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LOSS OF NUCLEAR ENVELOPE INTEGRITY IN AGING AND DISEASE

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Abstract

The nuclear envelope (NE) serves as central organizing unit for the eukaryotic cell. By virtue of its highly selective, semi-permeable barrier function, the NE shields the enclosed genetic material, while at the same time ensuring its regulated transcription, replication and repair. The NE has long been considered to only dismantle during mitosis. However, in recent years it has become clear that in a variety of pathologies, NE integrity becomes compromised during interphase as well. Loss of NE integrity, or briefly NE stress, is manifested in various ways, ranging from a gradual reduction in nucleocytoplasmic transport function, over selective loss and degradation of NE components, to catastrophic rupture events that provoke illegitimate molecular fluxes between nucleus and cytoplasm. Although cells manage to cope with such forms of NE stress, the different insults to nuclear compartmentalization alter gene regulation and jeopardize genome stability. Hence, loss of NE integrity is emerging as a broad-spectrum pathogenic mechanism. In this review, we discuss the relevance of nuclear compartmentalisation and the loss thereof for aging and disease development.

The nuclear envelope safeguards nuclear compartmentalization

The nuclear envelope (NE) is a complex structure composed of three main components: the nuclear lamina, a double lipid bilayer and nuclear pore complexes (Fig. 1). The double lipid bilayer consists of an inner (INM) and outer nuclear membrane (ONM), which together enclose a perinuclear space that is contiguous with the lumen of the endoplasmic reticulum. Directly underneath the INM lies the nuclear lamina, a dense meshwork of intermediate filaments, composed of A-type and B-type lamins, the ratio of which is determined by the cell type and extracellular matrix (ECM), more specifically, collagen-dependent tissue stiffness (Swift et al., 2013). Irrespective of its composition, the nuclear lamina plays a crucial role in nuclear organization and gene regulation. It does this inter alia, by tethering chromatin regions and transcription factors (Broers et al., 2006). Across the NE, well-defined sets of interconnected proteins, known as Linker of Nucleo- and Cytoskeleton (LINC) complexes, attach the nuclear lamina to the cytoskeleton. With cytoskeletal components being in direct contact with the ECM via integrins, this nucleo-cytoskeletal coupling assures full mechanical continuity between the intra- and extracellular environment (Crisp et al., 2006; Lombardi & Lammerding, 2011).

Although the genetic content is physically separated from the cytoplasm, bidirectional communication between the two compartments remains possible. Nucleocytoplasmic communication is tightly regulated by an array of adaptive turnstiles which are embedded in the NE, the nuclear pore complexes (NPCs) (Strambio-De-Castillia, Niepel, & Rout, 2010). NPCs dictate transport between the nucleus and cytoplasm during interphase by assuring that only small molecules ($< 40\text{kDa}$ in mass, $< 5\text{nm}$ in diameter (Wente & Rout, 2010)) and larger protein complexes carrying appropriate signal sequences (Nuclear Localisation Sequence, NLS or Nuclear Export Sequence, NES) pass through. This meticulous control over nucleocytoplasmic compartmentalization offers several advantages. For instance, spatial

separation of transcription and translation provides eukaryotes with additional layers of gene regulation that are based on alternative splicing (Keren et al., 2010) and conditional transcription factor shuttling (Cohen et al., 2008; Purvis & Lahav, 2013). In addition, the NE hosts a range of NPC-mediated quality control mechanisms to ascertain regulated gene expression (Ibarra et al., 2016) or selective export of correctly processed mRNA (Bonnet et al., 2015). Moreover, the encapsulation of DNA by the NE in eukaryotic cells offers an additional means for detecting ongoing infections: the innate immune system has developed cytosolic DNA sensor proteins that respond to the presence of pathogen- or host-derived DNA in the cytoplasm of the interphase cell by activating downstream signalling cascades that promote cell death and inflammation (Dempsey & Bowie, 2015; Xiao, 2015).

Historically, the sole moment in a cell's life cycle the NE barrier was considered to be broken, was during mitosis, when duplicated DNA becomes equally distributed among two new daughter cells. This spatiotemporally coordinated process is triggered by the phosphorylation of lamins (Gerace & Blobel, 1980) and relies on chromatin condensation (Antonin & Neumann, 2016). However, an accumulating body of evidence is now pointing towards processes that impinge on NE integrity throughout interphase as well. These perturbations arise under degenerative conditions such as aging, viral infection, cancer and laminopathies, suggesting that loss of NE integrity, also referred to as NE stress (Di Micco et al., 2016), is an emerging broad-spectrum hallmark of pathology. We define three different, but mutually non-exclusive levels of NE stress that vary in nature and extent (Fig. 1). One form of NE stress is NE erosion, a gradual loss of nuclear compartmentalization that is due to dysregulated NPC component turnover and/or localization. NE shedding is a second more direct form of NE stress caused by localized degradation of NE components. The most instant form of NE stress is NE rupture, a process that involves complete, but often transient disruption of the NE barrier and which leads to large-scale bidirectional exchange of nuclear and cytoplasmic

components. Here below, we summarize the molecular details of all three events and we discuss their contribution to pathology development.

NE erosion compromises transport selectivity

Across its surface, the NE is studded with NPCs. Every NPC consists of a stable core of scaffold nucleoporins (e.g., of the Y-complex), surrounding a central channel and a larger set of dynamic peripheral nucleoporins, such as the phenylalanine–glycine-repeat (FG-) containing nucleoporins of the Nup62 complex (D'Angelo & Hetzer, 2008; Kabachinski & Schwartz, 2015). While peripheral nucleoporins have a high turnover at the NPC, scaffold nucleoporins only dislodge and reassemble during mitosis (Daigle et al., 2001; Rabut et al., 2004). This implies that NPCs remain stably integrated within the NE of terminally differentiated cells, which makes them particularly vulnerable to damage accrual. In line with this, long-lived NPCs have been shown to deteriorate with age in post-mitotic rodent myotubes and neurons, due to oxidative damage to and selective loss of peripheral nucleoporins, thereby causing increased nuclear permeability (D'Angelo et al., 2009). At the same time, fibroblasts from elderly patients display decreased concentrations of various nuclear transport factors (karyopherin $\alpha 2$, CAS, and RanBP) and experience reduced nuclear import (Pujol et al., 2002). Together, these observations suggest that aging affects the selectivity of the NE barrier by increasing NPC leakiness, whilst at the same time diminishing directional transport (Fig. 2). A reduction in nuclear import rate has also been observed in Hutchinson-Gilford Progeria Syndrome (HGPS) patient cells, which accumulate the toxic, farnesylated lamin A intermediate, termed progerin (Kelley et al., 2011), as well as in cells overexpressing laminopathy-causing prelamin A variants (including progerin) (Busch et al., 2009). In late-passage HGPS cells, NPCs become clustered in small regions of the NE (Goldman et al., 2004). Furthermore, prelamin A build-up was found to disrupt the RAN

gradient and to mislocalize the NPC components NUP153 and TPR (Cobb et al., 2016; Kelley et al., 2011). This is not unexpected, given the pivotal role of lamins in the spatial distribution and anchoring of NPCs (Goldman et al., 2004; Guo & Zheng, 2015; Maeshima et al., 2006). Interestingly, prelamin A variants also accumulate during normal aging (Ragnauth et al., 2010; Scaffidi & Misteli, 2006), suggesting their potential involvement in aging-associated NE erosion. In an aging model of vascular smooth muscle cells, it was recently found that prelamin A-induced NE invaginations (also known as nucleoplasmic reticulum (Goulbourne et al., 2012)) trap NUP153 nucleoporins thereby compromising the RAN gradient and preventing larger cargo from efficiently being translocated to the nucleus (Cobb et al., 2016). One such molecule that becomes jammed in the cytoplasm is 53BP1, key factor in the repair of double strand-breaks (DSBs) (Panier & Boulton, 2014). Reduced nuclear import of 53BP1 resulted in increased levels of DNA damage in aged VSMCs (Cobb et al., 2016). This phenotype could not be reversed by treatment of aged VSMCs with a farnesyltransferase inhibitor, suggesting that it is not solely determined by the build-up of farnesylated prelamin A. The presence of non-farnesylated prelamin A and/or depletion of mature lamin A may thus equally provoke this defect. This is reinforced by the fact that the N-acetyl-transferase (NAT10) inhibitor Remodelin, which acts upstream of lamin A/C, could rescue the aged phenotype (Cobb et al., 2016; Larrieu et al., 2014).

The aforementioned examples show that both normal and accelerated aging are associated with an impaired NPC functionality and/or abnormal NPC localization, possibly due to prelamin A and/or ROS inflation. This leads to a loss in nuclear transport selectivity. As exemplified for the repair protein 53BP1, perturbed nucleocytoplasmic transport compromises the activity of proteins that demand regulated and timely transport and it thereby contributes to the build-up of genome instability. Given the functional versatility of nucleoporins, it is likely that NPC malfunction will directly affect many other tasks next to transport, including

regulation of gene expression, RNA processing and gene gating (Bonnet et al., 2015; Ibarra et al., 2016; Ikegami & Lieb, 2013).

NE shedding bypasses NPC control

Whilst the majority of nuclear transport is governed by NPCs, certain side-roads exist that do not rely on NPC-mediated quality control (Fig. 3). For instance, in *Drosophila melanogaster* muscle cells, large ribonucleoprotein (RNP) granules become directly trafficked across the NE via a NPC-independent or parapore transport process (Speese et al., 2012). RNP granules become engulfed by the inner nuclear membrane, forming vesicles in the perinuclear space. These vesicles subsequently fuse with the outer nuclear membrane to release their contents into the cytoplasm (Fig. 3a). Failure of this parapore transport mechanism has been linked to the neurological movement disorder dystonia, underlining its relevance as natural nuclear export pathway (Jokhi et al., 2013). Although at first sight, parapore transport does not directly compromise nucleocytoplasmic compartmentalization, it does affect the composition and turnover of the nuclear membranes. In addition, it is conceivable that random nuclear proteins become co-transported through these buds (bystander effect), putting an additional burden on cellular homeostasis. Moreover, a very similar process has been documented during nuclear egress of herpesvirus capsids, suggesting that this pathway is susceptible to abuse (Johnson & Baines, 2011). In both cases, the parapore process relies on PKC-mediated phosphorylation to promote local lamina disassembly (Klupp et al., 2007; Park & Baines, 2006). It is exactly this feature that also primes senescent cells for a more severe form of NE remodelling, which we term NE shedding (Fig. 3b), and which relies on localized bleb formation and subsequent budding of NE-derived vesicles. During NE shedding, chromatin fragments are extruded from the nucleus to the cytoplasm to become targeted to the lysosomes for autophagic degradation (Ivanov et al., 2013). Such cytoplasmic chromatin

fragments are generated during both oncogene-induced as well as replicative senescence, and contain histones and lamin B1. Recently, it was shown that interactions between lamin B1 and LC3 are the driving force behind the NE shedding process (Dou et al., 2015; Lenain et al., 2015). Considering the low turnover of lamins in the nuclear lamina (Broers et al., 1999), it might represent a pathway for targeted lamin breakdown; a means to literally shed off the lamina from the NE. Supporting this notion, lamin B1 levels decline upon senescence (Freund et al., 2012; Shimi et al., 2011). Since forced knockdown of *LMNB1* causes premature senescence, and overexpression of *LMNB1* delays the onset of senescence (Freund et al., 2012; Shimi et al., 2011), selective degradation of lamins by autophagy may thus represent a tumour-suppressive degradation mechanism (Dou et al., 2015).

An exaggerated form of NE shedding, termed nucleophagy, was also observed in A-type lamin and emerin deficient cells, with giant LC3-positive autophagosomes tethering to the NE (Park et al., 2014). Since inhibition of autophagy aggravated nuclear dysmorphism and compromised viability in these cells, it was suggested that nucleophagy serves to clean up damaged parts of the nucleus (Park et al., 2014).

Not all cytoplasmic chromatin-containing vesicles are degraded. In contrast with senescence-associated cytoplasmic chromatin fragments, many cancer cells are typified by the presence of persistent micronuclei (MN). While MN usually arise from dicentric chromosome breaks during mitosis (Fenech et al., 2011), similar extra-chromosomal elements can form during interphase via a process of local NE bleb formation (Utani et al., 2011) (Fig. 3c). A striking difference between both types of MN is that mitotic MN usually contain lamins, whereas interphase MN do not. The latter are generated at sites of the NE where the lamina is locally interrupted and they can remain connected to the nucleus by a thin chromatin fiber or eventually bud off. Yet, alike mitotic MN, their formation may contribute significantly to the build-up of genome instability.

In brief, the NE is susceptible to a variety of remodelling processes that enable NPC-independent transport of nuclear components. Although apparently active as a bonafide, native export strategy, the parapore transport pathway can be hijacked by viruses. A severe and irreversible form of NE remodelling, coined NE shedding, contributes to loss of nuclear material, including lamins and chromatin. In doing so, NE shedding increases nuclear permeability (Ivanov et al., 2013) and may promote genome instability.

NE rupture triggers illegitimate nucleocytoplasmic exchange

By reorganizing and degrading the NE, both NE erosion and NE shedding gradually increase nuclear permeability. However, certain pathological conditions provoke instantaneous and complete loss of NE barrier function. This occurs through transient ruptures of the NE (NERs) (de Noronha et al., 2001; De Vos et al., 2011; Denais et al., 2016; Raab et al., 2016; Robijns et al., 2016; Vargas et al., 2012) (Fig. 4). NERs were first observed in HIV infections, where they were proposed to facilitate nuclear entry of the viral pre-integration complex (de Noronha et al., 2001). Later on, NERs were also observed in a variety of laminopathy patient cells (De Vos et al., 2011), as well as in different types of cancer cells (Denais et al., 2016; Raab et al., 2016; Vargas et al., 2012). In addition, similar, yet irreversible rupture events have also been documented in mitotic MN (Hatch et al., 2013). The broad occurrence of this event fuels the hypothesis that NER plays a generic role in pathology.

Causes of NERs

A weakened nuclear lamina predisposes the nucleus to rupture. Indeed, there is a strong inverse correlation between the levels of A-type or B-type lamins and NER frequency, and NE regions that are depleted of lamins are more prone to bleb formation and rupture (De Vos et al., 2011; Irianto et al., 2016; Robijns et al., 2016; Vargas et al., 2012). In HIV infections

for instance, the virion-associated, nucleocytoplasmic shuttling protein Vpr was found to locally perturb the nuclear lamina network leading to NE herniations that eventually ruptured (de Noronha et al., 2001). By definition, laminopathy patient cells suffer from defects in the nuclear lamina (De Vos et al., 2011), and a variety of cancer cells demonstrate aberrant lamin levels (Chow et al., 2012; Foster et al., 2010). However, fibroblasts from laminopathy patients that produce different prelamin A variants but similar levels of mature lamin A, demonstrate vastly different NER frequencies (De Vos et al., 2011). This may be due to the different efficiency with which certain prelamin A variants incorporate into the nuclear lamina network. The importance of proper integration of A-type lamins for lamina integrity was elegantly demonstrated using designed ankyrin repeat proteins (DARPs) that specifically bind lamin A/C, thereby hindering their polymerization and subsequent incorporation into the lamina (Zwerger et al., 2015). Likewise, the assembly of the lamina will be compromised by the accumulation of prelamin A variants with different conformations. As the abundance of prelamin A increases with age (Ragnauth et al., 2010; Scaffidi & Misteli, 2006), aging itself may be a risk factor for increased NER, but this has not yet been proven.

It is clear that fragile sites of the NE that are devoid of lamins (weak spots) are most susceptible to bleb formation and rupture. Yet, lamin deficiency is not the sole determinant for NER. By growing cells on soft substrates, it was shown that even in lamin A/C-deficient cells, NER frequency can be reduced to zero (Tamiello et al., 2013). This suggests that mechanical forces exerted by the ECM through the cytoskeleton on the nucleus play a defining role in NER induction. Reinforcing this notion, cancer cells that migrate through confining spaces experience significantly more NERs (Denais et al., 2016; Raab et al., 2016). Moreover, the frequency of both spontaneous (unconfined) and confined migration-induced NERs could be abolished by blocking actomyosin contractility (Denais et al., 2016; Robijns et al., 2016). Recently, it was demonstrated that in lamin B1-depleted cancer cells, NER

induction indeed depends on a fully functional contractile LINC-anchored actin assembly (Hatch & Hetzer, 2016). Since weak spots are devoid of LINC complex proteins, it is not likely that NERs arise from cytoskeletal pulling forces. Rather, NER appears to be a result of compressive forces that are exerted on the nucleus by perinuclear actin bundles (Hatch & Hetzer, 2016).

Next to the overt involvement of mechanical forces, other factors may contribute to NER induction as well. For example, pro-apoptotic stimuli have been found to promote NER. In a Bax-regulated process that precedes the morphological hallmarks of apoptosis, nuclear protein-containing vesicles bud at the NE. These buds subsequently rupture, thereby releasing their content into the cytosol. Interestingly, targeting of Bax to the NE (using a KASH-domain) proved sufficient to induce lamin remodelling and nuclear vesicle formation, suggesting that the budding/bursting event is not-lethal, but may contribute to a regulated dismantling of the NE (Lindenboim et al., 2014). Since NE breakdown during mitosis has been shown to require several phosphorylation events (Heald & McKeon, 1990; Ottaviano & Gerace, 1985) and various viruses such as herpesvirus and parvovirus have been shown to promote local lamin phosphorylation by activating host kinases (e.g. PKC α , Cdk-2) (Hatch & Hetzer, 2014; Porwal et al., 2013), yet another trigger for NER induction may reside in activation of similar enzymatic activities. However, evidence for such mechanism has yet to be provided.

NE repair

It is important to note that rupture-prone cells do not die. On the contrary, even after repetitive rupture, cells continue to divide (De Vos et al., 2011; Robijns et al., 2016). This implies they are able to efficiently repair the damaged NE. Cytoskeletal forces and entropic barriers preclude spontaneous resealing of broken membranes (Cooper & McNeil, 2015), suggesting

the involvement of a dedicated machinery. Very recently, a tip of the veil was lifted by identifying the endosomal sorting complexes required for transport III (ESCRT-III) as a putative NE repair factor: upon NER, the ESCRT-III component CHMP4B is relocated to the site of rupture (Fig. 4). Moreover, depletion of CHMP4B extends NER repair times (Denais et al., 2016; Raab et al., 2016; Robijns et al., 2016). Although it was shown that the inner nuclear membrane protein LEM 2 recruits ESCRT factors to the NE (Gu et al., 2017), as yet, it is not fully clear what drives ESCRT-III to the rupture site, nor how the broken membrane is repaired. Moreover, since ESCRT-III mainly repairs small membrane injuries, it is most likely not the only factor involved (Jimenez et al., 2014). Because the ESCRT machinery was first found to reseal the plasma membrane, it is conceivable that other plasma membrane repair processes also act at the level of the NE. The main plasma membrane repair mechanism relies on calcium-induced exocytosis, a process in which lysosomes fuse with the membrane at the wounded site, thereby supplying membrane material for repair (Reddy, Caler, & Andrews, 2001). This fusion process is mediated by calcium-dependent lipid-binding proteins such as annexins, dysferlins and SNAREs (Andrews et al., 2014; Davenport et al., 2016; Draeger et al., 2014). Given the continuity between the NE and the ER and the presence of nuclear Ca^{2+} stores (Echevarría et al., 2003), a smaller yet significant calcium flux may initiate a similar trigger for NE repair. Although purely speculative, the presence of cytoplasmically exposed DNA or nuclear transcription factors in the cytosol may equally well serve as putative activators.

Consequences of NER

Clearly, the sudden loss of nuclear compartmentalization perturbs cellular homeostasis in various ways. For one, NERs lead to an uncontrolled bidirectional translocation of proteins between the nucleus and cytoplasm. Several of these proteins are transcription factors or

regulatory complexes, such as Oct-1, cyclin B and RelA (De Vos et al., 2011). Depending on the extent of rupture and the duration of repair, these shifts are bound to alter gene regulatory programs. It can also be expected that proteins close to the nuclear membrane on both sides are more susceptible to delocalisation. Examples are transcription factors that are known to interact with the nuclear lamina, such as pRB, cFos, SREBP1, GCL, AP-1, ERK1/2, Mok2 and Oct1 (Heessen & Fornerod, 2007; Malhas et al., 2009). It has been shown that the expression of downstream Oct1 targets become dysregulated in NER-prone cells (De Vos et al., 2011) but direct causal effects have yet to be demonstrated. Moreover, the presence of inhibitory binding proteins (such as I κ B in the case of RelA) may still prevent illicit gene activation.

Ruptures are not only accompanied by transient shifts in regulatory protein distribution, but also seem to provoke more permanent translocations of macromolecular complexes such as PML bodies (De Vos et al., 2011; Houben et al., 2013) and intact organelles such as mitochondria (De Vos et al., 2011; Vargas et al., 2012) (Fig. 4). PML bodies are versatile stress sensors and DNA processing factories that should normally be confined to the nuclear interior (Dellaire & Bazett-Jones, 2004; Lallemand-Breitenbach & de The, 2010). Thus, their cytoplasmic translocation and subsequent fragmentation will impinge on a variety of processes that govern cell physiology, including proper DNA maintenance.

It has been demonstrated that NERs also directly affect genome integrity. Interphase DNA that becomes exposed to the cytoplasmic environment is more susceptible to DNA damage as evidenced by increased γ -H2AX staining and 53BP1 activity (Denais et al., 2016; Raab et al., 2016). This may be a result of exposure to cytoplasmic nucleases. It has been shown that cells with dicentric chromosomes form chromatin bridges that persist during interphase and rupture. During those events, the cytoplasmic nuclease TREX1 was found to relocate to the exposed DNA regions, thereby possibly promoting chromothripsis (catastrophic localized

genome rearrangements) (Maciejowski et al., 2015). Alternatively, the relocation of mitochondria during NER, may represent a direct source of ROS that damages the DNA (Sieprath et al., 2012). Thirdly, a depletion of repair factors from ruptured or constricted (in case of confined migration) sites may promote damage accumulation (Irianto et al., 2017).

Immune cells dispose of a set of sensor proteins that trigger inflammasome activation upon recognition of cytoplasmic DNA (Xiao, 2015). The AIM2 inflammasome for example, promotes inflammation and also activates pyroptosis (Fernandes-Alnemri et al., 2009), a lytic form of programmed cell death induced by inflammatory caspases (Kroemer et al., 2008). It has recently been shown that treatment of bone marrow-derived macrophages with the HIV protease Nelfinavir promotes cytoplasmic DNA release and activates the AIM2-inflammasome (Di Micco et al., 2016). An important implication of this observation is that NE stress may even arise as side-effect of therapeutic strategies. As Nelfinavir blocks the ZMPSTE24 metalloprotease, an enzyme that catalyses the crucial final cleavage step during lamin A maturation (Bergo et al., 2002), it directly interferes with lamin A maturation and therefore contributes to the loss of NE integrity. However, whether the cytoplasmic DNA in these cells is a result of NER or rather a consequence of some form of NE shedding mechanism has yet to be examined.

Taken together, NERs force the cell to mobilise membrane repair machinery, redistribute mislocalized proteins, and even repair DNA damage. Irrespective of the source, it is clear that NERs present a major handicap to cells that can alter gene expression and may ultimately endanger genome stability.

Conclusion

The NE offers selective access to the nuclear content. With this, a remarkable wealth of gene regulatory mechanisms has come to exist. However, it also presents an Achilles' heel, which

becomes exposed in a variety of hereditary and acquired diseases. Indeed, loss of NE integrity, or briefly NE stress, is emerging as a broad-spectrum pathogenic mechanism that is particularly catalysed by a deregulated lamin metabolism. We have defined three non-exclusive forms of NE stress, which all compromise cellular homeostasis, yet with varying severity. Although it is clear that defective NE compartmentalization contributes to changes in gene regulation and genome stability, many questions remain to be answered. For example, it is unknown whether all NE stress pathways truly drive disease development or, if they rather reflect the consequences of other pathogenic processes. Equally unanswered are the questions as to whether there is cross-talk between NE stress pathways (e.g., localized bleb formation) and whether they trigger dedicated cellular counter-mechanisms (such as NE repair). Therefore, identification of the upstream and downstream events of NE stress pathways, and the molecular players involved, will be essential. Further, it remains to be determined whether NE stress phenomena aggravate over time and with age, and whether there is a critical threshold above which cells are beyond rescue. In this context, it is interesting to note that several elements of NE stress are part of normal physiological processes within the cell (e.g., parapore transport or NE breakdown during mitosis), but which become derailed or abused in pathology. This raises the question as to whether certain forms of NE stress that have so far only been connected to pathological conditions, might also have relevance for non-pathological processes. Indeed, several cells in the body (such as neutrophils or stem cells (Constantinescu et al., 2006; Rowat et al., 2013) have strongly reduced levels of lamin A/C and migrate through narrow spaces, rendering them vulnerable to NER. Whether this would provide them with a selective advantage or rather an additional burden however, remains unknown and requires further investigation.

In summary, a better understanding of the different molecular mechanisms that underlie NE stress will significantly contribute to a fundamental understanding of a variety of diseases,

357 including accelerated aging and cancer, but may also shed light on general cell physiology.

358 This in turn, may open avenues for the discovery of multi-purpose therapeutic strategies.

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594 **Figures**

595
596 **Figure 1.** Simplified model of the healthy nuclear envelope (upper left) and major pathways
597 that lead to loss of nuclear integrity or NE stress.

Figure 2. Molecular mechanisms of NE erosion. Aging and lamin defects result in oxidative damage, degradation and clustering of NPCs, and affect the RAN gradient. This increases all-over NE permeability, but reduces directional import of large molecules such as 53BP1, thereby compromising proper DNA damage repair.

a. Parapore Transport

b. NE Shedding

c. MN Formation

Figure 3. Different forms of NE remodelling that bypass normal NPC function: a) Parapore transport allows trafficking of macromolecules such as ribonucleoprotein (RNP) complexes across the NE, but may also be used by Herpes Simplex Viruses (HSV) for their nuclear egress; b) NE shedding is associated with nuclear budding, cytoplasmic chromatin fragment (CCF) formation and autophagic degradation of lamins and chromatin; and c) Local NE bleb formation may also result in the formation of persistent micronuclei (MN) during interphase.

Figure 4. Molecular mechanisms of nuclear envelope rupture (NER). Under cytoskeletal pressure, the NE forms blebs at weak spots (often devoid of A-type lamins), which rupture. This results in bidirectional exchange of soluble components, macromolecular components and whole organelles, some of which become permanently translocated. Although NE breaks are repaired quickly (e.g. by components of the ESCRT-III complex), NER can result in DNA damage at the sites of rupture.