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Poor old pores

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Nederlandse samenvatting

Achtergrond

In eukaryoten (schimmels, planten, dieren en dus ook de mens) zit DNA in een apart compartiment, de celkern, die omgeven wordt door een dubbel membraan, dat kernmembraan genoemd wordt. Vele moleculen moeten tussen de celkern en het cytoplasma getransporteerd worden. Hiervoor zitten in de kernmembraan grote eiwitcomplexen (Kernporiecomplexen). Kleine moleculen diffunderen vrij door deze kernporiecomplexen. Bovendien reguleert de kernporiecomplex ook de gecontroleerde uitwisseling van macromoleculen met een lokalisatiesignaal. De lokalisatiesignalen werken net zoals een postcode op een brief: de lokalisatiesignalen worden herkend door transportfactoren, die aan het kernporiecomplex binden en daardoor transport mogelijk maken. Het kernlokalisatiesignaal (NLS) is het lokalisatiesignaal voor transport naar de celkern, terwijl het kernexportsignaal (NES) gebruikt wordt voor transport van de celkern naar het cytoplasma.

De eiwitten van het kernporiecomplex (Nups) kunnen ingedeeld worden in twee groepen. Een groep van Nups maakt een stabiele donutvormige structuur. De andere groep van Nups zijn ontvouwen eiwitten (eiwitten zonder een stabiele vorm) in het centrum van de donut. Er is niet veel bekend over hoe kernporiecomplexen gemaakt worden en of een kapotte kernporiecomplex gerepareerd kan worden, maar het is waarschijnlijk dat deze processen ingewikkeld zijn. In dit proefschrift deden we onderzoek naar de veranderingen van het kernporiecomplex en naar veranderingen in het transport tussen de celkern en het cytoplasma tijdens veroudering van bakkersgist. Voor bakkersgist is elke cel een volledig organisme. Wetenschappers onderscheiden twee soorten van cellulaire veroudering. Als een cel asymmetrisch deelt, wordt de moedercel met elke deling ouder. Dit noemen wij replicatieve veroudering, en de leeftijd van een cel wordt geteld als het aantal voltooide delingen. Cellen die niet delen, verouderen chronologisch en hun leeftijd wordt gemeten in tijd.

Resultaten

Een deel van dit proefschrift beschrijft hoe we voor het eerst de oorzaken en de gevolgen van de veranderingen aan het kernporiecomplex tijdens de replicatieve veroudering in bakkersgist onderzocht hebben. Sommige eiwitten die belangrijk zijn voor het maken van het kernporiecomplex dalen in hoeveelheid tijdens het verouderen van de cellen en replicatief verouderde cellen hebben structuren in

hun kernmembraan die lijken op verkeerd gemaakte kernporiecomplexen. We zien in oude cellen dat de uitwisseling van eiwitten tussen de celkern en het cytoplasma langzamer is dan in jonge cellen, en daarom vermoeden wij dat de verkeerd gemaakte kernporiecomplexen niet in staat zijn de uitwisseling van eiwitten mogelijk te maken.

Bovendien wijzen onze en andere studies erop, dat de kernporiecomplexen in chronologisch verouderende cellen anders veranderen dan in replicatief verouderende cellen. Omdat sommige onderdelen van het kernporiecomplex extreem langlevend zijn, vermoeden wij dat niet het maken van nieuwe kernporiecomplexen, maar het onderhoud van de beschikbare kernporiecomplexen een ingewikkeld proces is voor chronologisch verouderende cellen. Onze resultaten zijn niet alleen van belang voor bakkersgist, maar ook voor hogere organismen zoals de muis of misschien zelfs de mens. Onze analyse van beschikbare proteoom data van oude ratten en muizen toont aan dat de veranderingen van de hoeveelheid aan Nups in de lever van oude ratten, lijken op de veranderingen in de hoeveelheden aan Nups, die in replicatief verouderde gist gevonden wordt. Tegelijk lijken de veranderingen in de hoeveelheden aan Nups van chronologisch verouderde gist op de veranderingen die in de hersenen van oude muizen gevonden worden.

Belang

Onderzoekers kennen vier processen die veroudering kunnen veroorzaken: Het verlies van de controle over hoeveel eiwitten er van elke type eiwit gemaakt wordt (loss of proteostasis), de toegenomen instabiliteit van het genoom, het korter worden van de einden van de chromosomen (telomeren) en veranderingen in het epigenoom. Het kernporiecomplex is waarschijnlijk medeverantwoordelijk voor het verlies van de controle over hoeveel eiwitten gemaakt worden, en kan dit proces misschien amplificeren. Bovendien is het kernporiecomplex ook betrokken bij de organisatie van de structuur van het genoom. Daarom is het mogelijk dat veranderingen aan het kernporiecomplex door veroudering ook belangrijk zijn voor de stabiliteit van het genoom. Het resultaat dat de samenstelling en functie van het kernporiecomplex verschillend veranderen tijdens replicatieve en chronologische veroudering is misschien de oorzaak waarom de veranderingen van de kernporie erg verschillend zijn tussen verschillende organismen en weefsels tijdens het verouderen. We denken dat dit misschien ook voor andere langlevende eiwitcomplexen van belang is.

Deutsche Zusammenfassung

Hintergrund

Eukaryoten (Pilze, Pflanzen und Tiere, inklusive dem Menschen) werden charakterisiert durch die Anwesenheit eines Zellkerns, der die Erbinformation (DNA) vom Rest der Zelle trennt. Der Zellkern wird gebildet durch eine Doppelmembran, die Kernhülle genannt wird. In der Kernhülle befinden sich große Proteinkomplexe (Kernporen), die den Austausch zwischen dem Zellkern und dem Rest der Zelle ermöglichen und regulieren. Kernporen ermöglichen den freien Austausch von kleineren Proteinen zwischen dem Zellkern und dem Zytoplasma. Gleichzeitig, regulieren Kernporen den schnellen und energiebetriebenen Transport von Makromolekülen. Makromoleküle, die zum Zellkern transportiert werden, haben ein Kernlokalisierungssignal (NLS), während Makromoleküle, die vom Zellkern zum Zytoplasma transportiert werden ein Kernexportsignal (NES) haben. Diese Signalsequenzen werden von Transportrezeptoren (NTRs) erkannt, die mit der Kernpore interagieren und somit den Transportprozess ermöglichen.

Die Proteine, die die Kernpore bilden (Nups), können vereinfacht dargestellt in zwei Gruppen unterteilt werden. Die eine Gruppe Nups bildet eine Donut förmige Struktur, die als Rahmen für zweite Gruppe von Nups fungiert. Diese Gruppe von Nups (FG-Nups) sind intrinsisch ungeordnete Proteine, die das Zentrum dieses Rahmens füllen. Wie Kernporen gebildet werden und ob eventuelle Schäden an der Kernpore von der Zelle erkannt und repariert werden können ist weitestgehend unklar, aber es liegt nahe, dass diese Prozesse Kompliziert sind. In dieser Doktorarbeit habe ich Änderungen in der Zusammensetzung und der Funktion der Kernpore während des Alterns untersucht. Für meine Studien habe ich einen relativ simplen Organismus benutzt, die Bäckerhefe. Bei einzelligen Lebewesen, wie der Bäckerhefe, unterscheiden wir zwischen zwei verschiedenen Formen des Alterns: Zellen die sich asymmetrisch teilen altern replicativ und das Alter der Zelle wird bestimmt anhand der vollendeten Zellteilungen. Zellen die sich nicht teilen, altern chronologisch und ihr Alter wird in Zeit gemessen.

Ergebnisse

Als Teil dieser Arbeit haben wir als Erste die Ursachen und Auswirkungen, der Änderungen an der Kernpore während des replicativen Alterns in Bäckerhefe untersucht. Wir konnten zeigen, dass einige Proteine, die die Kernpore bilden sich in ihrer Menge verringern und dass replicativ gealterte Zellen Hinweise auf

fehlerhaft gebildete Kernporen zeigen. Wir beobachten, dass in alten Zellen der Austausch von Proteinen zwischen dem Zellkern und dem Zytoplasma langsamer abläuft als in jungen Zellen, darum vermuten wir, dass die fehlerhaft gebildeten Kernporen nicht in der Lage sind den Austausch von Molekülen zu ermöglichen.

Weiterhin weisen unsere und andere Studien darauf hin, dass die Kernporen in chronologisch alternden Zellen distinkt sind von denen replicativ alternder Zellen. Da einige Teile der Kernpore extrem langlebig sind, lässt sich vermuten, dass nicht das Bilden neuer Kernporen, sondern eher die Instandhaltung der vorhandenen Kernporen problematisch ist für chronologisch alternde Zellen. Die hier beschriebenen Mechanismen sind wahrscheinlich relevant sind für das Altern von höheren Organismen und nicht beschränkt auf die einfache Hefezelle. Der Vergleich von bereits vorhandenen Proteom Daten von alternden Mäusen und Ratten deutet darauf hin, dass die Änderungen die wir an der Kernpore in replicativ gealterten Hefezellen finden den Änderungen ähnlich sind, die in der Leber von gealterten Ratten gefunden wurden. Die Änderungen, die an der Kernpore in chronologisch alternden Hefezellen gefunden wurden weisen Ähnlichkeiten auf mit den Änderungen, die im Hirn von gealterten Mäusen aufzufinden sind.

Bedeutung

Wir kennen vier Prozesse, die potentiell ursächlich für das Altern sind: Verlust von Proteinhomöostase, Instabilität des Genoms, das kürzer werden der Telomere und Änderungen im Epigenom. Die Kernpore hat das potential für den Verlust von Proteinhomöostase verantwortlich zu sein, oder diesen zu beschleunigen. Außerdem ist die Kernpore auch involviert in die Organisation der Struktur de Genoms, daher könnten alters bedingte Änderungen an der Kernpore auch die Stabilität des Genoms beeinflussen. Das Ergebnis, dass die Zusammensetzung und Funktion der Kernpore unterschiedlich durch replikatives und chronologisches altern beeinflusst wird, könnte erklären, warum Altersbedingte Änderungen an der Kernpore sehr abhängig sind vom Gewebe und Organismus, in dem sie untersucht wurden, dies trifft möglicherweise auch auf andere langlebige Proteinkomplexe zu.

English summary

Background

Nuclear pore complexes (NPCs) are among the largest protein complexes in the eukaryotic cell and are evolutionary conserved. NPCs are embedded in the nuclear envelope (NE), a double membrane that encloses the nucleus and separates nuclear- and cytoplasmic content. The proteins that form the NPC (Nups) can be distinguished into two groups. Scaffold Nups form the stably folded doughnut shaped scaffold of the NPC and intrinsically disordered Nups (FG-Nups) fill the center of the scaffold. The key function of NPCs is to facilitate nucleocytoplasmic exchange, as the main gateways to the nucleus. NPCs acts as a size dependent diffusion barrier, but also enable the directed, rapid and energy driven exchange of macromolecules between the nucleus and the cytoplasm. Macromolecules that are transported via the NPC have a so called nuclear localization signal (NLS), or a nuclear export signal (NES). The NLS and NES signal sequences are recognized by nuclear transport receptors that mediate the transport via the NPC.

Very little is known about how NPCs are assembled and how they are maintained, but it has long been speculated, that this is a difficult task. In this thesis, we have studied NPCs, NPC assembly, NPC maintenance and nucleocytoplasmic transport in the context of aging in baker's yeast. Here, we distinguish between two distinct kinds of aging: the aging of non-dividing cells (chronological aging) and the aging of asymmetrically dividing cells (replicative aging). The chronological lifespan is measured as the survival time of a cell, while replicative lifespan is measured as the limited number of divisions that a cell undergoes before it dies.

Results

In this thesis we describe the distinct changes in the whole cell abundance of Nups during replicative and chronological aging. We find, that replicative aging cells show a decreased abundance of FG-Nups at the whole cell level and at the NE. We further analyze the abundance of proteins that assist in NPC assembly in aging and find, that several proteins decrease in abundance in aging. This decrease in the NPC assembly machinery, and potentially the FG-Nups is probably sufficient to cause the signs of NPC assembly problems that we observe during replicative aging. Subsequently, we analyzed nucleocytoplasmic transport in replicative aging cells and find that nucleocytoplasmic exchange is slowed down during replicative aging, suggesting, that misassembled NPCs are

not able to facilitate nucleocytoplasmic exchange. We conclude that the NPC assembly machinery is particularly challenged in replicative aging cells. Chronologically aging cells have little requirement to assemble NPCs as NPCs are long-lived structures. Our analysis of existing proteome data from chronologically aged cells suggest, that NPCs change in different ways during replicative and chronological aging. Our studies on nucleocytoplasmic transport in chronologically aged cells shows that distinct changes in steady state localization of GFP-NLS reporter proteins during replicative and chronological aging.

Taken together, we have established a model, where replicative and chronological aging pose distinct challenges on the structure of the NPC. NPCs are difficult to assemble and in replicative aged cells NPCs misassemble more frequently causing a decrease in nuclear transport dynamics as the cells age. Chronologically aged cells on the other hand face the challenge to maintain their NPCs functional over a long period of time. NPCs in chronological and replicative aged cells show distinct functional changes.

Significance

Four hallmarks have been described to be causal for aging: loss of proteostasis, epigenetic alteration, genomic instability and telomere attrition. NPCs have the potential to be causal, or at least contribute to further loss of proteostasis in aging, but they are also involved in other processes relevant in aging, i.e. NPC are involved in genome organization and potentially have a role in the loss of genome stability in aging. Therefore, we conclude, that NPCs remain a valuable studying target in aging, due to the plethora of cellular processes, that they are involved in. The finding that replicative and chronological aging impact NPCs differently might explain, why age-related changes of the NPC found in different tissues or model systems are so divers and might also be applicable to other long-lived protein complexes.

Curriculum Vitae

2009 *Abitur, Ganztagsgymnasium Klosterschule, Hamburg, Germany*

2009 - 2012 *B.Sc. Biology, Philipps University Marburg, Germany*

Research project: “Studies on cleavage of Msb2 through aspartic proteases in *Ustilago maydis*” at the Max Planck institute for terrestrial microbiology in the laboratory of Professor Regine Kahmann.

2012 - 2014 *M.Sc. Molecular biology and biotechnology, University of Groningen, Netherlands*

Two research projects: “Single molecule analysis of nisin modification machinery” in the laboratory of Professor Oscar Kuipers and “The role of long unfolded linkers in inner nuclear membrane transport of membrane proteins” in the laboratory of Dr. Liesbeth Veenhoff.

2014 - 2019 *PhD candidate, ERIBA, University Medical Center Groningen, Netherlands*

In the laboratory of Dr. Liesbeth Veenhoff, the results of this PhD are described in this thesis.

List of author contributions

Chapter 2

ILR designed, performed and analysed experiments in Figure 1,2,4,5 and Supplementary Figures 2,4,5,6. AS designed, performed and analysed experiments in Supplementary Figures 3a,b and Figure 4c. AM, EG and PRO developed models in Figure 2a,b and Supplementary Figure 3cde & 8. DJT and CPL performed and analysed the experiments in Figure 3. MMC and MK designed, performed and analysed experiments in Figure 5 and Supplementary Figure 7 and designed the microfluidic chips. DPNJ, GEJ, AA and PP were involved in generating strains and preliminary data for Figure 4. LMV and ILR wrote the manuscript with input from all authors.

Chapter 3

ILR and PP performed and analysed experiments for Figure 1, 2, 3abc, 4 and 5, with help of MK. Simulations for figure 1 and 5 were performed by AM and AG supervised by PRO. PP, AHGW performed and analysed immuno EM in Figure 3d, supervised by BNNG. AS oversaw molecular biology and biochemistry aspects of the project and performed and analysed the experiments for Figure 6. ACM generated original data re-used in figure 1 from Meinema et al., Science 2011, and ACM and BP were involved in early stages of the project. All authors contributed to the design of experiments. ILR, PP and LMV wrote the manuscript with input from all authors.

A previous version of this manuscript also appeared in the thesis of Petra Popken

Chapter 4 and 5

ILR and LMV designed the study for chapter 4 and wrote the manuscripts. ILR performed the experiments for chapter 4 and analyzed the data.

Abbreviations

ILR – Irina L. Rempel; AS – Anton Steen; AM – Ankour Mishra; EG – Erik van der Giessen; PRO – Patrick R. Onck; DJT – David J. Thaller; CPL – C. Patrick Lusk; MMC – Matthew M Crane; MK – Matt Kaerberlein; DPNJ – Daniel P. N. Jansen; GEJ – Georges E Janssens; PP – Petra Popken
 ACM – Anne C. Meinema; MKI – Marindy Klaassens; BNGG - B.N.G. Giepmans; AHGW – Anouk H. G. Wolters; AG – Ali Ghavami

List of publications and manuscripts

Rempel, I.L., Veenhoff, L.M. Poor old pores – The challenge of making and maintaining nuclear pore complexes in aging (Manuscript in preparation)

Rempel, I.L., Veenhoff, L.M. Replicative and chronological aging differently impact nuclear transport in baker's yeast (Manuscript in preparation)

Rempel, I.L. †, Popken, P. †, Ghavami, A., Mishra, A., Wolters, A.H.G, Klaassens, M., Meinema, A.C., Poolman, B., Giepmans, B.N.G., Onck, P.R., Steen, A., Veenhoff, L.M. - Flexible and extended linker domains support efficient targeting of Heh2 to the inner nuclear membrane (Submitted)

†equal contributions

Rempel, I.L., Crane, M.M., Thaller, D.J., Mishra, A., Jansen, D.P.N., Janssens, G.E., Popken, Akşit, A., P., Kaeberlein, M., Van der Giessen, E., Steen, A., Onck, P.R., Lusk, C.P., Veenhoff, L.M. (2019) Age-dependent deterioration of nuclear pore assembly in mitotic cells decreases transport dynamics. *eLife* 2019;8:e48186

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Novarina, D., Mavrova, S.N., Janssens, G.E., **Rempel, I.L.**, Veenhoff, L.M., Chang, M. (2017). Increased genome instability is not accompanied by sensitivity to DNA damaging agents in aged yeast cells. *DNA Repair* 54:1-7.

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Addendum

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Everyone likes to find their names in the acknowledgements and many more names deserve to be mentioned in this section, but in the following sentences I would like to be concise and inclusive. So I will keep names to a minimum. I would like to thank all the collaborators that worked together with me on projects that resulted in publications. However, I am just as grateful to everyone that invested time, money and/or efforts to work with me on projects that did not result in a publication. Further, I would like to thank all past and present members of the band Quiet Room, for many fond memories of dinners and music together. I would like to thank all past and present members of the Chang and the Veenhoff lab for valuable input into my work and for being such great colleagues. I am going to miss the fantastic lunch conversations awfully. At times when nothing worked, you were the reason why I still wanted to go to work.