Chapter 5

Poor old pores – The challenge of making and maintaining nuclear pore complexes in aging

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Highlights

- The comparison of age-related changes of the Nuclear pore complex (NPC) shows tissue specific and organism specific changes.
- Aging dividing or non-dividing cells likely face different challenges in assuring faithful assembly and maintenance of NPCs.
- The changes in the abundances of the NPC components in replicative aging yeast cells are most similar to those in aging tissues that are turned over regularly, like liver, while those in chronological aging yeast mimic the changes in long-lived tissues, like brain.
- NPC quality control is likely an important node in aging, where NPCs are impacted by the loss of proteostasis in aging, while changes in NPC function potentially drive further loss of proteostasis in aging.
Abstract
The nuclear pore complex (NPC) is the sole gateway to the nuclear interior and its function is essential to all eukaryotic life. Controlling the functionality of NPCs is a tremendous challenge for cells. First, NPCs are composed of over 522 proteins, and the assembly is not trivial, and goes awry occasionally. In addition, once formed, specific parts of the NPC exist for extremely long periods of time, and as a result, they are susceptible to accumulate damage. Lastly, a significant part of the NPC is composed of, so-called, intrinsically disordered proteins that are prone to aggregation. In this review, we summarize the current knowledge of the fate of NPCs during normal aging in different tissues and different organisms. Our analysis of the abundances of the NPC components shows distinct changes in the stoichiometry in different model organisms and in different tissues. We then summarize the current knowledge of how the quality of NPCs is guarded in young cells and hypothesize that NPCs are poorly maintained during aging of non-dividing cells, while in dividing cells the main challenge seems related to the assembly of new NPCs. In this light we discuss the use of baker’s yeast as model organism to study NPCs in aging. Our survey of the current knowledge point at NPC quality control being an important node in aging where NPCs are impacted by the loss of proteostasis in aging and, vice versa, changes in NPC function potentially drive further loss of proteostasis in aging.

1. Introduction
One of the nine described universal hallmarks of aging is the loss of proteostasis (López-Otín et al., 2013). Loss of proteostasis can result from changes in the biogenesis, folding, trafficking and degradation of proteins. The cell’s ability to assemble and maintain functional protein complexes in aging is indeed compromised as changes in protein complex stoichiometry were highlighted as one of the prominent changes found across different aging organisms (Janssens et al., 2015; Ori et al., 2015). In baker’s yeast the nuclear pore complex (NPC) is among the most substoichiometric complexes in replicative aging cells (Janssens et al., 2015).

NPCs are among the largest protein complexes in the eukaryotic cell (Box 1). A prominent function of NPCs is to facilitate transport of macromolecules from the cytoplasm to the nucleus and vice versa (Aitchison and Rout, 2012; D’Angelo and Hetzer, 2008; Fiserova and Goldberg, 2010; Wente and Rout, 2010). The proteins of the core scaffold of NPCs are extremely long lived in Caenorhabditis elegans and in rat neurons (D’Angelo et al., 2009; Savas et al.,
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In this review, we compare the age-related changes found in different model organisms and different tissues and we further discriminate between aging in non-dividing cells (chronological aging) and aging in dividing cells (replicative aging). For age-related phenotypes caused by mutations to the NPC, or its supporting machinery we refer to the recent review by (Sakuma and D’Angelo, 2017). We compare specifically age-related changes in the abundances of the components of the NPC (Nucleoporins or Nups) as found in different proteome studies. The differences in Nup abundance changes during yeast replicative and chronological aging indicate that the dividing and non-dividing cells potentially face different challenges in NPC assembly and maintenance respectively. Based on our analysis of Nups in aging, we discuss to what extent replicative and chronological aging in baker’s yeast are suitable models for aging of tissues with faster turnover rates, like liver, or long lived tissues, like the brain. We further speculate on what could be causal for the age-related changes at the NPC. For this, we summarize the current knowledge of the mechanisms of NPC assembly and maintenance and point out open questions. In the last part of this review, we summarize how NPCs could contribute to the hallmarks of aging.
Box 1: The structure and function of the nuclear pore complex at a glance

Each NPC is composed of ~30 different proteins, called Nucleoporins or Nups, that are present in multiple copies (Figure 1). Each NPC is composed of eight spokes, that are arranged to form a cylindrical structure embedded in the nuclear envelope (NE) membranes. Each spoke is interconnected by flexible linker elements that give the NPC strength and flexibility at the same time. The core of the NPC is organized by stably folded proteins in symmetric inner and outer rings and is anchored to the NE by a membrane ring, made of just four transmembrane proteins. The outer rings are mainly formed by a protein complexes called the Y-shaped complex. Attached to the cytoplasmic side of the symmetric core is a structure called the RNA export platform (formerly called cytoplasmic filaments). Attached to the nuclear side of the symmetric core is a structure called the nuclear basket, which is involved in nucleocytoplasmic transport, RNA processing and RNA export, but also served as a multifunctional platform for various nuclear processes (e.g. transcriptional and chromatin organization). Both structures, RNA export platform and nuclear basket are involved in RNA processing and RNA export. In the center of the core scaffold are ID proteins that contain Phenylalanine-Glycine-rich nucleoporins (FG-Nups) that form the selective barrier of the NPC. A decrease in the concentration of FG-Nups in the center of the NPC compromises the permeability barrier and active transport rates of the NPC (Popken et al., 2015; Strawn et al., 2004; Timney et al., 2016).

Rapid and energy dependent transport across the NE is facilitated by the NPC and several transport factors (NTRs) (Aramburu and Lemke, 2017; Lemke, 2016; Li et al., 2016 and reviewed in: Fiserova and Goldberg, 2010). The energy dependent translocation of macromolecules between the nucleus and the cytoplasm is facilitated by nuclear transport receptors (NTRs) and the GTP binding Ras-related Nuclear protein (RAN). There are 17 different NTRs known in yeast (Allen et al., 2002), and about 30 NTRs are known in humans (Mackmull et al., 2017). For many proteins that are either transported to the nucleus or the cytoplasm, their respective NTRs remain to be identified.

Proteins that require nuclear import encode a nuclear localization signal (NLS) and are recognized in the cytoplasm by NTRs called importins. Proteins that require nuclear export encode a nuclear export signal (NES) and are recognized in the nucleoplasm by NTRs called exportins. For all proteins without a NLS/NES targeting signal, the NPC acts as a diffusion barrier. Generally, it can be stated that the size and surface properties of a molecule determine how easily a molecule can diffuse between nucleus and cytoplasm (Frey et al., 2018; Popken et al., 2015; Timney et al., 2016).
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In the center of the core scaffold are ID proteins that contain Phenylalanine-Glycine-Rich motifs. The outer rings and is anchored to the NE by a membrane ring, made of just four subunits, that are present in multiple copies (Figure 1). Each NPC is composed of eight spokes, that are arranged to form a cylindrical structure embedded in the inner rings and nuclear basket. The outer rings and the membrane ring are involved in RNA processing and RNA export. The nuclear basket, which is involved in RNA export, is anchored to the nuclear side of the symmetric core. The RNA export platform (formerly called the cytoplasmic filaments) is attached to the cytoplasmic side of the symmetric core. Both structures, RNA export platform and nuclear basket, are involved in RNA processing and RNA export, but also transcriptional and chromatin organization. Both structures, RNA export platform and nuclear basket, are involved in RNA processing and RNA export, but also transcriptional and chromatin organization.

Figure 1 Cartoon representation, adapted from Kim et al., 2018, of main structural features of NPCs and conserved proteins between yeast and humans. The main structural components (RNA export platform, outer rings, membrane ring, inner rings and nuclear basket, as well as the eight fold rotational symmetry of these structures) are conserved from yeast to humans (Shav-Tal and Tripathi, 2018). The outer rings are composed of Y-shaped complexes, yeast NPCs have a total two outer rings, one on the cytoplasmic and one on the nuclear side respectively. Humans have a total of four outer rings, two on the cytoplasmic side and two on the nuclear side. Human NPCs are considerably larger in their dimensions (not shown) and their molecular mass than yeast NPCs. Functional homologs that do not share a common name between yeast and humans have the same color, within their respective structural components. Nups in black font that are only mentioned for one organism are proteins, which are unique for either yeast or human. Proteins that also function in other protein complexes than NPC, or that associate only very transiently with NPCs are indicated with a star (*).

2. On the search for common age-related changes of NPCs in different cell types
2.1 General considerations for the comparison of aging profiles
In general there is a large degree of conservation of age-associated changes across different eukaryotic species (Fontana et al., 2010; Janssens and Veenhoff, 2016a; Kirkwood, 2008; López-Otín et al., 2013; Pitt and Kaeberlein, 2015; Reichard, 2017). To investigate if changes to the NPC are also conserved across species, we extracted Nups from six published proteome datasets, that analyzed age-related changes in three different model organisms, namely budding yeast (Binai et al., 2014; Janssens et al., 2015), rat (Ori et al., 2015) and mouse (Cutler et al., 2017)). The budding yeast studies addressed specifically proteome changes during aging in non-dividing cells (chronological aging; CA; Figure 2a) (Binai et al., 2014) and aging in dividing cells (replicative aging; RA; Figure 2b) (Janssens et al., 2015). The two proteomes from rat, studied age-related changes in brain and liver tissues, and those from...
mouse studied age-related changes in brain and muscle tissues. All three tissues are primarily composed of postmitotic cells. The majority of brain cells are extremely long lived and the brain has very limited regenerative capacity (Case and Tessier-Lavigne, 2005). The liver on the other hand, has high regenerative capacity and liver cells are turned over regularly (Magami et al., 2002; Malato et al., 2011). Skeletal muscle is predominantly composed of polynucleated muscle fibers, that can regenerate when injured (Tedesco et al., 2010).

Figure 2 Schematic representations of aging on the population and on the single cell level.

a) Cartoon of a chronological aging of a bakers yeast cell. Chronological aging, is the kind of aging that non-dividing cells experience in time, before they eventually die of old age. Chronological aging in *Saccharomyces cerevisiae* is induced through the depletion of nutrients. Commonly used protocols to achieve nutrient depletion include growing a culture to stationary phase, or transferring an exponential culture to water (Hu et al., 2013). Due to the prolonged starvation, chronological aging is characterized by upregulation of autophagy related pathways. Prolonged starvation is obviously a stress that postmitotic cells in higher eukaryotes do not experience and this should be taken into account when translating results from chronological aging studies in *S. cerevisiae* to higher eukaryotes. A more involved way to induce chronological aging, which overcomes the limitation of severe nutrient depletion in yeast, is to provide the cells with just too little nutrients to divide, called near-zero growth and is performed in a retentostat (Binai et al., 2014).

b) Cartoon of a replicative aging baker’s yeast cell. Dividing cells age with each completed division, therefore their age is measured in the number of completed divisions. Replicative aging
mouse studied age-related changes in brain and muscle tissues. All three tissues are primarily composed of postmitotic cells. The majority of brain cells are extremely long lived and the brain has very limited regenerative capacity (Case and Tessier-Lavigne, 2005). The liver on the other hand, has high regenerative capacity and liver cells are turned over regularly (Magami et al., 2002; Malato et al., 2011). Skeletal muscle is predominantly composed of polynucleated muscle fibers, that can regenerate when injured (Tedesco et al., 2010).

The challenge of making and maintaining nuclear pore complexes in aging in S. cerevisiae is triggered by the asymmetric retention of aging factors, which causes the daughter cell to be born young, while the mother cell retains the age-associated features including damage. This damage can occur in the form of damaged and nonfunctional organelles, extrachromosomal rDNA circles (Sinclair and Guarente, 1997), asymmetrically retained transmembrane proteins and protein aggregates.

c) Cartoon of a typical survival curve of a cohort of aging cells or organisms. Aging is associated with an increased risk of mortality and there is intrinsic variation in lifespan of individual cells or organisms within one cohort. As aging occurs at very different timescales, cross species comparisons of changes associated in aging, as well as comparisons between chronological and replicative aging cells can be based on the survival of the cohort.

The comparison of aging between different model organisms is not trivial for several reasons. First, replicative and chronological aging are measured in different units and different model organisms have different maximal lifespans. Therefore, a fair way to compare the different aging trajectories is to align them based on the average survival of the population (Figure 2c). For the specific proteome studies of aging yeast, mouse and rats (Binai et al., 2014; Cutler et al., 2017; Janssens et al., 2015; Ori et al., 2015) that we compare in figures 3-5, the time points of the aged samples are roughly taken at similar points in the respective lifespan curves. We estimate, that the viability of the yeast replicative aging population is about 55% at the last time point measured (72 hours) (Janssens et al., 2015), the viability of the yeast chronologically aged population is approximately 60% (after 21 days) (Boender et al., 2009), and the mice and rat should have a survival of approximately 50-70 % at the latest age time points (24 months) (Ori et al., 2015; Rowlatt et al., 1976; Smith et al., 2010). Rat and mouse strains used in the mentioned study have similar lifespans and both aged samples were analyzed after 24 month, however the young mouse sample was taken after 3 month (Cutler et al., 2017), and the rat sample was taken after 6 month (Ori et al., 2015). Second, aging is a highly individual process and analyzing only two or three animals per sample might not cover the full spectrum of aging changes in a population. Third, the age related changes in protein abundance might be caused by aging related changes in the abundances of specific cell types that compose a tissue (Frontera et al., 2000; Schmucker, 2005; Yankner et al., 2008). Lastly, different methods were used to pre-fractionate the mouse and rat samples, to do the mass spectrometry and to analyze the data. Most significantly, the mouse and rat proteomes are fractionated, and specifically the nuclear fraction was analyzed, while the yeast samples contain whole cell extracts. Even taking these limitations into account there are interesting similarities and differences in the aging trajectories of NPCs that we will highlight in the following sections.
2.2 Overall loss of complex stoichiometry

Loss of protein complex stoichiometry in general is a conserved phenotype of aging (Janssens et al., 2015; Ori et al., 2015), and NPCs have been shown among the most substoichiometric protein complexes in replicative aged yeast cells (Janssens et al., 2015). The comparison of the proteome datasets shows that loss of Nup stoichiometry is most pronounced in yeast replicative aging (Figure 3a and 3b), followed by yeast chronological aging. Furthermore, we observe that the loss of NPC stoichiometry is two-fold higher in the brain (mouse and rat) than in the rat liver, indicating that loss of NPC stoichiometry is tissue specific.

Another approach to analyze the data for conserved changes is to ask, whether certain Nups show similar changes in abundance throughout different model systems. A major conclusion of Ori et al., was that the age related changes in protein abundance are tissue specific, based on their proteome analysis of liver
2.3 Distinct Nup abundance changes in replicative and chronologically aging yeast

Also the comparison of Nup abundance changes in yeast replicative and chronological aging samples shows distinct changes in protein abundances. The majority of non FG-Nups, as well as the cytoplasmic FG-Nup, Nup159, show similar changes in abundance during replicative and chronological aging (Figure 4 c). It should be noted, that the proteins that show the most pronounced decrease in abundance during chronological aging do not uniquely function at the NPC (Dyn2 and Sec13). The decrease in abundance of Sec13 during chronological aging is possibly related Sec13’s role in CopII coated vesicle transport (Reviewed by Gomez-Navarro and Miller, 2016), which is required for cell cycle progression and cytokinesis. Dyn2 also acts as microtubule motor protein that is required for cell division (Reviewed in Laan et al., 2012). Therefore, it is likely that these proteins are downregulated in non-dividing cells. The strong loss of nuclear and central FG-Nups is specific to replicative aging. Chronologically aged cells show even a slight increase in abundance of FG-Nups, with the exception of Nsp1 and Nup49, which show a slight decrease in abundance. Overall the comparison of the changes in Nup abundances in aging mice, rats and replicative and chronological aging yeast cells reveals that the components of the NPC become most sub-stoichiometric in replicative aging yeasts and the specific changes in Nup abundances are distinct in each dataset.
Figure 4 Comparison of selected Nup abundances from different proteome datasets

a) Comparison of age-related changes in Nup abundance of rat brain and rat liver

b) Comparison of age-related changes in Nup abundance of mouse muscle and mouse brain.

c) Comparison of age-related changes in Nup abundance of yeast chronological at a timepoint of ~60% population viability and yeast replicative aging at timepoints representing ~75 and 55% viability (45h and 72h). The replicative aging proteome does not include information on the abundance of Mlp2, Nup57, Nup49, Nup59, Gle1, Nup42 and Dyn2, represented by an x on the x-axes.

2.4 Yeast as a model system to study aging of NPCs in higher eukaryotes

Yeast has been a powerful model in aging research for many practical reasons (Denoth Lippuner et al., 2014; Fontana et al., 2010; Janssens and Veenhoff, 2016a; Longo et al., 2012). Specifically for the here discussed proteomics studies the advantages are that the coverage of the Nups is higher in yeast due to the vastly reduced complexity of the proteome. Also, the methods for cultivation of aging yeast cells (Binai et al., 2014; Janssens et al., 2015) allow the assessment of many time points in the aging trajectory (12 in the case of Janssens et al. 2015), which increases the confidence of observed changes. Indeed, the comparison of the replicative aging proteome, with more targeted studies on the yeast NPC in replicative aging (summarized in Table 1) is overall
consistent (Janssens et al., 2015; Lord et al., 2015; Rempel et al., 2018). Such a level of consistency is currently not seen for the analysis of rodent brain samples. The mouse brain proteome and the rat brain proteome show no correlation in age related Nup abundance changes and neither dataset shows a decrease in the abundance of Nup93, that was shown for rat brain cells by D’Angelo et al., 2009. It is however important to address the question to what extent yeast is suitable for aging studies of the NPC.

The most direct comparison between the replicative and chronological aging regimes in yeast would be with asymmetrically dividing mitotic cells, like stem cells, and postmitotic cells, like neurons. Indeed, it is often stated that stem cells are subject to replicative aging, while post mitotic cells such as neurons are subject to chronological aging (Denoth Lippuner et al., 2014; Longo et al., 2012). With respect to the comparison in replicative aging, the immortal yeast daughter lineage should be compared to the self-renewing stem cell lineage. The yeast mother cells and the differentiated cells both are mortal and retain damaged components (Bufalino et al., 2013; Denoth Lippuner et al., 2014; Mortimer and Johnston, 1959; Rossi et al., 2008; Schultz and Sinclair, 2016). However, neither stem cells nor terminally differentiated cells experience solely replicative or chronological aging, but rather a mix. E.g. blood stem cells may stay quiescent for years before starting their replicative lifespan and, vice versa post mitotic cells were derived from stem cells that themselves experience aspects of replicative aging. In contrast, aging experiments with yeast can be performed such that they report more pure forms of replicative or chronological aging and hence the differences observed in chronological and replicative aging in yeast are at its most extreme compared to those observed for human cells. Going to the tissue level, it has been suggested that organs with high turnover rates, such as intestine or liver are likely to show signs of replicative aging (Moore and Jessberger, 2017; Rossi et al., 2008), while organs with low turnover rates (i.e. brain) would more likely show signs of chronological aging (Longo and Fabrizio, 2011). While the liver is mainly composed of postmitotic cells, the fact that it is a highly regenerative tissue could indicate that one might expect some resemblance between replicative aged cells and aged liver tissue.
Figure 5 Correlation of age-related changes in Nup abundance in different model systems.

(a) Pearson correlations of pairwise comparisons of changes in Nup abundances in samples from different aging proteomes. The number on top of the bars indicates the sample size of Nups that were used for the comparison. ** indicates a significant correlation with p<0.01. The correlation coefficient ranges from −1 to 1. Values close to −1 indicate negative, and values close to 1 indicate positive linear relationships between two samples. Values close to 0 indicate that there is no linear relationship between two samples.

(b) Comparison of age-related fold changes in Nup abundance between rat liver and yeast replicative aging cells. * indicates there are two yeast homologues for one metazoan Nups, here the third bar in dark grey shows the abundance change for Nup170.

(c) Comparison of age-related fold changes in Nup abundances between mouse brain and yeast near-zero growth (chronological aging). * Third bars for Nups with additional homologues, in darker grey, show abundance changes for Nup100, Nup59 and Nup170 in yeast chronological aging, which are additional homologues of Nup98, Nup35 and Nup155, respectively.

(d) Fold changes in yeast replicative aging, plotted against fold changes in rat liver. The dotted line indicates the best linear fit, individual Nups are annotated in yeast nomenclature.

(e) Fold changes in yeast chronological aging, plotted against fold changes in mouse brain. The dotted line indicates the best linear fit, individual Nups are annotated in yeast nomenclature.
### Table 1: Key findings related to NPC composition and transport function in aging.

| Study                    | Model organism                  | Nup protein abundances | Nup transcripts abundances | Transport Phenotype                                                                 |
|-------------------------|--------------------------------|------------------------|-----------------------------|-------------------------------------------------------------------------------------|
| D’Angelo et al., 2009   | C. elegans rat brain (24–28 month) | Nup93 ↓ FG-Nups ↓ Nup107- Nup93, Nup153 carbonylated. | Downregulation of scaffold nups in differentiated cells. | Increased passive permeability of the NE of isolated nuclei and intranuclear tubulin bIII *in vivo.* |
| Kim et al., 2010        | Senescent human fibroblast (>66 doublings) | Nup88↓ Nup107↓ Nup155↓ Nup50↓ | Decreased transcript levels of Nups, but also importin α/β and Ran system. | Fewer NPCs, decreased nucleocytoplasmic transport, irresponsive to cell stimuli. |
| Lord et al., 2015       | S. cerevisiae (6–9 divisions)   | Nup100 – Nup53 – Nup116 ↓ Nsp1↓ | Stable transcript levels of Nup100, Nup53, Nup116 and Nsp1. | Decreased nuclear accumulation of different NLS-GFP reporters at steady state.     |
| Rempel et al., 2018     | S. cerevisiae (several timepoints and ages) | Nup100 ↓ Nup133 – Nup116 ↓ Nup2↓ Nup120 - | | Increased nuclear accumulation of NLS-GFP reporters and RCC1 at steady state and decreases exclusion of NES-GFP reporters, decreased transport dynamics of Msn2 shuttling. |
| Janssens, Meinema et al., 2015 | S. cerevisiae (12 timepoints and ages) | Nup116 ↓↓ Nup2↓↓ Nsp1↓↓ Nup100 ↓ Nup60 ↓ Nup1 ↓ Mlp1 ↓ Gle2 ↓ 15 others - | Stable transcript levels for all Nups | |
These general considerations of how yeast replicative and chronological aging may relate to aging in rodent tissues are indeed broadly reflected in the analysis of age related changes of NPC components as follows: The pairwise comparison of correlation between age-related changes in Nup abundance shows that changes in Nup abundances in yeast replicative aging are significantly correlated to those in aged rat liver ($R=0.74$, $p=1.46\times10^{-3}$) (Figure 5a). The loss of the basket Nups is shared in both datasets of which especially the shared decrease of Nup2 (Nup50) abundance in aging is the main contributor to this correlation being significant (Figure 5b, d). However, we also see distinct differences, between replicative aged yeast cells and aged liver tissue. Aged liver tissue shows very little loss of protein complex stoichiometry and the overall decrease in the abundance of Nups points directly towards an overall reduction in the number of NPCs in aged liver cells. Yeast chronological aging shows the best correlation to mouse brain (Figure 5a). The p-value for this correlation between mouse brain and yeast chronological aging is not significant, most likely because the sample size of Nups which can be compared is too small (Figure 5a, c, e). Furthermore, Figure 5 shows low correlation between different tissues, as well as low correlation in age-related changes between replicative and chronological aging yeast. Overall, proteome changes in aging are tissue specific (Ori et al., 2015) and distinct from those observed in yeast, which might be related to different turnover rates of different tissues, and related, the different contributions of chronological and replicative aging.

3. Potential causes for age-related changes of NPCs, assembly and maintenance of NPCs

3.1 Unique biochemical properties set NPCs at risk in aging and in aggregation pathologies

To discuss what could be the cause for the distinct changes in the NPCs in different aging regimes and cell types we discuss how the NPC may be at risk for age related decline. Based on the fact that the transcript levels of many Nups is stable in aging (see Table 1) we discuss mostly the posttranscriptional aspects of NPC stability. First, we summarize the current knowledge about NPC assembly in the context of aging. The assembly of NPCs is a complex process that has occurs through two different mechanisms. In higher eukaryotes, NPCs and the NE disassemble at the start of mitosis. Consequently, all NPCs need to be reassembled into the reforming NE (postmitotic assembly) after each division (Figure 6a). Organisms with closed mitosis such as budding yeast, S. cerevisiae, and higher eukaryotes during interphase, need to assemble NPCs into the intact NE (de novo or interphase assembly; Figure 3b) (Reviewed in:
Otsuka and Ellenberg, 2018). Second, once NPCs are functionally formed their maintenance is a challenge. Parts of the NPC are long lived and thus susceptible to the accumulation of damage over time. We discuss the importance of oxidative damage in this context. Also, the intrinsically disordered FG-Nups readily self-associate and aggregate (Ader et al., 2010; Halfmann et al., 2012; Milles et al., 2013) and here we discuss the recently established connection between NPCs and aggregation pathologies. Lastly, we discuss the current knowledge about the mechanism for quality control of NPCs, an exciting new area of research (Toyama et al., 2019; Webster et al., 2014, 2016).

### 3.2 Two mechanisms of NPC assembly: interphase assembly

Mechanistic information on interphase assembly is still very limited. The assembly of NPCs during interphase occurs rather sporadic and takes considerably longer than postmitotic assembly (Dultz and Ellenberg, 2010). A major challenge in the study has been to distinguish NPC assembly sites, from the majority of fully assembled NPCs inside the cell. Single NPCs are too small to observe with normal fluorescence microscopy, which adds to the challenge of NPC assembly site identification (Otsuka et al., 2016). A scanning electron microscopy (EM) study using *Xenopus* egg extract suggested that that assembly begins with the formation and stabilization of a hole (pore) through the NE (Goldberg et al., 1997). A more recent study showed transmission EM images of NPC interphase assembly intermediates in mammalian cell lines (Otsuka et al., 2016). These revealed that interphase assembly occurs through the evagination of the inner nuclear membrane (INM), which further deforms, until it fuses with the flat outer nuclear membrane (ONM). The at the site of the deformed membrane is a mushroom shaped, electron dense mass of growing size (Otsuka et al., 2016).

It remains to be determined, in which order the nucleoporins exactly assemble, how the invagination of the INM is achieved, which non-NPC components might be involved in stabilizing the assembly intermediates and which mechanism ultimately fuses INM and ONM. The transmembrane proteins Sun1 and Pom121 establish where a new NPC is assembled, and Pom121 is part of the early assembly intermediate (Talamas and Hetzer, 2011). Nup153 and Nup53 are associated with these early assembly intermediates (Otsuka et al., 2016). The Y-shaped complexes probably join the assembly later, potentially after the fusion of INM and ONM (Talamas and Hetzer, 2011). The RNA export complex was shown to join only later in NPC assembly (Otsuka et al., 2016). The RNA export complex also joins only later in NPC assembly (Otsuka
et al., 2016), and assembly problems are often associated with mislocalization of proteins of the RNA export complex, while other Nups still localize to the NE (Figure 6 b).

Figure 6 NPC assembly mechanisms.

Our knowledge concerning NPC assembly is derived from different model organisms. The order in which Nups assemble during postmitotic assembly has been studied in Xenopus egg extract and in fixed human cells at different cell-cycle stages (Antonin et al., 2005; Bodoor et al., 1999; Eisenhardt et al., 2014; Mansfeld et al., 2006; Rasala et al., 2008). Additional structural insight comes from in vivo studies of rat and human cell lines (Dultz et al., 2008; Haraguchi et al., 2000; Lu et al., 2011; Otsuka et al., 2018). Interphase assembly factors have been studied in yeast (Dawson et al., 2009; Webster et al., 2014, 2016; Zhang et al., 2018) and structural insight into assembly intermediates comes from mammalian cell lines (Otsuka et al., 2016).

a) Postmitotic assembly takes place at the end of mitosis, into small openings in the reforming NE. (I) As the holes in the NE become smaller, Nups are released from Importin β by RAN-GTP. Proteins in the depicted assembly intermediates are mentioned. (II) The next step involves assembly of major parts of the symmetric core structure of the NPC (outer rings, inner rings and transmembrane ring), followed by part of the basket. (III) The incorporation of Nup62 complexes and Nup93 into the assembly intermediates stabilizes the NPC assembly intermediate and increases the FG-Nup density in the central channel. At this point, the assembly intermediates
become competent for nuclear import. (IV) The last steps of postmitotic NPC assembly involve the assembly of the RNA export platform and finishing the nuclear basket structure.

b) Interphase assembly occurs into the intact nuclear envelope. (I) The assembly starts with the evagination of the INM, which is observed as an electron dense mass localizes to the electron dense membrane. Proteins that are known to be part of this early assembly structure are shown, although the exact order of recruitment of the proteins is unknown. Early assembly structures already show eight fold rotational symmetry. Several yeast proteins that are known to assist in the assembly process are shown as ‘tools’ of the assembly process, it is not known in which steps of NPC assembly these proteins are employed to the NPC assembly site. (II) The INM further deforms, while the electron dense mass is growing. The proteins involved in this stage of NPC assembly remain to be shown. (III) At the point, where the INM contacts the ONM, the two membranes fuse. (IV) The final steps of NPC assembly involve the RNA export platform protein Nup358 and potentially also other proteins of this complex.

How many NPCs are assembled during interphase is regulated by the protein levels and phosphorylation state of the basket protein Tpr. Extracellular signal-regulated kinase (ERK) phosphorylates Tpr at the NPC and while resident at the NPC it phosphorylates Nup153. Phosphorylation of Nup153 then prevents Nup153’s association with the y-complex and blocks further NPC assembly (McCloskey et al., 2018; Vomastek et al., 2008). Cancer cells often have more NPCs (Lewin et al., 2007) and there is evidence suggesting that NPC numbers also change in aging (Denoth-Lippuner et al., 2014; Ori et al., 2015). Interestingly both yeast aging datasets and the rat liver proteomes show relative decreases levels of the Mlp1/Tpr proteins (and the other basket Nups human Nup50 and yeast Nup2). As the absence of Tpr is reported to increase NPC numbers (McCloskey et al., 2018) this could indicate that more NPCs are assembled in aging. This is not in line with the decline in NPC number reported in rat liver (Ori et al., 2015), but it is in-line with the overall increase in Nup levels at the NE in yeast (Denoth-Lippuner et al., 2014; Rempel et al., 2018).

The effects of having too many or too few NPCs are not well understood. Changes in the numbers of NPCs will primarily influence passive diffusion of molecules over the NE and is expected to increases with increased numbers of NPCs at the NE. The rate limiting step for energy dependent transport is the formation of a complex between the NTR and its cargo complex: it takes time for the NTR and cargo to find each other in the crowded cytosol while the actual translocation through the NPC is fast (Hodel et al., 2006; Riddick and Macara, 2005; Timney et al., 2006). We thus speculate that moderate changes in NPC numbers won’t have an effect on energy dependent transport. However, when NPC numbers are greatly reduces (~50%), the number of NPCs will become rate limiting for energy dependent transport as well, causing a drop in these transport rates (Makio et al., 2009).
In *S. cerevisiae*, we know a number of proteins that assist and monitor in NPC assembly, but their mechanism of action is only beginning to be understood. Misassembled NPCs are recognized by the inner nuclear membrane proteins Heh1 and Heh2 and cleared from the NE by the NE-specific ESCRT-III adaptor Chm7 and the ESCRT-III/Vps4 system (see Box 2) (Webster et al., 2014, 2016), and broken down by the proteasome. Interference with this quality control mechanism, results in the accumulation of misassembled NPCs (Webster et al., 2014). The proteins that guard NPC assembly in *S. cerevisiae* are conserved in higher eukaryotes. In higher eukaryotes Chmp7 and the ESCRT-III system additionally have the important function of resealing the NE after at the end of mitosis. In *S. cerevisiae* several proteins that assist and control the quality of NPC assembly decrease in abundance during aging, and there are indications that aged yeast cells experience problems with NPC assembly (more details in Box 3).

**Box 2: NPC quality control in *S. cerevisiae***

The data from Lusk and coworkers currently available supports the following model for the clearance of misassembled NPCs: The NE specific Endosomal Sorting Complexes Required for Transport (ESCRT) adaptor, Chm7, binds to Heh1/Heh2, which allows the ESCRT machinery to assemble at the site of the misassembled NPC (Webster et al., 2014, 2016). The subsequent clearance of the misassembled NPC from the NE depends on several proteins of the ESCRT-III complex. Vps2, Vps24 and Snf7 are certainly involved, Vps20 does not seem to be part of the nuclear ESCRT-III complex (Webster et al., 2014), although it was previously reported to be part of the core ESCRT-III complex (Hurley, 2010). Snf7 binds directly to Heh2 and Chm7, where it assembles into a polymer. The polymer is capped by Vps24. Vps24 then recruits Vps2 to the complex. Vps2 promotes the assembly of a Vps4 hexamer at the site of the misassembled NPC. Vps4 disassembles the ESCRT-III complex, while hydrolyzing ATP. What kind of membrane remodeling is needed to remove the misassembled NPC from the NE is currently still unknown (Thaller and Patrick Lusk, 2018), but membrane remodeling is achieved through the disassembly of the Snf7 filament (Hurley, 2010). Surprisingly, this process seems to be independent of the Vps4 cofactor, Vta1, based on the absence of synthetic genetic interactions between Vta1 and various tested Nups (Webster et al., 2014). This suggests, that the disassembly of the filament is relatively ineffective (Azmi et al., 2006). Ultimately, the misassembled NPC is degraded by the proteasome (Webster et al., 2014).
3.3 Two mechanisms of NPC assembly: Postmitotic assembly

Postmitotic assembly of NPCs happens in organisms with open mitosis upon mitotic exit, in the telophase. All NPCs reassemble simultaneously into small openings at the reforming NE envelope (Dultz et al., 2008; Otsuka et al., 2018). Initiation of NPC assembly starts in late anaphase with the association of ELYS with the decondensing chromatin at the nuclear periphery (Rasala et al., 2008). The RAN-GTP dependent release of Nups from importin β (Walther et al., 2003) allows the assembly of Y-shaped complexes, which are then bound to chromatin by ELYS at NPC assembly sites (Rasala et al., 2008). At this stage, Ndc1 and Pom121 are recruited to the assembly site to establish contact with the reforming NE. Subsequently, the Nup93-complex assembles at the prepore, which is followed by the Nup62 complex and several (other) FG-Nups (Sachdev et al., 2012). The last components to join the reassembled NPCs are the parts of the nuclear basket and the assembly of the RNA export platform (cytoplasmic filaments) (Figure 6a) (Bodoor et al., 1999; Dultz et al., 2008).

It was earlier noted, that in dividing cells NPCs might be renewed during reassembly after each division (D’Angelo and Hetzer, 2008; D’Angelo et al., 2009). Rat liver cells were characterized by decreased levels of several Nups suggesting an overall reduction NPCs. One explanation for the reduced number of NPCs in the liver is, that the postmitotic assembly of NPCs might be an opportunity for cells to clear up substoichiometric protein complexes, in line with this interpretation is also the low IQR of the aged rat liver sample (Figure 4). Such an opportunity to does not apply to aging in baker’s yeast, where the NE and NPCs remains intact during the entire division. Indeed, there is evidence in baker’s yeast, that replicative aged cells have problems to correctly assemble NPCs, and potentially also to clear misassembled NPCs from the NE (Box 3) (Rempel et al., 2018).

3.4 Mechanisms of NPC maintenance

Nups have vastly different turnover times. Among the group of particularly long lived Nups are Nup93, Nup96, Nup107, and Nup205, while Nup133 has intermediate turnover times, and Pom121, as well as FG-Nups are replaced regularly (Savas et al., 2012; Toyama et al., 2013, 2019). The first insights into NPC maintenance in post mitotic cells were only published recently (Toyama et al., 2019). This study revealed two different mechanisms of NPC maintenance. Quiescent muscle cells maintain their NPCs by the removal of whole NPCs from the NE in an ESCRT dependent manner, which are subsequently replaced by newly assembled NPCs. Terminally differentiated muscle cells maintain
their NPCs by piecemeal replacement of subunits, resulting in NPC composed of Nups with different ages (Toyama et al., 2019).

In addition to these ways of maintaining a functional cohort of NPCs, we speculate that the same mechanisms that guard NPC assembly and target NPCs for proteosomal degradation (Webster et al., 2014, 2016), could also be used to identify damaged NPCs. Alternatively, whole damaged NPCs might be able to be cleared through autophagy, specifically through microautophagy at nuclear vacuolar junctions or by selective autophagy. The possibility that damaged NPCs are not cleared from the NE at all in some cell types, cannot be ruled out either. The fact that at least two NPC maintenance mechanisms exist, combined with the possibility that different cells might favor different mechanisms of NPC maintenance might contribute to the age-related differences in Nup abundance across different model organisms and/or organ tissues. Other outstanding questions remain on how the cell decides which NPCs/NPC subunits need replacement, and what causes NPCs to become damaged in time.

3.5 What could be sources of damage to NPCs in aging?
The most frequent source of protein damage considered in aging is oxidative damage (Berlett and Stadtman, 1997). Replicative and chronologically aging cells of diverse origins have high levels of reactive oxygen species (ROS) and show signs of oxidative stress (Jakubowski et al., 2000; Laun et al., 2004). Also levels of carbonylated proteins, which are caused by ROS, are increased in those aged cells (Reviewed by Levine, 2002). In yeast, carbonylated proteins are retained by the mother cell during division (Aguilaniu et al., 2003) contributing to her aging.

Oxidation of the amino acid side chains of lysine, arginine, proline and threonine, causes the side chains to be replaced by carbonyl groups. Carbonylation of those amino acids is irreversible and consequently carbonylated proteins are altered in their charge and hydrophobicity impacting their synthesis, stability and functionality (Stadtman and Levine, 2003). On the other hand, ROS are also important intracellular signaling molecules that can trigger protective responses (Landry and Cotter, 2014; Møller and Sweetlove, 2010; Morgan and Liu, 2011). The main source of intracellular ROS stems from mitochondrial respiration (Morgan and Liu, 2011). Other sources of intracellular ROS include NAD(P)H oxidases at the plasma membrane, peroxisomes, D-amino acid oxidases in the cytoplasm, and disulfide bond formation at the ER (Morgan and Liu, 2011; Tyo et al., 2012). In yeast the NADH oxidase orthologue Yno1 additionally contributes to ROS formation at
the ER/NE network (Rinnerthaler et al., 2012) and may be closest to the NPCs. So, while it is clear that ROS and carbonylation contribute to age dependent changes of cellular systems, should we expect the NPC to be vulnerable to oxidative damage and if so, how would this affect nucleocytoplasmic transport?

D’Angelo et al., show in their 2009 paper, that carbonyl groups can be detected on Nup93 and Nup153, but not on Nup107 isolated from old rat brains. The proteins that form the scaffold of the NPC might thus be somewhat protected against oxidative damage. Especially carbonylation of the long-lived linker Nup, Nup93, might reduce the structural integrity of the NPC and cause NPCs to become more leaky with aging (Bley et al., 2015; D’Angelo et al., 2009; Fischer et al., 2015; Kim et al., 2018; Savas et al., 2012; Toyama et al., 2013). A direct effect of carbonyl modification of the FG-Nups is less likely, as a recent study failed to carbonyl-modify Nsp1 under conditions where other ID proteins do, such as when culturing cells in strong oxidative conditions (Rempel et al., 2018). Moreover, based on coarse-grained molecular dynamics simulations (Ghavami et al., 2014), carbonyl modified FG-Nups show little conformational changes compared to FG-Nups without carbonyl modifications, suggesting that protein carbonylation of FG-Nups has only a minor impact on the permeability of the NPC (Rempel et al., 2018). Apart from the impact that oxidative stress may have on the structure of the NPC there are effects on the Ran-GDP/GTP gradient in the cell (Chatterjee and Paschal, 2015) that cause nucleocytoplasmic transport rates to decrease under oxidative stress. Altogether, oxidative stress impacts nuclear transport (Chatterjee and Paschal, 2015) and carbonylation of Nup93 might reduce the structural integrity of NPCs in aged cells, but direct carbonylation of FG-Nups is is not likely to play a role in aging.

Instead we suggest that the unique interior of the NPC, with ultra-high concentrations of the disordered FG-Nups should be considered in the context of aging. The FG-Nups may be at risk for aggregation in aging as intrinsically disordered proteins, including FG-Nups, are known to be aggregation prone (Dölker et al., 2010; Milles et al., 2013) and protein aggregation in general increases during aging (David et al., 2010; Lindner et al., 2008). Intrinsically disordered proteins (IDPs), like the FG-Nups, do not have a stable secondary or tertiary structure. Instead they exist in a large set of readily interchangeable conformations. While we know well how cells guard the structure of stably folded proteins (Balchin et al., 2016; Kampinga and Bergink, 2016; van Oosten-Hawle and Morimoto, 2014; Rousseau and Bertolotti, 2018; Sontag et al., 2017), we know virtually nothing about the mechanisms that guard IDPs. IDPs,
including IDPs related to degenerative diseases such as Huntington's disease amyloid lateral sclerosis (ALS) and FG-Nups, can phase separate to form liquid-liquid demixed droplets (Lemke, 2016) or hydrogels (Frey and Görlich, 2007; Frey et al., 2006; Labokha et al., 2012) or aggregate to form amyloid fibres (Ader et al., 2010; Halfmann et al., 2012; Lee et al., 2016; Milles et al., 2013; Peskett et al., 2018; Schmidt and Görlich, 2015; Yamada et al., 2010; Zhang et al., 2015).

During transport, NTRs modulate the biophysical state of the disordered FG-Nups inside the NPC by engaging in rapid binding and unbinding events (Aramburu and Lemke, 2017; Hayama et al., 2018; Lim et al., 2015; Milles et al., 2015; Tetenbaum-Novatt et al., 2012). Recent data suggests that NTRs can also modulate the biophysical state and toxicity of several IDPs related to neurodegenerative diseases (Boeynaems et al., 2016; Freibaum et al., 2015; Grima et al., 2017; Jovičić et al., 2015; Loureiro et al., 2016; Schmidt and Görlich, 2015; Zhang et al., 2016). Moreover, several repeat-proteins that are associated with neurodegenerative diseases are known to disrupt nucleocytoplasmic transport (Chiu et al., 2015; Eftekharzadeh et al., 2018; Freibaum et al., 2015; Jovičić et al., 2015; Woerner et al., 2016; Zhang et al., 2016) (Reviewed by Kim and Taylor, 2017). Altogether these recent findings suggest that the FG-Nups may be at risk in aging if NTR levels become limiting, or if cells have a larger load of aggregation prone proteins that may sequester NPC components into aberrant phase separated states or aggregates. A full understanding of the stability of the disordered phase in normal aging is not available at present but considering the tight connection with aggregation pathologies and NPCs, we consider this a valuable research area for the future.

### Box 3: NPCs in replicative aging – a yeast perspective

Most of the available information on NPCs in aging stem from studies performed in replicatively aged S. cerevisiae. Here, several studies could show that NPC components are present in substoichiometric amounts in replicative aged cells. More specifically, a strong decrease in abundance (at the whole cell level and at the NE) was observed for the FG-Nups Nup116, Nsp1 and nuclear basket protein Nup2 (Janssens et al., 2015; Lord et al., 2015; Rempel et al., 2018) (Figure 7a). On the single cell level, the age dependent decrease in abundance of Nup116 and Nup100 at the NE was correlated to the lifespan of the cell, where decreased levels correlated with less remaining lifespan (Rempel et al., 2018). Interestingly, the deletion of Nup100 was previously described to extend the replicative lifespan, through an increase in cellular Gcn4 levels (Lord et al., 2015; Lord et al., 2017). This data demonstrates that
the full deletion of a gene in young cells can have a very different outcome than the age dependent decrease in abundance in old cells. Taken together, the changes in NPC stoichiometry during replicative aging are well documented and changes are consistent between different studies.

Studies of the NPC assembly and quality control machinery show that the aging cells lose several components, which normally ensure that NPCs are assembled correctly, or broken down, if this is not the case. Namely, Apq12, Brl1, Heh2 and Vps4 show decreased abundance in aging. How these proteins influence NPC assembly is still not fully understood (Figure 7b). Previous studies suggested, that Apq12, Brl1 and Brr6 are primarily involved in lipid homeostasis (Hodge et al., 2010; Lone et al., 2015) and suggested that changes in lipid composition could cause NPC assembly defects. A more recent study suggested a more direct involvement in NPC assembly (Zhang et al., 2018). The decrease of those proteins strongly suggests, that NPCs in old cells misassemble more frequently and are less effectively cleared from the NE (Figure 7c). In aged cells Chm7 foci, representing NPC assembly problems, appear almost three times as frequently as young cells. Misassembled NPCs and those that have extrachromosomal r-DNA circles (ERCs) tethered to their scaffold are asymmetrically retained by the mother cell (Denoth-Lippuner et al., 2014; Shcheprova et al., 2008; Webster et al., 2014). Apq12, Brl1 and Brr6 are conserved proteins in eukaryotes with closed mitosis. Although these proteins are not conserved on the sequence level in higher eukaryotes, it has been speculated that functional homologues do exist (Laudermilch et al., 2016; Thaller and Lusk, 2018).

It is unclear, whether the altered nucleoporin levels in replicative aged cells are a cause or a consequence of misassembled NPCs, or even of problems with NPC maintenance. The average nucleoporin abundance at the NE represents fully assembled functional NPCs, as well as potentially damaged NPCs and/or misassembled NPCs with altered nucleoporin composition. The loss of the FG-Nups Nsp1, Nup116 and Nup2, may thus reflect that there is a subset of misassembled NPCs that partially or fully lack those FG-Nups. These NPCs likely do not contribute to transport, as NPCs lacking these specific Nups would increase the passive permeability of the NE, and no such change is observed in aging (Rempel et al., 2018). Instead, misassembled NPCs, can be covered by membranes making them transport incompetent. The NE at those NPCs that are covered by membrane is often herniated. Indeed herniations have been observed in specific mutants, eg. mutants lacking Nup116 (Wente and Blobel, 1993), and are enriched in aged cells (unpublished data).

The changes in the populations of NPCs at the NE has functional consequences for the cell that are not fully understood. We report that they observe increased nuclear compartmentalization of GFP-NLS and GFP-NES
reporter proteins, as well as increase in nuclear localization of Rcc1-GFP. Consistently, the shuttling transcription factor Msn2 shows decreased shuttling dynamic during aging and the decrease in shuttling is correlated to lifespan. Lord et al., reports loss of nuclear localization of GFP based reporters with different NLSs in aging. Apart from technical differences (different yeast strains and methods of aging) we speculate that the results may also reflect different stages of the aging process. E.g., a moderate reduction of NPCs early in life would cause an increased nuclear compartmentalization, as this may primarily decrease passive diffusion of the reporter proteins over the NE. Only late in life the number of NPCs may become rate limiting for active transport. Several studies indicate that changes in the permeability barrier and the steady state localization of proteins are quite well tolerated by the cell (Lord et al., 2015; Strawn et al., 2004). In contrast, even moderate changes in nuclear transport dynamics correlated to remaining lifespan suggesting that changes in dynamics are detrimental for the cell as it interferes with their ability to react to its constantly changing environment (Rempel et al., 2018).

Figure 7 Model of age-related changes at the NPC during yeast replicative aging
a) Summary of the measured changes found in (Janssens et al., 2015; Lord et al., 2015; Rempel et al., 2018).
b) Schematic representation of NPC assembly and nuclear transport dynamics in young cells.
c) Model: In old cells, the decrease in abundance of several proteins that assist in NPC assembly (indicated by faded cartoons) causes the accumulation of misassembled NPCs in aged mother cells. Misassembled NPCs are covered with membrane and do not participate in nucleocytoplasmic exchange. In other words, the overall number of transport competent NPCs is reduced in aged mother cells.

4. Outlook
In this review, we have shown that age related changes at the NPC and nucleocytoplasmic transport are diverse in different tissues and model organisms and we have tried to find potential reasons why the conserved aging
process might be so diverse at the level of the NPC. We explain how NPC assembly might be challenging for replicative aging cells and that NPC maintenance might be challenging for chronologically aging cells. In aging tissues a mix of both will be observed depending on the regenerative capacity of the tissue. Additionally to this, there are two different NPC assembly mechanisms, and at least two different mechanisms of NPC maintenance, adding another explanation, why age related changes at the NPC might be diverse in different cells. NPC interphase assembly and NPC maintenance mechanisms are currently least understood. In this context, the ESCRT system is particularly interesting to study, because it is involved in NPC assembly (Webster et al., 2014) and NPC maintenance (Toyama et al., 2019). Age dependent changes in ESCRT function could therefore impact NPC assembly in aging dividing cells and NPC maintenance in chronological aging cells. At least in replicative aging, ESCRT function is likely to be compromised by the drastically reduced abundance of Vps4 and reduced levels of Heh2 at the NE (Rempel et al., 2018), but virtually nothing is known about ESCRT function during aging in other replicative aging cells.

Nucleocytoplasmic transport is influenced by the structure and numbers of NPCs, but also by the abundance of NTRs and the RanGDP/GTP gradient. We consider it likely, that changes in nucleocytoplasmic transport or passive diffusion during aging will cause changes in the localization of proteins during aging. The overall changes in nucleocytoplasmic transport might be moderate, because a full complement of nonfunctional NPCs is not compatible with life. More research is needed in order to understand how the nucleocytoplasmic transport network operates and how changes in protein localization influence the aging process. It remains to be established if a widespread mislocalization of nuclear proteins could be causal to the loss of protein homeostasis observed in many aggregation pathologies.

The cell also uses NPCs as an anchoring point for various processes. Outside of the nucleus, NPCs are connected to the cytoskeleton and the protein synthesis machinery (Reviewed by Goldberg, 2017) and inside of the nucleus, proteasomes and ERCs tether to the NPC (Albert et al., 2017b; Denoth-Lippuner et al., 2014). NPCs are also used as sites of gene activation (Casolari et al., 2004; Dieppois et al., 2006; Taddei et al., 2006)(Reviewed by Towbin et al., 2009), anchoring or eroded telomeres and DNA double strand breaks are repaired at the site of NPCs (Khadaroo et al., 2009; Lemaître et al., 2012; Nagai et al., 2008). Consequently, NPCs play an important role in genome stability.
and genome organization (Sood and Brickner, 2014). Changes in NPC architecture or NPC numbers, might change the availability of NPCs as anchoring points for these processes. We conclude, that nuclear pores, the physical gatekeepers to the nuclear interior may well represent an important gatekeeper in aging, and boosting its quality control may provide opportunities to increase resilience to aging and age-related diseases.