Cell

Age-Dependent Deterioration of Nuclear Pore Complexes Causes a Loss of Nuclear Integrity in Postmitotic Cells

Maximiliano A. D'Angelo,[1] Marcela Raices,[1] Siler H. Panowski,[1] and Martin W. Hetzer[1,*]
[1]Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
*Correspondence: hetzer@salk.edu
DOI 10.1016/j.cell.2008.11.037

SUMMARY

In dividing cells, nuclear pore complexes (NPCs) disassemble during mitosis and reassemble into the newly forming nuclei. However, the fate of nuclear pores in postmitotic cells is unknown. Here, we show that NPCs, unlike other nuclear structures, do not turn over in differentiated cells. While a subset of NPC components, like Nup153 and Nup50, are continuously exchanged, scaffold nucleoporins, like the Nup107/160 complex, are extremely long-lived and remain incorporated in the nuclear membrane during the entire cellular life span. Besides the lack of nucleoporin expression and NPC turnover, we discovered an age-related deterioration of NPCs, leading to an increase in nuclear permeability and the leaking of cytoplasmic proteins into the nucleus. Our finding that nuclear "leakiness" is dramatically accelerated during aging and that a subset of nucleoporins is oxidatively damaged in old cells suggests that the accumulation of damage at the NPC might be a crucial aging event.

INTRODUCTION

Nuclear pore complexes (NPCs) are aqueous channels formed by multiple copies of ~30 different proteins known as nucleoporins. Pores have an 8-fold symmetrical structure consisting of a nuclear envelope (NE)-embedded scaffold, which surrounds the central channel through which all nucleocytoplasmic transport occurs, and a cytoplasmic and nuclear ring to which eight filaments are attached (Figure 1A). Whereas the cytoplasmic filaments have one loose end, the nuclear filaments are attached to a distal ring forming a structure known as a nuclear basket. NPCs span the double-lipid bilayer of the NE at sites where the inner and outer nuclear membranes are fused (Alber et al., 2007; Beck et al., 2004; Kiseleva et al., 2004; Reichelt et al., 1990). This unique membrane topology requires scaffold nucleoporins such as the Nup107/160 complex to stabilize the two fused membrane leaflets (Harel et al., 2003; Walther et al., 2003). To accommodate the selective transport of cargo across the NE, additional nucleoporins are attached to the membrane-embedded scaffold (Rabut et al., 2004a). Most of the peripheral nucleoporins, such as Nup153, contain FG-repeats, interact with nuclear transport receptors, and provide a selective barrier for the diffusion of molecules larger than ~60 kDa (Rabut et al., 2004a; Weis, 2003).

In proliferating cells, the formation of new pores occurs during mitosis and interphase (D'Angelo et al., 2006; Maul et al., 1972; Rabut et al., 2004b) and requires the expression of the Nup107/160 complex members (Sec13, Seh1, Nup37, Nup43, Nup75, Nup96, Nup107, Nup133, and Nup160) (Harel et al., 2003; Walther et al., 2003), suggesting a general role for scaffold nucleoporins in establishing and maintaining the NPC structure. Whereas most peripheral nucleoporins are constantly exchanged at the NPC, the pore scaffold is stable during interphase and only disassembles during the M phase of dividing cells (Daigle et al., 2001; Rabut et al., 2004b). This raises the question of how the structural and functional integrity of NPCs is maintained throughout the life span of nondividing cells in which this mitotic renewal cycle is absent.

By using *C. elegans* and a mammalian differentiation system, we found that the expression of the NPC scaffold members is strongly downregulated when the cells exit the cell cycle. Furthermore, we observed that the scaffold nucleoporins are extremely stable and do not exchange once they are incorporated into the NE, persisting for the entire life span of a differentiated cell. We discovered that, in postmitotic cells, NPCs deteriorate with time, losing nucleoporins responsible for maintaining the pore diffusion barrier. Strikingly, we found that nuclei of old rat neurons containing deteriorated NPCs show an increased nuclear permeability and the intranuclear accumulation of cytoplasmic tubulin. The findings that oxidative stress accelerates the age-related "leakiness" of pores and that the proteins that are lost from NPCs can be found carbonylated in old cells, a result of oxidative protein damage, suggest that the deterioration of nuclear selectivity is a consequence of accumulated damage in old NPCs.

RESULTS

Life-Long Stability of *C. elegans* Scaffold Nucleoporins

As a first approach to characterize how NPCs are maintained in differentiated cells, we decided to analyze whether there were

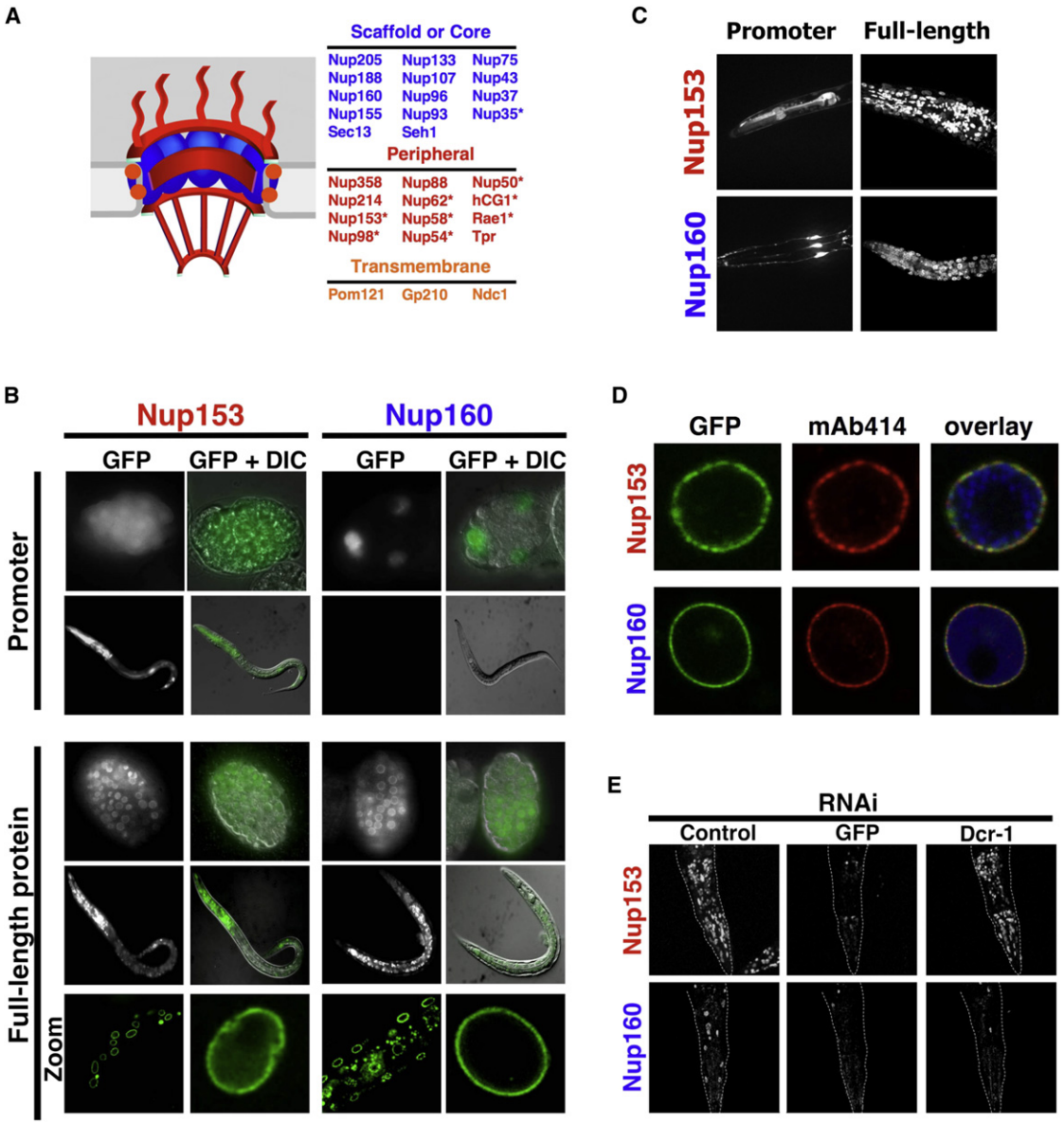

Figure 1. ceNup160 Scaffold Nucleoporin Shows Life-Long Stability

(A) Scheme of the NPC structure and composition. Asterisks denote dynamic nucleoporins.

(B) *C. elegans* worms expressing either GFP under the control of either the *ceNup153* promoter or the *ceNup160* promoter (Promoter) or ceNup153-GFP or ceNup160-GFP under their endogenous promoters (full-length protein) were analyzed by fluorescence microscopy. The GFP signal was merged with differential interference contrast images (DIC). Correct localization of ceNup153-GFP and ceNup160-GFP fusion proteins to the NE was analyzed by confocal microscopy (Zoom).

(C) The activity of the nucleoporin promoters and the localization of full-length proteins in the head of adult worms were analyzed by confocal microscopy. Image shows the maximal projection of 30 Z stacks.

(D) NPC insertion of ceNup160-GFP and ceNup153-GFP (green) was confirmed by colocalization with the NPC antibody mAb414 (red). Chromatin is shown in blue.

(E) ceNup153-GFP- and ceNup160-GFP-expressing worms were subjected to *GFP* RNAi until no fluorescent signal was detected. RNAi against *C. elegans dicer* (*DCR-1*) was used to release adult worms from the *GFP* RNAi. Adults were fed *DCR-1* RNAi for 6 days before the GFP signal was analyzed. Dashed lines outline worms' heads.

differences in the expression of scaffold nucleoporins between dividing and postmitotic cells. We reasoned that, if new pores are assembled in nondividing cells, scaffold nucleoporins that are essential for NPC formation into the NE, such as the Nup107/160 complex (D'Angelo et al., 2006), should be expressed. In contrast, if pore assembly is restricted to dividing

cells, the expression of scaffold nucleoporins could be repressed as soon as cells exit the cell cycle.

To distinguish between these two scenarios, we analyzed nucleoporin expression levels during the development of *Caenorhabditis elegans*, a well-characterized nematode from which different developmental stages can be separated manually. Importantly for this study, embryos and larvae contain dividing cells, whereas somatic cells of adult worms are entirely postmitotic. We generated stable worm lines expressing GFP under the promoter of a peripheral nucleoporin, ceNup153, or under the promoter of a scaffold nucleoporin, ceNup160. Driven by the ceNup153 promoter, GFP was expressed in most if not all cells of embryos and adult worms (Figures 1B and S1A available online). In contrast, the ceNup160 promoter-driven expression of GFP was restricted to the embryo and, except for a few neurons in the head (Figure 1C), was not detectable in adult worms (Figure 1B). Strikingly, even in embryos, the ceNup160 promoter was only active in a few cells that likely represent dividing cells, as evidenced by the coexpression with cyclin B (Figures S1B and S1C). It is important to note that the lack of promoter activity for both nucleoporins in the dividing germline is likely due to transgene silencing associated with this technique and not to the lack of promoter activity in the cells. These experiments indicate that expression of the ceNup160 scaffold nucleoporin is tightly regulated during development and absent in postmitotic cells.

The lack of ceNup160 expression in adult worms raised the question of whether the protein was present in the NPCs of adult cells. To test this, we generated stable worm lines expressing full-length ceNup160 or ceNup153 fused to GFP, again under their own promoters, and found that both nucleoporins were present in all embryonic, larval, and adult nuclei (Figure 1B). The correct incorporation of the GFP-tagged nucleoporins into *C. elegans* NPCs was verified by colocalization with endogenous nucleoporins (Figure 1D).

To directly confirm that Nup160 was not expressed in postmitotic cells, we fed worms that stably expressed ceNup160-GFP or ceNup153-GFP with bacteria expressing GFP RNAi for several generations until we obtained transgenic adult worms with no detectable GFP signal. When RNAi-mediated gene knockdown of ceNup160-GFP and ceNup153-GFP was reversed in these adult worms by RNAi against *C. elegans dicer* (*DCR-1*), an essential component of the RNAi machinery (Ketting et al., 2001), only ceNup153-GFP expression was restored (Figure 1E), further supporting the idea that ceNup160 is not expressed in postmitotic cells. Taken together, these results suggest that ceNup160 protein found in adult worms originates from embryonic expression and is extremely stable.

To analyze whether other scaffold nucleoporins showed a similar behavior, we obtained worm lines expressing tomato under ceNup133 promoter or ceNup133-tomato under its own promoter. Similar to our findings for ceNup160, the promoter of ceNup133 was restricted to the pharynx cells and a few neurons in the postmitotic adult worms, whereas the ceNup133 protein was present in every nucleus (Figure S1D). These results indicate that the life-long stability is a common feature of scaffold nucleoporins.

Expression of Scaffold Nucleoporins Is Strongly Downregulated during *C. elegans* Development

The dramatic difference in the transcriptional regulation of ceNup153 and the scaffold components ceNup160 and ceNup133 in differentiated cells prompted us to analyze the mRNA levels of other nucleoporins. Because wild-type worms contain a large number of dividing cells in their gonads, we used *C. elegans glp-4* mutant animals (strain SS104), which have normal morphology and brood size at 15°C but lack gonads and are unable to produce germ cells at 25°C (Beanan and Strome, 1992) (Figure 2A). Consistent with this phenotype, adult *glp-4* mutant animals were essentially free of dividing cells as indicated by the absence of cyclin B mRNA (Figure S2A). Whereas the mRNA levels of all tested nucleoporins and lamin were lower in adult worms compared to embryos, only the scaffold nucleoporins, such as ceNup107/160 complex, ceNup205, and ceNup155, were reduced to similar levels as cyclin B (Figure 2B). An exception was the ceNup107/160 complex member ceSec13, which remained highly expressed in adults, consistent with its additional function in COPII-mediated vesicular transport (Salama et al., 1993). Nucleoporin downregulation was dependent on cell-cycle exit since adult worms containing gonads with dividing cells exhibited high levels of nucleoporin expression (Figures 2A and 2C). These results suggest that the expression of scaffold nucleoporins, in contrast to peripheral NPC components, is either strongly downregulated or absent in nondividing cells.

Expression of Scaffold Nucleoporins Is Not Required during Adulthood

Despite the low amounts of scaffold nucleoporin mRNAs in postmitotic cells and the absence of detectable GFP expression from the ceNup160 promoter, it was still possible that low levels of nucleoporin production were sufficient to maintain nuclear integrity in postmitotic cells. To directly test this, we knocked down nucleoporin mRNAs by RNAi in N2 (wild-type) and *daf-2(e1370)* mutant worms, the latter having extended life spans due to a mutation in the insulin/IGF-1 receptor (Kenyon et al., 1993; Kimura et al., 1997), and analyzed their life spans. The mRNA levels of all tested knockdowns were further reduced by > 60%–90% (Figure S2B). If low levels of scaffold nucleoporins were required for nuclear function, we expected the knockdown to either reduce the life spans of adult worms and/or their nucleoporin protein levels. Consistent with their essential role in pore assembly in dividing cells, RNAi against most of the nucleoporins tested were embryonic lethal (Table S1). However, when the RNAi was performed in postmitotic adult worms, only the knockdown of dynamic nucleoporins significantly reduced adult life span, whereas none of the scaffold nucleoporins had a measurable effect, even when depleted in long-lived worms (Figures 3A and S2C). Similar to previous results (Haithcock et al., 2005), knockdown of the single lamin gene was lethal to adult worms with lethality rates comparable to that of ceNup153 (Figure 3A). Notably, the protein levels of the scaffold ceNup107 and ceNup160 nucleoporins in postmitotic worms remained high during the persistent depletion of the corresponding mRNAs by RNAi (Figures 3B and S2D). In contrast, the protein levels of the dynamic ceNup153 and ceNup35 decreased when their

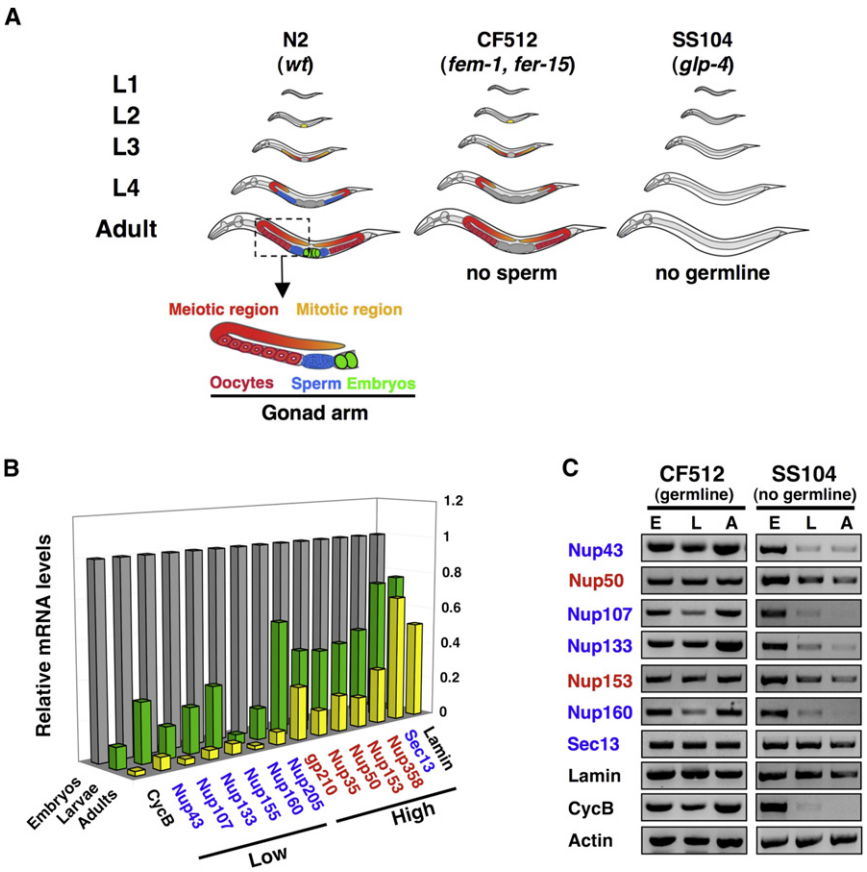

Figure 2. Expression of Scaffold Nucleoporins Is Downregulated during *C. elegans* Development

(A) Scheme of *C. elegans* strains used in this work (based on Crittenden et al., 2006). N2 is wild-type strain, CF512 (*fem-1*, *fer-15*) has thermosensitive mutations that inhibit male germline production at 25°C, and SS104 (*glp-4*) has a mutation that inhibits male and female germline production at 25°C. Thus, at the restrictive temperature, adult worm mutants are entirely postmitotic.

(B) Nucleoporin expression levels were analyzed in embryo, larva, and adult developmental stages of *C. elegans* (SS104 strain) by RT-PCR (n = 4) and normalized to embryonic levels. Cyclin B (CycB) was used as a marker for cell-cycle exit. Standard deviation is below 20%.

(C) Nucleoporin expression levels in embryos ("E"), larvae ("L"), and adult ("A") worms of *C. elegans* sterile strains CF5120 and SS104 were analyzed by RT-PCR (n = 4).

mRNAs were knocked down. When the RNAi was performed in reproductive worms containing the dividing cells of the germline, the total levels of all nucleoporins tested decreased (Figure 3C), consistent with the fact that new NPCs are constantly being assembled in proliferating cells. Taken together, these results suggest that the expression of scaffold nucleoporins is not required in the postmitotic stage, even in long-lived animals.

From the experiments described above, we conclude that (1) pore assembly is tightly regulated at the transcriptional level, (2) NPC assembly ceases upon cell-cycle exit, and (3) the NPC scaffold can last the entire life span of a metazoan organism.

Downregulation of Scaffold Nucleoporin Expression Is Conserved in Mammals

To test whether the transcriptional repression and the life-long stability of the NPC scaffold structure could also be observed in mammalian cells, we first compared mRNA levels of nucleoporins in dividing C2C12 mouse myoblasts and terminally differentiated myotubes (Figure S3A) by using Q-PCR. Consistent with our findings in *C. elegans*, all nucleoporins tested were highly expressed in dividing myoblasts. However, only the scaffold nucleoporins were strongly downregulated in postmitotic myotubes (Figure 4A).

In the C2C12 differentiation system, ~30% of the cells do not differentiate into myotubes and remain as quiescent cells that maintain the capability of re-entering the cell cycle when exposed to growth-factors-rich media. To investigate whether the downregulation of nucleoporin expression occurs during differentiation or when the cells exit the cell cycle, we separated differentiated myotubes from nondividing quiescent cells and analyzed the mRNA levels of different nucleoporins in comparison with dividing myoblasts. We observed that the shutdown of scaffold nucleoporin expression also occurred in quiescent cells and that releasing the cells into the cell cycle rapidly reactivated nucleoporin expression (Figure 4B). Our results indicate that the transcriptional downregulation of nucleoporins is conserved from worms to mammals and support the idea that, due to the lack of expression of essential scaffold nucleoporins, new pore formation ceases upon cell-cycle exit.

Despite the reduction of core nucleoporin expression, the nucleoporin protein levels and the overall number of NPCs (Figures 4C and 4D) remained high during differentiation, which can only be explained by life-long stability of these proteins.

Scaffold Nucleoporins Are Not Exchanged Once Inserted into the NE

One prediction from the life-long stability of scaffold nucleoporins is that these proteins would not be exchanged once they are incorporated into the NE. This hypothesis could not be experimentally tested by using fluorescence recovery after photobleaching (FRAP) measurements of GFP-tagged nucleoporins because the lifetime of nucleoporins exceeded the stability of GFP (we noticed a sharp decline in fluorescence intensity of ceNup160-GFP in adult worms, although the protein remained stable; data not shown). As an alternative approach, we mixed myoblasts expressing GFP-Nup107, a scaffold nucleoporin (Krull et al., 2004; Walther et al., 2003); Pom121-GFP, a structural transmembrane nucleoporin (Antonin et al., 2005); or Lamin B1-GFP, a component of the nuclear lamina (D'Angelo and

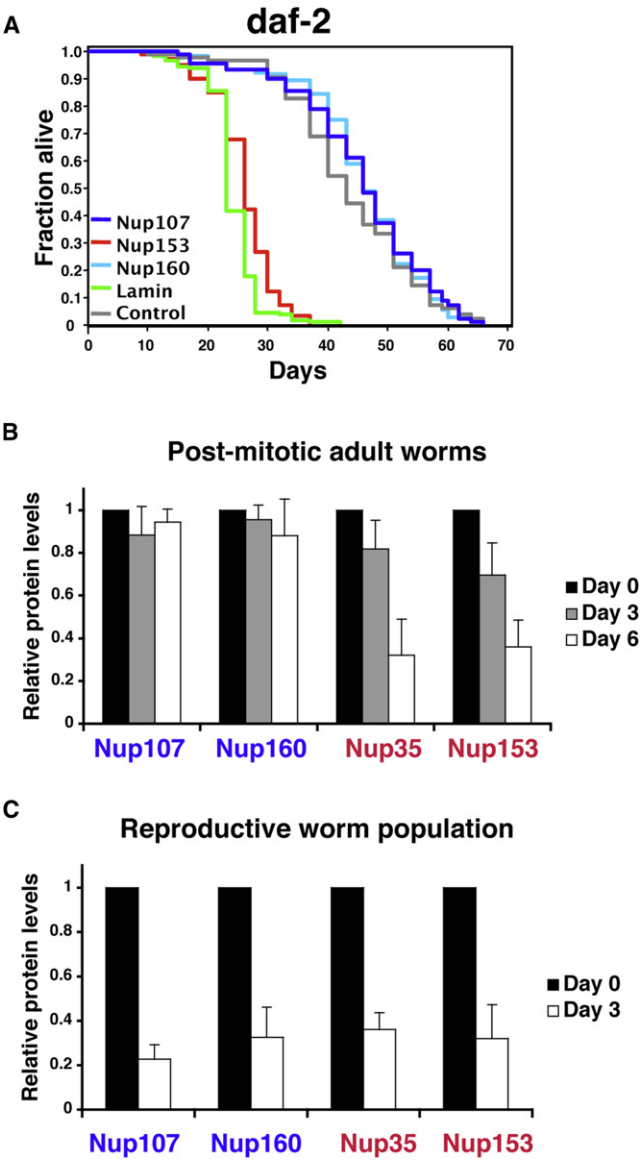

Figure 3. Expression of Scaffold Nucleoporins Is Not Required during Adulthood

(A) *daf-2(e1370)* mutant worms on day 1 of adulthood were fed bacteria expressing empty vector control (gray line), *ceNup153* RNAi (red line), *ceNup160* RNAi (light blue line), *ceNup107* RNAi (blue line), or *ceLamin* RNAi (green line). Life span was assessed as described (Raices et al., 2005). Statistical analyses can be found in Table S2.

(B) Total protein was extracted from sterile (25°C) adult SS104 worms subject to the indicated RNAis for 3 or 6 days, and protein levels were assayed by western blot analysis. Values represent average ± SD from three independent experiments.

(C) Total protein was extracted from a reproductive population (15°C) of SS104 worms subjected to the indicated RNAis for 3 days. Protein levels were assayed by western blot. Values represent average ± SD from three independent experiments.

Error bars represent SD.

Hetzer, 2006), under a constitutive cytomegalovirus (CMV) promoter with untransfected myoblasts and induced the fusion/differentiation into multinucleated myotubes by lowering the serum concentration. We then followed the incorporation of the GFP-tagged proteins into the nonfluorescent nuclei derived from untransfected cells (Figure 5A). As expected, we observed that Pom121-GFP, GFP-Nup107, and Lamin B1-GFP efficiently incorporated into the NPCs and lamina of dividing cells (Figure S3B). In postmitotic myotubes, Pom121 equilibrated at pores after ~30 hr (Figures 5B and 5C and Movie S1). In striking contrast, we did not detect any incorporation (i.e., replacement of endogenous Nup107) of GFP-Nup107 even after 2 days of imaging (Figures 5B and 5C and Movie S2). The lack of incorporation was also observed for all other scaffold nucleoporins tested, including Nup93, Nup43, and Seh-1, whereas all dynamic nucleoporins analyzed were replaced (Figure S3B). Supporting the fact that scaffold proteins cannot be incorporated at the NE of postmitotic myotubes, prolonged or high expression of these nucleoporins resulted in their cytoplasmic accumulation and the formation of NE-associated aggregates (Figures 5D and S3B). Interestingly, the incorporation of Lamin B1-GFP, which has been shown to have a residence time at the NE comparable to the Nup107/160 complex (Rabut et al., 2004a), was similar to Pom121-GFP (Figures 5B and 5C and Movie S3), indicating that the lamina turns over, albeit slowly.

The Scaffold Nucleoporin Nup107 Exhibits Extreme Stability

If scaffold nucleoporins are not exchanged once inserted in the NPC, we should expect these proteins to be present in postmitotic cells for long periods of time. To test this, we performed pulse-chase experiments by using isotope labeling of proteins in C2C12 cells. For this experiment, dividing myoblasts were incubated with an S^{35}-methionine/cysteine mix to allow the radioactively labeled scaffold nucleoporins to be incorporated into newly forming NPCs. Cells were then induced to differentiate, and nucleoporins were immunoprecipitated and analyzed at different times after differentiation. Consistent with an extreme stability of the NPC scaffold, we found that Nup107 showed no significant degradation over several weeks. In contrast, dynamic nucleoporins, such as Nup62 and Nup153, and structural proteins like tubulin and lamin A turned over rapidly (Figure 5E). Interestingly, histone H2B also showed high stability in postmitotic cells (Figure 5E), consistent with its reported half-life of 223 days (Commerford et al., 1982). But, unlike Nup107, histone H2B was exchanged from nucleosomes in postmitotic nuclei (Figure S4A). Thus, the observed extreme stability of scaffold nucleoporins is most likely due to the protective environment where these proteins are located, i.e., embedded in the NE and coated with peripheral nucleoporins, and not due to the intrinsic stability of proteins. This notion is supported by the finding that the entire nonameric Nup107/160 complex is rapidly degraded when one of its components is knocked down by RNAi in dividing cells (Walther et al., 2003). Additionally, we found that the cytoplasmic Nup107 protein that is not incorporated into NPCs in postmitotic myotubes is degraded (Figure S4B).

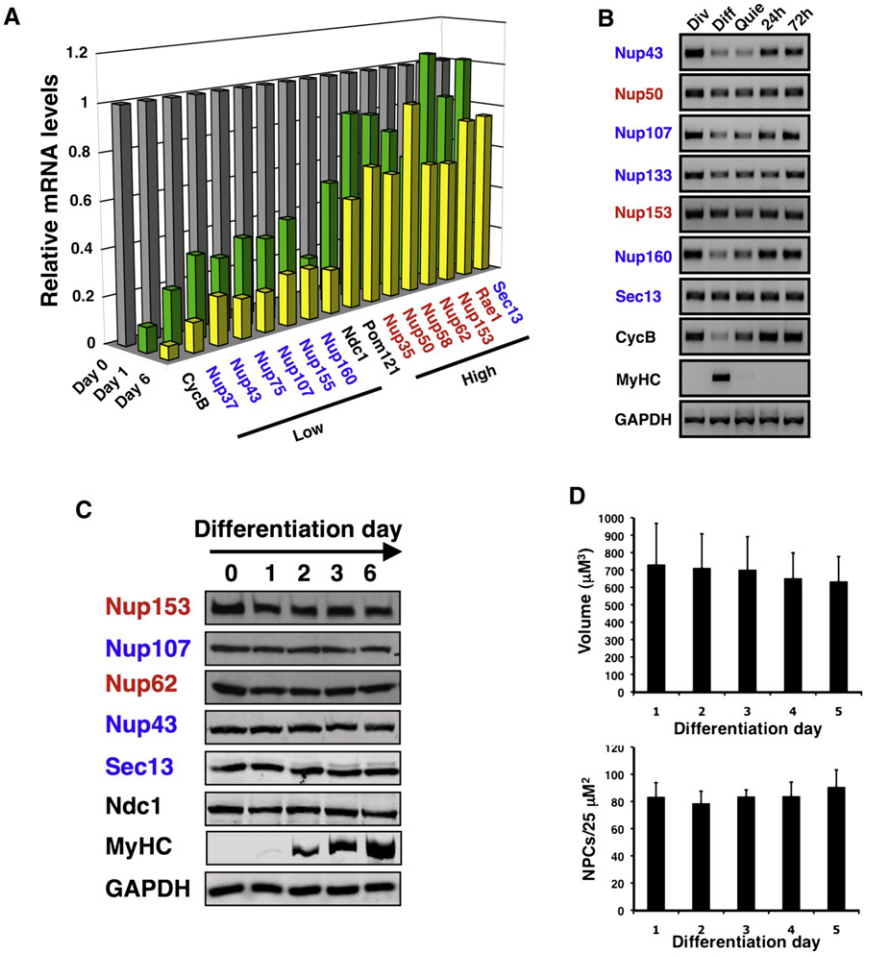

Figure 4. Downregulation of Scaffold Nucleoporin Expression Is Conserved in Mammals

(A) Nucleoporin expression levels in C2C12 myoblasts (day 0) or differentiated myotubes (day 3 and 6) were analyzed by Q-PCR (n = 3) and normalized to the expression of dividing cells. Cyclin B was used as a marker for cell-cycle exit. In all cases, standard deviation is below 20%.

(B) Nucleoporin expression levels in dividing (Div), differentiated (Diff) and quiescent (Quie) C2C12 cells were analyzed by RT-PCR. Cyclin B (CycB) and myosin heavy chain (MyHC) were used as controls for cell-cycle exit and differentiation, respectively.

(C) Nucleoporin protein levels in dividing myoblasts (day 0) or differentiated myotubes (days 1–6) were analyzed by western blotting. Myosin heavy chain (MyHC) was used as a differentiation control.

(D) C2C12 cells expressing 3GFP-NLS were induced to differentiate. Dividing myoblasts (day 0) and differentiated myotubes (days 1–5) were fixed, and NPCs were stained by using anti-Nup153 antibody. Nuclear volume and NPC density were analyzed by confocal microscopy and quantified.

Error bars represent SD.

Together with our findings in *C. elegans*, these results suggest that the NPC scaffold is built to last the lifetime of a cell, and, once embedded in the NE, its subcomponents are not replaced.

Defects in the Permeability Barrier of Old NPCs Lead to a Loss of Nuclear Integrity

Defects in nuclear organization and function have been reported in many old organisms (Haithcock et al., 2005; Scaffidi and Misteli, 2006). Our findings that pores do not turn over in nondividing cells raise the question of whether NPC function is compromised in old cells. Because a central role of the scaffold nucleoporins is to anchor other NPC components that are responsible for the maintenance of the nuclear permeability barrier (Alber et al., 2007; Frosst et al., 2002; Grandi et al., 1997), we decided to test whether the NPCs remain functional as cells age. Potential defects in nuclear membrane integrity and the permeability barrier of NPCs can be directly tested by using fluorescent dextrans with defined molecular weights because molecules larger than 60 kDa are excluded from intact nuclei (Lenart and Ellenberg, 2006) (Figure S5A). If the permeability barrier of NPCs is compromised, a 70 kDa dextran would be able to leak into the nucleus, whereas perturbations of the nuclear membrane are required to allow access of a 500 kDa dextran (Galy et al., 2003) (Figure S5A). It is important to note that dextran influx assays are more sensitive than measuring active transport through the NPC because even a single defective pore can be detected (Lenart et al., 2003). To test for functional alterations in old NPCs, we isolated nuclei from postmitotic adult worms at different ages and analyzed their nuclear permeability. We found that, whereas both dextrans were excluded from the nuclei of young worms, influx of the 70 kDa dextran, but not 500 kDa dextran, was observed in a significant percentage (~30%) of the old worm nuclei (Figure 6A), indicating that the NPC function becomes compromised with age.

To analyze whether these "leaky nuclei" could also be observed in mammals and to further determine whether this is a general phenomenon of aging, we isolated nuclei from nonproliferative regions of young (3 months) and old (28 months) rat brains and analyzed their permeability to dextrans. Similar to our observations in *C. elegans*, the ability to exclude 70 kDa dextran, but not 500 kDa, declined with age (Figure 6B). To confirm that the influx of dextran occurs through defective NPCs, we tested the effect of the wheat germ agglutinin (WGA), a lectin that binds to several glycosylated nucleoporins and can efficiently plug nuclear pores (D'Angelo et al., 2006) on the observed dextran influx. By using biotinylated WGA in combination with streptavidin, we could block the NPCs to an extent where even the diffusion of 10 kD dextran into the nucleus was impeded (Figure S5B). When the old nuclei were treated with WGA under these conditions, we found that the 70 kDa dextran no longer diffused inside

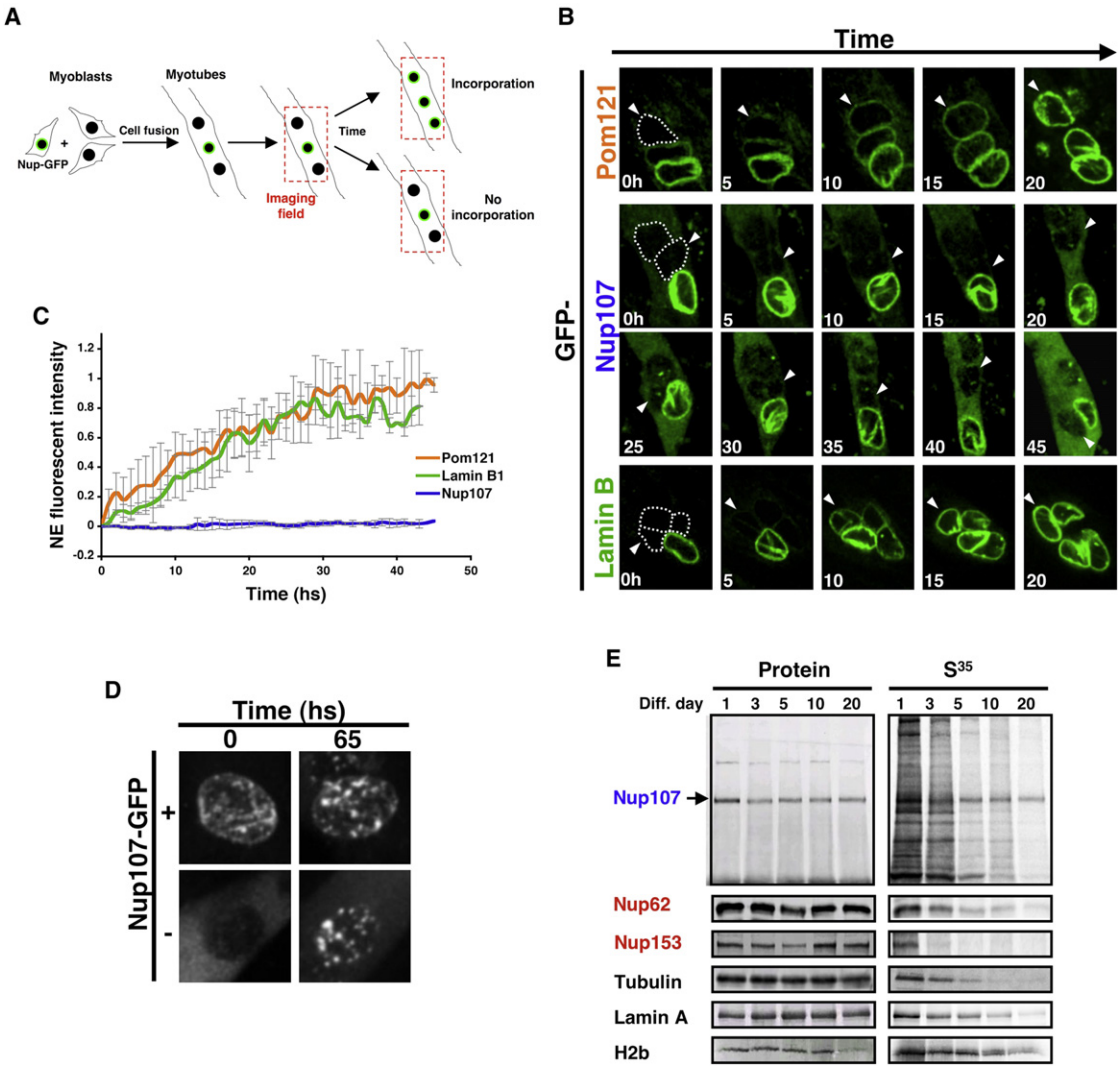

Figure 5. Nup107 Scaffold Nucleoporin Does Not Exchange Once Inserted into NPCs

(A) Scheme of the imaging setup used to analyze the incorporation of GFP-nucleoporins or Lamin B1-GFP fusion proteins into the NE of differentiated myotubes.

(B) Proliferating myoblasts were transfected with Pom121-GFP-, GFP-Nup107-, or Lamin B1-GFP-expressing vectors; diluted with untransfected cells; and induced to differentiate for 3 days. Fields containing GFP-positive and GFP-negative nuclei were selected and imaged using a spinning disk confocal microscope for at least 50 hr. Dotted lines show the position of GFP-negative nuclei at time 0, and arrowheads are used to follow the GFP-negative nuclei during time.

(C) Incorporation of Pom121-GFP, GFP-Nup107, or Lamin B1-GFP at the NE of GFP-negative nuclei was quantified using Image J.

(D) Proliferating myoblasts were transfected with a GFP-Nup107-expressing vector, diluted with untransfected cells, and induced to differentiate for 3 days. Fields containing nuclei that came from transfected cells (+) or untransfected cells (−) were imaged. The formation of Nup107 aggregates after 65 hr of overexpression is shown.

(E) Dividing C2C12 myoblasts were incubated with a S^{35}-Methonine/S^{35}-Cysteine mix for 24 hr (Pulse) and then switched to differentiating media (Chase). Total cell lysates were prepared from the indicated time points. Proteins were immunoprecipitated by using specific antibodies, separated by SDS-PAGE, and transferred to nitrocellulose membranes. The presence of S^{35}-labeled proteins was analyzed using a phosphoimager, and protein levels were determined by western blot.

Error bars represent SD.

of the nucleus (Figure S5C), demonstrating that the nuclear leakiness is a consequence of a defective NPC permeability barrier.

Interestingly, we observed that the time of dextran equilibration between cytoplasm and nucleoplasm varied considerably among nuclei, suggesting that the percentage of defective pores varied between them (data not shown). This fits well with the idea of a gradual loss of the pore permeability function over time. One consequence from such a progressive deterioration of pore function would be the leaking of cytoplasmic proteins into the nucleus, as happens when NPCs are partially dismantled during mitosis (Lenart et al., 2003). Supporting this idea, we found that the rat nuclei that failed to exclude 70 kDa dextran exhibited intranuclear tubulin βIII, a strictly cytoplasmic protein (Figure 6C). In many cases, nuclear tubulin had aggregated into large

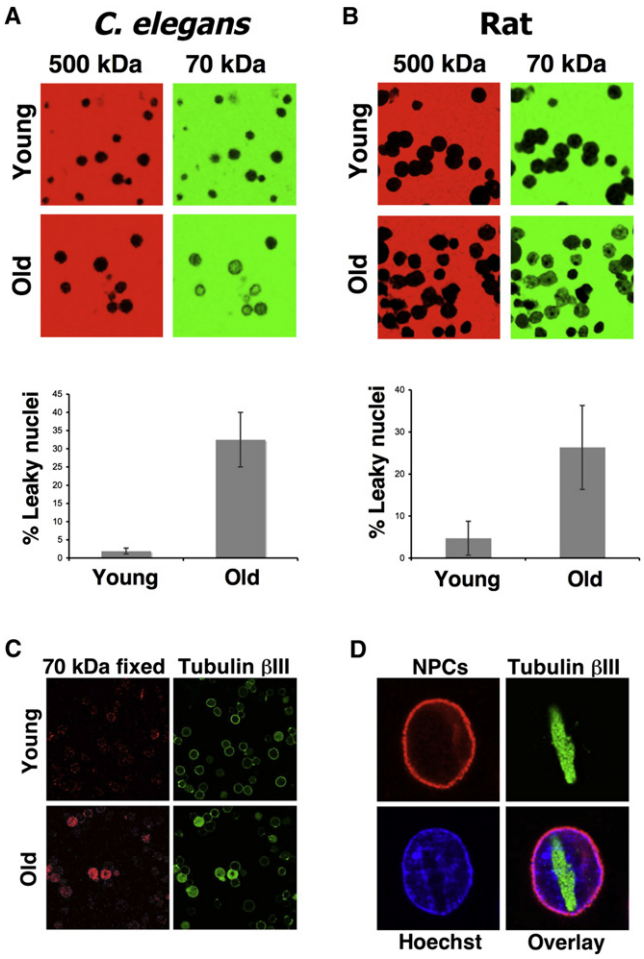

Figure 6. *C. elegans* and Mammals Show an Age-Related Increase of Nuclear Permeability

(A) Nuclei isolated from brains of young (3 months) and old (28 months) rats were incubated with green 70 kDa and red 500 kDa fluorescent dextrans, and nuclear influx was analyzed by confocal microscopy. Images show each dextran in its original color. The percentage of nuclei that showed nuclear influx of the 70 kDa dextran was determined using image processing software.

(B) Nuclear permeability was analyzed in nuclei prepared from *C. elegans* SS104 worms at different times of adulthood as described in (A).

(C) Young and old rat nuclei were incubated with the 70 kDa fluorescent dextran, fixed, and stained with an antibody against tubulin βIII.

(D) Brain nuclei from old rats were fixed and stained with the mAb414 antibody (NPCs) and an antitubulin βIII.

Error bars represent SD.

filamentous structures that caused severe morphological chromatin aberrations (Figure 6D). These extensive aggregates of a cytoplasmic protein are likely the result of a long-term accumulation process and support the idea that the loss of nuclear integrity occurred over time in vivo and not during nuclei isolation. These results further suggest that the observed permeability defects are directly linked to defective pores and not NE perturbations as observed in age-related diseases such as progeria, in which mutant lamins perturb the nuclear membrane (Scaffidi and Misteli, 2006). In agreement with this, we found no differences in the nuclear lamina structure, the inner nuclear membrane proteins, or the distribution of NPCs in intact and leaky nuclei, all phenotypes associated with alterations in NE and lamin proteins (Figures S5D and S5E) (D'Angelo and Hetzer, 2006).

Age-Related Deterioration of Nuclear Pore Complex Structure and Function

Taken together, our results suggest that the long-lived NPC structure deteriorates over time, leading to an increase in nuclear permeability with age. To investigate the presence of defects in the nuclear pore complexes from old nuclei, we stained leaky and intact nuclei with different antibodies against structural components. Strikingly, we noticed that Nup93, but not Nup107 or Pom121, was lost from the old leaky nuclei (Figures 7A, 7C, and S6A). Because Nup93 has been shown to be critical for establishing the NPC permeability barrier (Galy et al., 2003), the observed age-related loss of this nucleoporin provides a molecular explanation for the influx of 70 kDa in old nuclei. Interestingly, Nup93 has been shown to interact with FG-nucleoporins from the central channel, and it is believed to help establish the NPC diffusion barrier by recruiting these nucleoporins to the pore (Alber et al., 2007; Frosst et al., 2002; Grandi et al., 1997). Supporting this model, we found that the leaky nuclei also showed a decreased staining for mAb414, an antibody that recognizes several FG-nucleoporins such as Nup62 from the central channel (Davis and Blobel, 1987) (Figures 7B and 7C).

These results raise the question of why the scaffold Nup93, but not Nup107, is lost during aging. One possibility is that, due to their location in the NPC structure, the Nup107/160 complex is protected from cytosolic agents, whereas Nup93 is exposed to the central channel and might, therefore, be more accessible to damage that could alter its function. Oxidative protein damage caused by reactive oxygen species (ROS) has been shown to increase during aging and has been linked to a wide range of protein defects (Chakravarti and Chakravarti, 2007; Dalle-Donne et al., 2006). Consistent with this, we found a sharp increase in the levels of protein carbonyl groups, which are indicative of oxidative protein damage, in old versus young nuclei (Figure S7A). Interestingly, when we analyzed whether nucleoporins were targets of oxidative damage, we found that Nup93 and Nup153, but not Nup107, showed the presence of carbonyl groups (Figure 7D). These findings support the idea that the scaffold Nup107/160 complex, but not the Nup93/205 complex, are protected from damage and might explain why it is not lost from NPCs during aging.

If the observed NPC functional defects are, indeed, caused by ROS, we could expect that inducing oxidative stress in postmitotic cells would accelerate the appearance of leaky pores. To test this, we treated SS104 adult day 1 worms with N, N'-Dimethyl-4,4'-bipyridinium dichloride (paraquat), a known oxidative stress inducer that significantly increases ROS in worms (Kim and Sun, 2007; Oeda et al., 2001), for 6 days. Strikingly, we found that the worms treated with paraquat showed a higher percentage and earlier onset of leaky nuclei when compared to control worms (Figure 7E). In contrast, when we analyzed nuclei isolated from SS104 worms grown for 8 days on empty vector (control) or daf-2 RNAi, which increases life span and the tolerance to oxidative stress (Honda and Honda,

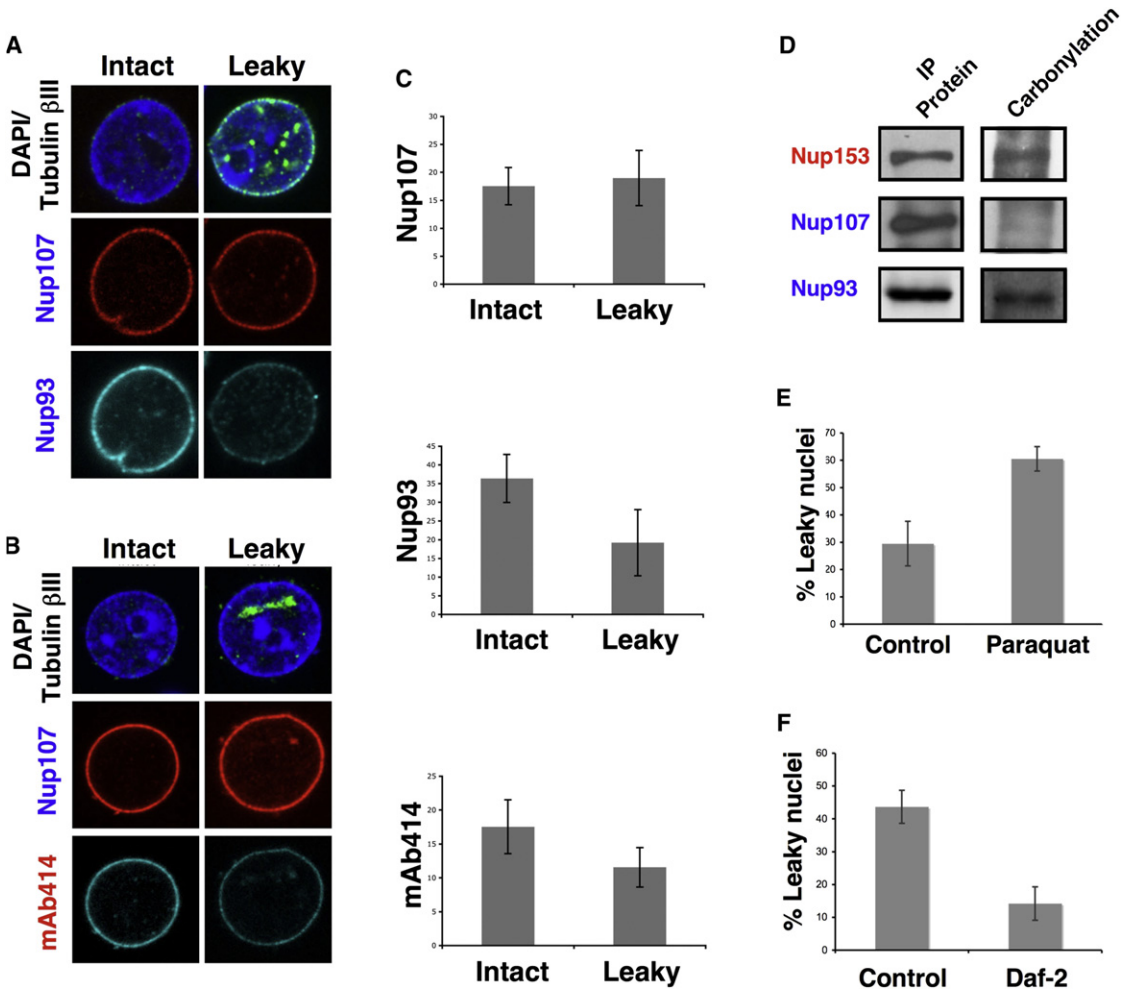

Figure 7. Nuclei with Increased Permeability Show Deteriorated NPCs

(A) Nuclei were isolated from brains of old (28 months old) rats, fixed, and stained with antibodies against tubulin βIII and the scaffold nucleoporins Nup107 and Nup133. The staining of intact and leaky nuclei (the latter identified by the intranuclear accumulation of intranuclear tubulin βIII) was compared.

(B) Intact and leaky nuclei isolated from old rat brains were stained with tubulin βIII, Nup107, and the mAb414 antibody. Intact and leaky nuclei were compared as described in (A).

(C) The fluorescent intensity of the nuclei stained in (A) and (B) was quantified using Image J.

(D) Total protein extracts were prepared from brains of old rats, and nucleoporins were immunoprecipitated (IP) using specific antibodies. The IP proteins were treated with 2,4-dinitrophenylhydrazine to derivatize carbonyl groups to 2.4-dinitrophenylhydrazone (DNP-hydrazone). The DNP-derivatized proteins were detected by western blot using an anti-DNP antibody.

(E) *C. elegans* SS104 worms on day 1 of adulthood were transferred to plates containing bacteria and buffer (Control) or Paraquat. On day 6, nuclei were isolated from worms, and nuclear permeability was analyzed by using the 70 kDa and 500 kDa dextrans and quantified by using Photoshop CS3 Extended.

(F) SS104 adult day 1 worms were grown in bacteria expressing empty vector (Control) or daf-2 RNAi (Daf-2) for 8 days. Nuclei were isolated from worms, and nuclear permeability was analyzed as in (E).

Error bars represent SD.

1999; Kim and Sun, 2007), we found the reciprocal effect, with the long-lived worms having a lower percentage of leaky nuclei than the control worms (Figure 7F).

To further confirm that the age-dependent increase in nuclear permeability occurs in vivo and is accelerated by oxidative stress, we injected worms of different days of adulthood with a 70 kDa dextran and analyzed its diffusion into the nucleus. Lamin-GFP served as a marker to identify the NE. Consistent with our data from isolated nuclei, we detected that old worms had an increased nuclear permeability compared to young worms and that nuclear leakiness was strongly increased by the treatment with paraquat (Figure S7B).

Together, these results suggest that oxidative protein damage can cause the deterioration of the pore permeability barrier and indicate that the appearance of pore defects is directly linked to the aging process. This may explain why the loss of nuclear selectivity occurs at different time scales in different organisms (i.e., days in worms versus months in rats).

DISCUSSION

The results presented in this work indicate that the biogenesis of nuclear pore complexes is restricted to mitotic stages and ceases upon cell-cycle exit. We demonstrate that NPC scaffold members do not turn over and show life-long residence at the NPC in nondividing cells. The finding that nuclear pore structure and function deteriorates with age suggests that the presence of the same nucleoporins during the entire life span of postmitotic cells comes at the physiological cost of aging-related pore defects that lead to a loss of nuclear integrity.

Why would cells keep the same NPC scaffolds during their entire life? Two reasons to explain this can be imagined. First, if new pores were to be inserted into the NE of postmitotic cells, then old pores would have to disassemble in order to maintain NPC number. As NPC disassembly is accompanied by an increase in nuclear permeability, nondividing cells would have to employ a disassembly mechanism that prevents a continuous loss of the nuclear permeability barrier. No evidence of such a mechanism has been found so far. Second, if no new pores were to be inserted in the NE but the subunits of pre-existing scaffolds were able to be replaced with newly synthesized ones, then cells would have to replace the components of the 8-fold symmetrical scaffold one by one in order to maintain the nuclear permeability barrier. Considering that the cores are embedded in the NE and covered by multiple layers of peripheral nucleoporins, it is difficult to imagine a molecular mechanism that would ensure such coordination and specificity based on the current knowledge of the pore. Therefore, NPC turnover appears to be restricted to mitosis, during which an orchestrated program of phosphorylation events leads to the rapid disassembly of the entire pore complex (Burke and Ellenberg, 2002).

Our findings imply that, in organisms that divide by closed mitosis (i.e., they divide without NE breakdown), such as *Saccharomyces cerevisiae*, the NPC scaffold should remain embedded in the NE for many generations. Consistent with this idea, it was recently shown that NPCs stay associated with the mother cell in a process that is mediated by the asymmetric segregation of NPCs during yeast mitosis (Shcheprova et al., 2008). It is, therefore, tempting to speculate that mother cells, which are already known to retain damaged proteins (Aguilaniu et al., 2003), also accumulate defective NPCs that might contribute to their overall aging process.

The lack of a replacement mechanism for scaffold nucleoporins in nondividing cells indicates that some differentiated cells, such as muscle fibers and neurons, would keep the NPC scaffolds for many years. These long-lived nucleoporins might, therefore, be particularly vulnerable to protein damage as cells age. Consistent with this idea, we found that Nup93, but not the Nup107 protein, can suffer from oxidative damage in old cells. As stated above, these differences may be a consequence of their location inside the NPC structure. Whereas the Nup107/160 complex is embedded in the nuclear membrane and shielded by several layers of proteins, the Nup93 complex, which acts as a linker between the Nup107/160 scaffold and the FG-nucleoporins from the central channel (Alber et al., 2007), is exposed to the cytoplasmic factors that can access the pore channel. Thus, Nup93, which, like Nup107, is not replaced in nondividing cells, might accumulate damage over time to a point where it can no longer remain bound to the NPC. The absence of the Nup93 protein linker would result in a loss of FG-nucleoporins and a deterioration of the permeability barrier. This provides a molecular explanation for the phenomenon of old leaky nuclei we observed, which potentially represents an early event in the previously described deterioration of old nuclei (Haithcock et al., 2005).

Interestingly, we discovered that nuclei purified from old differentiated cells show defects in the nuclear permeability barrier and the accumulation of cytoplasmic tubulin. Strikingly, tubulin-positive intranuclear filaments have been described in many mammals, and their occurrence has been linked to the aging process as well as to neurodegenerative diseases (Woulfe, 2007), all associated with elevated oxidative damage (Danielson and Andersen, 2008; Zhu et al., 2007). This raises the intriguing possibility that defective pores may be involved in the development of human pathologies.

EXPERIMENTAL PROCEDURES

Cells

C2C12 cells were obtained from ATCC. Proliferating myoblasts were maintained in DMEM + 20% FBS. Differentiation into myotubes was induced by shifting to 2% horse serum. Myotubes were separated from quiescent cells on day 6 of differentiation with 0.025% trypsin. Transfection of myoblast cells was performed using Optifect (Invitrogen).

Synchronization of Temperature-Sensitive Sterile Animals

Age-synchronized worms were obtained at 25°C by using the CF512 or SS104 temperature-sensitive sterile strains as described (Raices et al., 2005).

Generation of *C. elegans* Transgenic Lines

C. elegans transgenic worms were generated by using the N2 wild-type strain as previously described (Panowski et al., 2007). For details, see the Supplemental Data.

RNAi, Survival Analysis, and Embryonic Viability Assays

RNAi and survival analysis were performed as described by Raices et al. (2005). Life span assays were performed at 20°C, and RNAi treatments were initiated at day 1 of adulthood. RNAi clones used in this study are summarized in Table S2. pAD12 (Bluescript vector with opposing T7 promoters) was used as empty vector control. RNAi efficiency was controlled by determining embryonic lethality and by Q-PCR. For embryonic lethality assays, the total number of progeny born to a single worm under RNAi treatment was measured and compared to control worms. For details, see the Supplemental Data.

To determine the effect of RNAi on nucleoporin protein, ~5000 SS104 adult worms were grown at 25°C in the corresponding dsRNA and collected at the indicated time points. In parallel, synchronized worms were grown at 15°C on the same dsRNA bacteria plates to produce the reproductive population of worms used as mitotic control. Protein was prepared as described in the Supplemental Data.

For nuclei permeability assays, adult SS104 worms were transferred in day 1 of adulthood to plates containing control bacteria or bacteria expressing daf-2 RNAi. Worms were transferred to fresh plates every 2 days. After 6 days, nuclei were isolated and assayed for nuclear permeability.

RNAi Inhibition Using *DCR-1*

ceNup160-GFP and ceNup153-GFP-expressing worms were grown on *GFP* dsRNA for several generations until no GFP signal was detected. To lower the *GFP* RNAi activity, animals were shifted to bacteria expressing *DCR-1* dsRNA (Dillin et al., 2002) as day 1 adults. Empty vector was used in control animals (data not shown). Worms were grown for 6 days on *DCR-1* RNAi before imaged.

RNA Isolation, Semiquantitative RT–PCR, and Quantitative Q-PCR

C. elegans total RNA was isolated from synchronized populations by using TRIzol reagent (Invitrogen). C2C12 total RNA was prepared by using QIAGEN RNeasy kit. cDNA was created by using SuperScript III Reverse Transcriptase (Invitrogen) and used for RT-PCR and Q-PCR analysis. For details, see the Supplemental Data.

Pulse-Chase Experiments

Dividing C2C12 myoblasts were incubated with a 100 mCi/ml of S^{35}-Methonine/S^{35}-Cysteine mix (EasyTag EXPRESS35S Protein Labeling Mix, Perkin Elmer) for 24 hr (Pulse) and then switched to differentiating media (Chase). Total cell lysates were prepared at different days of differentiation. Proteins were immunoprecipitated by using specific antibodies, separated by SDS-PAGE, and transferred to nitrocellulose membranes. The presence of S^{35}-labeled proteins was analyzed using a phosphorimager, and protein levels were determined by western blot. For details, see the Supplemental Data.

Isolation of *C. elegans* and Rat Nuclei and Nuclear Permeability Assays

For *C. elegans* nuclear permeability assays, 60,000 worms at different days of adulthood were collected, and nuclei were isolated as described in the Supplemental Data.

For the preparation of rat nuclei, the brain cortexes of young (3 months old) and old (28 months old) Fischer 344 female rats were dissected and carefully minced. Nuclei were isolated as described by Lovtrup-Rein and McEwen (1966).

To analyze nuclear permeability, we incubated the isolated nuclei with a mixture of FITC-70 kDa and TRITC-500 kDa dextrans (Sigma) plus Hoechst for 15–30 min. Influx of dextrans inside the nuclei was analyzed in unfixed samples by confocal microscopy. The percentage of nuclei with alter permeability barrier was quantified by using Adobe Photoshop CS3 Extended.

In parallel, rat nuclei were incubated with the FITC-70 kDA dextran alone, fixed with 4% paraformaldehyde (PFA), and immunostained with an anti-Tubulin βIII antibody.

Oxidative Stress Treatment and Analysis

Protein carbonylation analysis was performed by using the Oxyblott Protein Detection Kit (Millipore) as described by the manufacturer. For details, see the Supplemental Data.

To analyze the effect of oxidative stress on the nuclear permeability of postmitotic worms, we transferred SS104 adult day 1 animals to plates containing bacteria or bacteria plus paraquat (0.5 mM final). Worms were transferred to fresh plates on day 3 and collected on day 6 to isolate nuclei and perform nuclear permeability assays. For studies of nuclear permeability in vivo, we grew adult worms for 6 days in the presence of 1 mM paraquat before injection.

Injection of Dextran in Adult Worms

Adult worms expressing Lamin-GFP were injected in the intestine with a TRITC-70 kDa dextran at the specified days of adulthood. After 20 min, worms in which the dextran had entered intestinal cells were separated and analyzed for nuclear permeability by confocal microscopy ($n \geq 10$).

Microscopy

Transgenic worms were analyzed using a Leica 6000B digital microscope and the Leica FW4000 software. For details, see the Supplemental Data. When comparing fluorescence intensity, nonsaturated maximal projections of 30–40 confocal sections were obtained by using an SP2 confocal microscope and were processed using Imaris software (Bitplane).

For nuclear volume and NPC density analysis, C2C12 myoblasts were transfected with a 3GFP-NLS-expressing vector and induced to differentiate. Cells were fixed and stained with anti-Nup153 antibody. Nuclear volume was determined from 3D reconstructions (~50 confocal sections) of nuclei that had imported fluorescently labeled 3GFP-NLS. NPC density was determined by counting the number of pores/μm^2.

The incorporation of GFP-tagged proteins into the NE of differentiated cells was analyzed by 4D confocal microscopy of myotubes expressing the corresponding fluorescent proteins. For details, see the Supplemental Data.

SUPPLEMENTAL DATA

The Supplemental Data include Supplemental Experimental Procedures, seven figures, three tables, and three movies and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(08)01512-2.

ACKNOWLEDGMENTS

We thank A. Dillin, P. Askjaer, B. Burke, J. Ellenberg, R. Foisner, M. Hengartner, Y. Gruenbaum, U. Kutay, and E. Schirmer for reagents; M. Downes for mammalian Q-PCR assays; J. Jepsen for isolation of rat brains; and the Hetzer lab, C. Hetzer-Egger, T. Hunter, W. Eckhart, F. Gage, and S. Pfaff for critical discussion and reading of the manuscript. This work was funded by the NIH R01 GM057438 grant.

Received: May 20, 2008
Revised: October 5, 2008
Accepted: November 21, 2008
Published: January 22, 2009

REFERENCES

Aguilaniu, H., Gustafsson, L., Rigoulet, M., and Nystrom, T. (2003). Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science *299*, 1751–1753.

Alber, F., Dokudovskaya, S., Veenhoff, L.M., Zhang, W., Kipper, J., Devos, D., Suprapto, A., Karni-Schmidt, O., Williams, R., Chait, B.T., et al. (2007). The molecular architecture of the nuclear pore complex. Nature *450*, 695–701.

Antonin, W., Franz, C., Haselmann, U., Antony, C., and Mattaj, I.W. (2005). The integral membrane nucleoporin pom121 functionally links nuclear pore complex assembly and nuclear envelope formation. Mol. Cell *17*, 83–92.

Beanan, M.J., and Strome, S. (1992). Characterization of a germ-line proliferation mutation in C. elegans. Development *116*, 755–766.

Beck, M., Forster, F., Ecke, M., Plitzko, J.M., Melchior, F., Gerisch, G., Baumeister, W., and Medalia, O. (2004). Nuclear pore complex structure and dynamics revealed by cryoelectron tomography. Science *306*, 1387–1390.

Burke, B., and Ellenberg, J. (2002). Remodelling the walls of the nucleus. Nat. Rev. Mol. Cell Biol. *3*, 487–497.

Chakravarti, B., and Chakravarti, D.N. (2007). Oxidative modification of proteins: Age-related changes. Gerontology *53*, 128–139.

Commerford, S.L., Carsten, A.L., and Cronkite, E.P. (1982). Histone turnover within nonproliferating cells. Proc. Natl. Acad. Sci. USA *79*, 1163–1165.

Crittenden, S.L., Leonhard, K.A., Byrd, D.T., and Kimble, J. (2006). Cellular analyses of the mitotic region in the Caenorhabditis elegans adult germ line. Mol. Biol. Cell *17*, 3051–3061.

D'Angelo, M.A., and Hetzer, M.W. (2006). The role of the nuclear envelope in cellular organization. Cell. Mol. Life Sci. *63*, 316–332.

D'Angelo, M.A., Anderson, D.J., Richard, E., and Hetzer, M.W. (2006). Nuclear pores form de novo from both sides of the nuclear envelope. Science *312*, 440–443.

Daigle, N., Beaudouin, J., Hartnell, L., Imreh, G., Hallberg, E., Lippincott-Schwartz, J., and Ellenberg, J. (2001). Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. J. Cell Biol. *154*, 71–84.

Dalle-Donne, I., Aldini, G., Carini, M., Colombo, R., Rossi, R., and Milzani, A. (2006). Protein carbonylation, cellular dysfunction, and disease progression. J. Cell. Mol. Med. *10*, 389–406.

Danielson, S.R., and Andersen, J.K. (2008). Oxidative and nitrative protein modifications in Parkinson's disease. Free Radic. Biol. Med. *44*, 1787–1794.

Davis, L.I., and Blobel, G. (1987). Nuclear pore complex contains a family of glycoproteins that includes p62: Glycosylation through a previously unidentified cellular pathway. Proc. Natl. Acad. Sci. USA *84*, 7552–7556.

Dillin, A., Crawford, D.K., and Kenyon, C. (2002). Timing requirements for insulin/IGF-1 signaling in C. elegans. Science *298*, 830–834.

Frosst, P., Guan, T., Subauste, C., Hahn, K., and Gerace, L. (2002). Tpr is localized within the nuclear basket of the pore complex and has a role in nuclear protein export. J. Cell Biol. *156*, 617–630.

Galy, V., Mattaj, I.W., and Askjaer, P. (2003). Caenorhabditis elegans nucleoporins Nup93 and Nup205 determine the limit of nuclear pore complex size exclusion in vivo. Mol. Biol. Cell *14*, 5104–5115.

Grandi, P., Dang, T., Pane, N., Shevchenko, A., Mann, M., Forbes, D., and Hurt, E. (1997). Nup93, a vertebrate homologue of yeast Nic96p, forms a complex with a novel 205-kDa protein and is required for correct nuclear pore assembly. Mol. Biol. Cell *8*, 2017–2038.

Haithcock, E., Dayani, Y., Neufeld, E., Zahand, A.J., Feinstein, N., Mattout, A., Gruenbaum, Y., and Liu, J. (2005). Age-related changes of nuclear architecture in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *102*, 16690–16695.

Harel, A., Orjalo, A.V., Vincent, T., Lachish-Zalait, A., Vasu, S., Shah, S., Zimmerman, E., Elbaum, M., and Forbes, D.J. (2003). Removal of a single pore subcomplex results in vertebrate nuclei devoid of nuclear pores. Mol. Cell *11*, 853–864.

Honda, Y., and Honda, S. (1999). The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. FASEB J. *13*, 1385–1393.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461–464.

Ketting, R.F., Fischer, S.E., Bernstein, E., Sijen, T., Hannon, G.J., and Plasterk, R.H. (2001). Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev. *15*, 2654–2659.

Kim, Y., and Sun, H. (2007). Functional genomic approach to identify novel genes involved in the regulation of oxidative stress resistance and animal lifespan. Aging Cell *6*, 489–503.

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science *277*, 942–946.

Kiseleva, E., Allen, T.D., Rutherford, S., Bucci, M., Wente, S.R., and Goldberg, M.W. (2004). Yeast nuclear pore complexes have a cytoplasmic ring and internal filaments. J. Struct. Biol. *145*, 272–288.

Krull, S., Thyberg, J., Bjorkroth, B., Rackwitz, H.R., and Cordes, V.C. (2004). Nucleoporins as components of the nuclear pore complex core structure and tpr as the architectural element of the nuclear basket. Mol. Biol. Cell *15*, 4261–4277.

Lenart, P., and Ellenberg, J. (2006). Monitoring the permeability of the nuclear envelope during the cell cycle. Methods *38*, 17–24.

Lenart, P., Rabut, G., Daigle, N., Hand, A.R., Terasaki, M., and Ellenberg, J. (2003). Nuclear envelope breakdown in starfish oocytes proceeds by partial NPC disassembly followed by a rapidly spreading fenestration of nuclear membranes. J. Cell Biol. *160*, 1055–1068.

Lovtrup-Rein, H., and McEwen, B.S. (1966). Isolation and fractionation of rat brain nuclei. J. Cell Biol. *30*, 405–415.

Maul, G.G., Maul, H.M., Scogna, J.E., Lieberman, M.W., Stein, G.S., Hsu, B.Y., and Borun, T.W. (1972). Time sequence of nuclear pore formation in phytohemagglutinin-stimulated lymphocytes and in HeLa cells during the cell cycle. J. Cell Biol. *55*, 433–447.

Oeda, T., Shimohama, S., Kitagawa, N., Kohno, R., Imura, T., Shibasaki, H., and Ishii, N. (2001). Oxidative stress causes abnormal accumulation of familial amyotrophic lateral sclerosis-related mutant SOD1 in transgenic Caenorhabditis elegans. Hum. Mol. Genet. *10*, 2013–2023.

Panowski, S.H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. Nature *447*, 550–555.

Rabut, G., Doye, V., and Ellenberg, J. (2004a). Mapping the dynamic organization of the nuclear pore complex inside single living cells. Nat. Cell Biol. *6*, 1114–1121.

Rabut, G., Lenart, P., and Ellenberg, J. (2004b). Dynamics of nuclear pore complex organization through the cell cycle. Curr. Opin. Cell Biol. *16*, 314–321.

Raices, M., Maruyama, H., Dillin, A., and Karlseder, J. (2005). Uncoupling of longevity and telomere length in C. elegans. PLoS Genet. *1*, e30.

Reichelt, R., Holzenburg, A., Buhle, E.L., Jr., Jarnik, M., Engel, A., and Aebi, U. (1990). Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. J. Cell Biol. *110*, 883–894.

Salama, N.R., Yeung, T., and Schekman, R.W. (1993). The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. EMBO J. *12*, 4073–4082.

Scaffidi, P., and Misteli, T. (2006). Lamin A-dependent nuclear defects in human aging. Science *312*, 1059–1063.

Shcheprova, Z., Baldi, S., Frei, S.B., Gonnet, G., and Barral, Y. (2008). A mechanism for asymmetric segregation of age during yeast budding. Nature *454*, 728–734.

Walther, T.C., Alves, A., Pickersgill, H., Loiodice, I., Hetzer, M., Galy, V., Hulsmann, B.B., Kocher, T., Wilm, M., Allen, T., et al. (2003). The conserved Nup107-160 complex is critical for nuclear pore complex assembly. Cell *113*, 195–206.

Weis, K. (2003). Regulating access to the genome: Nucleocytoplasmic transport throughout the cell cycle. Cell *112*, 441–451.

Woulfe, J.M. (2007). Abnormalities of the nucleus and nuclear inclusions in neurodegenerative disease: A work in progress. Neuropathol. Appl. Neurobiol. *33*, 2–42.

Zhu, X., Su, B., Wang, X., Smith, M.A., and Perry, G. (2007). Causes of oxidative stress in Alzheimer disease. Cell. Mol. Life Sci. *64*, 2202–2210.