**, de Bruin A, Reiberger T, Rohr-Udilova N, Villunger A. (2020) PIDDosome-induced p53-dependent ploidy restriction facilitates hepatocarcinogenesis. EMBO Rep.

Laboratory of Ageing Biology and Stem Cells

Group Leader: Gerald de Haan

Belderbos ME , Jacobs S, Koster TK, Ausema A , Weersing , Zwart E , de Haan G , **Bystrykh LV** (2020) Donor-to-Donor Heterogeneity in the Clonal Dynamics of Transplanted Human Cord Blood Stem Cells in Murine Xenografts. Biol Blood Marrow Transplant

Jacobs S, Ausema A, Zwart E, Weersing E, Kingma MJ, El Menshawi YAS, **de Haan G**, Bystrykh LV, Belderbos ME. (2020) Quantitative distribution of patient-derived leukemia clones in murine xenografts revealed by cellular barcodes. Leukemia.

Serrano Martinez P , Cinat D , van Luijk P, Baanstra M , **de Haan G**, Pringle S , Coppes RP (2020) Mouse parotid salivary gland organoids for the in vitro study of stem cell radiation response. Oral Dis

Luinenburg DG , **de Haan G** (2020) MicroRNAs in hematopoietic stem cell aging. Mech Ageing Dev

Jacobs S, Ausema A, Zwart E, Weersing E , Kingma MJ, El Menshawi YAS, **de Haan G**, Bystrykh LV, Belderbos ME (2020) Correction: Quantitative distribution of patient-derived leukemia clones in murine xenografts revealed by cellular barcodes. Leukemia

Jacobs S , Ausema A , Zwart E ,Weersing E , **de Haan G** ,Bystrykh LV , Belderbos ME (2020) Detection of chemotherapy-resistant patient-derived acute lymphoblastic leukemia clones in murine xenografts using cellular barcodes. Exp Hematol

Han Y , Nikolić M , Gobs M, Franzen J, **de Haan G**, Geiger H & Wagner W(2020) Targeted methods for epigenetic age predictions in mice, Scientific Reports.

Luinenburg DG , Bak Dinitzen A , Flohr Svendsen A , Cengiz R , Ausema A , Weersing E , Bystrykh L , **de Haan G**.(2020) Persistent expression of microRNA-125a targets is required to induce murine hematopoietic stem cell repopulating activity Exp Hematol

van Zeventer IA, Buisman SC, de Graaf AO, **de Haan G**, Jansen JH, Huls G. (2020) Klonale hematopoëse [Clonal hematopoiesis: a risk factor for leukemia and cardiovascular disease?]. Ned Tijdschr Geneeskd.

Laboratory of Macromolecules and Interactomes

Group Leader: John LaCava

Dou Y, Barbosa I, Jiang H, Iasillo C, Molloy KR, Schulze WM, Cusack S, Schmid M, Le Hir H, **LaCava J**, Jensen TH. (2020) NCBP3 positively impacts mRNA biogenesis. Nucleic Acids Research.

Dou Y, Kalmykova S, Pashkova M, Oghbaie M, Jiang H, Molloy KR, Chait BT, Rout MP, Fenyö D, Jensen TH, Altukhov I, **LaCava J**. (2020) Affinity proteomic dissection of the human nuclear cap-binding complex interactome. Nucleic Acids Research.

Laboratory of Cellular Biochemistry

Group Leader: Liesbeth Veenhoff

Rempel IL, Popken P, Ghavami A, Mishra A, Hapsari RA, Wolters AHG, Veldsink AC, Klaassens M, Meinema AC, Poolman B, Giepmans BNG, Onck PR, Steen A, **Veenhoff LM**. (2020) Flexible and Extended Linker Domains Support Efficient Targeting of Heh2 to the Inner Nuclear Membrane. Structure.

Rempel IL, Steen A, **Veenhoff LM**. (2020) Poor old pores-The challenge of making and maintaining nuclear pore complexes in aging. The FEBS Journal.

Mouton SN, **Veenhoff LM**, Boersma AJ. (2020) Macromolecular Crowding Measurements with Genetically Encoded Probes Based on Förster Resonance Energy Transfer in Living Cells. Methods in Molecular Biology.

Liu B, Mavrova SN, van den Berg J, Kristensen SK, Mantovanelli L, **Veenhoff LM**, Poolman B, Boersma AJ. (2020) Correction to "Influence of Fluorescent Protein Maturation on FRET Measurements in Living Cells". ACS Sensors.

Novarina D, Janssens GE, Bokern K, Schut T, van Oerle NC, Kazemier HG, **Veenhoff LM**, Chang M. (2020) A genome-wide screen identifies genes that suppress the accumulation of spontaneous mutations in young and aged yeast cells. Aging Cell.

Laboratory of Cellular Senescence and Age-related Pathologies

Group Leader: Marco Demaria

Alimirah F, Pulido T, Valdovinos A, Alptekin S, Chang E, Jones E, Diaz DA, Flores J, Velarde MC, **Demaria M**, Davalos AR, Wiley CD, Limbad C, Desprez PY, Campisi J. (2020) Cellular Senescence Promotes Skin Carcinogenesis through p38MAPK and p44/42MAPK Signaling. Cancer Research.

Lubberts S, Meijer C, **Demaria M**, Gietema JA (2020) Early ageing after cytotoxic treatment for testicular cancer and cellular senescence: Time to act. Crit Rev Oncol Hematol.

Soto-Gamez A, Chen D, Nabuurs AGE, Quax WJ, **Demaria M**, Boersma YL. (2020) A Bispecific Inhibitor of the EGFR/ADAM17 Axis Decreases Cell Proliferation and Migration of EGFR-Dependent Cancer Cells. Cancers (Basel)

van der Feen DE, Bossers GPL, Hagdorn QAJ, Moonen JR, Kurakula K, Szulcek R, Chappell J, Vallania F, Donato M, Kok K, Kohli JS, Petersen AH, van Leusden T, **Demaria M**, Goumans MTH, De Boer RA, Khatri P, Rabinovitch M, Berger RMF, Bartelds B. (2020) Cellular senescence impairs the reversibility of pulmonary arterial hypertension. Science Translational Medicine.

Casciaro F, Borghesan M, Beretti F, Zavatti M, Bertucci E, Follo MY, Maraldi T, **Demaria M**. (2020) Prolonged hypoxia delays aging and preserves functionality of human amniotic fluid stem cells. Mechanisms of ageing and development.

Woldhuis RR, de Vries M, Timens W, van den Berge M, **Demaria M**, Oliver BGG, Heijink IH, Brandsma CA. (2020) Link between increased cellular senescence and extracellular matrix changes in COPD. American journal of Physiology.

Nehme J, Borghesan M, Mackedenski S, Bird TG, **Demaria M**. (2020) Cellular senescence as a potential mediator of COVID-19 severity in the elderly. Aging Cell.

Wang B, Kohli J, **Demaria M**. (2020) Senescent Cells in Cancer Therapy: Friends or Foes? Trends Cancer.

Borghesan M, Hoogaars WMH, Varela-Eirin M, Talma N, **Demaria M**. (2020) A Senescence-Centric View of Aging: Implications for Longevity and Disease. Trends Cell Biol.

Peng X, Wu Y, Brouwer U, van Vliet T, Wang B, **Demaria M**, Barazzuol L, Coppes RP. (2020) Cellular senescence contributed to radiation-induced hyposalivation by affecting the stem/progenitor cell niche. Cell death and Disease

Mkrtchyan GV , Abdelmohsen K , Andreux P , Bagdonaite I, Barzilai N , Brunak S , Cabreiro F , de Cabo R , J Campisi J , Cuervo AM , **Demaria M** , Ewald CY , Fei Fang E , Faragher R , Ferrucci L , Freund A , Silva-García CG , Georgievskaya A, Gladyshev VN , Glass DJ , Gorbunova V , de Grey A , He WW , Hoeijmakers J , Hoffmann E , Horvath S , Houtkooper RH , Jensen MK , Jensen MB , Kane A , Kassem M , de Keizer P , Kennedy B , Karsenty G , Lamming DW , Lee K , MacAulay N , Mamoshina P , Mellon J , Molenaars M , Moskalev A , Mund A , Niedernhofer L , Osborne B , Pak HH , Parkhitko A , Raimundo N , Rando TA , Rasmussen LJ , Reis C , Riedel CG , Franco-Romero A , Schumacher B , Sinclair DA , Suh Y , Taub PR , Toiber D , Treebak JT, Valenzano DR , Verdin E , Vijg J , Young S , Zhang L , Bakula D , Zhavoronkov A , Scheibye-Knudsen M (2020) ARDD 2020: from aging mechanisms to interventions. Aging.

Fitsiou E, Pulido T , Campisi J, Alimirah F, **Demaria M**. (2020) Cellular Senescence and the Senescence-Associated Secretory Phenotype as Drivers of Skin Photo. Aging.

Laboratory of Telomeres and Genome Integrity

Group Leader: Michael Chang

Novarina D, Desai R, Vaisica JA, Ou J, Bellaoui M, Brown GW, **Chang M**(2020) A Genome-Wide Screen for Genes Affecting Spontaneous Direct-Repeat Recombination in Saccharomyces cerevisiae. G3 (Bethesda).

Novarina D, Janssens GE, Bokern K, Schut T, van Oerle NC, Kazemier HG, Veenhoff LM, **Chang M**. (2020) A genome-wide screen identifies genes that suppress the accumulation of spontaneous mutations in young and aged yeast cells. Aging Cell.

Preprint:

Rosas Bringas, F.R., Stinus, S., Wanders, L., de Zoeten, P., Cohn, M., and **Chang M**. (2020) Rap1, Rif2, and the Ku complex work in concert to cap chromosome ends. bioRxiv. doi: 10.1101/2020.12.30.424824.

Morita, A., Nusantara, A.C., Perona Martinez, F.P., Hamoh, T., Damle, V.G., van der Laan, K.J., Sigaeva, A., Vedelaar, T., **Chang, M**., Chipaux, M., and Schirhagl, R. (2020) Quantum monitoring the metabolism of individual yeast mutant strain cells when aged, stressed or treated with antioxidant. arXiv: arXiv:2007.16130v1

Laboratory of Genetic Instability and Ageing

Group Leader: Peter Lansdorp

Sladky VS, Knapp K , Soratroi C, Heppke J, Eichin F, Rocamora-Reverte L, Szabo TG, Bongiovanni L, Westendorp B, Moreno E , van Liere EA, Bakker B, **Spierings DCJ**, Wardenaar R, Pereyra D, Starlinger P , Schultze S, Trauner M, Stojakovic T, Scharnagl H, Fava LL, Foijer F, de Bruin A, Villunger A (2020) E2F-Family Members Engage the PIDDosome to Limit Hepatocyte Ploidy in Liver Development and Regeneration. Dev. Cel.

Schukken KM , Lin YC, Bakker PL , Schubert M, Preuss SF , Simon JE , van den Bos, H , Storchova Z , Colomé-Tatché M , Bastians H, **Spierings DCJ** , Foijer F (2020) Altering Microtubule Dynamics Is Synergistically Toxic With Spindle Assembly Checkpoint Inhibition. Life Science Alliance.

Tamminga M, de Wit S, van de Wauwer C, van den Bos H, Swennenhuis JF, Klinkenberg TJ, Hiltermann TJN, Andree KC, **Spierings DCJ**, Lansdorp PM, van den Berg A, Timens W, Terstappen LWMM, Groen HJM. (2020) Analysis of Released Circulating Tumor Cells During Surgery for Non-Small Cell Lung Cancer. Clinical Cancer Research

Middelkamp S, van Tol HTA, Spierings DCJ, Boymans S, Guryev V, Roelen BAJ, **Lansdorp PM**, Cuppen E, Kuijk EW. (2020) Sperm DNA damage causes genomic instability in early embryonic development. Science Advance.

Tamminga M, Andree KC, Hiltermann TJN, Jayat M, Schuuring E, van den Bos H, Spierings DCJ, **Lansdorp PM**, Timens W, Terstappen LWMM, Groen HJM. (2020) Detection of Circulating Tumor Cells in the Diagnostic Leukapheresis Product of Non-Small-Cell Lung Cancer Patients Comparing CellSearch® and ISET. Cancers (Basel).

Vollger MR, Logsdon GA, Audano PA, Sulovari A, Porubsky D, Peluso P, Wenger AM, Concepcion GT, Kronenberg ZN, Munson KM, Baker C, Sanders AD, Spierings DCJ, **Lansdorp PM**, Surti U, Hunkapiller MW, Eichler EE. (2020) Improved assembly and variant detection of a haploid human genome using single-molecule, high-fidelity long reads. Annals of Human Genetics.

Porubsky D, Sanders AD, Taudt A, Colomé-Tatché M, **Lansdorp PM**, Guryev V. (2020) breakpointR: an R/Bioconductor package to localize strand state changes in Strand-seq data. Bioinformatics.

van den Bos H , **Spierings DCJ** , Westendorp B, Curinha A , Stojakovic T, Scharnagl H , Timelthaler G , Tsuchia K , Pinter M , Semmler G , Foijer F , de Bruin A , Reiberger T , Rohr-Udilova N , Villunger A (2020) PIDDosome-induced p53-dependent ploidy restriction facilitates hepatocarcinogenesis. EMBO Rep. Online ahead of print. Nov 23.

Sladky VC, Knapp K, Szabo TG, Braun VZ, Bongiovanni L, van den Bos H, **Spierings DC**, Westendorp B, Curinha A, Stojakovic T, Scharnagl H, Timelthaler G, Tsuchia K, Pinter M, Semmler G, Foijer F, de Bruin A, Reiberger T, Rohr-Udilova N, Villunger A. (2020) PIDDosome-induced p53-dependent ploidy restriction facilitates hepatocarcinogenesis. EMBO Rep. Nov 23 Online ahead of print.

Saba KH, Cornmark L, Hofvander J, Magnusson L, Nilsson J, van den Bos H, **Spierings DC**, Foijer F, Staaf J, Brosjö O, Sumathi VP, Lam SW, Szuhai K, Bovée JV, Kovac M, Baumhoer D, Styring E, Nord KH.(2020) Loss of NF2 defines a genetic subgroup of non-FOS-rearranged osteoblastoma. J Pathol Clin Res.

Andersson N, Bakker B, Karlsson J, Valind A, Holmquist Mengelbier L, **Spierings DCJ**, Foijer F, Gisselsson D.(2020) Extensive Clonal Branching Shapes the Evolutionary History of High-Risk Pediatric Cancers. Cancer Res.

Nelson L, Tighe A, Golder A, Littler S, Bakker B, Moralli D, Murtuza Baker S, Donaldson IJ, **Spierings DCJ**, Wardenaar R, Neale B, Burghel GJ, Winter-Roach B, Edmondson R, Clamp AR, Jayson GC, Desai S, Green CM, Hayes A, Foijer F, Morgan RD, Taylor SS. (2020) A living biobank of ovarian cancer ex vivo models reveals profound mitotic heterogeneity. Nature Communications

Laboratory of Genome Structure Ageing

Group Leader: Victor Guryev

Boudewijn IM, Lan A, Faiz A, Cox CA, Brouwer S, Schokker S, Vroegop SJ, Nawijn MC, Woodruff PG, Christenson SA, Hagedoorn P, Frijlink HW, Choy DF, Brouwer U, Wisman M, Postma DS, Fingleton J, Beasley R, van den Berge M, **Guryev V**. (2020) Nasal gene expression changes with inhaled corticosteroid treatment in asthma. Allergy.

Lähnemann D, Köster J, Szczurek E, McCarthy DJ, Hicks SC, Robinson MD, Vallejos CA, Campbell KR, Beerenwinkel N, Mahfouz A, Pinello L, Skums P, Stamatakis A, Attolini CS, Aparicio S, Baaijens J, Balvert M, Barbanson B, Cappuccio A, Corleone G, Dutilh BE, Florescu M, **Guryev V**, Holmer R, Jahn K, Lobo TJ, Keizer EM, Khatri I, Kielbasa SM, Korbel JO, Kozlov AM, Kuo TH, Lelieveldt BPF, Mandoiu II, Marioni JC, Marschall T, Mölder F, Niknejad A, Raczkowski L, Reinders M, Ridder J, Saliba AE, Somarakis A, Stegle O, Theis FJ, Yang H, Zelikovsky A, McHardy AC, Raphael BJ, Shah SP, Schönhuth A. (2020) Eleven grand challenges in single-cell data science. Genome Biology

Middelkamp S, van Tol HTA, Spierings DCJ, Boymans S, **Guryev V**, Roelen BAJ, Lansdorp PM, Cuppen E, Kuijk EW. (2020) Sperm DNA damage causes genomic instability in early embryonic development. Science Advances.

Koopmans T, Hesse L, Nawijn MC, Kumawat K, Menzen MH, Sophie T Bos I, Smits R, Bakker ERM, van den Berge M, Koppelman GH, **Guryev V**, Gosens R. (2020) Smooth-muscle-derived WNT5A augments allergen-induced airway remodelling and Th2 type inflammation. Scientific Reports

Brandsma CA, **Guryev V**, Timens W, Ciconelle A, Postma DS, Bischoff R, Johansson M, Ovchinnikova ES, Malm J, Marko-Varga G, Fehniger TE, van den Berge M, Horvatovich P. (2020) Integrated proteogenomic approach identifying a protein signature of COPD and a new splice variant of SORBS1. Thorax.

Porubsky D, Sanders AD, Taudt A, Colomé-Tatché M, Lansdorp PM, **Guryev V**. (2020) breakpointR: an R/Bioconductor package to localize strand state changes in Strand-seq data. Bioinformatics.

Boudewijn IM, Roffel MP, Vermeulen CJ, Nawijn MC, Kok K, Terpstra MM, Koppelman GH, **Guryev V**, van den Berge M. (2020) A Novel Role for Bronchial MicroRNAs and Long Noncoding RNAs in Asthma Remission. Am J Respir Crit Care Med.

*71 publications out of which 12 are preprints.

People

EMPLOYEES

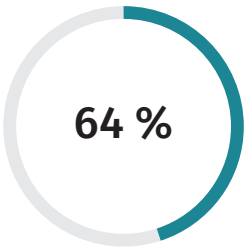
96

INTERNS

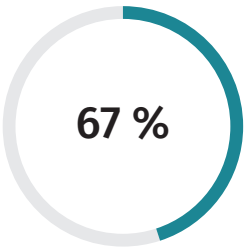
47

(12 of these students hosted by ERIBA under the GSMS Bursary Scheme)

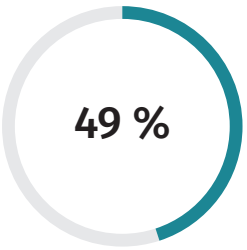
STAFF MEMBERS UNDER 40 (%)



FEMALE EMPLOYEES



INTERNATIONALS



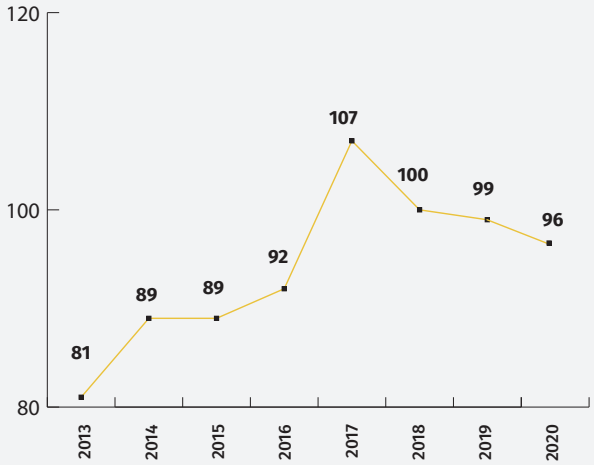
NUMBER OF NATIONALITIES

25

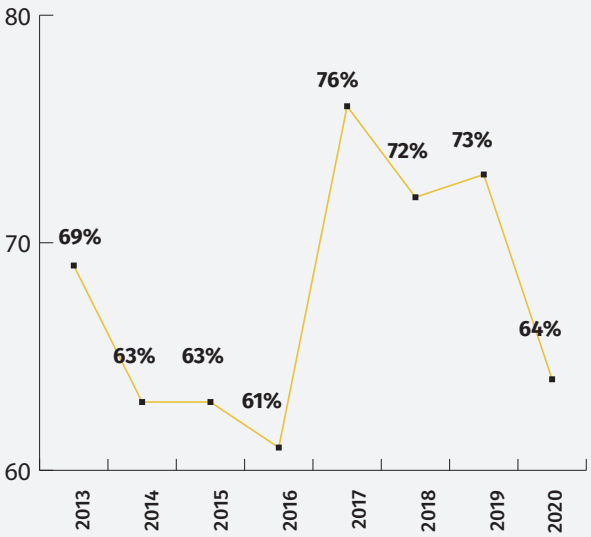
DOCTORAL STUDENTS HOSTED BY

34

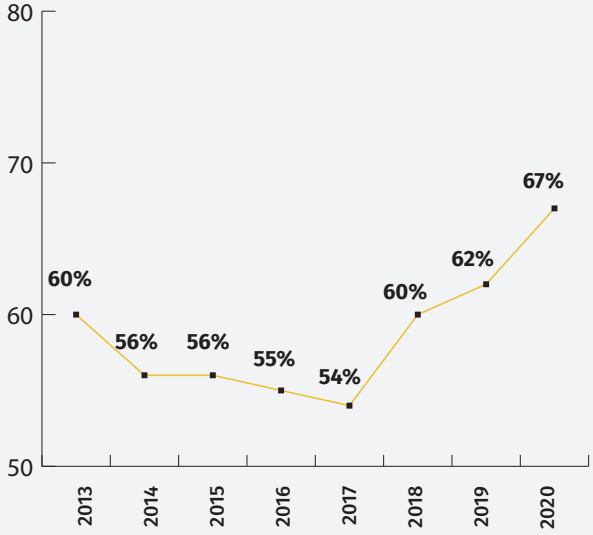
Employees



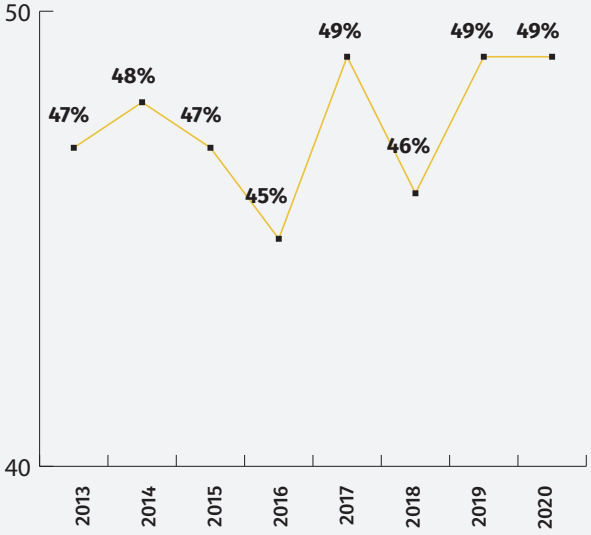
Staff under 40 (%)



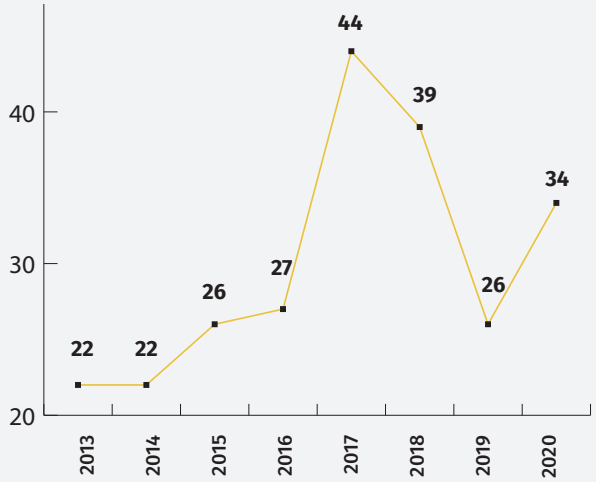
Female employees (%)



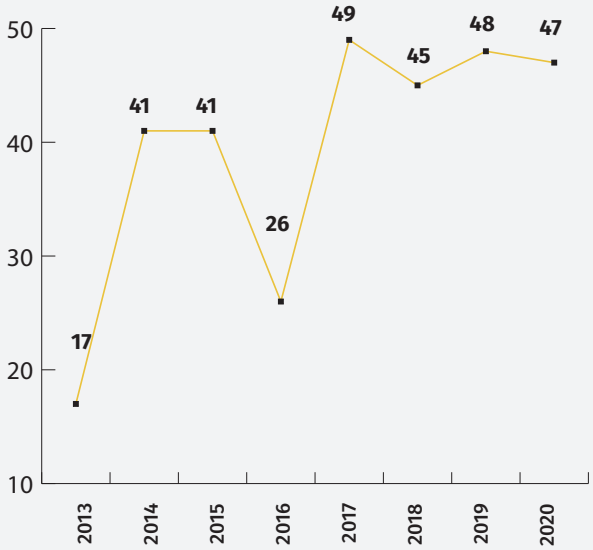
Internationals (%)



Doctoral Students Hosted by ERIBA

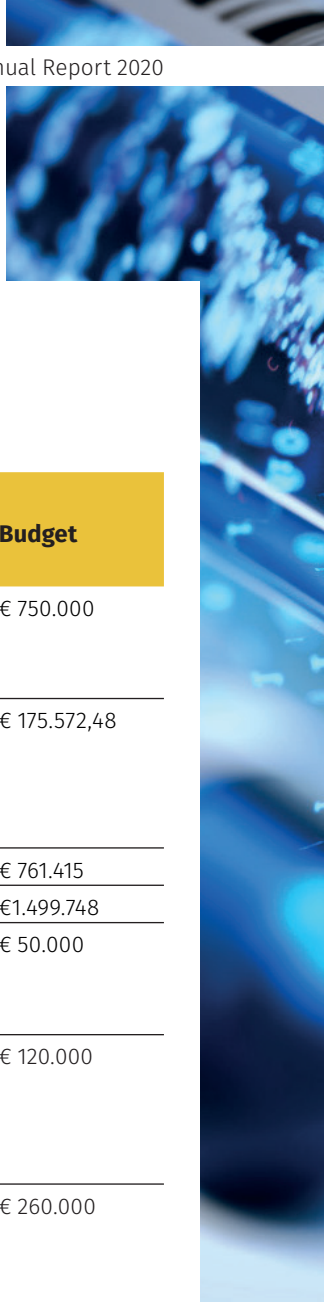


Interns



Management team ERIBA

Henk Heidekamp, Managing Director
Arnoud Rozema, Staff Advisor
Kevin Huizinga, Financial controller
Megha Upadhyay-Pandey, Research Coordinator
Annet Vos-Hassing, Secretary
Sylvia Hoks, Secretary
Alida de Haan, Secretary
Karin van Wageningen, Secretary
Yin Fai Chan, Technician (general support)



Funding/Grants

Proposals awarded in 2020

Principal Investigator/ Researchers	Role	Grant	Title	Budget
Eugene Berezikov	Applicant	ZonMw open competition	Molecular Engineering of Healthspan extension leveraging genetics of ageing-resilient animals	€ 750.000
Anna Ainslie (Ellen Nollen)	Applicant	H2020 MSCA-IF	Identifying neuronal or muscle cell-specific suppressors that rescue motility defects in the ALS model of the nematode worm Caenorhabditis elegans	€ 175.572,48
Liesbeth Veenhoff		NWO-groot (consortium grant)	Guardians of Protein disorder	€ 761.415
Liesbeth Veenhoff		NWO VICI	Quality control of nuclear pores	€1.499.748
Stijn Mouton/ Eugene Berezikov		NWO Open Competition-XS	Learning from an immortal animal how to improve protein homeostasis and slow down ageing	€ 50.000
Ellen Nollen		PPP	PPP-UniQure-Using the nematode worm C.elegans as a model to test gene therapy for Parkinson's disease and other neurodegenerative diseases	€ 120.000
Ellen Nollen		Stichting ParkinsonFonds	Deciphering how a functional circadian clock promotes proteostasis and protects against αSynuclein toxicity in C. elegans	€ 260.000
Judith Paridaen/ Nynke Oosterhof		NWO Open Competition-XS	Revealing the true colors of individual cells using barcodes	€ 50.000
John LaCava		PPP	Measuring and harnessing the druggabilityof the LINE-1 Interactome	€ 775.000
Arthur Flohr Svendsen		De Cock	Identification of Neogenin-1 extracellular and intracellular proteomic interactors in hematopoietic stem cells	€ 4.000
Floris Foijer/ Sahil Gupta		KRF	Mechanism of CIN-driven transformation of pre-invasive to invasive lesions	€ 3.000
Eugene Berezikov		NWO Open competition-XS	Towards improved DNA protection and repair: from flatworms to human cells	€ 50.000
Total				**€ 4.498.735,48**

NWO: Netherlands Organisation for Scientific Research
De Cock: Jan Kornelis de Cock Foundation
UMCG/KRF: University Medical Center Groningen/Cancer Research Funds
KRF: Cancer Research Funds
PPP: Health Holland Public-Private Partnerships
ZonMw: De Nederlandse organisatie voor gezondheidsonderzoek en zorginnovatie

Facilities:

iPSC/CRISPR

The discovery of protocols to reprogram somatic cells into induced pluripotent stem cells (iPSCs) is revolutionizing regenerative medicine. The therapeutic promise of iPSC technology includes the production of isogenic cell lineages and (in the future) tissues to replace body parts that can be autografted in patients when organs are failing. Importantly, when combined with CRISPR genome engineering technology, iPSC technology can be used to cure (mono) genetic diseases, by repairing the disease-causing mutation in patient-derived iPSCs and by differentiating the repaired cells into functional tissues and transplanting them back into the patient.

The iPSC/CRISPR centre at ERIBA aims to contribute to this therapeutic promise.
For this, we help UMCG and RUG employees with deriving iPSCs and establishing differentiated cultures from these iPSCs. Furthermore, we help our customers with CRISPR genome engineering, including making knockout cell lines, engineering point mutations, tagging endogenous genes, etc. in various cell lines, including iPSCs. Furthermore, we facilitate genome-wide CRISPR functional screens and together with the Netherlands Cancer Institute and Leiden University we make up the national CRISPR screen infrastructure ScreeninC supporting CRISPR screen at the national level. Since the start of the centre in 2014, we accommodated ~100 different projects for more than 50 different groups. We implemented a number of differentiation protocols and protocols to grow cerebral organoids and validated the reagents for genome-wide CRISPR screens. Importantly, we trained several PhD students in deriving and maintaining iPSCs and in differentiating iPSCs into various cell types.

Furthermore, we hosted MSc students for internships and we
organised the CRISPR genome engineering course for Biomedical Sciences MSc students as well as the Epigenetics and Gene Editing BSc course. As of 2020 our center's funding was renewed for another 6 years including an investment ina tissue culture robot to automate iPSC reprogramming, which will drastically improve our throughput in the years to come.

Who

Floris Foijer - Coordinator IPSC/CRISPR facility
René Wardenaar - Bioinformatician
Jonathan Seiler - Postdocoral Fellow
Narendra Chunduri – Postdoctoral fellow
Mathilde Broekhuis – Lab manager
Ohtman Alhazzaa – PhD student
Soraya Wobben – PhD student

Contact

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Eriba.umcg.nl/ipsc-crispr-facility/

Fish Facility

The Central Animal Facility (CDP) in the University Medical Center Groningen is one of the 13 academic experimental animal institutions in the Netherlands. The CDP is a facility where experimental animals are housed, and where animal experimentation is conducted. The CDP supports and facilitates research and education projects involving vertebrate experimental animals, such as rodents and fish.

In 2016, a fish facility was established within the CDP, where state-of-the-art housing is supplied for two species of small fish: killifish (*Nothobranchius furzeri*), and zebrafish (*Danio rerio*). Killifish are the shortest living vertebrate experimental animal system, which makes it very suitable for studying ageing processes. They were introduced by the Berezikov lab in the ERIBA as a new model organism to study the biology of aging in the ERIBA and UMCG. Currently, the CDP is the only facility in the Netherlands that houses killifish. Zebrafish are a very versatile vertebrate experimental animal system that is used extensively in biomedical research, and constitute a cheaper and easy-to-work-with alternative to rodents.

Key expertise and services

- Dedicated animal care-takers trained in breeding, rearing of fish larvae, general care and health services in small fish species
- Dedicated microinjection and epifluorescence stereomicroscopy setups for visual inspection, analysis, manipulation and microinjection of zebrafish and killifish embryos
- Dedicated incubators for housing fish embryos
- Availability of several strains of wild-type fish, such as AB, TL and *Casper* (transparent) zebrafish
- Breeding services to obtain embryos of wild-type, and if required, other strains
- Support and advice regarding genomic modification methods, such as transgenesis and CRISPR/cas9-mediated genomic modifications
- Training of new users in fish experimentation (next to obligatory course on Laboratory Animal Science)
- Breeding and care taking, biotechnical support, micro-surgical support, imaging support and animal welfare monitoring

Who

Eugene Berezikov – ERIBA PI (killifish expert)
Joscha Muck – Postdoctoral fellow, ERIBA (killifish expert)
Judith Paridaen – ERIBA PI (zebrafish expert)
Nynke Oosterhof – postdoctoral felow, ERIBA (zebrafish expert)
Alex Kluppel – Manager CDP
Catriene Thuring – Animal Welfare Officer, Deputy Head CDP

Contact

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9713 AV Groningen
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c.a.kluppel@umcg.nl
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Sequencing Facility

Next generation sequencing (NGS) technology is revolutionizing medicine and life sciences and has become a routine tool to assess the genomes, epigenomes and transcriptomes of cultured cells, (liquid) biopsies, and primary tissue/disease samples. In May 2019, the Research Sequencing Facility was officially established within the ERIBA Technology Center, as a dedicated research infrastructure to provide support for NGS-based projects for UMCG and RUG research groups. For this, we will not only facilitate the expeditious sequencing of NGS libraries either prepared by research groups themselves or by the facility on behalf of the researcher, but also implement the latest NGS techniques used in medicine and life sciences research, and (co)develop and implement new state-of-the-art NGS techniques to keep NGS-dependent research in the UMCG at the forefront. Furthermore, we advise the researchers on the set-up of their NGS experiments and train researchers in the production of NGS libraries if they would prefer to do this themselves. As a spin-off from the Peter Lansdorp research group, we are experts in single-cell DNA sequencing and the only sequencing facility offering the Strand-seq technology as a service. Strand-seq is a powerful tool to identify besides copy number alterations also copy-number neutral structural genomic aberrations such at inversions and translocations, all at the single cell level thereby preserving tissue heterogeneity. In 2019, we have generated over 14,000 single-cell DNA-seq libraries derived from approximately 340 different samples. Moreover, we facilitated ~100 sequencing runs in total. Although we could only facilitate small NGS projects (RNA-seq, ATAC-seq, DNA-seq) in 2019, we expect to accommodate many new large-scale projects as we have invested in infrastructure to automate library preparation thereby drastically increasing our capacity for many NGS applications.

Who

Diana Spierings – Coordinator Research Sequencing Facility
Nancy Halsema – Technician
Karina Wakker-Hoekstra – Technician
Jennefer Beenen – Technician

Contact

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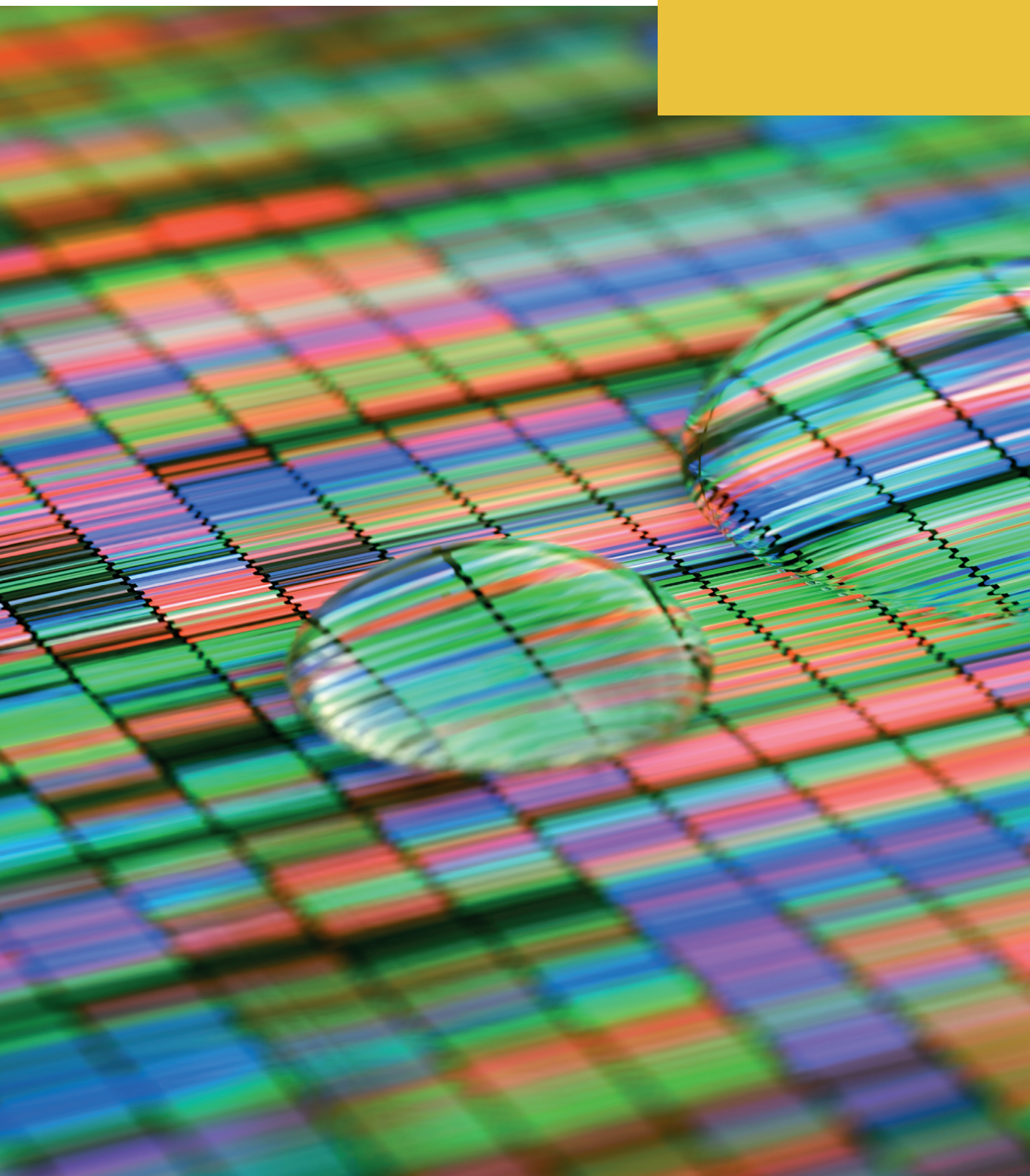


Education

ERIBA scientists are involved in multiple education activities. The list below is a selection of major contributions to teaching. It excludes a large number of individual lectures and undergraduate student internships.

"Molecular en Genetic Age Research ERIBA; coordinator. 18 BSC Biology and Life Science and Technology students"
10 ECTS, 18 BSc. Biology, and Life Science and Technology students

Coordinator: **Cor Calkhoven**

Objectives: A laboratory research course with research topics covering a broad range of techniques and model systems related to ageing, lifespan an dage-related disease. Topics may involve (stem) cells, yeast, worms, mice and cove the biological processes of signal transduction, transcription, translation, post-translation modification, protein homeostasis, energy metabolism, chromosome biology, genetics and epigenetics and bioinformatics.

"CRISPR genome engineering"
5 ECTS; 32 students

Coordinator: **Floris Foijer**

Objectives: In this course, MSc learn the basics of CRISPR engineering. Students learn about the history and various applications of CRISPR including knockouts, knockins, CRISPR I, CRISPRa, mutations and genome-wide screens. Furthermore, they get hands-on experience in the design of guide RNAs and genome editing tools and apply them in the lab. Finally, they combine their newly acquired expertise in an assignment in which they design a complete CRISPR strategy for a fictive project.

"Models systems in Ageing research"
3 ECTS; 12 students

Coordinator: **Floris Foijer**

Objectives: In this JSM course, third year medical students explore fundamental biology and are exposed to several of the model organisms we use at ERIBA for ageing-related research. Students discuss advantages and disadvantages of the model organisms with researchers on the lab. and study relevant papers that make use of the model organisms. In small groups, they compare the feasibility of 2-3 models Ito study (aspects of) a particular disease and discuss the advantages and disadvantages of these models in this setting.

"Epigenetics and Gene editing"
5 ECTS; 60 students

Coordinator: **Floris Foijer/Marianne Rots**

Objectives: In this course 2nd and 3rd year BSc students will learn the basics of epigenetics in the context of developmental biology: how do epigenetic processes lead to the differentiation of an organism? Furthermore, the students learn the basics of stem cell biology, pluripotency and induced pluripotent stem cells. Finally, the students are exposed to the beginnings of gene editing, including CRISPR genome engineering. The lectures contain basic knowledge about these topics and application lectures in which this basic knowledge is applied in current research illustrated by examples of UMCG researchers.

"Molecular Biology of Ageing and Age related Disease"
5 ECTS, 29 MSc biomedical students

Coordinator: **Liesbeth Veenhoff**

Objectives: In this course we focus on the molecular and cellular mechanisms by which tissue and organ function deteriorate and homeostasis fails, resulting in ageing and age related disease. We present the model systems and experimental strategies that are used in ageing research. This course is supported by a team of specialists in different fields of ageing who provide lectures and reading material. The course will be further supported by materials from an online course "Why do we age? The molecular mechanisms of ageing". The course unit is compulsory for the ageing track and is an elective in the other tracks of the programs.

"Current Themes in Healthy Ageing"
5 ECTS, 10 MSc Biomedical Sciences students

Coordinator: **Marco Demaria**

Objectives: Learn leading edge ageing research and interact with prominent aging scientists by following scientific seminars. Biomedical Science students attend 7- scientific seminars and report on content, scientific excellence and track records of the presenters. This course has 10-30 students/year.

"Genome biology"
May and September 2020, organized at Karolinska Institute in Stockholm, Sweden

15x2 doctoral students

Coordinators: **Dr. Leonid Bystrykh** and **Dr. Victor Guryev**

Objectives: Data retrieval from public databases, use of R and python scripts for the analysis of data. Gene expression data processing for microarrays, bulk RNAseq data and single-cell RNAseq. To increase the understanding of the basic principles of bioinformatics and to gain practical skills in bioinformatics analysis of sequence data.

Outreach Dissemination & Events

ERIBA is committed to outreach and dissemination activities. ERIBA personnel consistently take part in various events. This is to ensure that students and general public is well aware of the activities in ERIBA. Due to the pandemic, few activities were hampered but keeping in mind ERIBA's social responsibilities, our scientists still managed to perform various outreach activities. ERIBA seeks to connect a wide range of audiences outside the academia: general public, students, industry, decision makers, media, and patient organizations.

The activities/events are listed below:

Gerald de Haan

The official kick-off for Ride for the Roses. KWF Fundraising (Feb 2020)

Comenius Leergangen. 2 seminars on stem cells and aging as part of a leadership course

Marco Demaria

Institute for Biomedical Aging Research. Speaker at Institute seminar series. Innsbruck (Austria) (Jan 2020)

European Association for Cancer Research. Speaker at Conference 'A matter of life and death'. Bergamo (Italy) (Feb 2020)

Speaker at the 7th Aging Drug Discovery Meeting, online (Sep 2020)

Speaker at the Philippine Society for Cell Biology annual meeting, online (Oct 2020)

Rubedo lifesciences. Speaker at the Company seminar series, online (Nov 2020)

Organising webinars Aging series UMCG/ERIBA (~80 attendees/lecture)

CRCG PhD Day (Nov 2020, 120 attendees)

Judith Paridaen

Chair of Young Academy Groningen (YAG) Public

Engagement working group 2020-2021

VHTO Beeldenbreker – visit primary and high schools to talk about a career in life science (e.g. primary school in Hoogezand) (Oct 2020)

Floris Foijer

A seminar at Cambridge University (Feb 2020)

Hosted an online seminar from John van der Oost (Oct 2020)

A seminar on the Aneuploidy Online Zoom conference organised by Jason Sheltzer and Samuel Bakhoum hosted by Cold Spring Harbor Laboratories (December 2020)

ERIBA-IMB Retreat

Organizers: Gerald de Haan (ERIBA), Megha Upadhyay (ERIBA), and Christof Niehrs (IMB Mainz)

This virtual event was jointly held on 14th December 2020 by ERIBA and Institute of Molecular Biology, Mainz, Germany. The meeting included discussions on progress of collaborative projects. The scientists also did a brainstorming session to discuss and explore the potential future collaborations.

Science in a Box

Leading Staff Member: Stijn Mouton

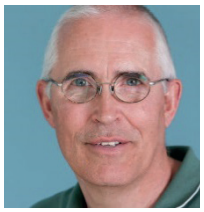
In 2019, "Science in a Box" was officially ,launched, and the first "Regeneration Boxes" were sold. These boxes provide a hands on experience to explore topics such as regeneration and stem cells in the class room.

Scientific Advisory Board

The Board is comprised of the following distinguished scientists:

Christine Mummery

Professor of Developmental Biology
Chair of the Department of Anatomy and Embryology
Leiden University Medical Center
The Netherlands



Johan Auwerx

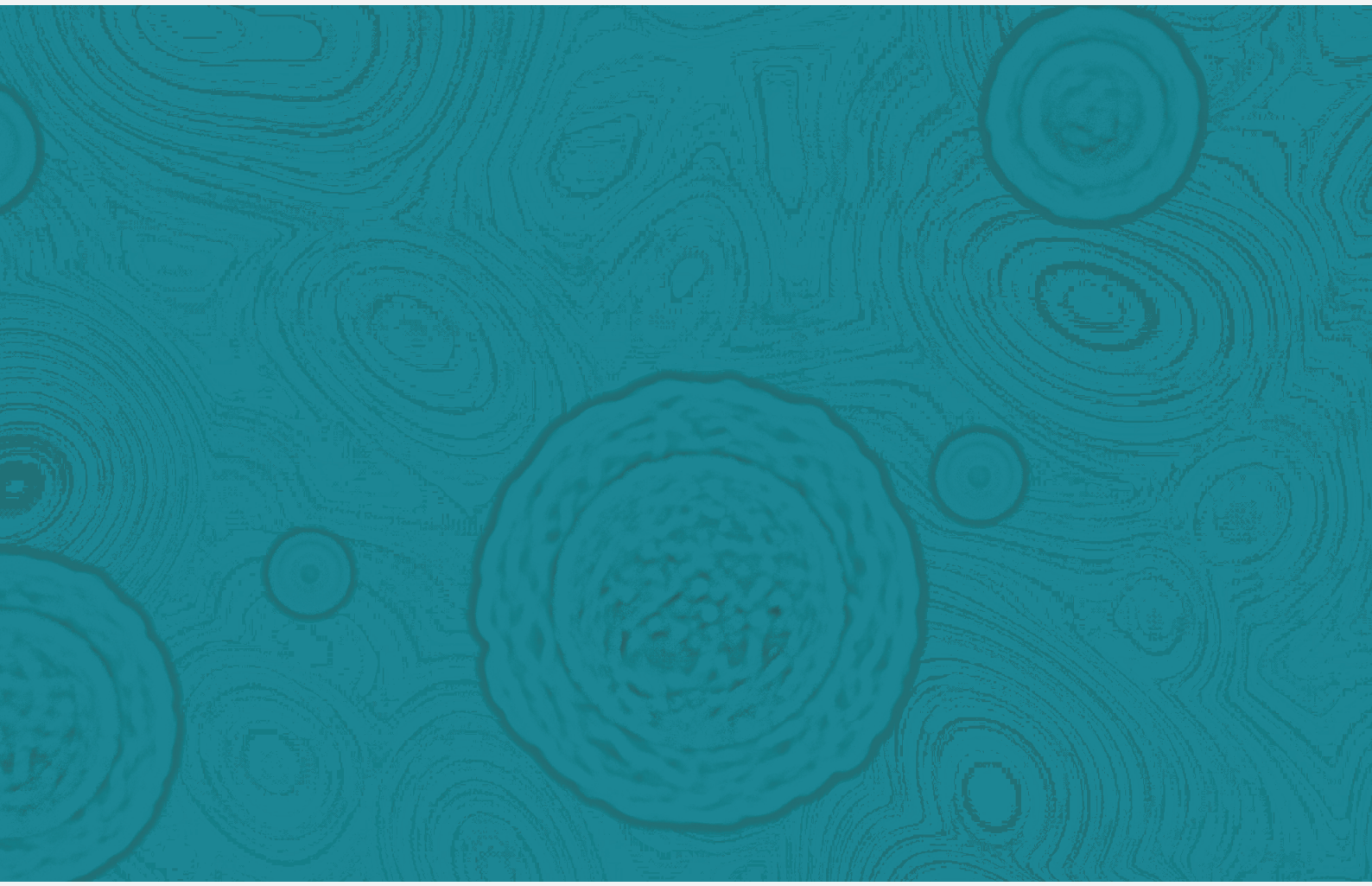
Professor and Nestlé Chair in Energy Metabolism
Ecole Polytechnique Fédérale in Lausanne
Switzerland

Helle Ulrich

Scientific Director of the Institute of Molecular Biology
Professor at the Faculty of Biology
University of Mainz
Germany

Yves Barral

Associate Professor of Biochemistry
Department of Biology
ETH Zurich
Switzerland



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