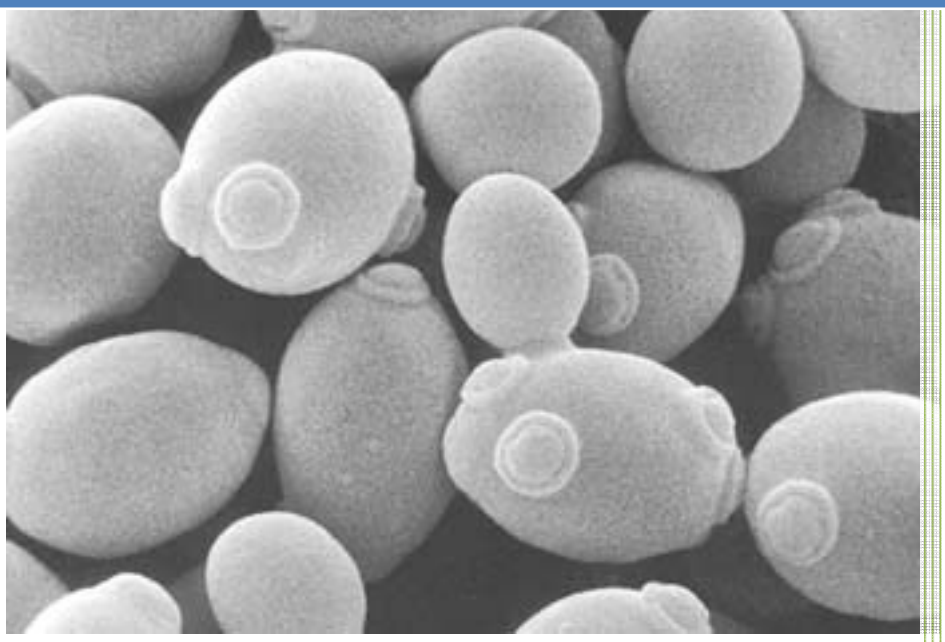
2012

The effects of aging on the nuclear envelope

Why is the nuclear position lost in aging *Saccharomyces cerevisiae*?



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February 2012

Content

Abstract

The Veenhoff group observed that aging *Saccharomyces cerevisiae* cells lose their nuclear position. Interestingly, there is no clear mechanism known that might explain this process in more detail. This paper provides an overview of the main components of the nuclear envelope (NE) and the current knowledge of their function, their role in aging and in nuclear positioning.

Nuclear pore complexes (NPCs) form the direct link between the cytoplasm and the nucleoplasm, since their function is actively transporting macromolecules across the nuclear membrane. During aging the NPCs occur to lose their transport function as they start to 'leak' due to damage to their peripheral nucleoporins. Whether or not aged baking yeast cells pass the damaged NPCs on to their buds is currently under debate.

The interaction of the nuclear envelope with the cytoskeleton may be of crucial importance in nuclear positioning in mammalian cells. The LINC complex, linking the SUN and KASH domains together, plays a key role in this process by the exertion of microtubule pushing. Damage resulting from aging may have an effect on the SUN-KASH interaction, leading to loss of attachment sites. Although baking yeast does not have SUN-KASH interactions, there is evidence suggesting an important role for homologous SUN domain proteins like Mps3. Also, the spindle pole body may be an important factor in the nuclear positioning in yeast.

The nuclear lamina are strongly associated with aging, since age accelerating diseases, like Hutchinson-Gilford progeria syndrome (HGPS), are a result of mutations in lamin encoding genes. Fibroblast of HGPS patients show a disrupted nuclear architecture, which include lobulation of the NE, thickening of the nuclear lamina and clustering of the NPCs.

With multiple players involved in the nuclear positioning it is unclear which mechanism specifically causes the loss of nuclear position in aging baking yeast cells. When one mechanism diminishes it time, it is reasonable to assume that other mechanisms may compensate for the loss.

Introduction

The nuclear envelope is a key player in integrating signals between the cytoplasm and the nucleoplasm. Using the model organism *Saccharomyces cerevisiae*, it was seen that the nuclear position is lost in aging yeast cells (G.E. Janssens, unpublished data). In young cells the nuclear position is defined, while in old cells the nucleus appears to be more mobile. This paper will discuss our current knowledge of the process of aging and particularly the effects on the nuclear envelope with its nuclear pore complexes and its associated nuclear lamins. I will then discuss which proteins and mechanisms may contribute to the observed changes in nuclear position in yeast cells during aging.

The nuclear pore complex

Nuclear pore complexes (NPCs) form the only link between the inner nuclear membrane (INM) and the outer nuclear membrane (ONM). Also, they are the sole mediators for the transport between the nucleoplasm and the cytoplasm. During aging, structural changes occur within the NPCs leading to 'leaking' of cytoplasmic proteins into the nucleoplasm (Hetzer et al., 2010).

Structure

The NPC is a structure consisting of ~450 proteins. Approximately 30 distinct proteins, named nucleoporins, form the multi-protein NPC complex with a size varying between 40 and 70 MDa between different species. Several classes of nucleoporins are needed for the NPC assembly; transmembrane nucleoporins span the nuclear membrane and are involved in anchoring the NPC to the NE. A computational model of the architecture of the yeast NPC shows that the 'core nucleoporins' form 2 ring-like structures consisting of the Nup84 complex (outer ring) and Nup170 complex (inner ring). Together both ring structures form the core scaffold within the NPC. The phenylalanine-glycine (FG) nucleoporins extend into the pore of the NPC, and are characterised by natively unfolded domains that include multiple FG dipeptide repeats (Fernandez-Martinez et al., 2009). The FG nucleoporins and the core scaffold are connected by linker nucleoporins. Figure 1 shows a schematic overview of how the NPCs may be assembled (Rout et al., 2009).
Previously, the mechanism underlying NPC formation was unclear, although several plausible mechanisms were proposed (Fernandez-Martinez et al., 2009): newly synthesized components could be inserted *de novo* into the NE without pre-existing NPCs. Secondly it was thought to be possible for NPC components to assemble with pre-existing NPCs, to form large complexes that later on split into two daughter NPCs. A third possible mechanism could be that NPCs would assemble in cytoplasmic membrane systems that later fuse with the NE. Data in support of the first hypothesis comes from the D'Angelo group and others; their data shows that in vertebrate cells the NPCs are formed *de novo*, and the new NPCs do not contain significant amounts of components taken from pre-existing NPCs (D'Angelo et al., 2006).
Besides nuclear factors like Ran, Ran cofactors, Kaps and nucleoporins also other proteins seem to play a role in the NPC assembly (Ryan et al., 2003; Lusk et al., 2002). Recently it was shown that the ER protein Apq12, reticulons (RTNs) and Yop1/DP1 protein families may also play a role in this process (Dawson et al., 2009). RTNs in yeast are essential for NPC formation, and in vertebrates they are needed for *in vitro de novo* NPC formation. Yop1/DP1

protein families might also play an important role in the NPC formation as they can bend membranes and have functions in post mitotic NE shaping (Anderson et al., 2008)

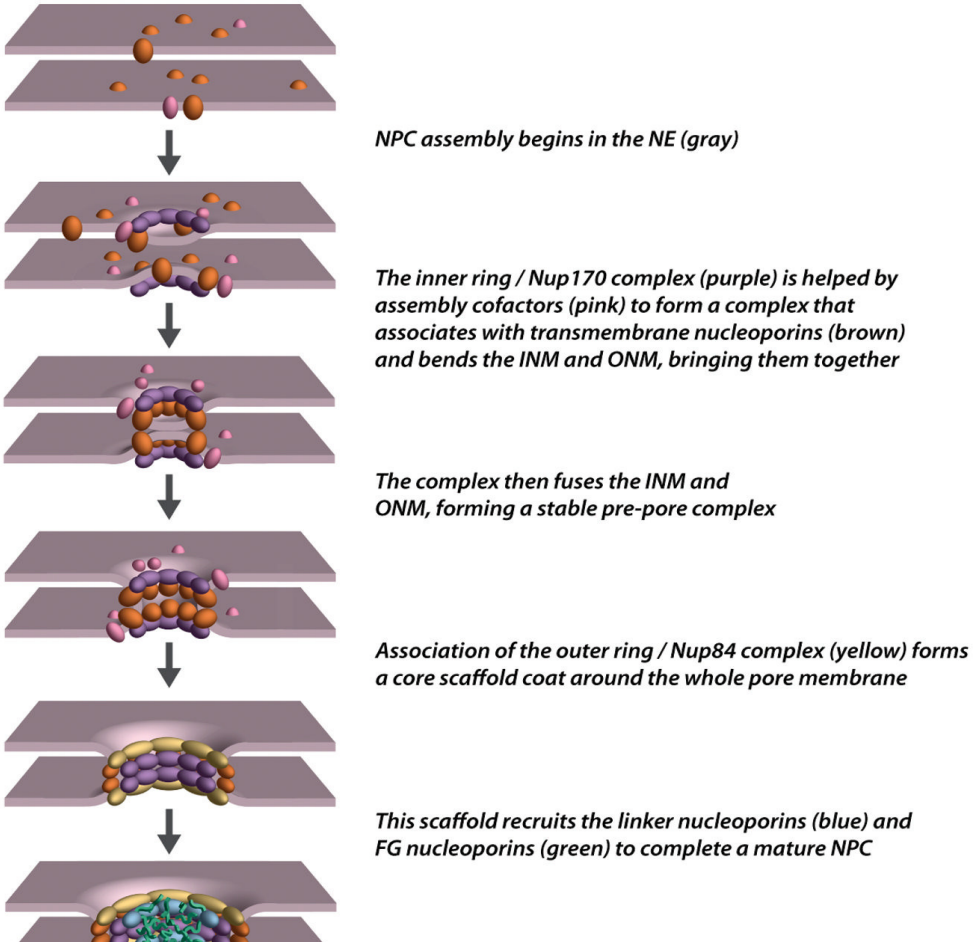

Figure 1: Schematic overview of the full NPC assembly by different types of nucleoporins. NPC assembly begins in the NE and the INM and the ONM are bended, bringing them together. Two ring complex structures are formed and the INM and the ONM are fused together. Finally linker nucleoporins are recruited and FG nucleoporins complete the mature NPC. (Figure adapted from Rout et al., 2009)

Function

Small molecules can diffuse freely through NPCs, but the transport of macromolecules is highly regulated. Transport across the NPC is fast, energy dependant and receptor mediated (Rout et al., 2009). Karyopherins (Kaps) are the main transport factors involved in transport across the NPC. Kaps bind to specific nuclear localization signals (NLS) or nuclear export signals (NES). Ran GTPase is the main energy source of this Kap mediated transport. Chromatin associated RanGEF in the nucleoplasm maintains Ran in its GTP bound state. Contrary, in the cytoplasm RanGAP stimulates GTP hydrolysis. Therefore the nucleotide-bound state of Ran provides a signal for the direction of the transport.
During transport into the nucleus a Kap binds to NLS-containing cargo in the cytoplasm. Once the cargo is present in the nucleus RanGTP binds to the Kap resulting in cargo release. During export, Kaps bind NES bearing cargo in the nucleoplasm, in the presence of RanGTP. When the cargo has been transported through the NPC, Ran returns to its GDP bound state and thereby the cargo is released.

NPCs during cell division
Saccharomyces cerevisiae undergo closed mitosis. During this closed mitosis the NE and NPCs remain intact throughout the entire cell cycle, indicating that there is almost no turnover of NPCs. Contrary, during open mitosis the NE and NPCs are disassembled during prophase and reassembled during telophase. The disassembly of the NPCs is a fast process, and the mechanism is not fully understood. However, it has been shown that the disassembly is highly synchronous and strictly coordinated. The disassembly of an NPC is not just the reverse process of the assembly. At the onset of the mitosis, key nucleoporins of the NPC get phosphorylated, which may be the trigger for destabilizing the protein structure of the NPC (Galy et al., 2008; Glavy et al., 2007; Macaulay et al., 1995). Mitosis specific kinases, like cyclin dependent kinase 1 (cdk1) and NIMA are involved in this phosphorylation (Lusk et al., 2007; Onischenko et al., 2005).

The nuclear envelope

The nuclear envelope (NE) consists of an inner nuclear membrane (INM) and an outer nuclear membrane (ONM) which are, as previously described, connected by NPCs. The NE forms a barrier between gene transcription and translation. The INM interacts with the chromatin and is involved in DNA replication, whereas the ONM interacts with the cytoskeleton and is involved in nuclear positioning (Mellad et al., 2011). Aging is associated with multiple cellular changes, processes in which the NE may play an important role.

The ONM
The ONM of mammalian cells contains a number of NE SPectRIN repeat proteins (nesprins). These are characterised by a central rod region of variable length, comprised of multiple spectrin repeats, and a C-terminal Klarsicht/ANC-1/Synehomology (KASH) transmembrane domain that facilitates NE localisation (Mellad et al., 2011). Also, nesprins posses variable N-terminal motifs, enabling interaction with different components of the cytoskeleton.
The Linker of the Nucleoskeleton and Cytoskeleton (LINC) complex links the KASH domain to the Sad1p-UNC84 (SUN) domain proteins Sun1 and Sun2 within the perinuclear space (Shimi et al., 2011). Figure 2 gives a schematic overview of the LINC complex with its SUN and KASH domains. Lamins in the lamina bind to INM proteins like emerin, lamina associated polypeptide 2β (LAP2β), lamin B receptor (LBR) and SUN domain proteins. Nesprins in the ONM interact with cytoskeletal filaments like actin and microtubules by binding plectin or kinesin. The LINC complex anchors the nuclear lamina to the cytoskeleton (Shimi et al., 2011). Shimini et al. showed that knocking out elements of the LINC system leads to uncoupling of the INM from the ONM, and detaching of the nucleus from the cytoskeleton.

Nuclear positioning
Tran et al. have demonstrated how fission yeast cells use dynamic microtubules as intracellular rulers to define the position of the nucleus in the cell (Tran et al., 2001). This study indicated that each microtubule structure actually consists of a bundle of microtubules, organised in an antiparallel configuration. Thus, microtubule plus ends facing the two cell tips, and minus ends near the nucleus. It was shown that the microtubules may have a direct active function in nuclear positioning, instead of functioning as tracks. The authors conclude that microtubules generate a pushing force on the nucleus via microtubule polymerization. This microtubule pushing results in a central positioning of a nucleus within the yeast cells. Microtubules are required for both nuclear positioning and nuclear movements.

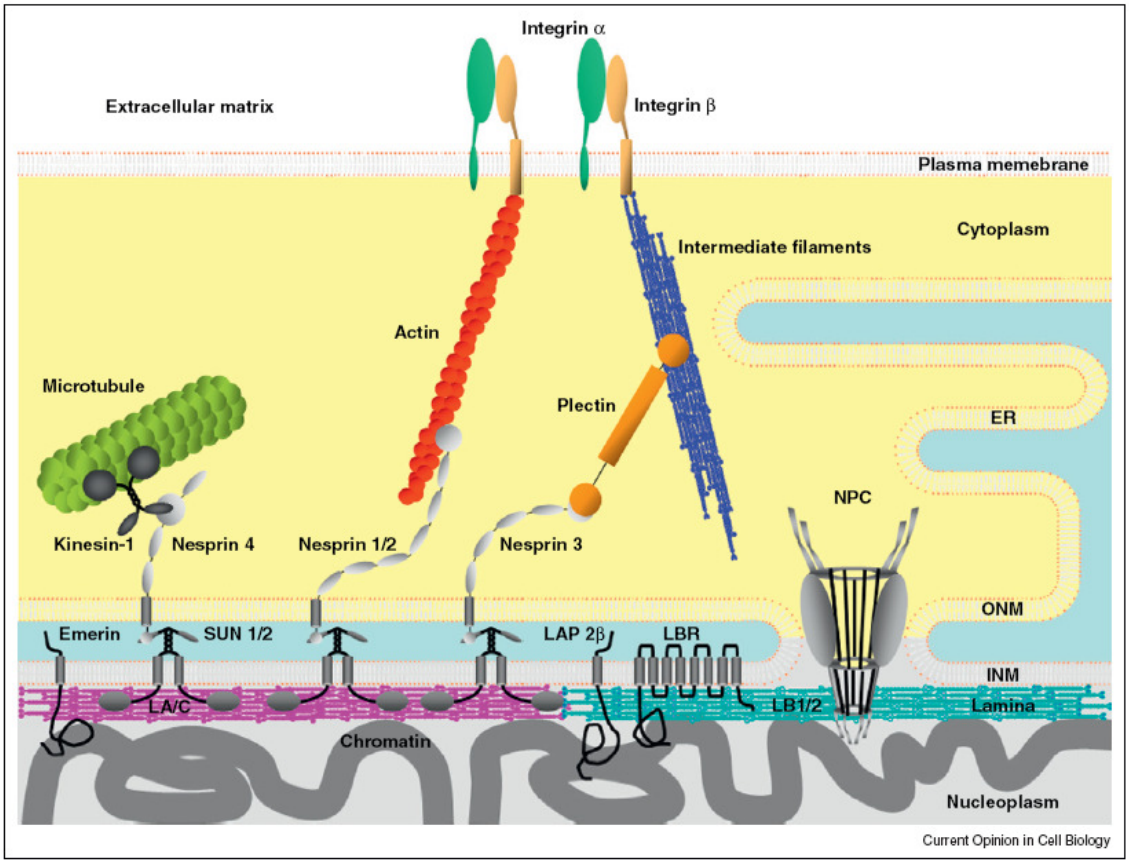

Figure 2: Schematic overview of the LINC complex. The KASH domain and SUN domain are linked together. This results in linkage of the nuclear lamina to the cytoskeleton, since the SUN domain interacts with the nuclear lamina. (Figure adapted from Shimi et al., 2011)

The nuclear lamina

On the inner side of the nucleus of eukaryotic cells a dense fibrillar network is present, which is called the nuclear lamina. It is composed of intermediate filaments and membrane associated proteins, and the nuclear lamina is associated with the INM. Mutations in nuclear lamina proteins, collectively called laminopathies, can cause accelerated aging, as seen in diseases like Hutchinson-Gilford progeria syndrome.

Architecture:
The nuclear lamina consists of two components; lamins and nuclear lamin-associated membrane proteins. Lamins are members of the type V intermediate filament (IF) superfamily. They have a characteristic tripartite organization consisting of a short N-terminal head domain, a central α-helicon rod domain and a C-terminal globular tail domain (Mattout et al., 2006). Based on their protein structure and expression patterns, lamins are divided in 2 types; A (lamin A and C) and B (lamin B1 and B2). Type B lamins are encoded by distinct genes (LMNB1, LMNB2), whereas A lamins are encoded by one single gene (LMNA); alternative splicing gives rise to the different A lamins. Nuclear lamin-associated membrane proteins are integral or peripheral membrane proteins. Because of their positioning they mediate the attachment of the nuclear lamina to the NE. The main lamin associated membrane

proteins include lamin associated polypeptide 1 and 2 (LAP1 and LAP2), emerin, lamin B receptor (LBR) otefin and MAN1 (Broers et al., 2006).

Function:

The general idea is that one function of the lamina is to provide structural support to the nuclear envelope (Worman et al., 2010). Also, there is a role for the lamins in chromatin organization, DNA replication and apoptosis. However, underlying mechanisms are not fully understood yet.

Aging

Each cell has a certain number of cell divisions before it is no longer able to survive and function properly. During its lifetime a cell accumulates damage, for instance mutations, double stranded DNA breaks or oxidative stress. This damage leads to loss of function and eventually to cell death.
One specific biomarker of chronological aging in mammalian cells is telomere length. Telomeres are heterochromatic domains at the end of chromosomes that are essential for chromosome protection and genomic stability (Donate et al., 2010). They consist of guanine rich, 6 to 8 base pair long repeats at the end of a chromosome. During each cell division the telomere ends shorten since DNA polymerase is unable to replicate the ends of linear chromosomes. This process is often referred to as the 'end replication problem'. In humans this process is targeted by telomerase. Telomerase is a cellular enzyme that compensates for the telomere shortening by adding *de novo* TTAGGG repeats to the chromosome ends (Greider et al., 1985). Telomere shortening below a certain threshold length can result in loss of telomeric protection, which may lead to end-to-end chromosome fusion, cell cycle arrest or apoptosis (Donate et al., 2010).
In baking yeast, *Saccharomyces cerevisiae*, two types of aging are defined: chronological aging and replicative aging. Replicative aging corresponds to the number of 'daughter' cells one 'mother' yeast cell has produced. Each time a 'mother' cell buds a 'daughter' cell this leaves a scar on the cell surface of the 'mother' cell. It has been shown that factors associated with aging, like extra ribosomal DNA circles (ERCs) and aggregated proteins largely remain present in the 'mother' cell (Shcheprova et al., 2008). 'Daughter' cells are thus rejuvenated, and indeed one can measure that 'daughters' of older 'mother' cells still have full replicative potential.

Effects of aging on the NPC

Recently it was suggested that NPCs in postmitotic cells are a dynamic structure where components are continuously renewed (D'Angelo et al., 2009). D'Angelo et al. showed that NPC proteins from the periphery (like Nup153 and Nup50) may have a higher turnover than nucleoporins from the core scaffold (like the Nup107/160 complex). However, this repair mechanism does not completely save the function of the NPCs. Over time, particularly damage to the linker nucleoporins that connect the FG nucleoporins may lead to an impaired transport function of the NPCs. It is possible that cells stop regenerating NPC components years before the cells die (D'Angelo et al., 2009).

NPCs in yeast
During mitosis in the yeast *Saccharomyces cerevisiae,* a 'mother' cell buds off a new 'daughter' cell. Interestingly, the new 'daughter' cells were reported not to inherit the old pre-existing NPCs from the 'mother' cell. Asymmetric segregation of NPCs leaves the old NPCs with the 'mother' cell (Shcheprova et al., 2008), while the daughter cells get new *de novo* formed NPCs. Shcheprova et al. showed that, during anaphase, septin proteins and Bud6 form a barrier between newly dividing cells and thereby facilitate the asymmetric segregation of the NPCs. They photo-bleached Nup49-GFP in the 'mother' lobe of early anaphase nuclei, and subsequently they monitored the recovery (Figure 3). For a period of at least 10 minutes no recovery was observed, indicating no *de novo* insertion of NPCs in the mother. However, during the recovery time the fluorescence increased in the buds (Figure 3B). Since the

fluorescence could not come from the 'mother' cells, the authors concluded that *de novo* pore insertion accounts for the fluorescence seen in the bud. Additional photo-bleaching of the bud fraction of the nuclei indeed showed recovery within less than 5 minutes, while no fluorescence was lost in the corresponding 'mothers'. Therefore, pore insertion might account for the fluorescence observed in the bud. Accordingly, the fluorescence of Nup49-GFP in 'daughter' nuclei of pore assembly mutations *nic96-1*and *nup133*Δ was decreased (Figure 3C).

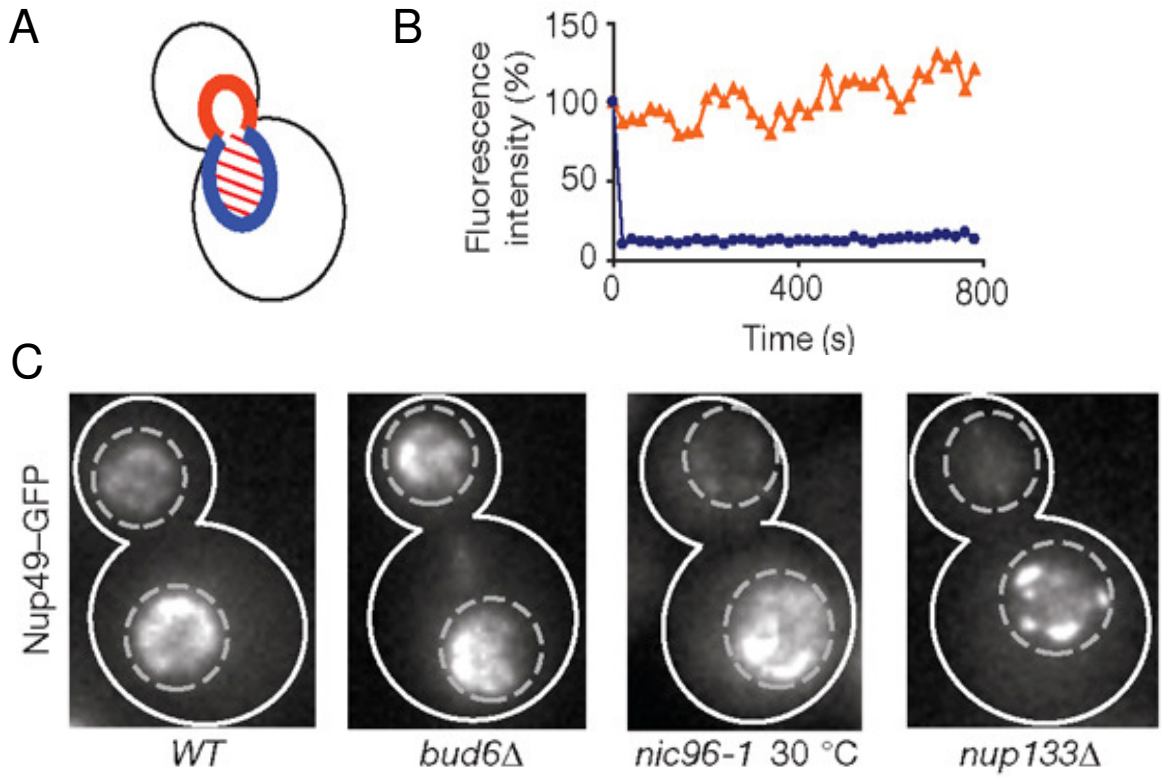

Figure 3: Asymmetrical segregation of NPCs in yeast. **A**: Schematic overview of nuclear division during telophase (blue 'mother' lobe and orange 'daughter' lobe). **B**: While photo-bleaching Nup49-GFP in the 'mother' cell inhibits fluorescence (blue), 'daughter' cells show increased fluorescence (orange). **C**: Pore assembly mutants show decreased fluorescence in the 'daughter' nuclei, while wildtype yeast cells show fluorescence in both 'mother' and 'daughter' cell. (Figure adapted from Shcheprova et al., 2008)

However, recently Khmelinskii et al. have challenged the hypothesis of asymmetrical segregation of NPCs in yeast, and they conclude that the 'daughter' cells also get old NPCs from the 'mother' cell (Khmelinskii et al., 2010). They used fluorescence time-lapse microscopy, and showed that the intensity of GFP- tagged core NPC components at the NE increased during G1 phase, S phase and early mitosis. Contrarily, during nuclear migration through the bud neck in anaphase the fluorescence remained constant. When the nucleus moved into the bud, the appearance of GFP was associated with a GFP intensity drop in the 'mother' cell. These results strongly suggest that the NPCs in the 'daughter' cell do originate, at least partly, from the 'mother' cells.

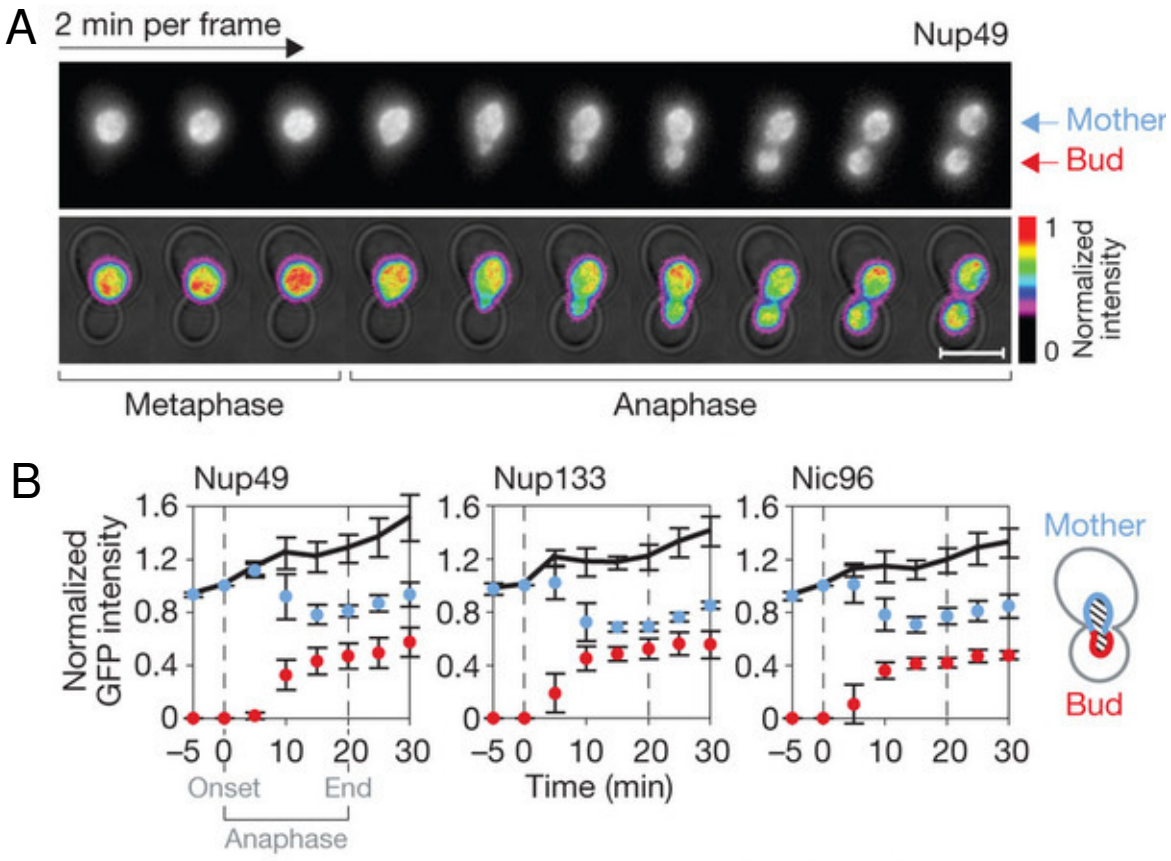

Figure 4: GFP expression of different NPC elements. **A+B**: During budding the intensity of GFP in the 'mother' cell decreases (blue line), suggesting NPCs from the 'mother' cell segregate to the 'daughter' cell (red line). The black line represents total GFP intensity. (Figure adapted from Khmelinskii et al., 2010)

In yeast, the NPCs are associated with circular DNA (cDNA) elements (Shcheprova et al., 2008). During the life span of a yeast cell more cDNA elements are formed. When a 'mother' cell is at the end of her reproductive life cycle, the accumulated amount of cDNA can be larger than the amount of chromosomal DNA present in the cell. This large amount of cDNA must have detrimental effects on the normal functioning of the cells since it is reasonable to assume that normal gene expression gets disrupted. Since the NPCs were thought to remain with the 'mother' cell during mitosis, this would also mean that the cDNA elements remain with the 'mother' cell. With this mechanism the 'daughter' cells would get their 'biological clock' set back to zero, meaning they would have as much cDNA as the 'mother' cell before she started to age. All produced 'daughter' cells would therefore be able to live long, and indeed 'daughter' cells formed during the early life of a yeast cell become just as old as 'daughters' formed near the end of the lifespan of the 'mother' cell.

Effect of aging on the nuclear envelope

As previously stated, the NE forms the direct link between the cytoplasm and the nucleoplasm. Aging related changes within cells are directly linked to the NE, since it forms the barrier between gene transcription and gene translation. Changes in the NE may have great consequences for the proper functioning of a cell.

Gene regulation

Lamins and integral INM proteins have been identified to interact with transcription factors in the nucleus (Heessen et al., 2007). Lamins will be discussed in more detail in the next chapter. Emerin is a single spanning INM protein which is encoded by the EMD gene. Together with LAP2β, a lamin associated protein, it is associated with several transcriptional regulators. This interaction can lead to gene silencing or activation (Markiewicz et al., 2006). The underlying mechanism is not fully understood yet since transcriptional regulators can do both. Heessen et al. suggested that if the transcription factor acts as a gene activator, a possible mechanism would be that the transcription factor is transported away from the target gene. If the transcription factor functions as a repressor it would be possible that a repressive environment for the target gene would be created at the nuclear periphery. One exception to this hypothesis would be the β-catenin–emerin interaction. This protein is a co-activator of Wnt signalling genes, which are involved in the formation of a network of proteins involved in embryogenesis. In the presence of Wnt, β-catenin accumulates in the nucleus. There it interacts with transcriptional factors of the T cell factor/ lymphocyte enhancing factor (TFC/LEF) family to form active transcriptional complexes. An overexpression of emerin however prevents this accumulation, and inhibits its transcriptional activity (Markiewicz et al., 2006). When emerin is not present near the INM it is unable to inhibit β-catenin activity.
It was described in a previous chapter that the NPCs lose their functionality over time, which may result in 'leakage' (Hetzer, 2010). The 'leakage' of the NPCs presumably leads to mixing of the nucleoplasm and the cytoplasm, and since the NE functions as a direct spatial barrier between gene transcription and gene translation, this mixing may lead to loss of transcriptional factors as the example of the β-catenin–emerin interaction describes.

Mechanotransduction

It has been suggested that the mechanical properties of the NE and the cytoskeletal systems may partly regulate the nuclear shape (Shimi et al., 2011). This hypothesis has been supported by the fact that mechanical stress from outside of the cell causes changes in nuclear shape. This may possibly be due to changes in the LINC complex (Maniotis et al., 2006; Lombardi et al., 2011). Ye et al. have made a speculative model of age related changes and their effect on the NE (Ye et al., 2011). A young healthy cell shows an intact NE, maintained by a proper membrane spanning. Both the NE and the lamina are still intact (Figure 5A). When comparing to an aged cell it can be observed that the NE has lost its healthy phenotype (Figure 5B). Defects in the lamina and the NE have an effect on the nuclear shape. Mutations of nuclear lamina elements may accelerate these effects, but this process will be discussed in the following chapters.

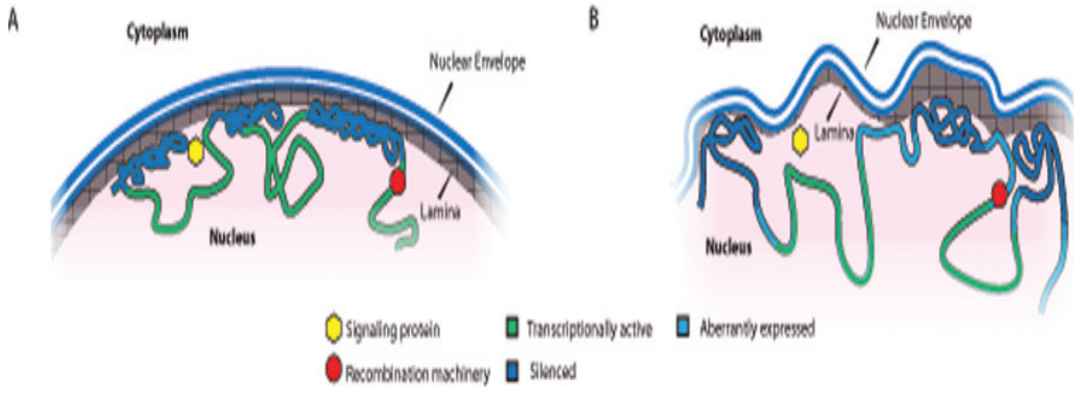

Figure 5: Model of age related changes of the NE. **A**: A young, healthy cell with intact nuclear lamina an NE. **B**: An old cell with a damaged NE. The NE has lost its integrity due to aging effect like lamin mutations, disrupted signalling or recombination errors. (Figure adapted from Yet al., 2011)

Effects of aging on the nuclear lamina

Mutations in lamina encoding genes have been associated with multiple heritable diseases. Interestingly, mutations in LMNB1 and LMNB2 have not been associated with human diseases. Down regulation of these genes in HeLa cells has shown to induce apoptosis, and therefore it is assumed that mutations in these genes are lethal (Mattout et al., 2006). Contrary, over 150 mutations in LMNA have been associated with multiple inherited diseased, collectively called 'laminopathies' (Hutchison et al., 2004; Worman et al., 2005). A large range of diseases has been associated with LMNA mutations (e.g. autosomal and recessive Emery Dreifuss muscular dystrophy [EDMD], Seip syndrome and recessive Charcot-Marie-Tooth disorder type 2). Premature aging diseases like Hutchinson Gilford progeria syndrome (HGPS), atypical Werner's syndrome and mandibuloacral dysplasia (MAD) are also linked to these LMNA mutations (Mattout et al., 2006). These diseases are studied extensively, and more details emerge continuously. It would be too complex to discuss multiple diseases in detail, and therefore I chose to show a shortened overview of HGPS and its effects on aging.

Hutchinson Gilford progeria syndrome:
Hutchinson Gilford progeria syndrome is a autosomal dominant rare disease with features of accelerate or premature aging. Individuals with this disease generally die within 20 years of life, due to myocardial infarction or stroke (Merideth et al., 2008). The observed phenotype is caused by mutations in lamina encoding genes. For the processing of lamin four steps are required. The first step involves farnesylation at the terminal cysteine by farnesyl transferase. Then, cleavage of the last three residues occurs, probably by Zmpste24. During the third step, carboxylmethylation of the terminal cysteine occurs by lcmt. The final step involves cleavage of 15 amino acids from the terminal cysteine by Zmpste24. In cells of HGCF patients the second cleavage site of lamin A is missing as a result of deletion of 50 amine acids and the LAΔ50 (progerin) retains the farnesylated cysteine (Mattout et al., 2006).

Aging effects in HGPS:
Mattout et al. have shown that fibroblasts derived from HGPS patients undergo early senescence. Derived fibroblasts (HGPS) were compared with fibroblasts of healthy individuals (control). The cells undergo significant changes in nuclear shape (Figure 6), which include lobulation of the nuclear envelope, thickening of the nuclear lamina, loss of peripheral heterochromatin and clustering of NPCs. *In vitro* studies, using HeLa cells, show comparable results. Al these nuclear changes correlate with LAΔ50 accumulation.

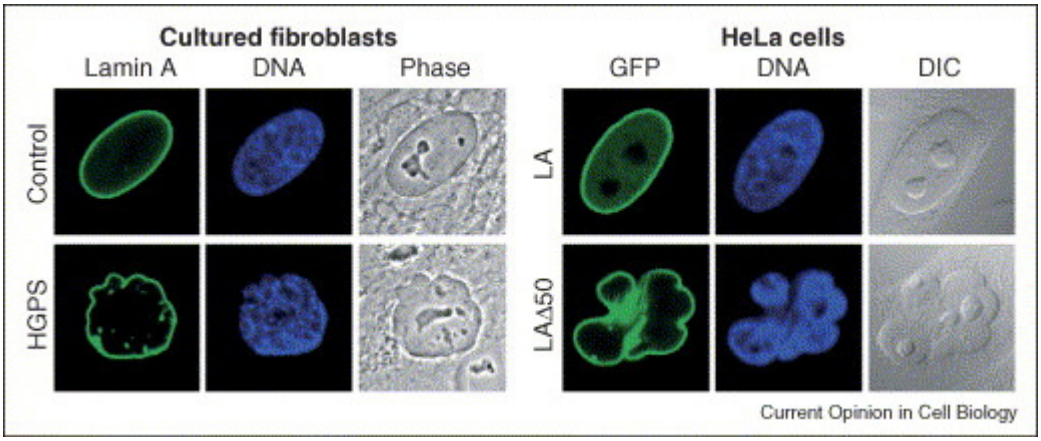

Figure 6: Cell study of fybroblasts collected from HGPS patients and healthy controls. Healthy controls show normal cell architecture. HGPS derived fibroblasts show disrupted nuclear architecture, including lobulation of the NE, thickening of the nuclear lamina and clustering of NPCs. Cultured fibroblasts and HeLa cells were compared, and similar results were seen. (Figure adapted from Mattout et al., 2006)

As discussed, research has proven the existing link between LMNA mutations and different laminopathies. Underlying molecular mechanisms are likely lamin structures, providing a scaffold that is used to assemble and regulate protein complexes involved in nuclear integrity, gene expression, DNA replication, nuclear positioning and cell cycle progression (Goldman et al., 2002).
Liu et al. have shown that cells derived from HGPS patients, or from Zmste24-null mice have an abnormal response to DNA damage (Liu et al., 2005). These cells are unable to recruit DNA repair factors 53PB1 and Rad51 which are involved in the repair of DNA breaks. The authors also conclude that HGPS fibroblasts are hypersensitive to heat stress and contain an abnormal stress response. The lack of these repair elements might explain the accelerated aging, as DNA mutations in healthy cells occur frequently, but are repaired properly to maintain normal cell function.

Outlook

In the previous paragraphs I have summarized the key components of the NE and our current knowledge of their function, how they are affected by the process of aging, and how this influences cell function. In the next paragraphs I will attempt to indentify potential molecular players that cause the loss of nuclear positioning with increased aging, observed recently in the Veenhoff group (G.E. Janssen, unpublished). I have chosen a set of parameters to determine which molecular players I will discuss. First of all there has to be a direct, unique link with the NE. Secondly, there has to be an association with the cytoskeleton. And finally, there have to be indications that the molecular player has a low turnover, so there is time for the accumulation of damage.

Oxidative stress, which is caused by the accumulation of reactive oxygen species (ROS), has been proven to have a direct affect on the cytoskeleton (Dalle-Donne et al., 2001). ROS can damage several cellular components including lipids, proteins and DNA (Oberdoerffer et al., 2007). Therefore, the accumulation of ROS during aging may be responsible for the loss of nuclear position. The accumulated ROS in aging cells may affect the cytoskeleton on different levels; (1) synthesis of cytoskeletal components, needed for proper cytoskeleton formation, may be lower, (2) attachment sites of the cytoskeleton to the NE may be affected leading to detachment of the cytoskeleton from the nucleus or (3) the direct interaction of the cytoskeleton with the chromatin may be disrupted leading to loss of 'anchor' elements.

A large range of proteins is needed for a proper cytoskeletal synthesis. Tang et al. for instance showed that a yeast cell needs Pan1p, End3p, and Sla1p for a proper cytoskeleton organisation and for a normal cell wall morphogenesis (Tang et al., 2000). Unfortunately, no proper data is available on the formation of a large range of these proteins in aging cells. Hence, it may be possible that oxidative stress reduces the formation of essential proteins leading to malfunctioning of the cytoskeleton.

As previously described, aging may have an effect on the LINC complex. It has been shown that mechanical stress, exerted at the outer cell surface, causes changes in the nuclear shape. These changes possibly occur through the LINC complex (Wang et al., 2009; Lammerding et al., 2004). Aging cells get damaged by 'wear and tear' which possibly causes mechanical stress. In aging cells this stress may become worse, and consequently the LINC complex may be targeted for a long period of time. This may eventually cause damage to the LINC complex leading to the loss of attachment of the cytoskeleton. Even though the number of attachment sites to the NE is unknown, this process may be involved in the loss of nuclear position.

Interestingly, baking yeast does not have a KASH-SUN interaction, but there is a homologous SUN domain protein; Mps3. Mps3 is involved in spindle pole insertion into the nuclear membrane (Friederichs et al., 2011). One of the main factors involved in nuclear positioning in yeast cells is the spindle pole body (SPB). It functions as the microtubule organizing centre, comparable with centrosome function in mammalian cells. In the fission yeast *Schizosaccharomyces pombe* it was shown that the SPB regulates the nuclear positioning; the SPB interacts directly with the cytoskeleton and after mitosis the SPB leads the migration of the nucleus back towards the centre of the cell (Hagan et al., 1997). It may be reasonable to propose that also in *Saccharomyces cerevisiae* the SPB plays a key role in nuclear positioning. Although the characteristics of the SPB are becoming clearer, there is no research published yet investigating the possible loss of the SPB function in aging cells. Since multiple components within a cell deteriorate over time, it may be reasonable to assume that also the SPB loses its function during aging resulting in loss of nuclear position.

However, it is not solely the PSB that determines the nuclear position in baking yeast; it is the interaction of the SPB with the cytoskeleton. Perturbation of microtubules proved that nuclear positioning required a functional microtubule cytoskeleton. (Hagan et al., 1997). King et al.

has shown that, in the fission yeast *S. pombe*, nuclei are actively positioned at the cell centre by microtubules (King et al., 2008). Their study showed that cytoplasmic microtubules are linked directly to the nuclear heterochromatin. Three proteins were thought to be involved in this process; Kms2, Sad1 and Ima1. Sad1 is another homologous SUN domain protein. These proteins form a framework for communication between cytoplasmic microtubules and chromatin. With the direct linkage of the heterochromatin to the cytoskeleton, the heterochromatin seems to function as an anchor for nuclear position. The cytoskeleton is anchored to the cell membrane and the nuclear envelope. The binding with the heterochromatin functions as an extra tight barrier for attachment to the nucleus. Unfortunately, since Ima1 has only recently been identified there is not much data available. It would be interesting to investigate these proteins in more detail, and see whether or not the protein levels degrade over time. This could be an explanation for the loss of nuclear position in aging yeast cells. If the anchoring function of the heterochromatin is lost, there may be a less strong binding site for the cytoskeleton to bind to the NE, and the cytoskeleton may detach easier over time.

Taking the previous into account, it indicates that there may not be one specific mechanism that is solely responsible for the loss of nuclear position in aging yeast cells. Multiple factors are involved in the positioning of the nucleus, and when one mechanism diminishes in time, the other mechanisms may still be strong enough to compensate for the loss.

Asymmetrical segregation in baking yeast may give new insights in the field of stem cell research. Stem cells undergo asymmetrical cell division, producing two distinct daughter cells; one daughter cell is identical to the mother cell, and the other daughter cell is programmed to differentiate. Understanding aging in yeast cells in more detail may reveal insights in mechanisms explaining asymmetrical aging.

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