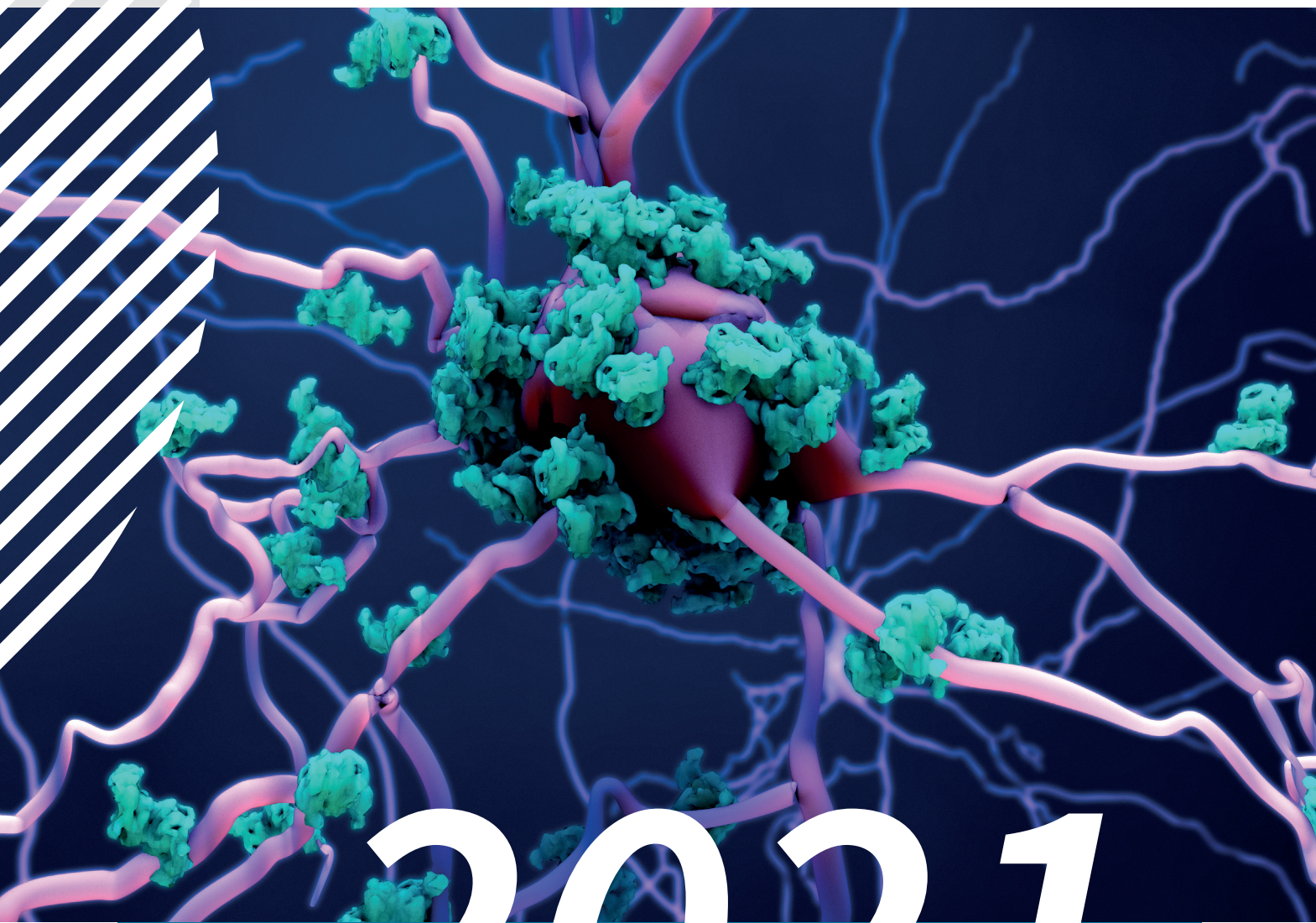
ERIBA European Research Institute for the Biology of Ageing

European Research Institute for the Biology of Ageing

umcg university of groningen

Annual Report



2021

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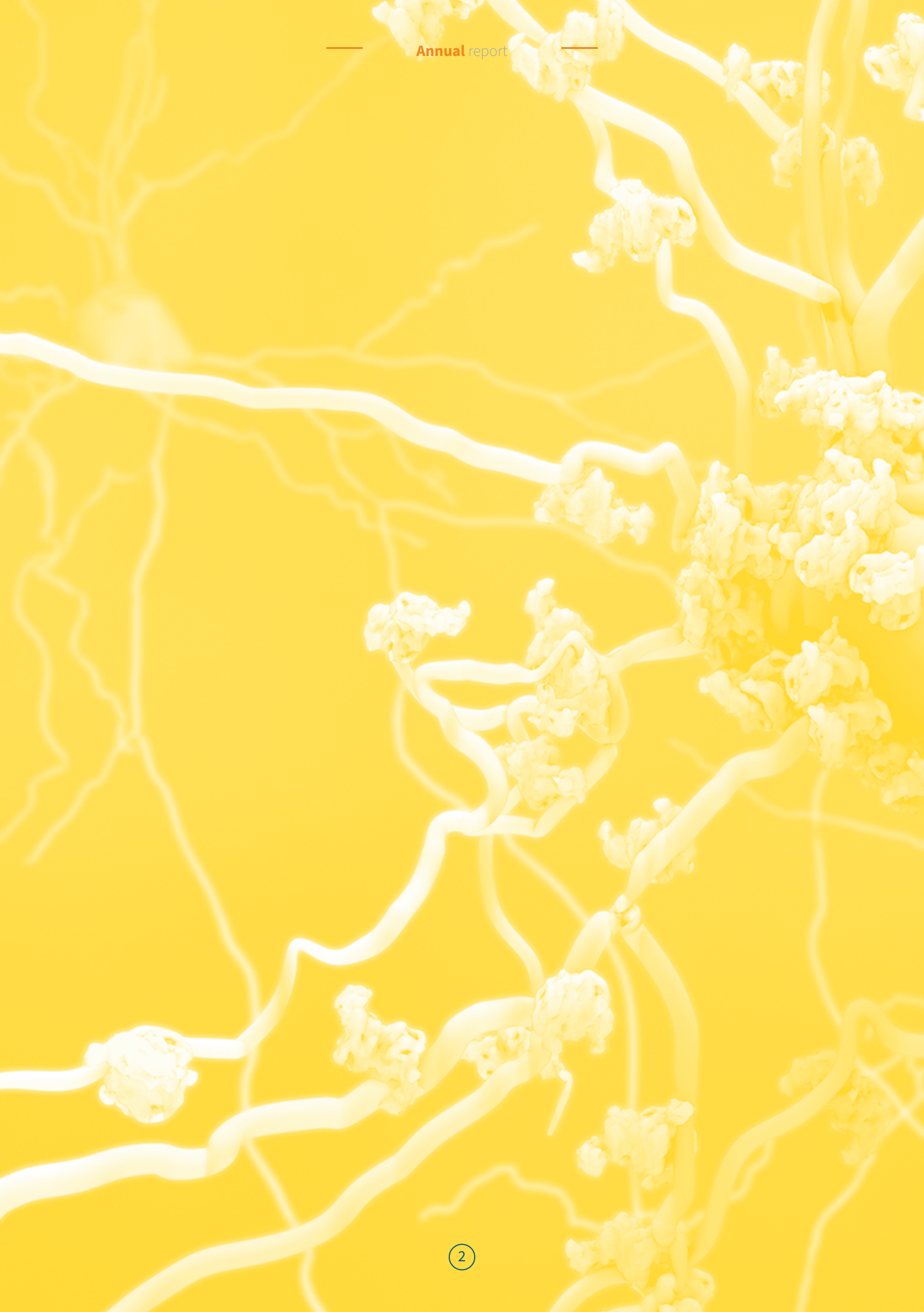

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Foreword by the Director

It is a great pleasure to present to you the 2021 annual report of the European Research Institute for the Biology of Ageing. This report provides an overview of all our activities and successes in science, education, knowledge utilization and outreach. We are proud to share with you all that has been accomplished in 2021.

We keep moving forward on our main mission to elucidate the mechanisms that drive ageing, with a view towards developing strategies to combat unhealthy ageing and development of age-related disease. We practice and promote our mission in all of our core activities, from our science and education to our outreach.

ERIBA's PIs, PhD students, postdocs, technicians, interns, and administrative staff did a remarkable job working their way through another year of COVID-19 restrictions. Collectively, we published 72 papers in international peer-reviewed scientific journals. Most of these publications resulted from long-standing collaborations with our local, national and international collaborators, both scientists and clinicians. We cherish and thank all of our colleagues for this continuous collaboration.

In 2021, our scientists were once again successful in the acquisition of research funding, which includes prestigious and competitive grants awarded by the Netherlands Organization for Scientific Research (NWO) and the Dutch Cancer Society (KWF). Apart from funds through these more traditional funding schemes, ERIBA's pioneering work, unique expertise and facilities have been increasingly recognized by international biotech companies, leading to financed collaborative research projects in public private partnerships. This continued recognition by academic scientists as well as by industry strengthens our belief in the power of our curiosity-driven research to find solutions for invalidating age-related diseases and, thereby, contribute to solutions to halt the increasing burden of continuously rising healthcare costs.

Finally, we have intensified our efforts to expose our work to the general public. In 2021, our presence on social media received a boost from an energetic committee of junior ERIBA scientists. Follow us on @UMCG_ERIBA and stay updated through beautiful images, announcements, movies and much more.

In April, our scientific director, Gerald de Haan left ERIBA to become the scientific director of the Sanquin Research Institute in Amsterdam. We are grateful to Gerald for his role in the foundation of ERIBA and putting us on the global map with other leading ageing research institutes. We are honored that Folkert Kuipers, who co-founded ERIBA in his former position as the Dean of the Faculty of Medical Sciences, will take over his role and head ERIBA starting from August 2022. We look forward to ERIBA's future under his leadership.

Best regards,

Ellen Nollen
Interim director

June 2022

Ageing Research at ERIBA

ERIBA is an internationally recognised European research centre on ageing. The institute focuses on fundamental biology to understand the causes of ageing. At ERIBA, studies are focused on the mechanisms that result in the loss of cells with age, and decline in function of old cells and tissues

Stem Cell Regulation and Mechanisms of Regeneration

Eugene Berezikov

INTRODUCTION

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*.

RESEARCH FOCUS

The flatworm *Macrostomum lignano* has an impressively advanced resilience, far beyond other animals (Figure). 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.

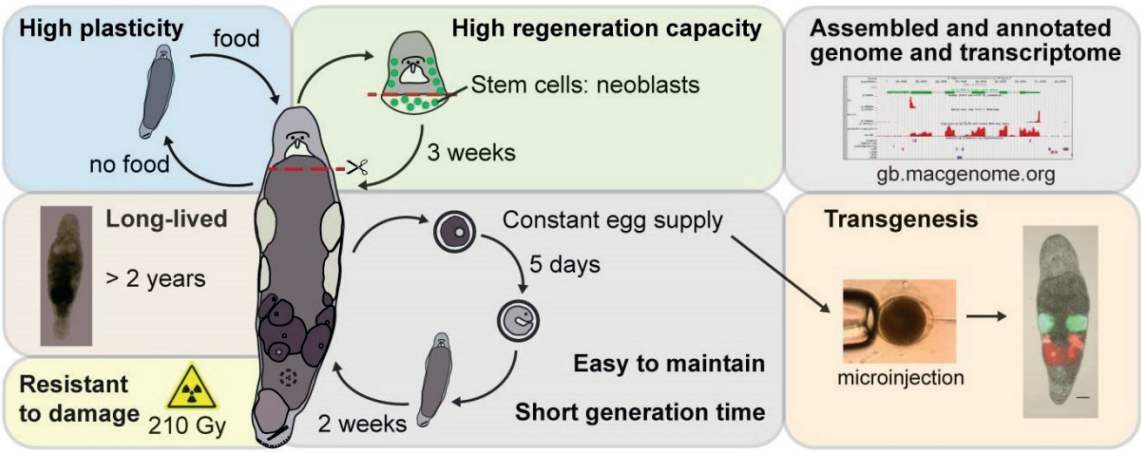


Figure. The flatworm *Macrostomum lignano* is a versatile model organism to study stem cells, regeneration, ageing and resilience mechanisms. Adapted from Wudarski et al., EvoDevo 11:5 (2020).

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.

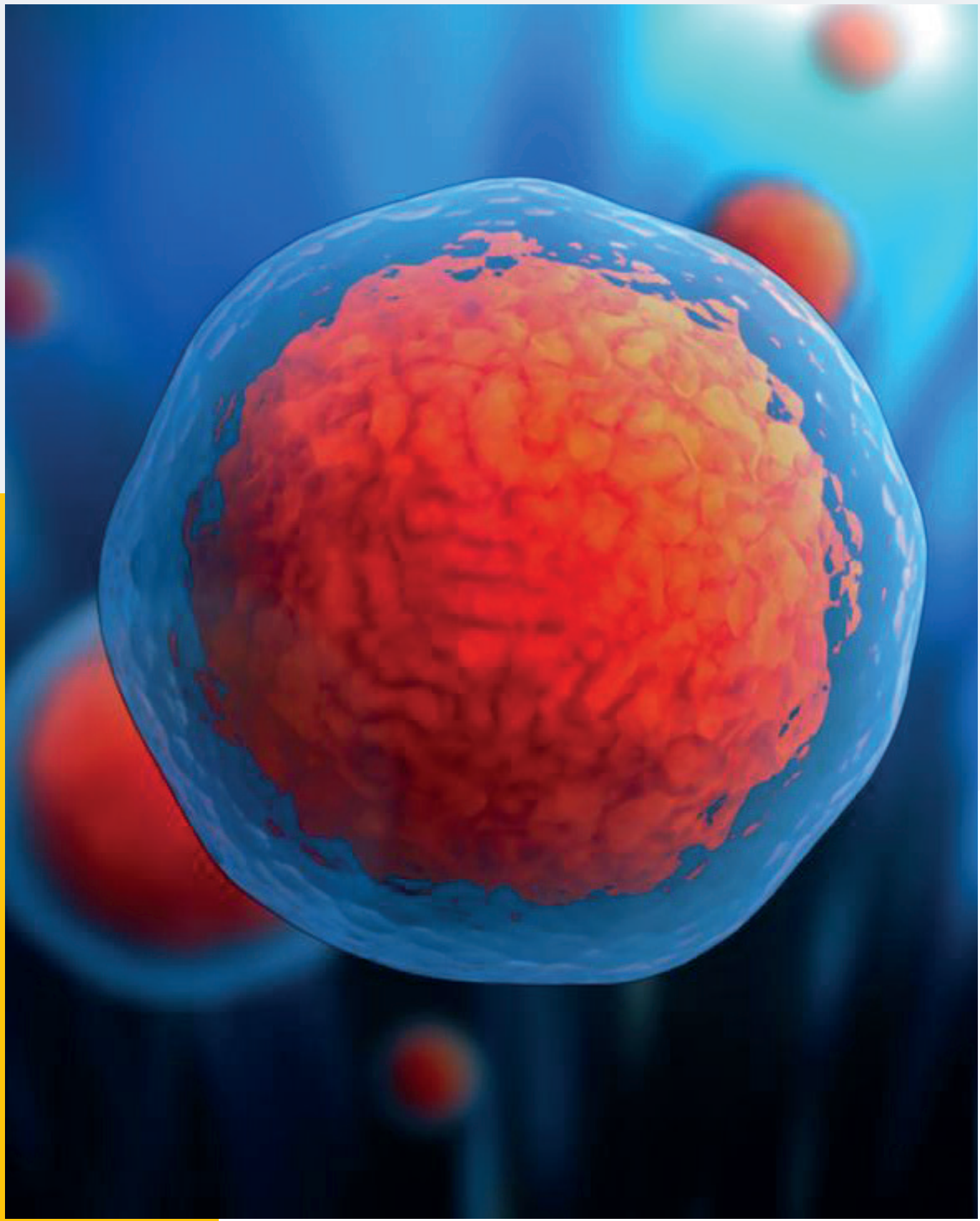
2. Mechanisms of DNA damage control in *M. lignano*
Damage to DNA is a major factor of ageing and cancer. Hence, preventing and repairing DNA damage is an important resilience strategy. We will investigate how *M. lignano* survives high doses of gamma- and UVC radiation by combining transgenesis, genomics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

Stem cells and resilience mechanisms



Gene Regulation In Ageing and Age-Related Diseases

Cor Calkhoven

INTRODUCTION

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 promoting cancer metabolism and cell migration. In another line of research, we aim to understand the pro-tumorigenic role of TSC-mTORC1 regulation in lung cancer.

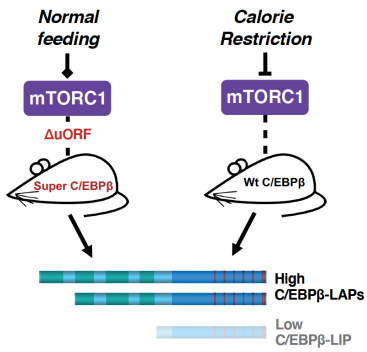
RESEARCH FOCUS

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 mTORC1 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.



Metababolic 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

We will investigate the role of mTORC1- and SIRT1-C/EBPα regulation in health and lifespan determination using genetic engineered mouse and killifish models. The role C/EBPβ in breast cancer development and cancer immune evasion will be studied. 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.

TOP 3 PUBLICATIONS

1. Niehrs, C. and **Calkhoven, C.F.** (2020) The emerging role of C/EBPβ and epigenetic DNA methylation in ageing. *Trends Genet*, 6, 71-80 DOI: 10.1016/j.tig.2019.11.005
2. Ackermann, T., Hartleben[$], G., Müller, C.[$], Mastrobuoni, G., Groth, M., Sterken, B.A., Zaini, M.A., Youssef, S.A., Zuidhof, H.R., Krauss, S.R., Kortman, G., de Haan, G, de Bruin, A., Wang, Z-Q., Platzer, M., Kempa, S., and **Calkhoven, C.F.** (2019) C/EBPβ-LIP induces metabolic reprogramming by regulating the let-7/LIN28B circuit. *Commun Biol*, 2, 1-15 DOI: 10.1038/s42003-019-0461-z
3. 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

Gene regulation in ageing and age-related diseases

Telomeres and Genome Integrity

Michael Chang

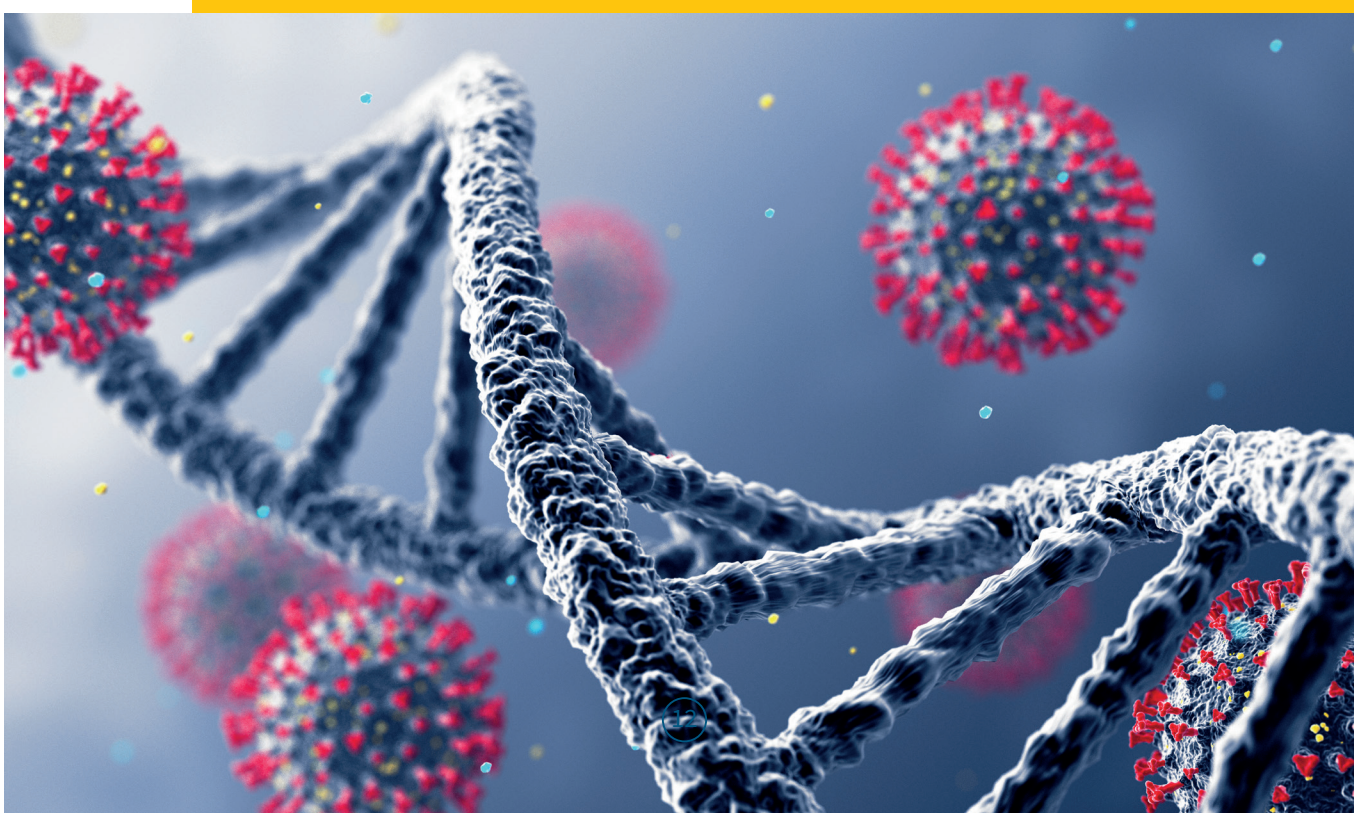
INTRODUCTION

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. Dysfunctional telomeres result in DNA damage checkpoint activation and cell cycle arrest. Recent work in our lab has found that telomere-specific depletion of Rap1, the main sequence-specific telomere binding protein in *Saccharomyces cerevisiae*, can be tolerated due to the presence of secondary telomere capping mechanisms, which may help explain the rapid evolution of budding yeast telomeres.

We have also developed a novel screening method that allows the identification of genes that affect low-frequency events. While yeast has been, and continues to be, an exceptionally powerful model organism in which to conduct high-throughput, genome-wide screens, it is very difficult to screen for genes that affect low-frequency events with established methodology. We have used our new method to identify genes that suppress the accumulation of mutations in aging yeast cells, and for genes that modulate homologous recombination of direct DNA repeats. Our future research will take advantage of this new screening approach.



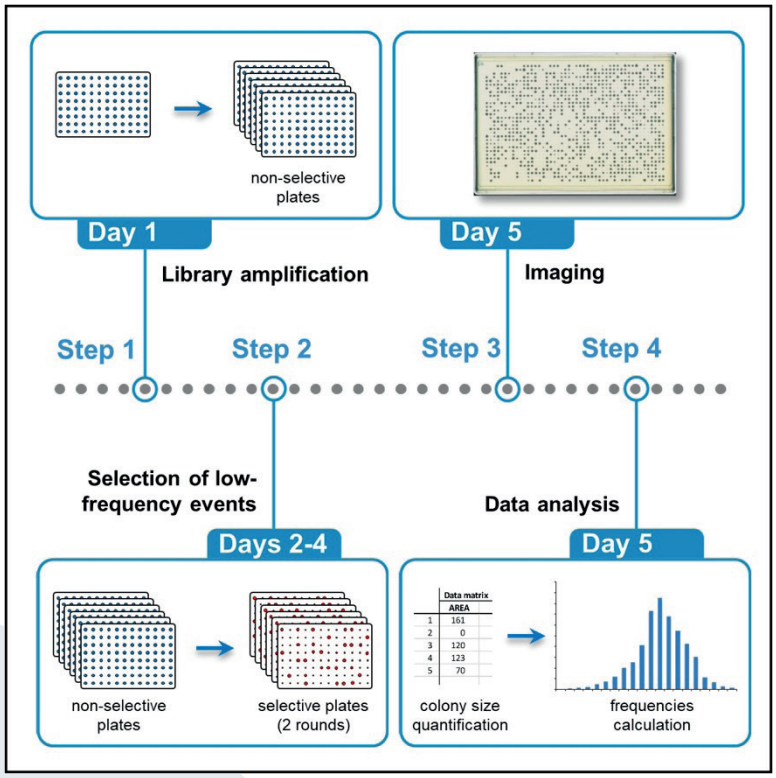


Figure. High-throughput replica-pinning approach to screen for yeast genes controlling low-frequency events (from Novarina, Rosas Bringas, Rosas Bringas, and Chang, 2022).

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 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., de Zoeten, P., Cohn, M., and **Chang, M.** (2022) Rif2 protects Rap1-depleted telomeres from MRX-mediated degradation in *Saccharomyces cerevisiae*. ***eLife***, doi: 10.7554/eLife.74090.

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. Strecker, J.[†], Stinus, S.[†], Pliego Caballero, M., Szilard, R.K., **Chang, M.**[*], and Durocher D.[*] (2017) A Pif1-dependent threshold separates DNA double-strand breaks and telomeres. ***eLife***, 6: e23783. [†]Co-first author, [*]Co-corresponding author

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

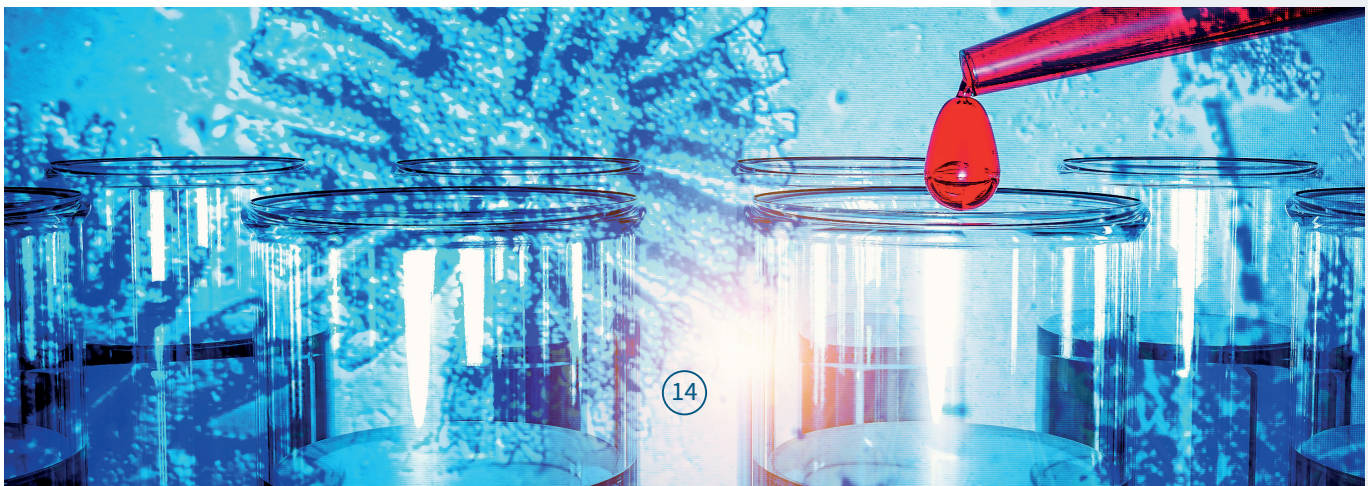
INTRODUCTION

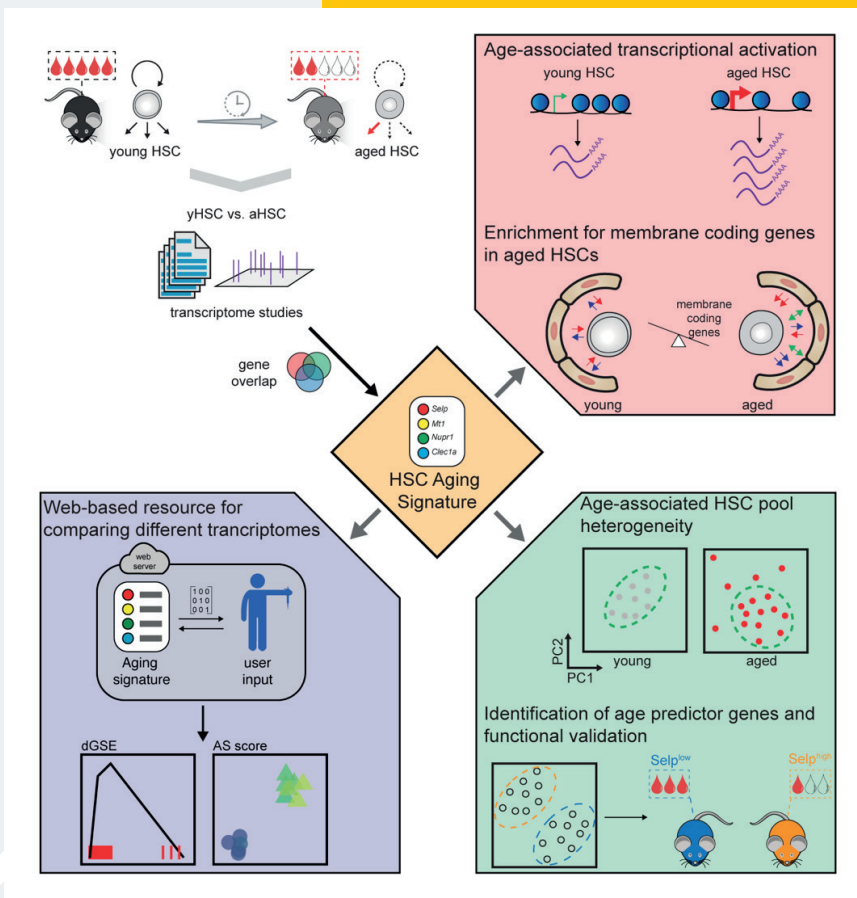
Our research is aimed to understand the molecular mechanisms that contribute to hematopoietic stem cell (HSC) development and aging. A small population of selfrenewing HSCs ensures that all the various types of blood cells are produced during the lifetime of an organism. Nevertheless, during aging blood 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 excessive selfrenewal of leukemic stem cells.

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 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). 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.





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.

TOP 3 PUBLICATIONS

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. Flohr Svendsen A, Yang D, Kim K, Lazare S, Skinder N, Zwart E, Mura-Meszaros A, Ausema A, von Eyss B, **de Haan G**, Bystrykh L. A comprehensive transcriptome signature of murine hematopoietic stem cell aging. Blood. 2021 Aug 12;138(6):439-451. doi: 10.1182/blood.2020009729

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.

Cellular Senescence and Age-related Pathologies

Marco Demaria

INTRODUCTION

Our research focuses on 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 but can cause inability to maintain important cell pools with age. Remarkably, most senescent cells secrete a collection of various cytokines, growth factors, matrix metalloproteases, lipids and nucleotides – a 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. However, when senescent cells aberrantly accumulate, such as during aging or under excessive stress, the SASP contributes to chronic low-level inflammation and aberrant tissue growth and remodeling. The identification of deleterious pro-disease senescent cells prompted the development of senolytic drugs able to induce selective death of senescent cells.

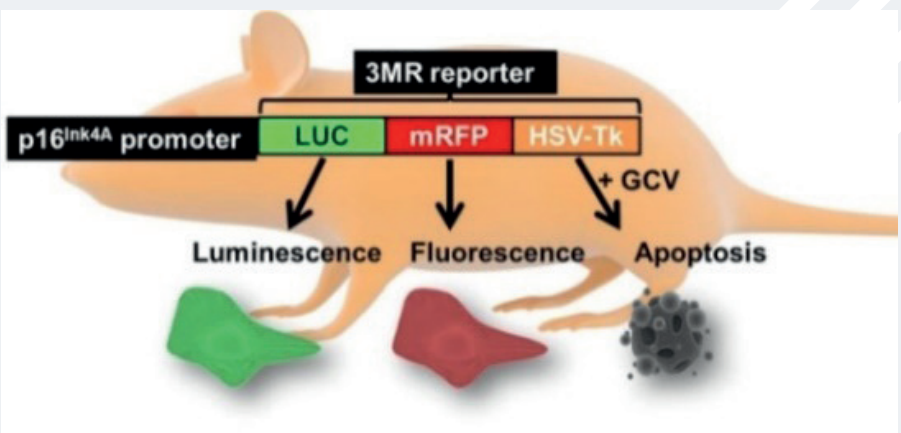
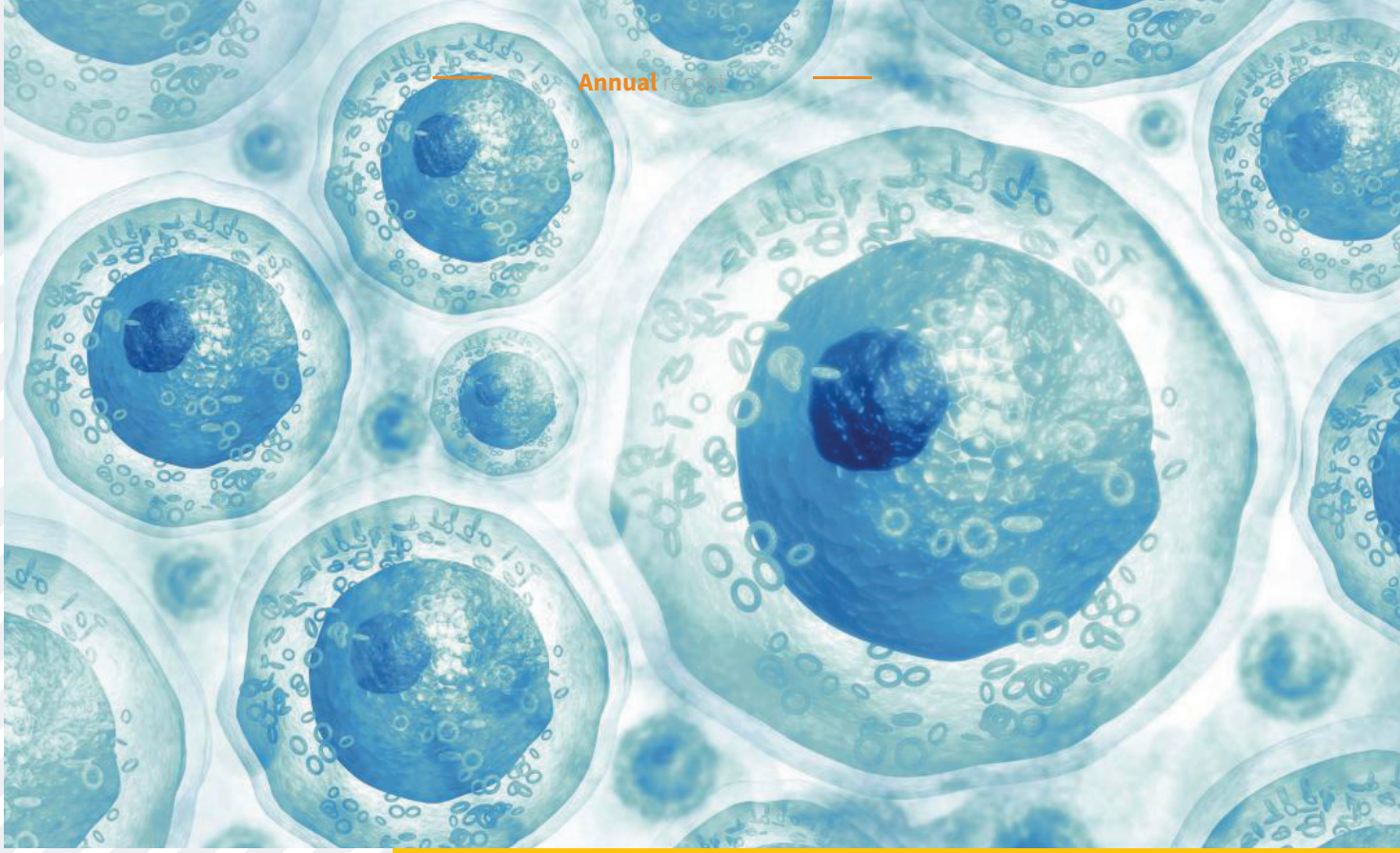


Figure 1. The p16-3MR mouse mouse.



RESEARCH FOCUS

Our laboratory has pioneered the concept of senescence heterogeneity by performing a comprehensive transcriptomic study to different senescence subsets. These data together with data from other laboratories have demonstrated that several intrinsic and extrinsic factors contribute to regulate induction and development of senescence-associated phenotypes. Importantly, we have defined that different subsets of senescent cells -- each characterized by particular combinations of senescence-associated phenotypes -- can co-exist *in vivo*, but specific molecular characteristics of these different populations remain largely unknown. Identification of senescence subset-specific molecular marks can help to unequivocally characterize physiological and pathological functions of cellular senescence. To reach this goal, we are using 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 1). New reporter lines are under development.

To dissect between beneficial and detrimental senescence programs we are using various models. For beneficial senescence, we use models of skin and kidney injury. For detrimental senescence, we are using a number of disease models including cancer, osteoarthritis and chronic kidney disease. We are also planning to exploit the knowledge acquired during this analyses to design more efficient and less toxic anti-aging therapeutic strategies that selectively target the 'bad' senescent cells.

In addition to understand cell-intrinsic features characterizing beneficial and detrimental senescent cells, our laboratory is exploring how environmental conditions influence establishment of senescence-associated features. Recently, we have demonstrated that conditions of low oxygen concentrations, also known as hypoxia, can interfere with the production of damaging SASP factors via repression of mTOR activity. We are currently exploring in pre-clinical models the anti-aging potential of intermittent exposure to hypoxia or to hypoxia-mimicking compounds (Figure 2). Diet is arguably one of the most powerful lifestyle factor to determine the balance between health and sickness. In collaboration with a number of laboratories we are characterizing senescence induction and development in mice and humans under specific diet regimens. We have already provided the proof-of-concept that diets rich in proteins and fats exacerbate senescence, whereas conditions of calorie restriction alleviates accumulation of age-associated senescent cells.

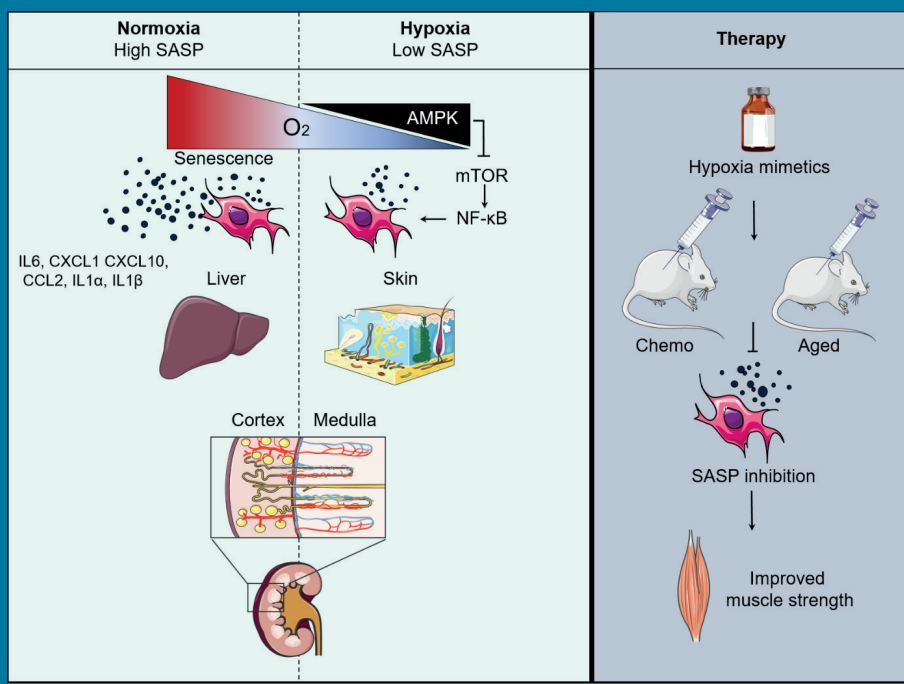


Figure 2. Hypoxia and hypoxia mimetics in regulation of senescence and SASP

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. To reach this goal, we have established collaborations with several clinical departments and with different biotech and pharma companies.

TOP 3 PUBLICATIONS

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

2. Kohli J, Wang B, Brandenburg S, Basisty N, Evangelou K, Varela-Eirin M, Campisi J, Schilling B, Gorgoulis V, **Demaria M**. Algorithmic assessment of cellular senescence in experimental and clinical specimens. 2021. Nature Protocols. 16:2471-2498

3. Vliet T, Wang B, Varela-Eirin M, Borghesan M, Brandenburg S, Evangelou K, Franzin R, Seelen M, Gorgoulis V, **Demaria M**. Physiological hypoxia restrains the Senescence-Associated Secretory Phenotype (SASP) via AMPK-mediated mTOR suppression. 2021. Molecular Cell. 81:2041-2052.

Heterogeneity of cellular senescence: from mechanisms to interventions

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.

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. 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 (ref 1 and earlier work). However, the tumors exhibiting chronic CIN displayed higher intratumor heterogeneity. 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.

In another collaborative project with the labs of Reneta Basto (Institute Marie Curie, Paris) and Zuzana Storchova (University Kaiserslautrn), we used scWGS to investigate the consequences of polyploidization for genomic integrity, which revealed that polyploidization coincides with replication stress, yielding further genomic instability (ref 2).

We furthermore investigate how aneuploid cells adopt a malignant fate. For this, 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 (ref 3).

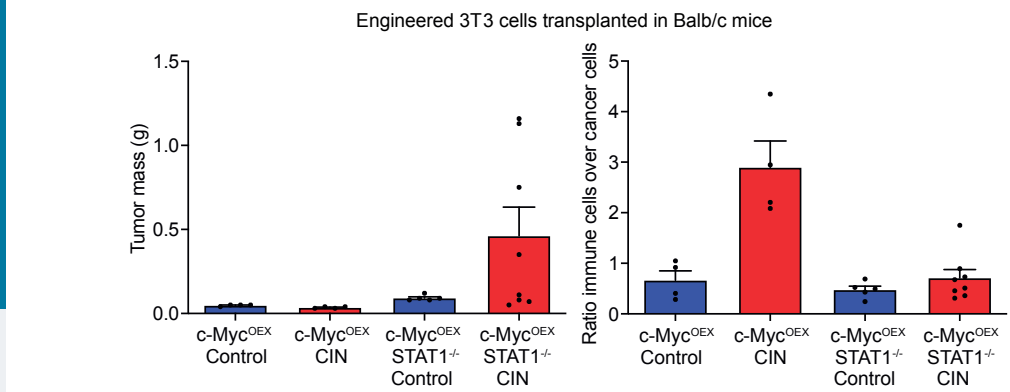
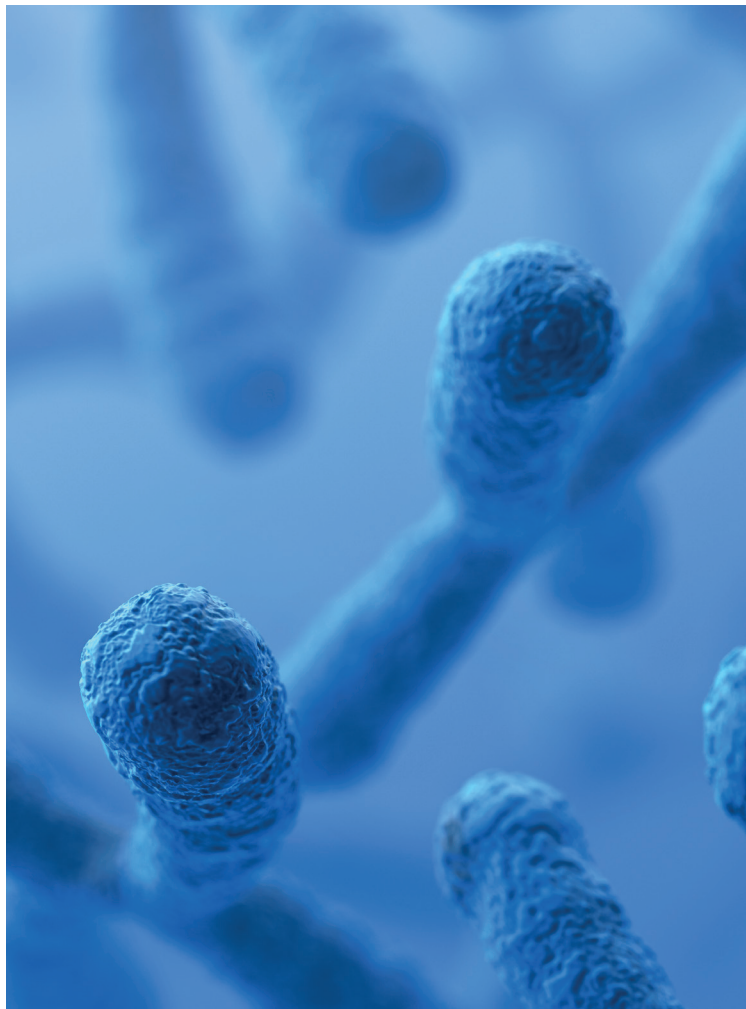


Figure. Tumor size and tumor infiltration as function of CIN and Stat1 signaling

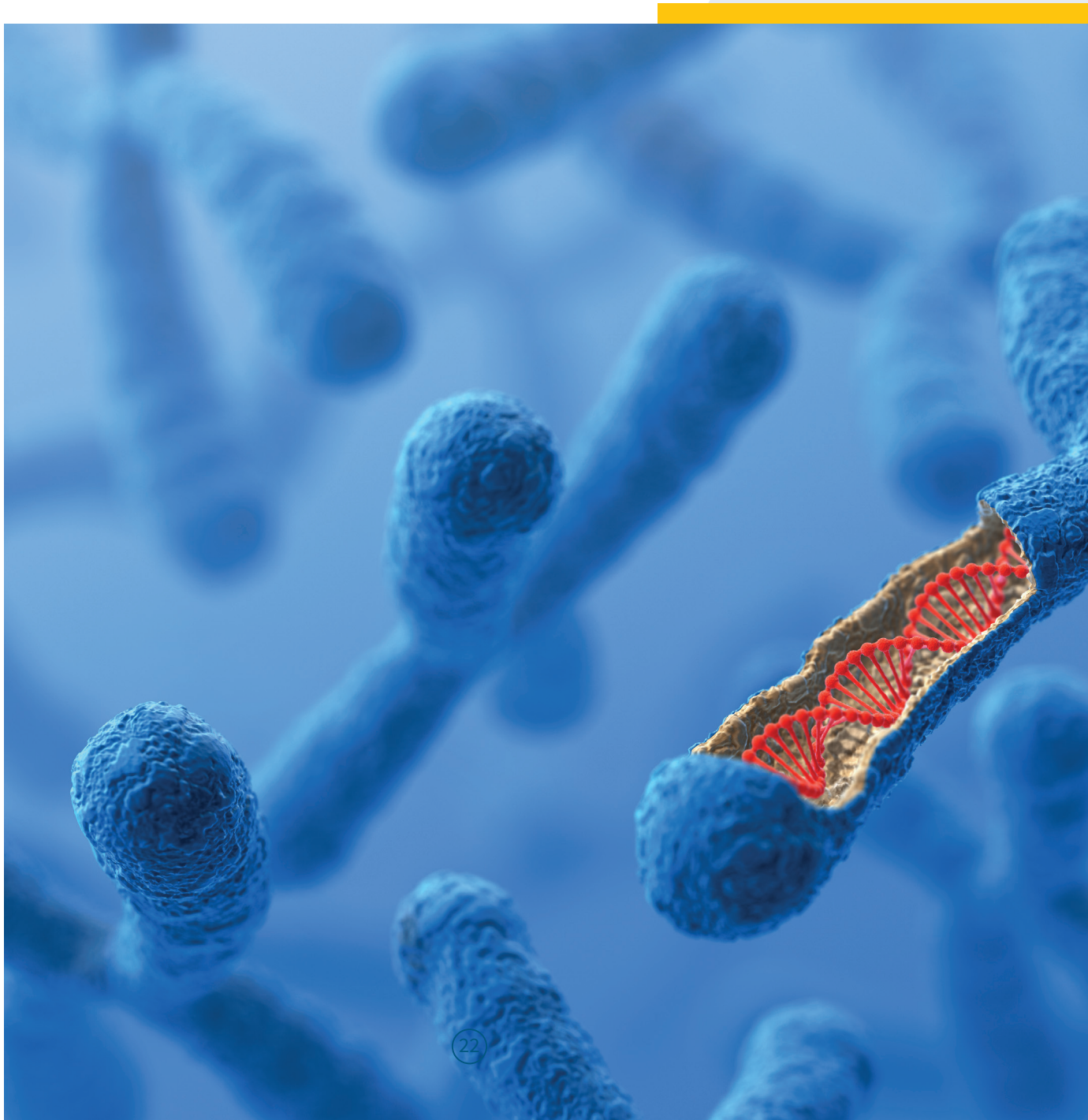
Our findings indicate that CIN triggers an inflammation response in cells that relies on Stat1 signaling. Intriguingly, while Stat1 inactivation promoted tumorigenesis in combination with CIN, we also found that alleviating the inflammatory response upstream of Stat1 is very toxic to chromosomal instable cells. We also found that the inflammatory response triggered by CIN critically relies on IL6 activity upstream of Stat1 and Stat3. 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, 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., Wang Y., Kim D.H., Maldonado M., Demarest M.A., Artates J., Zhengyu O., Mark A., Wardenaar R., Sasik R., Spierings D.C.J., Vitre B., Fisch K., **Foijer F.**[#], and Cleveland D.W.[#]. Transient genomic instability drives tumorigenesis through accelerated clonal evolution. Genes Dev. 2021 Aug 1;35(15-16):1093-1108., doi:10.1101/gad.348319.12
2. Gemble S., Wardenaar R.[*], Keuper K.[*], Srivastava N., Nano M., Macé A., Tijhuis A.E., Bernhard S.V., Spierings D.C.J., Simon A., Goundiam O., Hochegger H., Piel M., **Foijer F.**, Storchová Z., and Basto R. Genetic instability from a single S-phase after whole genome duplication bioRxiv 2021.07.16.452672. Nature, in press.
3. Schubert M.[*,#], Hong C.[*] Jilderda L.J.[*], Requesens M.[*], Tijhuis A.E., Simon J.E., Bakker P.L., Cooper J.L., Damaskou A., Wardenaar R., Bakker B., Gupta S., van den Brink A., Andrade Ruiz L., Koster M.H., Youssef S.A., Luinenburg D., Strong A., Engleitner T., Ponstingl H., de Haan G., de Bruin A., Rad R., Nijman H.W., Medema R.H., van Vugt M.A.T.M., de Bruyn M., Spierings D.C.J., Colomé-Tatché M., Vassiliou G.S., and **Foijer F#**. Cancer tolerance to chromosomal instability is driven by Stat1 inactivation in vivo. bioRxiv 2021.12.03.471107;

[*]Equal contributions, [#]corresponding authors.

Chromosomal instability in ageing and cancer

Genome Structure 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 a 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 of 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 and molecular phenotypes that manifest themselves at RNA and protein levels and potentially play roles in human diseases.

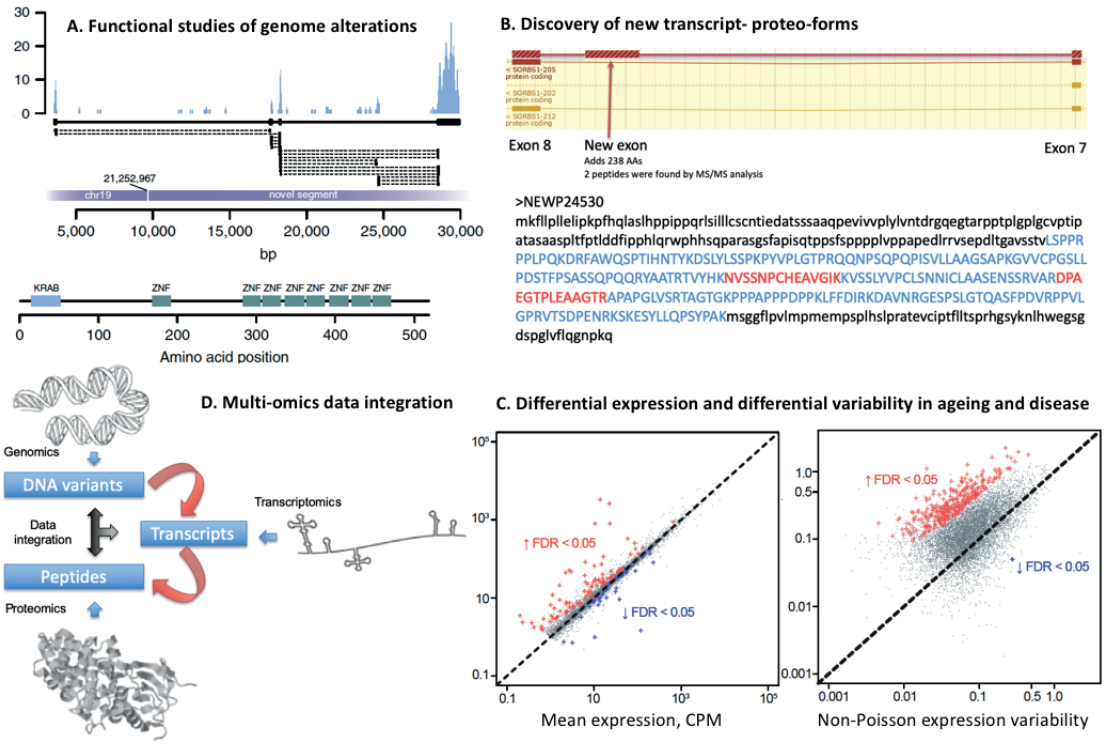
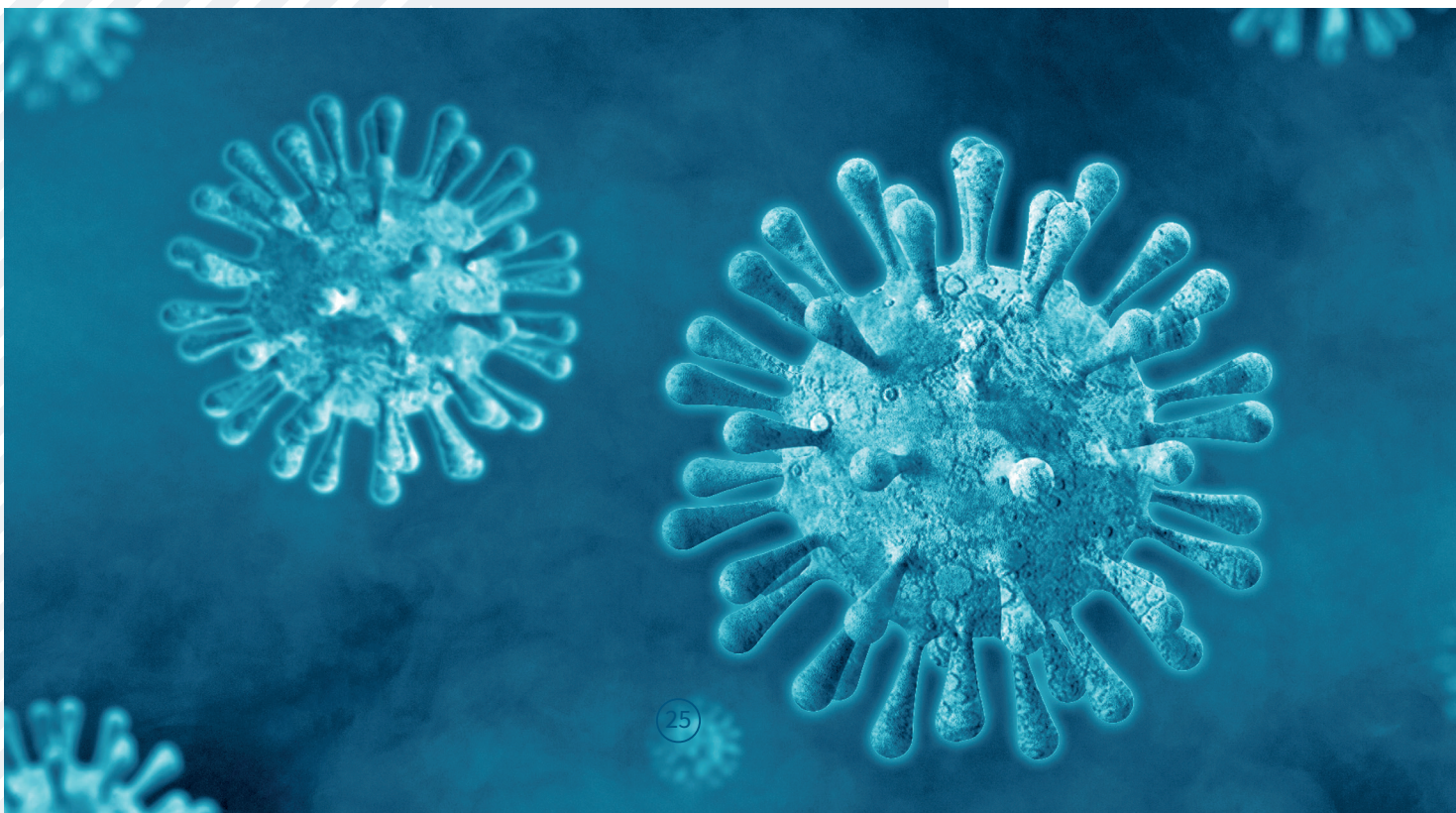


Figure 1. Major research directions. (A). Investigation and functional characterization of large genome alterations and their role in the onset of age-related diseases. An example of a long non-reference insert on chr19, that encodes for a new zinc finger gene. Transcript coverage by RNA-seq reads (top), 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.



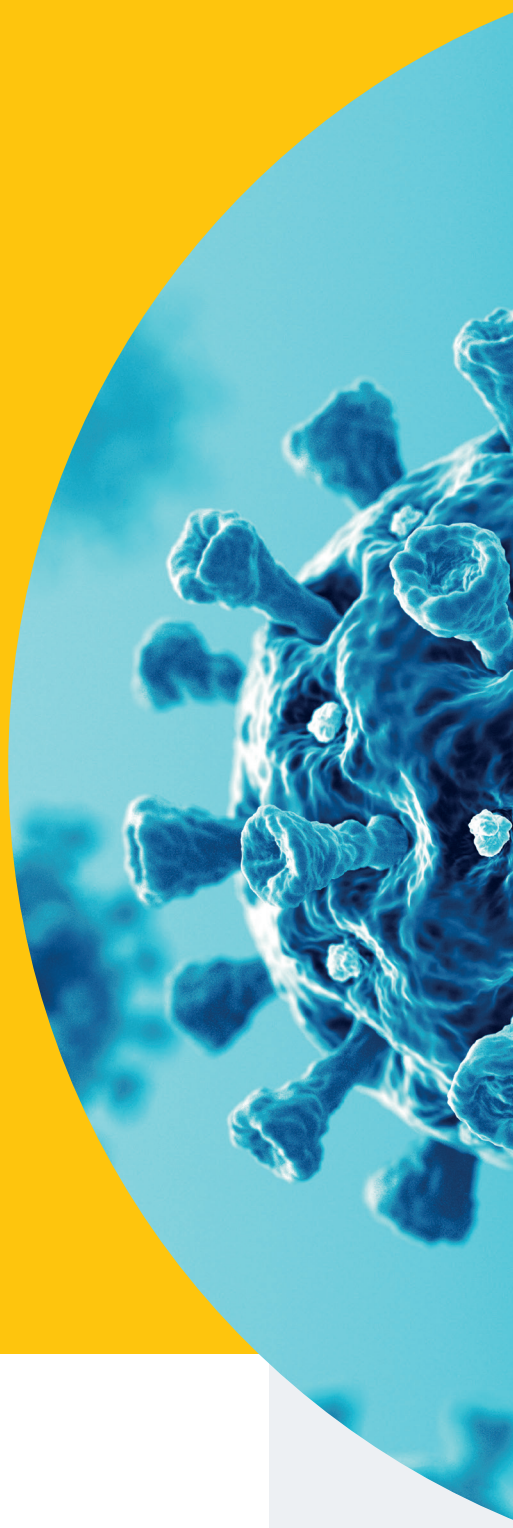
THE FUTURE

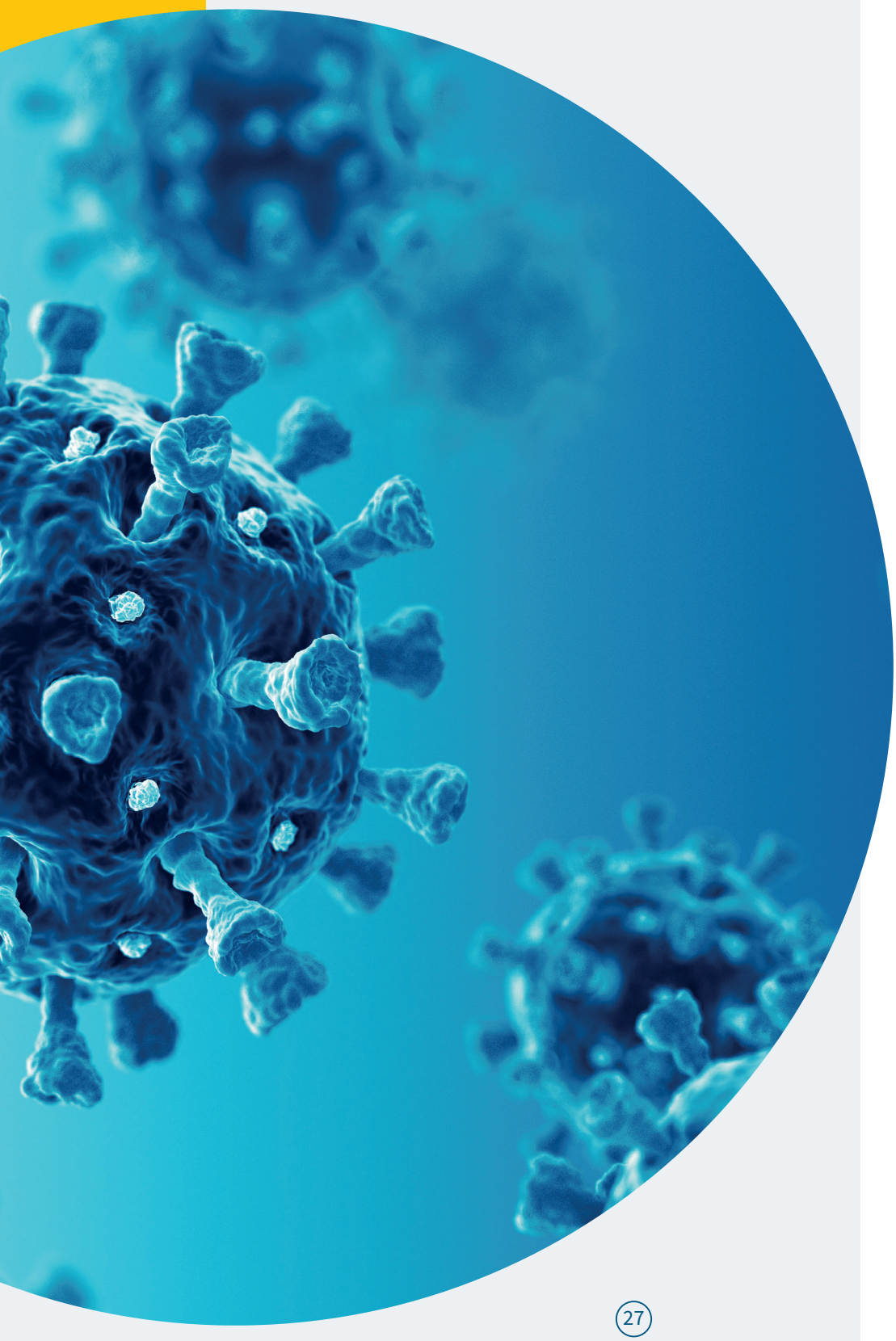
In the future, we aim to improve the prediction of functional consequences for large genome alterations in relation to human diseases. We plan to identify new transcriptional units and novel isoforms for known genes and link them to genome variation and dysregulated splicing factors (age- and disease-specific). 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

1. de Jong TV, Moshkin YM, **Guryev V**. 2019. Gene expression variability: the other dimension in transcriptome analysis. *Physiol Genomics*. 51:145-158.
2. Hehir-Kwa JY, Marschall T, [..], Ye K, **Guryev V**. 2016. A high-quality human reference panel reveals the complexity and distribution of genomic structural variants. *Nat Commun*. 7:12989.
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.

Multi-omics data integration for understanding ageing-related diseases





Molecular Neurobiology of Ageing

Ellen Nollen

INTRODUCTION

Loss of protein homeostasis during ageing is thought to accelerate aging and cause age-related neurodegenerative diseases, such as Parkinson's disease and amyotrophic lateral sclerosis (ALS). As a consequence, aggregation-prone proteins accumulate in abnormal inclusions and aggregates that characterize disease. The biological mechanisms that drive these protein transitions and their toxicity are poorly understood. Our research group aims to identify and characterize these mechanisms. With our results we hope to contribute to the development of interventions that prevent or delay protein toxicity in age-related diseases and promote healthy ageing.

RESEARCH FOCUS

Using genetic screens in the tiny and short-lived roundworm *C.elegans*, we have previously identified several drivers of protein toxicity that also exist in human, including the modifiers of aggregation MOAG-4/SERF and the tryptophan-metabolizing enzyme TDO.

Our recent research has focused on the mechanisms by which these modifiers act. For example, using a screen for recognition motifs on a panel of disease-related proteins and functional studies in human cell- and worm models, we have found that MOAG-4/SERF drives aggregation and toxicity via charge interactions[1]. Our results suggest that prevention of charge interactions between disease proteins and cellular factors like MOAG-4/SERF could be explored as a strategy to prevent or delay protein toxicity.

In addition, we developed a screening pipeline to study behavior and the function of individual neurons in a circuit. We used this pipeline to study aging of the motor circuit, in which acetylcholine neurons, GABA neurons and muscle cells together coordinate movement (Figure)[2]. We find that acetylcholine neurons age much faster than GABA neurons and muscle cells and that their aging rate corresponds to age-related motor impairment in the same animals. Why acetylcholine cells are more vulnerable and whether restoring their function can restore movement remains to be determined, which will be part of our future studies.

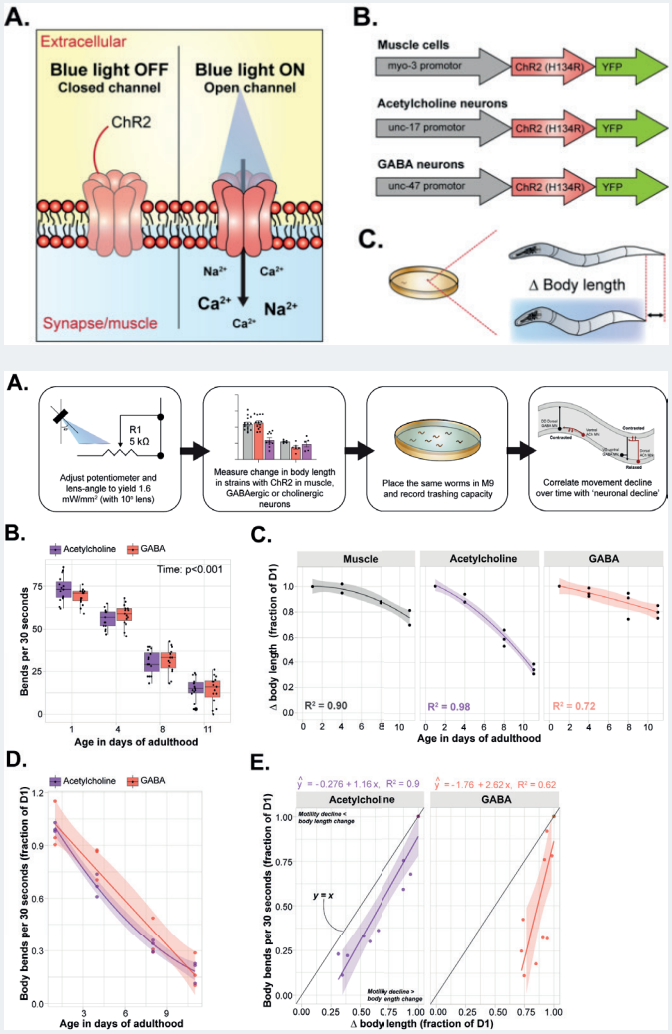
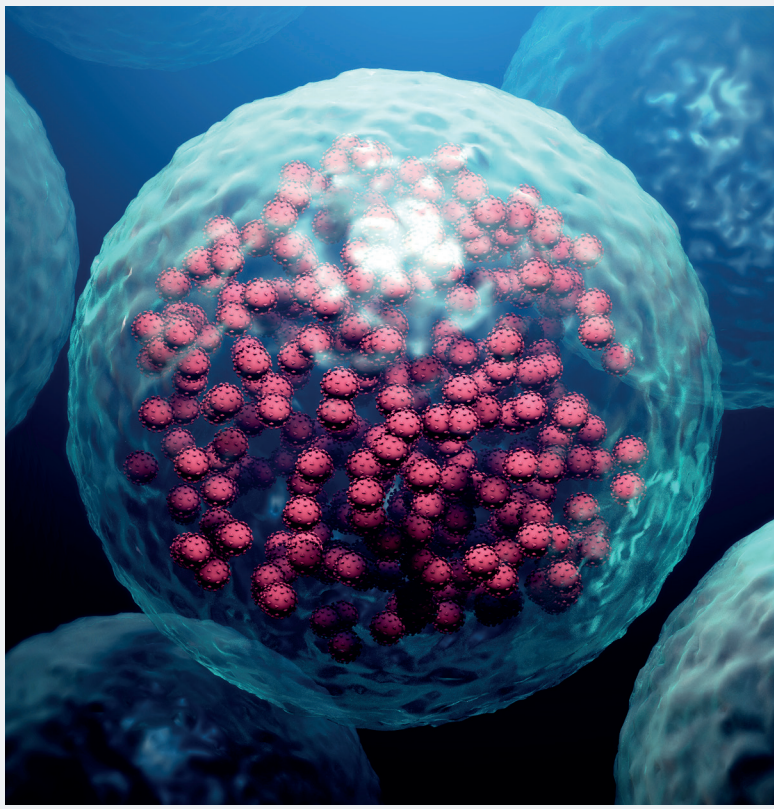


Figure. Light-induced approach to study function of individual neurons in the C.elegans motor circuit. A. *C. elegans* strains with light-activated ion channels in either muscle cells, acetylcholine neurons or GABA neurons are used to study the functional integrity of these cells and their contribution to movement. **B.** Functional integrity of individual motor circuit cell during aging, as measured by muscle contraction in response to light activation (from reference 1).



THE FUTURE

Using *C.elegans* as a model, we will continue to identify and characterize modifying mechanisms of age-related protein toxicity. We will explore the influence of organismal as well as external factors, such as microbiome and circadian rhythm. With our recently developed automated tracking pipeline we will zoom in on mechanisms that determine age-related loss of circuit integrity and screen for interventions that can boost their resilience to aging and age-related protein toxicity.

TOP 3 PUBLICATIONS

1. Pras A, Houben B, Aprile FA, Seinstra R, Gallardo R, Janssen L, Hogewerf W, Gallrein C, De Vleeschouwer M, Mata-Cabana A, Koopman M, Stroo E, de Vries M, Louise Edwards S, Kirstein J, Vendruscolo M, Falsone SF, Rousseau F, Schymkowitz J, **Nollen EAA**. The cellular modifier MOAG-4/SERF drives amyloid formation through charge complementation. EMBO J. 2021 Nov 2;40(21):e107568. doi: 10.15252/embj.2020107568. Epub 2021 Oct 7. PMID: 34617299.

2. Koopman M, Janssen L, **Nollen EAA**. An economical and highly adaptable optogenetics system for individual and population-level manipulation of Caenorhabditis elegans. BMC Biol. 2021 Aug 24;19(1):170. doi: 10.1186/s12915-021-01085-2.

3. Hardenberg MC, Sinnige T, Casford S, Dada ST, Poudel C, Robinson EA, Fuxreiter M, Kaminksi CF, Kaminski Schierle GS, **Nollen EAA**, Dobson CM, Vendruscolo M. Observation of an α-synuclein liquid droplet state and its maturation into Lewy body-like assemblies. J Mol Cell Biol. 2021 Aug 4;13(4):282-294. doi: 10.1093/jmcb/mjaa075. PMID: 33386842; PMCID: PMC8339365.

Asymmetric Cell Division and Ageing

Judith Paridaen

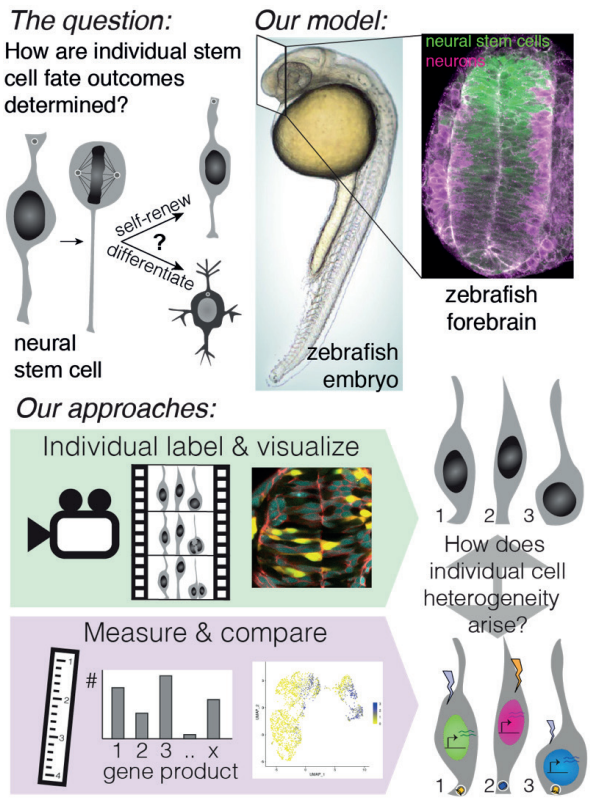
INTRODUCTION

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

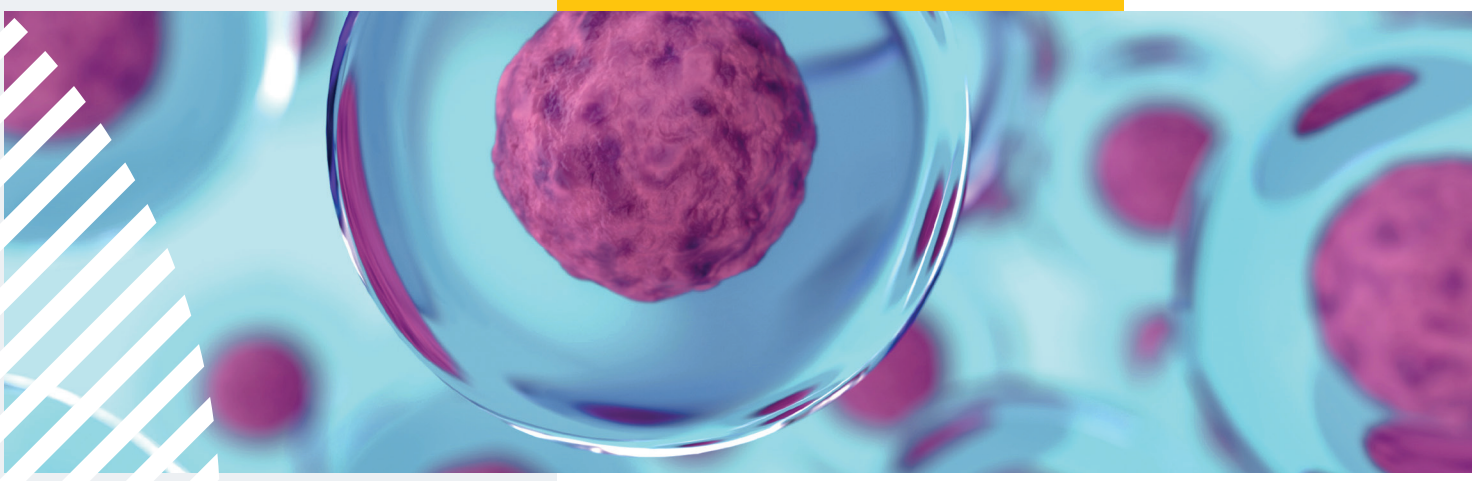
Despite individual stem cell heterogeneity, their functional output in normal development is very robust. However, it is unclear how this heterogeneity comes about, and what the contributions of truly noisy or truly random (stochastic) processes is. We hypothesize that hidden features of neural stem 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.

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 under-lying 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.

Neural stem cell biology at the single-cell level

Cellular Biochemistry

Liesbeth Veenhoff

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 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.

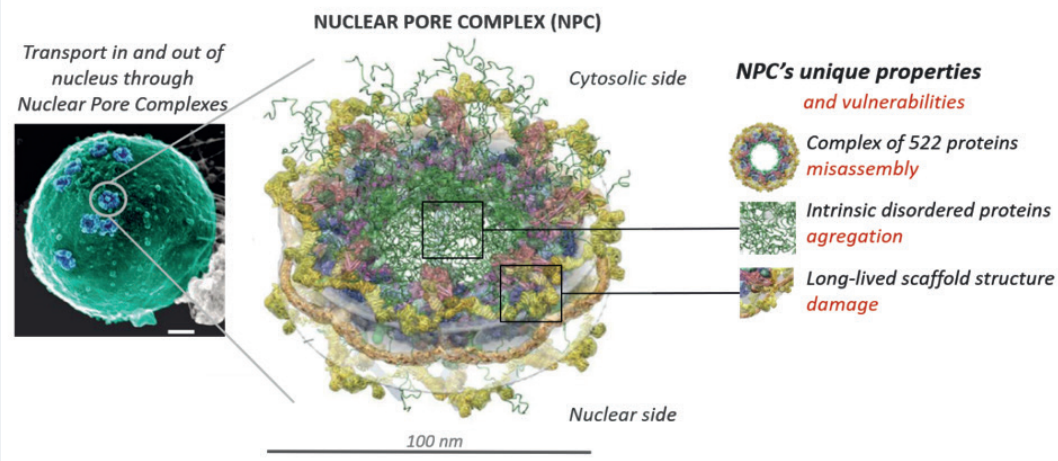


Figure 1. Left: field emission scanning electron micrograph of yeast nucleus with blue-colored NPCs (adapted from E. Kiseleva, Nature Cell Biology, 2004). Middle: structure of yeast NPC (adapted from Kim *et al.,* Nature, 2018), highlighting its size and complexity and the presence of intrinsically disordered proteins (in green) and long-lived proteins in the scaffold (other colors).

In order to better understand how the intrinsically disordered proteins of the NPCs are protected from forming dysfunctional aggregates, we embarked on biochemical studies following their condensation and aggregation. The intrinsically disordered domains of several yeast and human NPC proteins spontaneously form condensates that develop into aggregates (Fig 2AB).

In a collaborative effort with the department of Biomedical Sciences of Cells and Systems, we provided cell biological and biochemical evidence to support that the chaperone DNAJB6 can prevent aggregation of several intrinsically disordered proteins of the NPC (Fig 2C). We propose that the activity of DNAJB6 is needed to facilitate the assembly of the intrinsically disordered proteins into new NPCs.

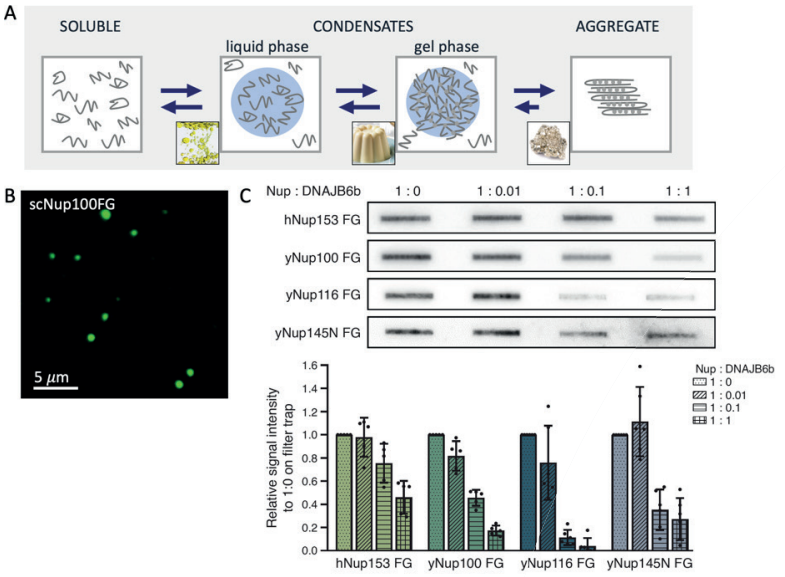


Figure 2. A) cartoon illustrating condensation and aggregation of intrinsically disordered proteins. B) fluorescent microscopy image of condensates formed by the intrinsically disordered FG-rich region of the yeast NPC proteins Nup100; purified protein fluorescently labelled. C) from Kuiper et al., 2021: quantitative assessment of aggregation by different intrinsically disordered domains of yeast and human NPC proteins in the absence and presence of the chaperone DNAJB6.

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 proteins of the NPC are interesting to study, as it appears that mechanisms that guard their structural state, 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. The molecular chaperone DNAJB6 provides surveillance of FG-Nups and is required for interphase nuclear pore complex biogenesis. Kuiper EF, Gallardo P, Bergsma T, Mari M, Kolbe Musskopf M, Kuipers J, Giepmans BNG, Steen A, **Veenhoff LM**, Kampinga HH, Bergink S. bioRxiv preprint doi: https://doi.org/10.1101/2021.10.26.465890
2. 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.
3. 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.

Understanding the role of nuclear pores in cellular ageing

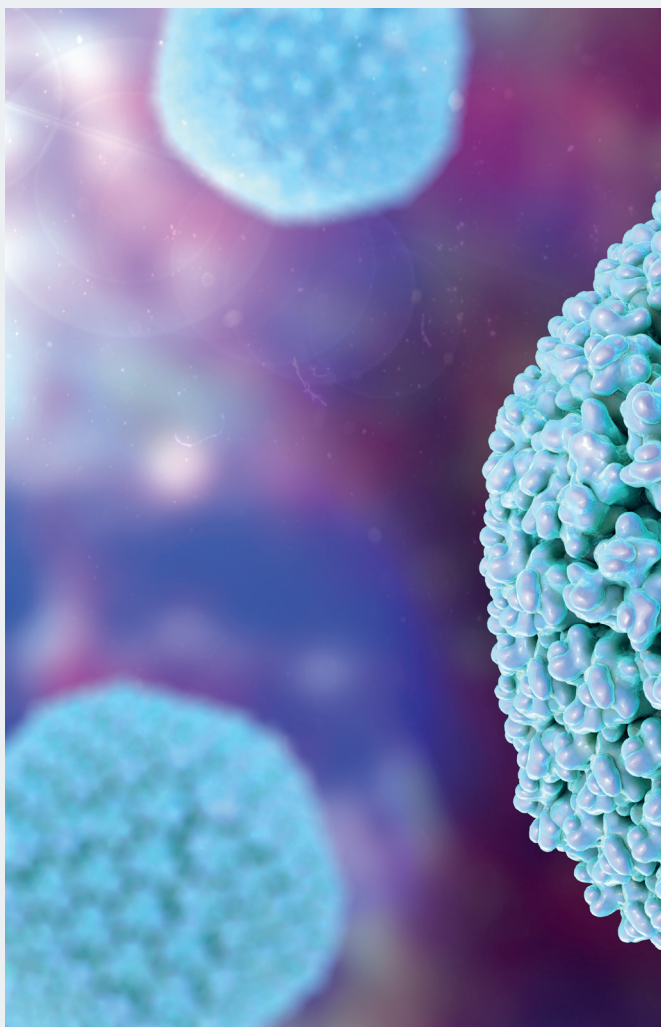
Macromolecules and Interactomes

John LaCava

INTRODUCTION

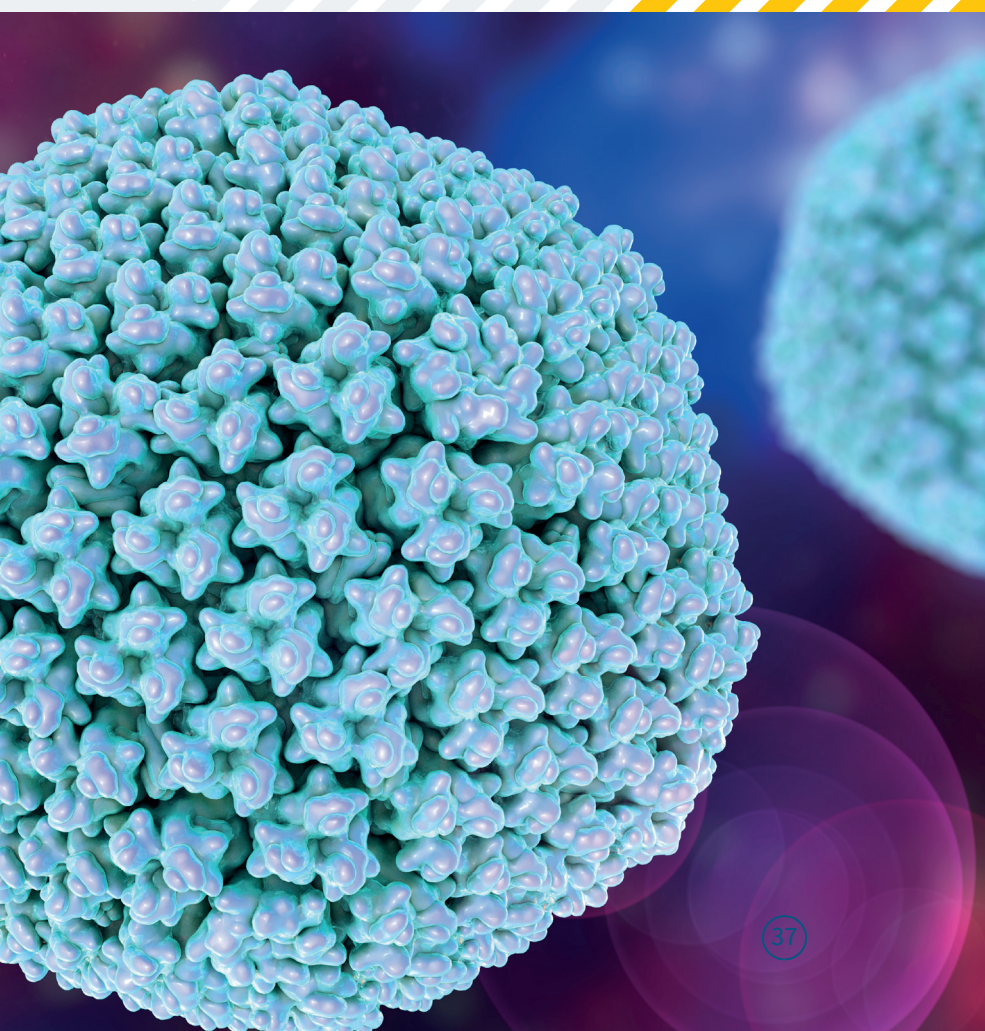
Our group has a specific technology focus: developing methods for interactome analyses. We specialize in affinity proteomic approaches. Presently, we aim to translate our research tools, which explore and characterize protein interactions within multi-component macromolecular complexes, towards the clinic: for example, identifying differences in protein complex constituents between healthy and diseased patient tissues. Several projects in the lab seek to apply our interactome analytical tools to diverse biological questions, typically (but not exclusively) with connections to human diseases. The characterization of human LINE-1 retrotransposons is central among our biological interests. 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 e.g., cancer, autoimmunity, and neurodegeneration. We continue to explore the roles of LINE-1 in colorectal cancers and systemic lupus erythematosus. Most recently, we have initiated studies into the emerging connections between LINE-1 and 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 proteomic 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 in affinity proteomics. We place special emphasis on approaches that also enable downstream structural and biochemical studies of enriched macromolecules. In the context of this technology focus, we are agnostic to the specific disease or underlying biology and collaborate widely on diverse projects with fundamental biologists and clinicians alike.

Long Interspersed Element 1 (LINE-1, L1), a retrotransposon, is a core biological interest of our lab. 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 L1 RNPs consist of different assortments of constituents, depending on the cell type, 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, considering these interactions. In doing so, we also explore L1 contributions to pathobiology.



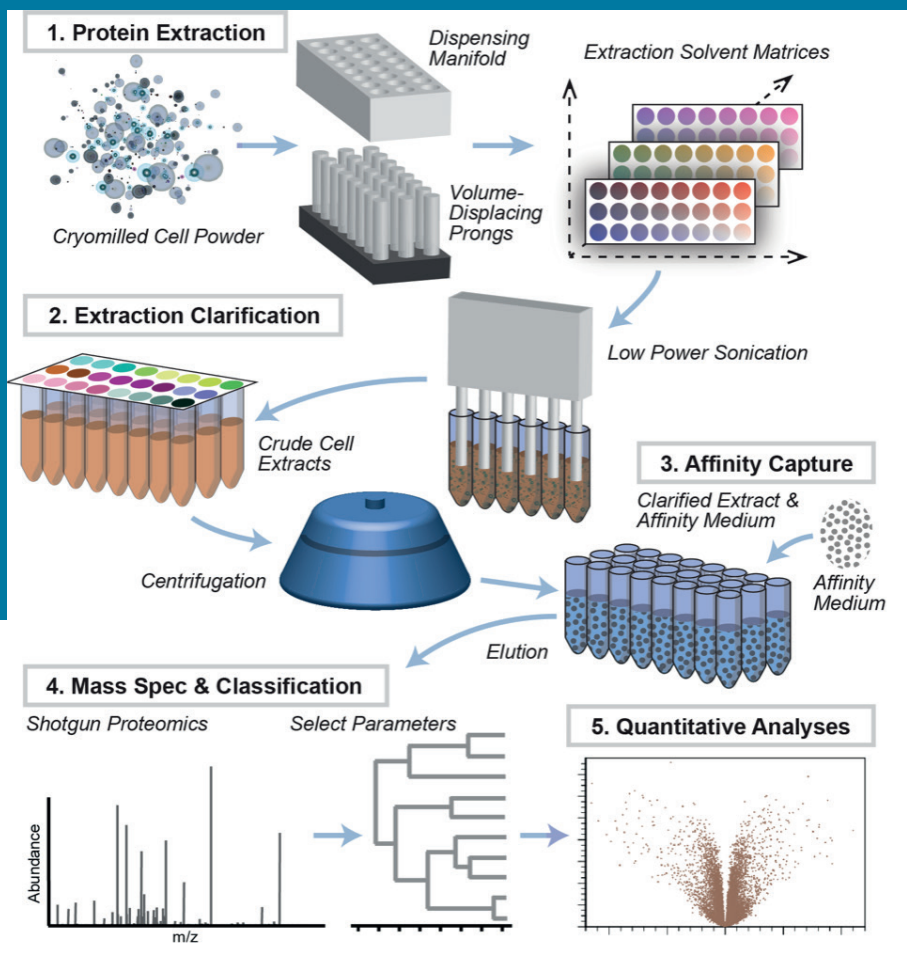


Figure. Methodological approach: Cryomilled cell powders are distributed with a dispensing manifold and macromolecules are extracted with different extraction solutions (1). Brief sonication is applied to disperse and homogenize the extracts (2). After clarifying the extracts by centrifugation, affinity 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.

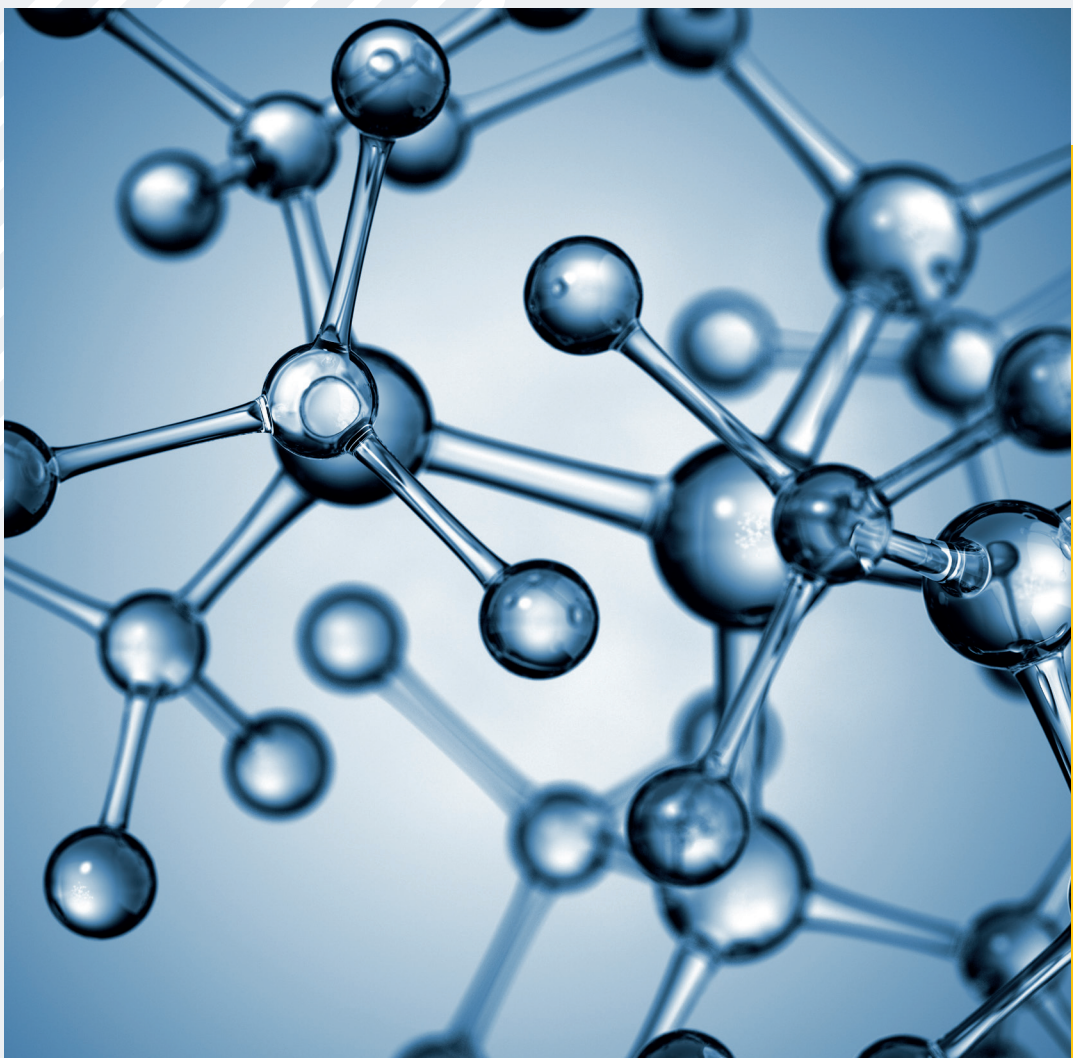
Our work with L1 is growing in numerous directions, chief among them, our development of a sensitive, quantitative biomarker assay for the detection of L1 ORF proteins in e.g., serum and cerebrospinal fluid. This assay is enabling us to explore diagnostic implications of L1 expression in cancers, autoimmunity, and neurodegeneration, which we are pursuing in on-going research.

TOP PUBLICATIONS

1. Ukadike, K.C., Ni, K., Wang, X. *et al.* IgG and IgA autoantibodies against L1 ORF1p expressed in granulocytes correlate with granulocyte consumption and disease activity in pediatric systemic lupus erythematosus. *Arthritis Res Ther* **23,** 153 (2021). https://doi.org/10.1186/s13075-021-02538-3

2. Repeats Mimic Immunostimulatory Viral Features Across a Vast Evolutionary Landscape.Petr Šulc, Alexander Solovyov, Sajid,A. Marhon, Siyu Sun, John LaCava, Omar Abdel-Wahab, Nicolas Vabret, Daniel D. De Carvalho, Rémi Monasson, Simona Cocco, Benjamin D. Greenbaum bioRxiv 2021.11.04.467016; doi: https://doi.org/10.1101/2021.11.04.467016

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



Highlights- 2021

This section reports a selected number of achievements that have been accomplished by ERIBA staff in 2021.

Scientific Publications

In 2021, ERIBA scientists published a record number of 72 papers* in scientific journals. Many of the published papers were the result of fruitful collaborations. Joint projects were initiated between research groups in ERIBA, between scientists in ERIBA with research groups at the University Medical Center Groningen and the Faculty of Science and Engineering of the University of Groningen, and with international partners.

*53 publications and 19 Preprints

Eugene Berezikov's group

Kirill Ustyantsev, Jakub Wudarski, Igor Sukhikh, Filipa Reinoite, Stijn Mouton, **Eugene Berezikov**. (2021). Proof of principle for piggyBac-mediated transgenesis in the flatworm *Macrostomum lignano.* Genetics 218:iyab076. doi: 10.1093/genetics/iyab076.

Regeneration-capable flatworms are informative research models to study the mechanisms of stem cell regulation, regeneration, and tissue patterning. The free-living flatworm *Macrostomum lignano* is currently the only flatworm where stable transgenesis is available, and as such it offers a powerful experimental platform to address questions that were previously difficult to answer. The published transgenesis approach relies on random integration of DNA constructs into the genome. Despite its efficiency, there is room and need for further improvement and diversification of transgenesis methods in *M. lignano*. Transposon-mediated transgenesis is an alternative approach, enabling easy mapping of the integration sites and the possibility of insertional mutagenesis studies. Here, we report for the first time that transposon-mediated transgenesis using piggyBac can be performed in *M. lignano* to create stable transgenic lines with single-copy transgene insertions.

Ellen Nollen's group

Koopman M, Janssen L, Nollen EAA. An economical and highly adaptable optogenetics system for individual and population-level manipulation of Caenorhabditis elegans. BMC Biol. 2021 Aug 24;19(1):170. doi: 10.1186/s12915-021-01085-2.

We developed a screening pipeline to study behavior and the function of individual neurons in a circuit. We used this pipeline to study aging of the motor circuit, in which acetylcholine neurons, GABA neurons and muscle cells together coordinate movement. We find that acetylcholine neurons age much faster than GABA neurons and muscle cells and that their aging rate corresponds to age-related motor impairment in the same animals. Why acetylcholine cells are more vulnerable and whether restoring their function can restore movement remains to be determined, which will be part of our future studies.

Floris Foijer's group

Shoshani O., Bakker B., Wang Y., Kim D.H., Maldonado M., Demarest M.A., Artates J., Zhengyu O., Mark A., Wardenaar R., Sasik R., Spierings D.C.J., Vitre B., Fisch K., **Foijer F.#**, and Cleveland D.W.#. Transient genomic instability drives tumorigenesis through accelerated clonal evolution. Genes Dev. 2021 Aug 1;35(15-16):1093-1108., doi:10.1101/gad.348319.12

Abnormal numerical and structural chromosome content is frequently found in human cancer. To test the role of aneuploidy in tumor initiation and progression, we generated mice with random aneuploidies by transient induction of polo-like kinase 4 (Plk4), a master regulator of centrosome number. Short-term chromosome instability (CIN) from transient Plk4 induction resulted in formation of aggressive T-cell lymphomas in mice with heterozygous inactivation of one *p53* allele and accelerated tumor development in the absence of p53. Transient CIN increased the frequency of lymphoma-initiating cells with a specific karyotype profile, including trisomy of chromosomes 4, 5, 14, and 15 occurring early in tumorigenesis. Tumor development in mice with chronic CIN induced by an independent mechanism (through inactivation of the spindle assembly checkpoint) gradually trended toward a similar karyotypic profile, as determined by single-cell whole-genome DNA sequencing. Overall, we show how transient CIN generates cells with random aneuploidies from which ones that acquire a karyotype with specific chromosome gains are sufficient to drive cancer formation, and that distinct CIN mechanisms can lead to similar karyotypic cancer-causing outcomes.

Gerald de Haan's group

Flohr Svendsen A, Yang D, Kim K, Lazare S, Skinder N, Zwart E, Mura-Meszaros A, Ausema A, von Eyss B, **de Haan G**, Bystrykh L.(2021) A comprehensive transcriptome signature of murine hematopoietic stem cell aging. Blood.

Multiple studies have revealed how murine hematopoietic stem cells lose functional activity upon aging, and have suggested the involvement of specific genes in this decline. However, a consistent gene expression pattern of aged HSCs has never been detected. In this paper we explored, using multiple available and newly established data sets whether such an aging signature actually exists, and if so, which genes would be affected. Using various bioinformatic approaches we were able to identify a comprehensive transcriptome signature of aged HSCs, and found that the collective expression of ~20 genes predicts the age of an HSC. Interestingly, many of these HSC aging genes encode for proteins that are located at the cell surface. We were able to show that in aged bone marrow, high expression of one of these aging genes, P-selectin, identifies HSCs with inferior repopulating ability. Our data suggest that communication of HSCs with their immediate bone marrow environment is altered during aging.

John LaCava's group

Repeats Mimic Immunostimulatory Viral Features Across a Vast Evolutionary LandscapePetr Šulc, Alexander Solovyov, Sajid A. Marhon, Siyu Sun, **John LaCava**, Omar Abdel-Wahab, Nicolas Vabret, Daniel D. De Carvalho, Rémi Monasson, Simona Cocco, Benjamin D. Greenbaum doi: https://doi.org/10.1101/2021.11.04.467016

An emerging hallmark across many human diseases - such as cancer, autoimmune and neurodegenerative disorders – is the aberrant transcription of typically silenced repetitive elements. Once transcribed they can mimic pathogen-associated molecular patterns and bind pattern recognition receptors, thereby engaging the innate immune system and triggering inflammation in a process known as "viral mimicry". Yet how to quantify pathogen mimicry, and the degree to which it is shaped by natural selection, remains a gap in our understanding of both genome evolution and the immunological basis of disease. Here we propose a theoretical framework that combines recent biological observations with statistical physics and population genetics to quantify the selective forces on virus-like features generated by repeats and integrate these forces into predictive evolutionary models. We establish that many repeat families have evolutionarily maintained specific classes of viral mimicry. We show that for HSATII and intact LINE-1 selective forces maintain CpG motifs, while for a set of SINE and LINE elements the formation of long double-stranded RNA is more prevalent than expected from a neutral evolutionary model. We validate our models by showing predicted immunostimulatory inverted SINE elements bind the MDA5 receptor under conditions of epigenetic dysregulation and that they are disproportionately present during intron retention when RNA splicing is pharmacologically inhibited. We conclude viral mimicry is a general evolutionary mechanism whereby genomes co-opt features generated by repetitive sequences to trigger the immune system, acting as a quality control system to flag genome dysregulation. We demonstrate these evolutionary principles can be learned and applied to predictive models. Our work therefore serves as a resource to identify repeats with candidate immunostimulatory features and leverage them therapeutically.

Liesbeth Veenhoff's group

Semmelink MFW, Steen A, **Veenhoff LM**.(2021) Measuring and Interpreting Nuclear Transport in Neurodegenerative Disease-The Example of C9orf72 ALS. Int J Mol Sci.

Transport from and into the nucleus is essential to all eukaryotic life and occurs through the nuclear pore complex (NPC). There are a multitude of data supporting a role for nuclear transport in neurodegenerative diseases, but actual transport assays in disease models have provided diverse outcomes. In this review, we summarize how nuclear transport works, which transport assays are available, and what matters complicate the interpretation of their results. Taking a specific type of ALS caused by mutations in *C9orf72* as an example, we illustrate these complications, and discuss how the current data do not firmly answer whether the kinetics of nucleocytoplasmic transport are altered.

Answering this open question has far-reaching implications, because a positive answer would imply that widespread mislocalization of proteins occurs, far beyond the reported mislocalization of transport reporters, and specific proteins such as FUS, or TDP43, and thus presents a challenge for future research.

Marco Demaria's group

Vliet T, Wang B, Varela-Eirin M, Borghesan M, Brandenburg S, Evangelou K, Franzin R, Seelen M, Gorgoulis V, **Demaria M**. Physiological hypoxia restrains the Senescence-Associated Secretory Phenotype (SASP) via AMPK-mediated mTOR suppression. 2021. Molecular Cell. 81:2041-2052.

Cellular senescence is a state of stable proliferative arrest triggered by damaging signals. Senescent cells persist during ageing and promote age-related pathologies, including cancer. Persistent senescent cells exert detrimental functions mostly by a heterogeneous and pro-inflammatory Senescence-Associated Secretory Phenotype (SASP) which regulation depends on environmental factors. *In vivo*, a major environmental variable is oxygenation, which vary among and within tissues. Here, we demonstrate that senescent cells expressed reduced levels of detrimental proinflammatory SASP factors in hypoxic compared to normoxic oxygen environments, in culture and in tissues. Mechanistically, hypoxic senescent cells show reduced mTOR and NF-kB activity compared to senescent cells under normoxia, with mTOR suppression resulting from enhanced AMPK activity. We additionally demonstrate that treatment with hypoxia mimetic compounds also achieve SASP inhibition leading to improve muscle strength in mice. Our findings highlight the importance of oxygen as a determinant for pro-inflammatory SASP expression, and offer a potential new strategy to reduce detrimental paracrine effects of senescent cells upon treatment with hypoxia or hypoxia-mimicking approaches.

Michael Chang's group

Fekete-Szücs, E., Rosas Bringas, F.R., Stinus, S., and **Chang, M**. (2021) Suppression of *cdc13-2*-associated senescence by *pif1-m2* requires Ku-mediated telomerase recruitment. ***G3***, doi: 10.1093/g3journal/jkab360.

In *Saccharomyces cerevisiae*, recruitment of telomerase to telomeres requires an interaction between Cdc13, which binds single-stranded telomeric DNA, and the Est1 subunit of telomerase. A second pathway involving an interaction between the yKu complex and telomerase RNA (TLC1) contributes to telomerase recruitment but cannot sufficiently recruit telomerase on its own to prevent replicative senescence when the primary Cdc13-Est1 pathway is abolished—for example, in the *cdc13-2* mutant. In this study, we find that mutation of *PIF1*, which encodes a helicase that inhibits telomerase, suppresses the replicative senescence of *cdc13-2* by increasing reliance on the yKu-TLC1 pathway for telomerase recruitment. Our findings reveal new insight into telomerase-mediated telomere maintenance.

Victor Guryev's group

de Jong TV, **Guryev V**, Moshkin YM. Estimates of gene ensemble noise highlight critical pathways and predict disease severity in H1N1, COVID-19 and mortality in sepsis patients. Sci Rep. 2021 May 24;11(1):10793. doi: 10.1038/s41598-021-90192-9. PMID: 34031464; PMCID: PMC8144599.

Finding novel biomarkers for human pathologies and predicting clinical outcomes for patients is challenging. This stems from the heterogeneous response of individuals to disease and is reflected in the inter-individual variability of gene expression responses that obscures differential gene expression analysis. Here, we developed an alternative approach that could be applied to dissect the disease-associated molecular changes. We define gene ensemble noise as a measure that represents a variance for a collection of genes encoding for either members of known biological pathways or subunits of annotated protein complexes and calculated within an individual. The gene ensemble noise allows for the holistic identification and interpretation of gene expression disbalance on the level of gene networks and systems. By comparing gene expression data from COVID-19, H1N1, and sepsis patients we identified common disturbances in a number of pathways and protein complexes relevant to the sepsis pathology. Among others, these include the mitochondrial respiratory chain complex I and peroxisomes. This suggests a Warburg effect and oxidative stress as common hallmarks of the immune host–pathogen response. Finally, we showed that gene ensemble noise could successfully be applied for the prediction of clinical outcome namely, the mortality of patients. Thus, we conclude that gene ensemble noise represents a promising approach for the investigation of molecular mechanisms of pathology through a prism of alterations in the coherent expression of gene circuits.

Facts and Figures

Grants

Eriba scientists secured 3.5 million in funding from various funding agencies and grants.

Graduations

In 2021, nine scientists graduated from ERIBA as PhD students and since have moved to their next position:

Name	PI	Currently Located
Thijmen van Vliet	Marco Demaria	Clinical Study Manager bij ICON (PRA Health Sciences), Netherlands
Irena Bockaj	Sophia Bruggeman	Scientist at UniQure, Amsterdam, Netherlands
Inna Armandari	Sophia Bruggeman	University lecturer at the University of Gadjah Mada, Indonesia
Bjorn Bakker	Floris Foijer	Post-doctoral training fellow at The Francis Crick Institute, London, England
Britt Sterken	Cor Calkhoven	Postdoctoral Researcher at Cancer Research UK Beatson Institute, Glasgow, Schotland
Anita Pras	Ellen Nollen	Nature teacher at Apoidea gGmbH, Hamburg, Germany
Sabrina Jacobs	Gerald de Haan	Data Steward - Cohort and Biobank Coordination Hub · UMCG, Groningen, Netherlands
Danielle Luinenburg	Gerald de Haan	Clinical Study Manager at the ICON (PRA Health Sciences), Netherlands
Sonja Buisman	Gerald de Haan	ANIOS Internal Medicine, Martini hospital, Groningen, Netherlands

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 various outreach events. Our scientists participated in the Young Academy Groningen Science fair at Noorderzon Arts Festival where they presented the research work. 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 55.

Publications

NUMBER OF SCIENTIFIC PUBLICATIONS IN 2021: 72 *

(53 Publications+19 preprints)

Publications:

Publications per Research Group

Laboratory of Gene Regulation in Ageing and Age-related Diseases

Group Leader: Cor Calkhoven

de Leeuw AJM, Oude Luttikhuis MAM, Wellen AC, Müller C, **Calkhoven CF**. (2021) Obesity and its impact on COVID-19. J Mol Med (Berl)

Laboratory of Molecular Neurobiology of Ageing

Group Leader: Ellen Nollen

Hardenberg MC, Sinnige T, Casford S, Dada ST, Poudel C, Robinson EA, Fuxreiter M, Kaminksi CF, Kaminski Schierle GS, **Nollen EAA**, Dobson CM, Vendruscolo M. (2021)Observation of an α-synuclein liquid droplet state and its maturation into Lewy body-like assemblies. J Mol Cell Biol.

Pras A, **Nollen EAA**. (2021) Regulation of Age-Related Protein Toxicity. Front Cell Dev Biol.

Pras A, Houben B, Aprile FA, Seinstra R, Gallardo R, Janssen L, Hogewerf W, Gallrein C, De Vleeschouwer M, Mata-Cabana A, Koopman M, Stroo E, de Vries M, Louise Edwards S, Kirstein J, Vendruscolo M, Falsone SF, Rousseau F, Schymkowitz J, **Nollen EAA**. (2021)The cellular modifier MOAG-4/SERF drives amyloid formation through charge complementation. EMBO J.

Perni M, van der Goot A, Limbocker R, van Ham TJ, Aprile FA, Xu CK, Flagmeier P, Thijssen K, Sormanni P, Fusco G, Chen SW, Challa PK, Kirkegaard JB, Laine RF, Ma KY, Müller MBD, Sinnige T, Kumita JR, Cohen SIA, Seinstra R, Kaminski Schierle GS, Kaminski CF, Barbut D, De Simone A, Knowles TPJ, Zasloff M, **Nollen EAA**, Vendruscolo M, Dobson CM. (2021) Comparative Studies in the A30P and A53T α-Synuclein C. elegans Strains to Investigate the Molecular Origins of Parkinson's Disease. Front Cell Dev Biol.

Koopman M, Janssen L, **Nollen EAA.** (2021) Publisher Correction to: An economical and highly adaptable optogenetics system for individual and population-level manipulation of Caenorhabditis elegans. BMC Biol.

Preprints:

Brain deletion of Serf2 shifts amyloid conformation in a mouse model E. Stroo, L. Janssen, O. Sin, W. Hogewerf, M. Koster, L. Harkema, S.A. Youssef, N. Beschorner, A.H.G. Wolters, B. Bakker, A. Thathiah, F. Foijer, B. van de Sluis, J. van Deursen, M. Jucker, A de Bruin, **E.A.A. Nollen** bioRxiv 2021.01.05.423442;

doi: https://doi.org/10.1101/2021.01.05.423442 3. Preserving protein homeostasis prevents motor impairment in DNA Damage Response-compromised C. elegans Wouter Huiting, Alejandra Duque-Jaramillo, Renée I. Seinstra, Harm. H. Kampinga, **Ellen A.A. Nollen**, Steven Bergink. bioRxiv 2021.12.22.473820; doi: https://doi.org/10.1101/2021.12.22.473820

Laboratory of Stem cell regulation and mechanisms of regeneration

Group Leader: Eugene Berezikov

Mouton S, Ustyantsev K, Beltman F, Glazenburg L, **Berezikov E.** (2021)TIM29 is required for enhanced stem cell activity during regeneration in the flatworm Macrostomum lignano. Sci Rep.

Ustyantsev K, Wudarski J, Sukhikh I, Reinoite F, Mouton S, **Berezikov E.** (2021) Proof of principle for piggyBac-mediated transgenesis in the flatworm Macrostomum lignano. Genetics.

Rinkevich B, Ballarin L, Martinez P, Somorjai I, Ben-Hamo O, Borisenko I, **Berezikov E**, Ereskovsky A, Gazave E, Khnykin D, Manni L, Petukhova O, Rosner A, Röttinger E, Spagnuolo A, Sugni M, Tiozzo S, Hobmayer B. (2021) A pan-metazoan concept for adult stem cells: the wobbling Penrose landscape. Biol Rev Camb Philos Soc.

Bomer N, Pavez-Giani MG, Deiman FE, Linders AN, Hoes MF, Baierl CLJ, Oberdorf-Maass SU, de Boer RA, Silljé HHW, **Berezikov E**, Simonides WS, Westenbrink BD, van der Meer P.(2021) Selenoprotein DIO2 Is a Regulator of Mitochondrial Function, Morphology and UPRmt in Human Cardiomyocytes. Int J Mol Sci.

Laboratory of Genomic Instability in Development and Disease

Group Leader: Floris Foijer

Phan TP, Maryniak AL, Boatwright CA, Lee J, Atkins A, Tijhuis A, Spierings DC, Bazzi H, **Foijer F**, Jordan PW, Stracker TH, Holland AJ. (2021) Centrosome defects cause microcephaly by activating the 53BP1-USP28-TP53 mitotic surveillance pathway. EMBO J.

Schukken KM, Zhu Y, Bakker PL, Koster MH, Harkema L, Youssef SA, de Bruin A, **Foijer F**. (2021) Acute systemic loss of Mad2 leads to intestinal atrophy in adult mice. Sci Rep.

Jilderda LJ, Zhou L, **Foijer F**. (2021) Understanding How Genetic Mutations Collaborate with Genomic Instability in Cancer. Cells.

Cohen-Sharir Y, McFarland JM, Abdusamad M, Marquis C, Bernhard SV, Kazachkova M, Tang H, Ippolito MR, Laue K, Zerbib J, Malaby HLH, Jones A, Stautmeister LM, Bockaj I, Wardenaar R, Lyons N, Nagaraja A, Bass AJ, Spierings DCJ, **Foijer F**, Beroukhim R, Santaguida S, Golub TR, Stumpff J, Storchová Z, Ben-David U. (2021) Aneuploidy renders cancer cells vulnerable to mitotic checkpoint inhibition. Nature.

Shoshani O, Bakker B, de Haan L, Tijhuis AE, Wang Y, Kim DH, Maldonado M, Demarest MA, Artates J, Zhengyu O, Mark A, Wardenaar R, Sasik R, Spierings DCJ, Vitre B, Fisch K, **Foijer F**, Cleveland DW. Transient genomic instability drives tumorigenesis through accelerated clonal evolution. Genes Dev. 2021 Aug 1;35(15-16):1093-1108. doi: 10.1101/gad.348319.121. Epub 2021 Jul 15. PMID: 34266887; PMCID: PMC8336898.

Bronder D, Tighe A, Wangsa D, Zong D, Meyer TJ, Wardenaar R, Minshall P, Hirsch D, Heselmeyer-Haddad K, Nelson L, Spierings D, McGrail JC, Cam M, Nussenzweig A, **Foijer F**, Ried T, Taylor SS.(2021)TP53 loss initiates chromosomal instability in fallopian tube epithelial cells. Dis Model Mech.

Ippolito MR, Martis V, Martin S, Tijhuis AE, Hong C, Wardenaar R, Dumont M, Zerbib J, Spierings DCJ, Fachinetti D, Ben-David U, **Foijer F**, Santaguida S. (2021) Gene copy-number changes and chromosomal instability induced by aneuploidy confer resistance to chemotherapy. Dev Cell.

Coulson-Gilmer C, Morgan RD, Nelson L, Barnes BM, Tighe A, Wardenaar R, Spierings DCJ, Schlecht H, Burghel GJ, **Foijer F**, Desai S, McGrail JC, Taylor SS. (2021) Replication catastrophe is responsible for intrinsic PAR glycohydrolase inhibitor-sensitivity in patient-derived ovarian cancer models. J Exp Clin Cancer Res.

Bočkaj I, Martini TEI, de Camargo Magalhães ES, Bakker PL, Meeuwsen-de Boer TGJ, Armandari I, Meuleman SL, Mondria MT, Stok C, Kok YP, Bakker B, Wardenaar R, Seiler J, Broekhuis MJC, van den Bos H, Spierings DCJ, Ringnalda FCA, Clevers H, Schüller U, van Vugt MATM, **Foijer F**, Bruggeman SWM. (2021) The H3.3K27M oncohistone affects replication stress outcome and provokes genomic instability in pediatric glioma. PLoS Genet.

Zhou L, Zheng S , Rosas Bringas FR , Bakker B , Simon JE, Bakker PL , Kazemier HG, Schubert M , Roorda M , van Vugt MATM, Chang M , **Foijer F**.(2021)G3 (Bethesda) A synthetic lethal screen identifies HDAC4 as a potential target in MELK overexpressing cancers.

Preprints:

Dorine C. Hintzen, Mar Soto, Michael Schubert, Bjorn Bakker, Diana C.J. Spierings, Karoly Szuhai, Peter M. Lansdorp, **Floris Foijer**, René H. Medema, Jonne A. RaaijmakersbioRxiv 2021.08.31.458318; doi: https://doi.org/10.1101/2021.08.31.458318

Mechanisms of genetic instability in a single S-phase following whole genome doubling. Simon Gemble, Sara Vanessa Bernhard, Nishit Srivastava, René Wardenaar, Nano, Anne-Sophie Macé, Andréa E. Tijhuis, Kristina Keuper, Diana C.J. Spierings, Helfrid Hochegger, Matthieu Piel, **Floris Foijer**, Zuzana Storchová, Renata Bastodoi: https://doi.org/10.1101/2021.07.16.452672

Laboratory of Ageing Biology and Stem Cells

Group Leader: Gerald de Haan

Yang D, **de Haan G.**(2021) Inflammation and Aging of Hematopoietic Stem Cells in Their Niche. Cells.

Luinenburg DG, Dinitzen AB, Flohr Svendsen A, Cengiz R, Ausema A, Weersing E, Bystrykh L, **de Haan G.** (2021) Persistent expression of microRNA-125a targets is required to induce murine hematopoietic stem cell repopulating activity. Exp Hematol.

Serrano Martinez P, Cinat D, van Luijk P, Baanstra M, **de Haan G**, Pringle S, Coppes RP. (2021) Mouse parotid salivary gland organoids for the in vitro study of stem cell radiation response. Oral Dis.

Flohr Svendsen A, Yang D, Kim K, Lazare S, Skinder N, Zwart E, Mura-Meszaros A, Ausema A, von Eyss B, **de Haan G**, Bystrykh L.(2021) A comprehensive transcriptome signature of murine hematopoietic stem cell aging. Blood.

Renders S, Svendsen AF, Panten J, Rama N, Maryanovich M, Sommerkamp P, Ladel L, Redavid AR, Gibert B, Lazare S, Ducarouge B, Schönberger K, Narr A, Tourbez M, Dethmers-Ausema B, Zwart E, Hotz-Wagenblatt A, Zhang D, Korn C, Zeisberger P, Przybylla A, Sohn M, Mendez-Ferrer S, Heikenwälder M, Brune M, Klimmeck D, Bystrykh L, Frenette PS, Mehlen P, **de Haan G**, Cabezas-Wallscheid N, Trumpp A. (2021)

Laboratory of Macromolecules and Interactomes

Group Leader: John LaCava

Ukadike KC, Ni K, Wang X, Taylor MS, **LaCava J**, Pachman LM, Eckert M, Stevens A, Lood C, Mustelin T. (2021) IgG and IgA autoantibodies against L1 ORF1p expressed in granulocytes correlate with granulocyte consumption and disease activity in pediatric systemic lupus erythematosus. Arthritis Res Ther.

Preprints:

Targeting DNA topoisomerases or checkpoint kinases results in an overload of chaperone systems, triggering aggregation of a metastable subproteome. Wouter Huiting, Suzanne L. Dekker, Joris C. J. van der Lienden, Rafaella Mergener, Gabriel V. Furtado, Emma Gerrits, Maiara K. Musskopf, Mehrnoosh Oghbaie, Luciano H. Di Stefano, Maria A.W.H. van Waarde-Verhagen, Suzanne Couzijn, Lara Barazzuol, **John LaCava**, Harm H. Kampinga, Steven Bergink doi: https://doi.org/10.1101/2021.06.04.447142

Repeats Mimic Immunostimulatory Viral Features Across a Vast Evolutionary LandscapePetr Šulc, Alexander Solovyov, Sajid A. Marhon, Siyu Sun, **John LaCava**, Omar Abdel-Wahab, Nicolas Vabret, Daniel D. De Carvalho, Rémi Monasson, Simona Cocco, Benjamin D. Greenbaum doi: https://doi.org/10.1101/2021.11.04.467016

LINE-1 expression in cancer correlates with DNA damage response, copy number variation, and cell cycle progression Wilson McKerrow, Xuya Wang, Paolo Mita, Song Cao, Mark Grivainis, Li Ding, **John LaCava**, Jef Boeke, David Fenyö. doi: https://doi.org/10.1101/2020.06.26.174052

Laboratory of Asymmetric Cell Division and Ageing

Group Leader: Judith Paridaen

Preprint:

The developmental stage of the medulloblastoma cell-of-origin restricts Hedgehog pathway usage and drug sensitivity

Marlinde J. Smit, Inna Armandari, Irena Bockaj, Tosca E.I. Martini, Walderik W. Zomerman, Zillah Siragna, Tiny G.J. Meeuwsen, Frank J.G. Scherpen, Mirthe H. Schoots, Martha Ritsema, Wilfred F.A. den Dunnen, Eelco W. Hoving, **Judith T.M.L. Paridaen**, Gerald de Haan, Victor Guryev, Sophia W.M. Bruggeman
bioRxiv 2021.01.22.427790; doi: https://doi.org/10.1101/2021.01.22.427790

Laboratory of Cellular Biochemistry

Group Leader: Liesbeth Veenhoff

Semmelink MFW, Steen A, **Veenhoff LM**.(2021) Measuring and Interpreting Nuclear Transport in Neurodegenerative Disease-The Example of C9orf72 ALS. Int J Mol Sci.

Preprints:

A precise and general FRET-based method for monitoring structural transitions in protein self-organization. Qi Wan, Sara N. Mouton, **Liesbeth M. Veenhoff**, Arnold J. Boersma. bioRxiv 2021.02.25.432866; doi:https://doi.org/10.1101/2021.02.25.432866

The molecular chaperone DNAJB6 provides surveillance of FG-Nups and is required for interphase nuclear pore complex biogenesis. E. F. Elsiena Kuiper, Paola Gallardo, Tessa Bergsma, Muriel Mari, Maiara Kolbe Musskopf, Jeroen Kuipers, Ben N. G. Giepmans, Anton Steen, **Liesbeth M. Veenhoff**, Harm H. Kampinga, Steven Bergink. bioRxiv 2021.10.26.465890; doi: https://doi.org/10.1101/2021.10.26.465890

Laboratory of Cellular Senescence and Age-related Pathologies

Group Leader: Marco Demaria

Fitsiou E, Pulido T, Campisi J, Alimirah F, **Demaria M**. Cellular Senescence and the Senescence-Associated Secretory Phenotype as Drivers of Skin Photoaging. J Invest Dermatol. 2021 Apr;141(4S):1119-1126. doi: 10.1016/j.jid.2020.09.031. Epub 2020 Dec 19. PMID: 33349436.

Casciaro F, Zia S, Forcato M, Zavatti M, Beretti F, Bertucci E, Zattoni A, Reschiglian P, Alviano F, Bonsi L, Follo MY, **Demaria M**, Roda B, Maraldi T. (2021) Unravelling Heterogeneity of Amplified Human Amniotic Fluid Stem Cells Sub-Populations. Cells.
Fitsiou E, Soto-Gamez A, **Demaria M**. (2021) Biological functions of therapy-induced senescence in cancer. Semin Cancer Biol.

Varughese FM, **Demaria M**. (2021) A novel transcriptomic-based classifier for senescent cancer cells. Trends Cancer.

Zhang M, Serna-Salas S, Damba T, Borghesan M, **Demaria M**, Moshage H.(2021) Hepatic stellate cell senescence in liver fibrosis: Characteristics, mechanisms and perspectives. Mech Ageing Dev.

Talma N, Gerrits E, Wang B, Eggen BJL, **Demaria M**. (2021) Identification of distinct and age-dependent p16High microglia subtypes. Aging Cell.

Wiley CD, Sharma R, Davis SS, Lopez-Dominguez JA, Mitchell KP, Wiley S, Alimirah F, Kim DE, Payne T, Rosko A, Aimontche E, Deshpande SM, Neri F, Kuehnemann C, **Demaria M**, Ramanathan A, Campisi J. (2021)Oxylipin biosynthesis reinforces cellular senescence and allows detection of senolysis. Cell Metab.

Kohli J, Veenstra I, Demaria M.(2021) The struggle of a good friend getting old: cellular senescence in viral responses and therapy. EMBO Rep.

van Vliet T, Varela-Eirin M, Wang B, Borghesan M, Brandenburg SM, Franzin R, Evangelou K, Seelen M, Gorgoulis V, **Demaria M**. (2021) Physiological hypoxia restrains the senescence-associated secretory phenotype via AMPK-mediated mTOR suppression. Mol Cell.

Soto-Gamez A, Wang Y, Zhou X, Seras L, Quax W, **Demaria M**. (2021) Enhanced extrinsic apoptosis of therapy-induced senescent cancer cells using a death receptor 5 (DR5) selective agonist. Cancer Lett.

Wang B, **Demaria M**. (2021) The quest to define and target cellular senescence in cancer. Cancer Research.

Zampetidis CP, Galanos P, Angelopoulou A, Zhu Y, Polyzou A, Karamitros T, Kotsinas A, Lagopati N, Mourkioti I, Mirzazadeh R, Polyzos A, Garnerone S, Mizi A, Gusmao EG, Sofiadis K, Gál Z, Larsen DH, Pefani DE, **Demaria M**, Tsirigos A, Crosetto N, Maya-Mendoza A, Papaspyropoulos A, Evangelou K, Bartek J, Papantonis A, Gorgoulis VG.(2021) A recurrent chromosomal inversion suffices for driving escape from oncogene-induced senescence via subTAD reorganization. 2021. Molecular Cell.

Kohli J, Wang B, Brandenburg S, Basisty N, Evangelou K, Varela-Eirin M, Campisi J, Schilling B, Gorgoulis V, **Demaria M**.(2021) Algorithmic assessment of cellular senescence in experimental and clinical specimens.Nature Protocols.

Laboratory of Telomeres and Genome Integrity

Group Leader: Michael Chang

G3 (Bethesda) A synthetic lethal screen identifies HDAC4 as a potential target in MELK overexpressing cancers. (2021) Zhou L, Zheng S , Rosas Bringas FR , Bakker B, Simon JE, Bakker PL , Kazemier HG , Schubert M , Roorda M , van Vugt MATM, **Chang M** , Foijer F.

Enikő Fekete-Szücs, Fernando Rosas Bringas, Sonia Stinus, **Michael Chang** (2021) Suppression of *cdc13-2*-associated senescence by *pif1-m2* requires Ku-mediated telomerase recruitment.

Preprints:

Zhou, L., Zheng, S., Rosas Bringas, F.R., Bakker, B., Simon, J.E., Bakker, P.L., Kazemier, H.G., Schubert, M., Roorda, M., van Vugt, M.A.T.M., **Chang, M**.*, and Foijer, F.* (2021) A synthetic lethal screen identifies HDAC4 as a potential target in MELK overexpressing cancers. bioRxiv, doi: 10.1101/2021.07.16.452653.

Fekete-Szücs, E., Rosas Bringas, F.R., Stinus, S., and **Chang, M**. (2021) Suppression of cdc13-2-associated senescence by pif1-m2 requires Ku-mediated telomerase recruitment. bioRxiv. doi: 10.1101/2021.03.31.437837.

Laboratory of Genome Structure Ageing

Group Leader: Victor Guryev

Hanlon VCT, Mattsson CA, Spierings DCJ, **Guryev V**, Lansdorp PM. (2021) InvertypeR: Bayesian inversion genotyping with Strand-seq data. BMC Genomics.

Bernal V, Bischoff R, Horvatovich P, **Guryev V**, Grzegorczyk M. (2021)The 'un-shrunk' partial correlation in Gaussian graphical models. BMC Bioinformatics.

Hills M, Falconer E, O'Neill K, Sanders AD, Howe K, **Guryev V**, Lansdorp PM. (2021) Construction of Whole Genomes from Scaffolds Using Single Cell Strand-Seq Data. Int J Mol Sci.

Zwinderman MRH, Lobo TJ, van der Wouden PE, Spierings DCJ, van Vugt MATM, Lansdorp PM, **Guryev V**, Dekker FJ.(2021) Deposition Bias of Chromatin Proteins Inverts under DNA Replication Stress Conditions. ACS Chem Biol.

Ditz B, Boekhoudt JG, Aliee H, Theis FJ, Nawijn M, Brandsma CA, Hiemstra PS, Timens W, Tew GW, Grimbaldeston MA, Neighbors M, **Guryev V**, van den Berge M, Faiz A. (2021) Comparison of genome-wide gene expression profiling by RNA Sequencing versus microarray in bronchial biopsies of COPD patients before and after inhaled corticosteroid treatment: does it provide new insights? ERJ Open Res.

Faiz A, Rathnayake SNH, Ten Hacken NHT, **Guryev V**, van den Berge M, Pouwels SD. (2021) Single-nucleotide polymorphism rs2070600 regulates AGER splicing and the sputum levels of the COPD biomarker soluble receptor for advanced glycation end-products. ERJ Open Res.

Ditz B, Sarma A, Kerstjens HAM, Liesker JJW, Bathoorn E, Vonk JM, Bernal V, Horvatovich P, **Guryev V**, Caldera S, Langelier C, Faiz A, Christenson SA, van den Berge M. (2021)The sputum transcriptome better predicts COPD exacerbations after the withdrawal of inhaled corticosteroids than sputum eosinophils. ERJ Open Res.

de Jong TV, **Guryev V**, Moshkin YM. (2021) Estimates of gene ensemble noise highlight critical pathways and predict disease severity in H1N1, COVID-19 and mortality in sepsis patients. Sci Rep.

de Jong TV, Kim P, **Guryev V**, Mulligan MK, Williams RW, Redei EE, Chen H. (2021)Whole genome sequencing of nearly isogenic WMI and WLI inbred rats identifies genes potentially involved in depression and stress reactivity. Sci Rep.

Zverinova S, **Guryev V**. (2021) Variant calling: Considerations, practices, and developments.Hum Mutat.

Pandey S, Hajikazemi M, Zacheja T, Schalbetter S, Baxter J, **Guryev V**, Hofmann A, Heermann DW, Juranek SA, Paeschke K. (2021)Telomerase subunit Est2 marks internal sites that are prone to accumulate DNA damage. BMC Biol.

Pre Sister chromatid exchanges induced by perturbed replication are formed independently of homologous recombination factorsAnne Margriet Heijink, Colin Stok, David Porubsky, Eleni M. Manolika, Yannick P. Kok, Marieke Everts, H. Rudolf de Boer, Anastasia Audrey, Elles Wierenga, **Victor Guryev**, Diana C.J. Spierings, Puck Knipscheer, Arnab Ray Chaudhuri, Peter M. Lansdorp, Marcel A.T.M. van Vugt. bioRxiv 2021.09.17.460736; doi: https://doi.org/10.1101/2021.09.17.460736

The developmental stage of the medulloblastoma cell-of-origin restricts Hedgehog pathway usage and drug sensitivity. Marlinde J. Smit, Inna Armandari, Irena Bockaj, Tosca E.I. Martini, Walderik W. Zomerman, Zillah Siragna, Tiny G.J. Meeuwsen, Frank J.G. Scherpen, Mirthe H. Schoots, Martha Ritsema, Wilfred F.A. den Dunnen, Eelco W. Hoving, Judith T.M.L. Paridaen, Gerald de Haan, **Victor Guryev**, Sophia W.M. Bruggeman

Proteogenomics Reveals how Metastatic Melanoma Modulates the Immune System to Allow Immune Evasion. Jeovanis Gil, Yonghyo Kim, Beáta Szeitz, Viktória Doma, Uğur Çakır, Natália Pinto de Almeida, Yanick Paco Hagemeijer, **Victor Guryev**, Jenny G Johansson, Yogita Sharma, Indira Pla Parada, Zsolt Horvath, Jéssica de Siqueira Guedes, Gustavo Monnerat, Gabriel Reis Alves Carneiro, Fábio CS Nogueira, Boram Lee, Henriett Oskolas, Enikő Kuroli, Judit Hársing, Yutaka Sugihara, Magdalena Kuras, Roger Appelqvist, Elisabet Wieslander, Gilberto B Domont, Bo Baldetorp, Runyu Hong, Gergely Huszty, Laura Vizkeleti, József Tímár, David Fenyö, Lazaro Hiram Betancourt, Johan Jakobsson, Johan Malm, Aniel Sanchez, A. Marcell Szász, Peter Horvatovich, Melinda Rezeli, Sarolta Kárpáti, György Marko-Varga

Sequencing facility
Diana Spierings

Tamminga M, Andree KC, van den Bos H, Hiltermann TJN, Mentink A, **Spierings DCJ**, Lansdorp P, Timens W, Schuuring E, Terstappen LWMM, Groen HJM.(2021) Leukapheresis increases circulating tumour cell yield in non-small cell lung cancer, counts related to tumour response and survival. Br J Cancer.

Preprints:

Sister chromatid exchanges induced by perturbed replication are formed independently of homologous recombination factors. Anne Margriet Heijink, Colin Stok, David Porubsky, Eleni M. Manolika, Yannick P. Kok, Marieke Everts, H. Rudolf de Boer, Anastasia Audrey, Elles Wierenga, Victor Guryev, **Diana C.J. Spierings**, Puck Knipscheer, Arnab Ray Chaudhuri, Peter M. Lansdorp, Marcel A.T.M. van Vugt. bioRxiv 2021.09.17.460736; doi: https://doi.org/10.1101/2021.09.17.460736

Monosomies, trisomies and segmental aneuploidies differentially affect chromosomal stability. Dorine C. Hintzen, Mar Soto, Michael Schubert, Bjorn Bakker, **Diana C.J. Spierings**, Karoly Szuhai, Peter M. Lansdorp, Floris Foijer, René H. Medema, Jonne A. Raaijmakers. bioRxiv 2021.08.31.458318; doi: https://doi.org/10.1101/2021.08.31.458318

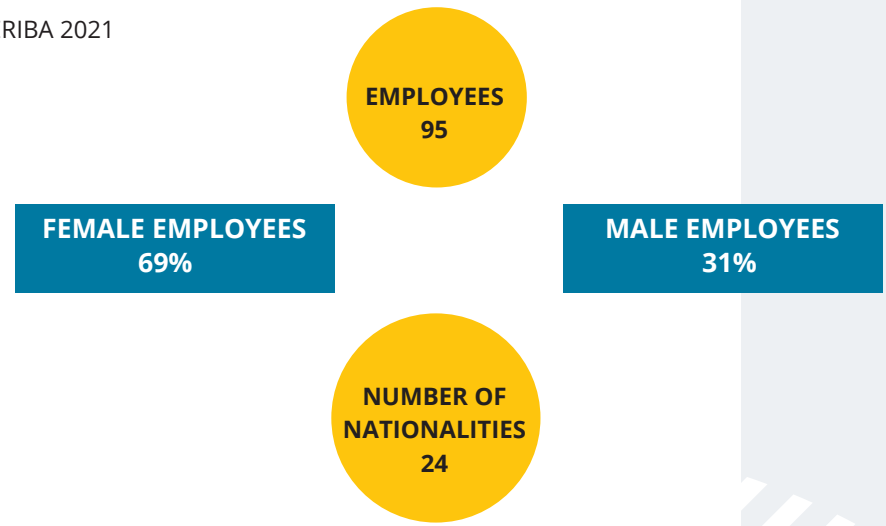
Mechanisms of genetic instability in a single S-phase following whole genome doubling. Simon Gemble, Sara Vanessa Bernhard, Nishit Srivastava, René Wardenaar, Maddalena Nano, Anne-Sophie Macé, Andréa E. Tijhuis, Kristina Keuper, **Diana C.J. Spierings**, Helfrid Hochegger, Matthieu Piel, Floris Foijer, Zuzana Storchová, Renata Basto. bioRxiv 2021.07.16.452672; doi: https://doi.org/10.1101/2021.07.16.452672

Inhalable textile microplastic fibers impair lung repair. F. van Dijk, S. Song, G.W.A van Eck, X. Wu, I.S.T. Bos, D.H.A. Boom, I.M. Kooter, **D.C.J. Spierings**, R. Wardenaar, M. Cole, A. Salvati, R. Gosens, B.N. Melgert. bioRxiv 2021.01.25.428144; doi: https://doi.org/10.1101/2021.01.25.428144

People

FACTS AND FIGURES ERIBA 2021

FACTS AND FIGURES ERIBA 2021



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Management team ERIBA
Henk Heidekamp, Managing Director
Megha Upadhyay-Pandey, Research Coordinator
Arnoud Rozema, Staff Advisor
Kevin Huizinga, Financial controller
Sylvia Hoks, Secretary
Karin van Wageningen, Secretary
Yin Fai Chan, Technician (general support)

Invited Speakers

In 2021, our scientists were invited to give talks and lectures globally.

Speaker	Date	Topic	Location
Michael Chang	March 2021	within the Nucleic Acid Secondary Structures: G4s and Beyond Webinar Series	Online
Eugene Berezikov	October 2021	Analysis of cell hierarchies in the regenerative flatworm Macrostomum lignano using single-cell transcriptomics and stem cell-specific transgenic lines	MARISTEM Meeting, Padova, Italy
Floris Foijer	January 2021	Oncology Research Meeting	UMCG
	May 2021	Invited seminar New York University	host Teresa Davoli (online)
	June 2021	Aneuploidy NL symposium	online
	July 2021	Invited lecture Nadja Mada University	online
	September 2021	Invited seminar Karolinska University	host Christian Riedel (online)
	October 2021	Invited lecture CRISPR editing	University Milan
	October 2021	Invited speaker Groningen	Jena Ageing meeting
Ellen Nollen	October 2021	Modifiers of age-related protein toxicity	Groningen-Jena Aging Meeting (G-JAM) 2021
Cor Calkhoven	October 2021	FLI colloquium, FLI, Jena, Germany Title: mechanisms of health- and lifespan regulation by C/EBP transcription factors	online
	March 2021	co-organiser van de Groningen-Jena Aging Meeting (G-JAM), Last autor of poster Christine Müller	Jena Ageing meeting
Marco Demaria	January 2021	The Evolving Tumor Microenvironment in Cancer Progression. AACR.	Online
	April 2021	Mechanisms of Aging, Hellenic Society of Biochemistry and Molecular Biology	Online
	June 2021	44th Annual meeting of the Japan Society for Biomedical Gerontology. University of Nagoya, Japan.	Online
	June 2021	ResetAgeing Conference. University of Coimbra, Portugal	Online
	August 2021	European Society for Photobiology Photoaging Symposium. Salzsburg. Austria.	Online
	November 2021	Gerontological Society of America 2021 Annual Scientific Meeting. Phoenix, USA.	Online
	December 2021	International Cell Senescence Association annual meeting. University of Osaka, Japan	Online
	March 2021	invited by Dr M. Pirisi, University of Novara	Online
	May 2021	invited by Dr A. Sapino, Oncological Institute Candiolo (IRCC)	Online
	May 2021	invited by Dr L. Pietrocola, Karolinska Institute	Online
	May 2021	invited by Dr R. Perez-Lorenzo, Columbia University	Online
	July 2021	invited by Dr C. Billault, Gerotalks, University of Santiago del Chile	Online
	November 2021	Nice (France), invited by Dr E. Gilson, IRCAN	Online
Victor Guryev	August 2021	a keynote talk at 16th International Hibernation Symposium	Groningen
John LaCava	April 2021	Helsinki, Hilive webinars	Online
Judith Paridaen	June 2021	UMC Utrecht – Seminar in Stem Cell course (host: Koen Braat & Paul Coffer)	Online
	June 2021	Workshop speaker yearly PhD Day, University of Groningen	Online

Funding/Grants

Proposals awarded in 2021

PI	Call	Title of the project	Budget
Marco Demaria	NWO-VIDI	Characterizing and targeting detrimental senescence to promote healthy aging	€ 799,600
Nynke Oosterhof/ Judith Paridaen	NWO-XS	Revealing the true colors of individual cells using barcodes	€ 50,000
Pilotfonds Wetenschapscommunicatie	KNAW	ERIBA Outreach team	€ 10,000
KRF Spierings	KRF	Integrating single-cell DNA sequencing and high-resolution imaging to link cell-based reporter readout to genomic integrity	€ 35,000
De Cock Hong	DCH	Determining the sub-type(s) of immune cells involved in the clearance of chromosomally instable (CIN) tumors and how CIN tumors evolve to evade them	€ 4,000
De Cock Wobben	DCH	Developing and validating a fluorescent barcode cell model for tracking clonal growth dynamics both visually and using sequencing	€ 4,000
De Cock Alhazzaa	DCH	Mechanism of intrinsic vs induced chromosome instability tolerance in stem cells	€ 4,000
NWO-ENW XS Berezikov-2	NWO	Feeding on light: genetic mechanisms of photosymbiosis in a highly regenerative animal	€ 50,000
NWO Chang	NWO	Replication of repetitive DNA—telomere rescue at nuclear pores	€ 641,575
NWO-ENW XS Mouton-2	NWO	Slowing down ageing: from immortal planarians to short-lived yeast	€ 50,000
Marco Demaria	Oisin Biotechnologies	Collaborative Research with Oisin Biotechnologies	€ 158,632
Marco Demaria	KRF	Identification of senescence-associated mechanisms induced by chemotherapy treatment in testicular cancer patients	€ 30,000
Marco Demaria	Impetus Grants	Hold your breath: understanding the anti-aging effects of hypoxia mimicking compounds	€ 145,654.57
John LaCava	PPP-Refeyn extension	Mass photometry combined with mass spectrometry to assess sample heterogeneity and predict macromolecular topologies	€ 69,400
Diana Spierings	NWO-XS	Single cell quantification of extrachromosomal and chromosomal DNA to study tumor evolution	€ 50,000
Marco Demaria	KWF	Understanding how cellular senescence contributes to the sexual dimorphism of hepatocellular carcinomas'	€ 786,825
Cor Calkhoven	KWF	Translationele opregulatie van C/EBPβ-LIP in Triple-Negatieve Borstkanker (TNBC) en haar rol in de ontwijking van tumorafweer.	€ 661,825
Total			€ 3,550,511.57

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

Functional Genomics Centre - iPSC/CRISPR facility

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 form a national KWF-funded CRISPR screen infrastructure – ScreeninC - supporting CRISPR screens 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 organized 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 in a tissue culture robot to automate iPSC reprogramming, which will drastically improve our throughput in the years to come. This robot was purchased in 2021 and is currently being implemented in our reprogramming pipeline.

Facilities

Who

Floris Foijer - Coordinator Functional Genomics Centre
Mathilde Broekhuis – Lab manager
Petra L. Bakker – Project manager
Narendra Chunduri – Postdoctoral fellow
Laura Kempe – Research technician
René Wardenaar - Bioinformatician

Contact

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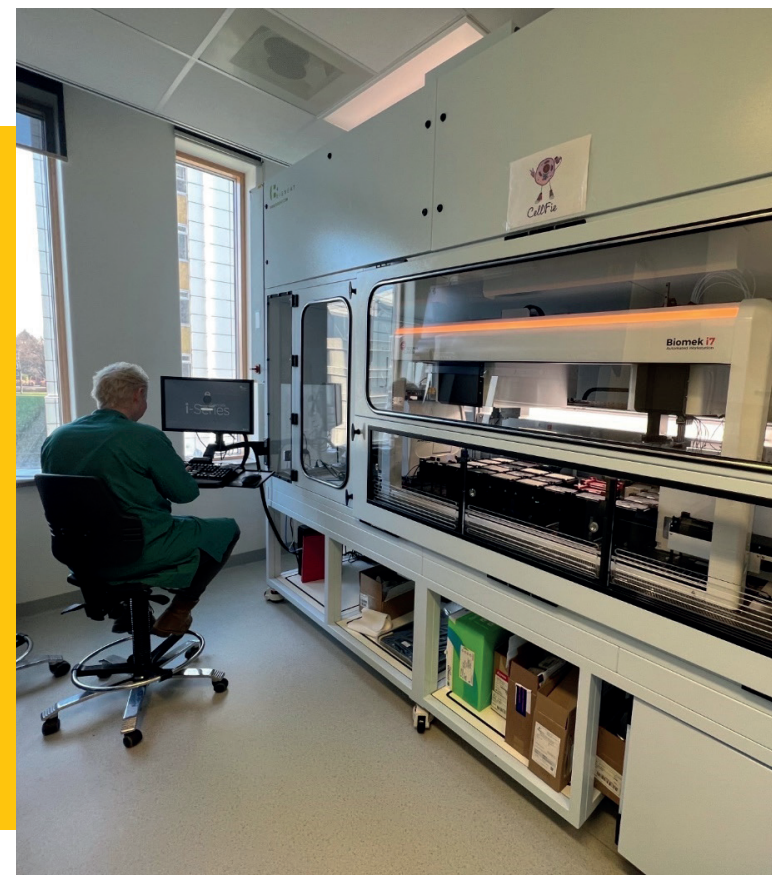

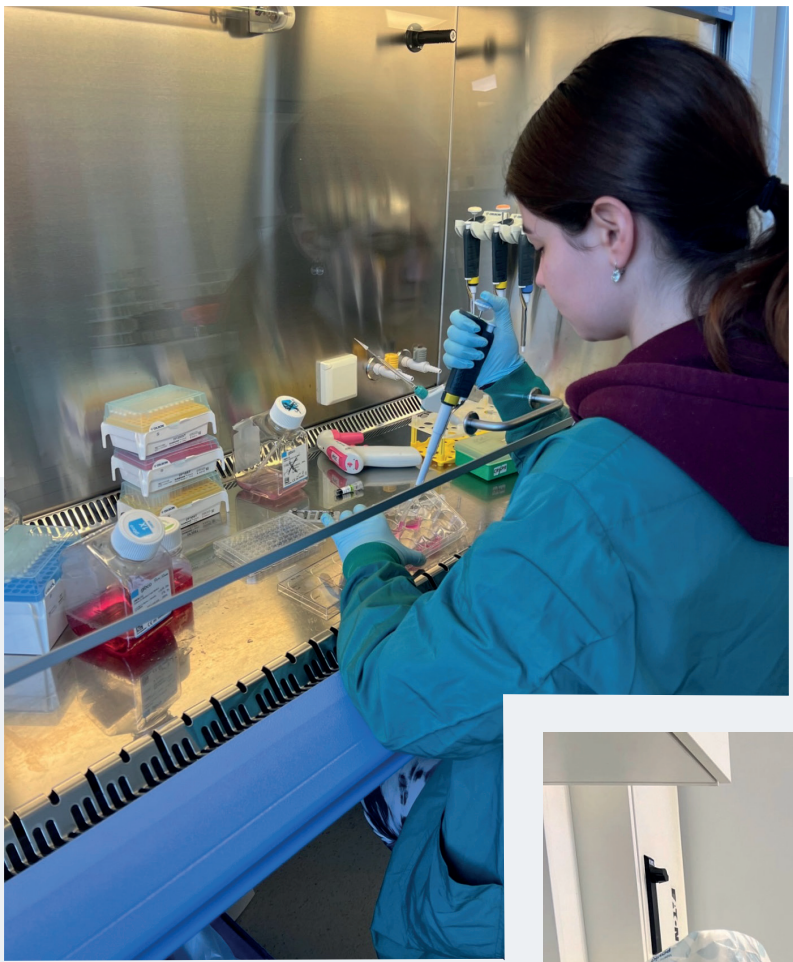


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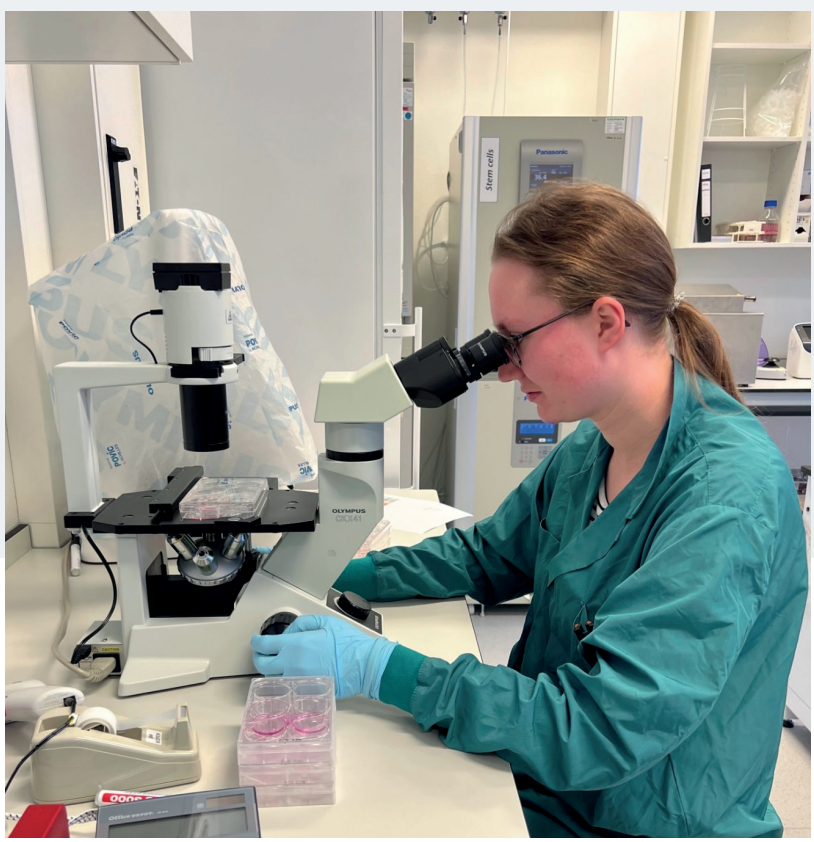
Stem cells
Panasonic
OLYMPUS

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
The Netherlands
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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.

Despite the Covid-19 lock-down and restricted working conditions in 2020, we have generated over 9000 single-cell DNA-seq libraries derived from approximately 346 different samples. Moreover, we facilitated ~80 sequencing runs in total. Although we could only facilitate small NGS projects (RNA-seq, ATAC-seq, DNA-seq), 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
Jennefer Beenen – Technician
Rianna Arjaans – 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.

"The Current Themes in Healthy Ageing cursus BMS master FSE – coordinators"
Coordinators: Judith Paridaen and Marco Demaria
5 ECTS & 19 Students

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

MSc CRISPR Course
Coordinator: Floris Foijer
5 ECTS & 30 Students

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.

JSM BSc course: Model Organisms in Ageing Research
Coordinator: Floris Foijer
3 ECTS & 14 Students

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.

BSc course LST Epigenetics and Gene editing
Coordinator: Floris Foijer
5 ECTS & 80 Students

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
Coordinator: Liesbeth Veenhoff
5 ECTS & 27 Students

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 unit is compulsory for the ageing track and is an elective in the other tracks of the programs.

Molecular and Genetic Age Research
Coordinators: Cor Calkhoven & Sophia Bruggeman
10 ECTS & 18 Students

Objectives: A laboratory research course with research topics covering a broad range of techniques and model systems related to ageing, lifespan and age-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

Macromolecular Interactions in Human Disease
Coordinator: John LaCava
3 ECTS & 6 Students

Objectives: This course will we be an opportunity to consider the challenges associated with mapping PPIs in clinical samples as well as to form an opinion on the value of this kind of information in biomedical studies. We will focus on teaching methods and concepts related to immunoprecipitation and proximity labeling in conjunction with mass spectrometry (among other proteomic approaches) to map changes in macromolecular interactions between health and disease.

Outreach Dissemination & Events

Principal Investigator	Outreach Activities
Floris Foijer	Lecture on CRISPR genome engineering at Oncology School Amsterdam
	Invited speaker at New York University (online) - Hosted by Teresa Davoli
	Cancer biology lecture at Gadjah Mada University (online)
	Invited speaker at Karolinska University (online) - Hosted by Christian Riedel
	CRISPR lecture Universty Milan (online)
	Speaker Groningen/Jena Ageing meeting
	Hosted highschool students to show labwork and help with their projects
Judith Paridaen	Invited speaker at PhD Day 2021: Make it in the North! University of Groningen
	Elected Member of Young Academy Groningen (YAG); chair of YAG-Public Engagement Group
	Chair of UMCG Medische Publieksakademie
	Contributor to Pre-university academy (Scholierenakademie) University of Groningen on Knowlands project – outreach to high school class 3/4
	Article on UMCG research using zebrafish model in Kennis in Zicht
John LaCava	Lecture HILIFE WEBINARS – 26th April 2021, Helsinki University
	Scientific consultant for the Waag society in Amsterdam: advised / worked with a couple of their artists on projects; lecture at the Waag society on 4th April 2021
Eugene Berezikov	Lecture at the MARISTEM (Stem cells of marine/aquatic invertebrates) consortium meeting (Padova, Italy)
Liesbeth Veenhoff	Guiding 3 high school students with their profile assignment

Outreach activities other ERIBA Scientists/ERIBA Outreach team

KNAW Gewaardeerd! Prize for Science communication activities (€10.000) to ERIBA Outreach Team 2021

Young Academy Groningen Science fair at Noorderzon Arts Festival, Groningen, Science Fair, presented an experiment on DNA '*Kraak de code van het leven: wat weet jij over DNA?*' Involved ERIBA scientists: Judith Paridaen, Anton Steen, Nynke Oosterhof, Andréa Tijhuis, Soraya Wobben

Zpannend Zernike. Activities at the Energy barn, on the Zernike complex. Children performed experiment to isolate DNA from bananas and strawberries. Anton Steen Amarins Blaauwbroek, Soraya Wobben and Annemiek Veldsink contributed.

Science in a box by Stijn Mouton

Stijn Mouton gave Interview related to the Gewaardeerd!-price

Scientific Advisory Board

The Board is comprised of the following distinguished scientists:

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