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BYPASSING BORDER CONTROL: NUCLEAR ENVELOPE RUPTURE IN DISEASE

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Summary for table of contents: nuclear envelope rupture is emerging as a broad-spectrum pathogenic mechanism that compromises nuclear compartmentalization and genome stability

Abstract

Recent observations in laminopathy patient cells and cancer cells have revealed that the nuclear envelope (NE) can transiently rupture during interphase. NE rupture leads to an uncoordinated exchange of nuclear and cytoplasmic material, thereby deregulating cellular homeostasis. Moreover, concurrently inflicted DNA damage could prime rupture-prone cells for genome instability. Thus, NE rupture may represent a novel pathogenic mechanism that has far-reaching consequences for cell and organism physiology.

Introduction

The nuclear envelope (NE) is a defining feature of the eukaryotic cell (FIGURE 1). In the most reductionist view, the NE consists of a set of lipid bilayers or membranes, of which the outer membrane is continuous with that of the endoplasmic reticulum (ER). However, the complete NE is much more complex. Both the inner (INM) and the outer nuclear membrane (ONM) contain diverse groups of proteins that are typically not found in the ER (42). INM proteins include integral (also referred to as NE transmembrane proteins or NETs (84)) and peripheral membrane proteins, some of which – such as the LEM domain proteins (Emerin, MAN1 and lamina-associated polypeptide 1 (LAP1)) – directly interact with chromatin and lamins (2, 27). Lamins are filament-forming proteins that constitute a meshwork underneath the INM known as the nuclear lamina. In mammalian cells, there are two types of lamins, the A-type and B-type lamins, which form separate but densely intertwined networks of unusually thin (3.5 nm) type V intermediate filaments (87, 95). The A-type lamins, lamin A and C are alternative splice products of the *LMNA* gene (60, 61), which are virtually absent in embryonic stem cells, only to become expressed during differentiation (15). Lamin B1 and lamin B2 are encoded by separate genes, *LMNB1* (59) and *LMNB2 (4)* respectively, and they are expressed throughout the embryonic stem cell lineage. Both mammalian lamin types also have tissue-specific splice variants (lamin B3, lamin AΔ10 and lamin C2) (33, 34). The nuclear lamina is an integral, structural part of the NE, it organizes the genome, and it is connected to elements of the cytoskeleton through membrane-spanning Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes (17), composed of cytoplasmic Nesprins and nucleoplasmic SUN proteins, which interact in the perinuclear space (9, 73) (FIGURE 1). To date, more than 300 disease-causing mutations have been identified in genes encoding NE proteins (67). The majority (>250) of these mutations has been mapped to the *LMNA* gene, and is associated with a collection of disorders known as laminopathies (71, 100). This class

of nuclear envelopathies predominantly targets cardiac or skeletal muscle, but also entails systemic disorders such as Hutchinson-Gilford Progeria Syndrome. Other nuclear envelopathies are caused by defects in lamin processing (*e.g.,* ZMPSTE24) or lamin interacting (*e.g.,* Emerin, Nesprin 1…) proteins.

Although the NE physically separates the genetic content from the cytoplasm, it is not completely impervious. Aqueous protein channels called Nuclear Pore Complexes (NPCs), which are embedded and immobilized within the NE (19), facilitate regulated bidirectional nucleocytoplasmic transport. Only very small metabolites and molecules (< 40kDa in mass, < 5nm in diameter) can pass freely, whereas larger protein complexes require appropriate signal sequences (nuclear localisation signal (NLS) or nuclear export signal (NES)) to be transported through the NPC (47). This selective gating feature is essential for conditional gene regulation and genome maintenance (44). Indeed, gene expression can be regulated via temporally-controlled import of transcription factors into the nucleus (106), as well as the regulated export of mRNA (99). Moreover, the physical separation of the genome from the cytoplasm has facilitated the development of an innate immune recognition system for detecting cytoplasmic DNA from unwanted invaders (103, 108) or nuclear damage (40, 49). Because of this, it was long thought that nuclear compartmentalization is vital for mammalian interphase cells and that the NE only dismantles in a highly-regulated manner during mitosis. However, it has now become apparent that the NE can also transiently lose its barrier function in interphase, during so-called nuclear envelope rupture (NER) events (Box 1). Under various conditions, the NE has been shown to rupture, resulting in an instantaneous and uncoordinated exchange of cytoplasmic and nucleoplasmic components (20, 22, 24, 77, 79, 97). This phenomenon can occur several times within the same cell and is non-lethal, as cells are still able to divide after repetitive NER (FIGURE 2a,b). Our group discovered the occurrence of spontaneous NERs in laminopathy patient cells (22), but a similar phenomenon was also

observed a decade earlier in cells artificially expressing the HIV-associated nucleocytoplasmic shuttling protein Vpr (20). More recently, NERs have also been detected in cancer cells (97) and cells migrating through narrow channels (24, 77). Thus, NER is emerging as a novel hallmark for a variety of pathological conditions. Here, we will describe the most recent insights into the causes and consequences of NERs for cell and organism physiology, and we will discuss the opportunities for future research and translational science.

BOX 1. Detecting NER in living cells

NERs are accompanied by a transient loss of nuclear compartmentalization. This can be visualized microscopically, using fluorescent markers that are sequestered to either the cytoplasmic or nuclear compartment in properly compartmentalized cells (*i.e.* cells that have an intact NE). To reveal true molecular fluxes that are independent of any cellular signaling pathway, such a molecule should ideally be as inert as possible. Hence, one approach is to load the cytoplasm with fluorescently labeled dextran molecules of high molecular weight (> 70kDa), which should not traverse the NPCs (7, 22, 48, 57). Upon NER, the fluorescent signal will change from an exclusively cytoplasmic location to a pan-cellular one. A disadvantage of this approach is the need for physical perturbation of the plasma membrane (through micro-injection or scrape loading) to load the cells with dextran. This problem can be avoided by using a genetically encoded fluorescent protein, such as GFP, coupled to a nuclear localization signal (NLS) of the SV40 large T antigen (^{126}PKKKRKV^{132}) (22, 79, 97). Under normal conditions, the signal sequence is recognized by importins, causing the NLS-tagged protein to become transported into the nucleus (54). When the NE barrier is broken, the fluorescent fusion protein will no longer be contained within the nucleus and will flow into the cytoplasm. This leads to a quantifiable decrease of the nuclear signal, and concurrent increase in cytoplasmic signal (FIGURE 2a). Upon restoration of nuclear

compartmentalization, the signals restore to baseline levels. Importantly, not all nuclear proteins delocalize during NER. For instance, most histones remain tightly bound to the DNA when the NE breaks (FIGURE 2c). This specific feature can be used to facilitate automated quantification of NERs (79). Whereas the histone 2B (H2B) signal can be used to consistently track nuclei in successive time frames, the ratio of the NLS to H2B signal serves as a robust readout for NER events, and allows for the removal of false positives that may arise from out-of-focus movements (FIGURE 2c).

Molecular mechanisms of NER

Recent work has uncovered the first molecular details of the NER sequence (FIGURE 3). The most supported model thus far suggests that mechanical stress is exerted onto the NE, and thereby triggers a local remodeling. This is often accompanied by the formation of a nuclear extrusion or bleb. Above a certain threshold level, the NE breaks, especially at sites that are more fragile, for instance due to lamin depletion. Concurrent loss of compartmentalization leads to exchange of soluble proteins, and also of larger macromolecules and even whole organelles. Shortly after, the ruptured site is resealed and nuclear compartmentalization is restored.

a) NER is caused by mechanical stress and promoted by lamin defects

Unassailably, mechanical stress is an important elicitor of NER. Physical compression by entrapment of cells in between a glass and polymer plate or by single cell indentation has been shown to trigger transient or permanent NER (6, 56). Likewise, cells that migrate in microfluidic channels exhibit NER when their nucleus squeezes through narrow pore openings (25 μm^2 cross-section) (24, 77). The forces that act to induce NER are believed to be, at least in part, conveyed by the actin cytoskeleton, since depletion of actomyosin contractility significantly reduces NER frequency (24, 41, 79) and loss of antagonizing

myosin phosphatases (PPP1R12A and PPP1CB1) promotes NER (91). Compressive rather than pulling forces are considered to be the principal modus operandi of actomyosin-driven NER, as mechanical confinement of the nucleus in cells that have been treated with actin-depolymerizing drugs proved to be sufficient for inducing NER (41). This is further supported by the observation that LINC-complex components are absent from lamin-depleted regions of the NE (41), which are known to be preferential initiation sites of NER (22). However, the involvement of other forms of mechanical stress should not be ruled out. For instance, pulling forces might mediate NERs that occur during the anaphase of cells with long chromatin bridges formed by dicentric chromosomes (62). And, next to actin filaments, microtubules might also contribute to NER propensity. Indeed, much alike actin bundles, microtubule associated motor proteins dynein and kinesin-1 can generate tension on the NE via LINC complexes, whilst pulling the nucleus in opposite directions (90). Moreover, abnormal microtubule bundling has been linked to apoptotic NER events (101), and Remodelin, a compound affecting microtubule organization (55), has been found to reduce NER frequency under conditions of lamin deficiency (79).

In most healthy cells grown in tissue culture, NER frequency is low. However, defects of the nuclear lamina render cells much more prone to NER (6, 45). Indeed, NER occurs more frequently in laminopathy patient fibroblasts than it does in cells from healthy individuals (22), and experimental depletion of A-type or B-type lamins significantly increases NER frequency in a variety of settings (24, 41, 45, 79, 98). It is known that A-type lamin depleted cells grown on glass display much higher nuclear plasticity (21, 52) and at the same time exhibit more pronounced stress fibers and higher traction forces (16), suggesting that these cells are subjected to higher levels of mechanical stress. Moreover, growing laminopathy patient cells on soft substrates abolishes the occurrence of NERs, supporting their increased sensitivity towards cytoskeletal tension (93). The impact on NER susceptibility seems to be

independent of the type of lamin since depletion of lamin B1 raises NER probability to a similar level as depletion of A- and B-type lamins combined, and lamin B2 overexpression rescues the NER phenotype triggered by lamin B1 depletion (97). However, the exact contribution of different lamin types is still unclear. Specific mutant (pre-)lamins may contribute as well, since laminopathy patient fibroblasts with different mutations in the *LMNA* gene demonstrate variable NER frequencies (22). This may point to the presence of more subtle, localized defects in the nuclear lamina meshwork. Indeed, in several laminopathy patient cells, NERs are often preceded by the formation of herniations or blebs in the NE, indicative of local perturbations of the nuclear lamina (22, 24, 97) (Fig. 2b). Fluorescence microscopy has revealed that such fragile sites are devoid of other NE components, such as B-type lamins and NPCs (22, 75). In cells with normal lamin levels, similar blebs arise during confined migration. These blebs originate at the leading edge where the curvature of the NE is highest. Based on a viscoelastic model of the nuclear lamina and chromatin, it has been proposed that holes in the nuclear lamina can lead to outflow of nuclear content, thereby inflating the bleb (25). Thus, local fragility in combination with external forces is sufficient for rupture. Yet, lamin depletion greatly enhances sensitivity to NER events.

b) The NE is rapidly resealed by a dedicated repair machinery

Despite the severe impact on nuclear compartmentalization, cells are able to cope with NER events. Live cell imaging of rupture-prone cells labelled with NLS-tagged fluorescent proteins (BOX 1, FIGURE 1) shows that the nuclear signal recovers to its' original level after NER (22, 79, 97). Moreover, a single cell can undergo repetitive rounds of NER whilst still maintaining division potential (FIGURE 2b). *A fortiori*, it was elegantly demonstrated that *C. elegans* embryos can survive a transient loss of compartmentalization during early embryogenesis (75). This indicates the presence of an efficient molecular repair machinery. Accumulating evidence supports a role herein for the endosomal sorting complex required for

transport (ESCRT-III). This filament-forming complex promotes membrane remodeling (65) and has known functions in plasma membrane resealing and NE restoration (46, 83) after mitosis (72, 98). Knockdown of the ESCRT-III component CHMP4B results in a significant increase in repair half-time (24, 77, 79). Moreover, NE breaks induced by confined migration, compression or laser ablation (FIGURE 4), are followed by the rapid (< 2 min) recruitment of core ESCRT-III components and the AAA+ ATPase VPS4 to the sites of NE damage (24, 77). Based on its canonical role, it can be hypothesized that ESCRT-III subunits assemble into heteropolymeric filaments to form concentric spirals at the inner neck of membrane invaginations, which, fueled by VPS4, eventually bud off to repair the wounded area (1). ESCRT-III components are recruited to NE lesions by the site-specific INM adaptor protein LEM2, an inner nuclear membrane protein (10). Recruitment most likely occurs through a direct interaction between the C-terminal nucleoplasmic domain of LEM2 and CHMP7, an ESCRT-II/ESCRT-III hybrid protein that functions as an early-response factor for recruitment of other ESCRT-III proteins. However, the factors and cellular events that cause the recruitment of CHMP7 by LEM2 have yet to be elucidated. Despite the rapid mobilization of the ESCRT-III machinery, there is a large variation in recovery half times after NER (varying from 5 to 110 minutes) (79). This might due to the varying severity of NER, with larger ruptures, multiple rupture sites or rapidly following rupture events contributing to increased repair times (79). The exact cause however, remains to be determined. It is also not known whether alternative repair mechanisms exist.

Cellular consequences of NER

NERs are accompanied by a temporary loss of nuclear compartmentalization. This has direct repercussions for cellular homeostasis. For instance, it instantly provokes an uncoordinated exchange of nuclear and cytoplasmic components, as has been demonstrated for the

transcription factors Oct-1 and p65 (22), regulatory factor cyclin B (22), and mRNA processing proteins eIF4AIII and UPF1 (97). Although these fluxes are transient and proteins are transported back to their respective locations after the NE is resealed, temporary delocalization may influence gene regulatory programs (22). Furthermore, NERs also cause more persistent translocations of macromolecular complexes such as PML bodies (22, 43) and intact organelles such as mitochondria (22, 97). PML bodies are normally confined to the nuclear volume where they regulate key processes such as DNA replication, transcription, recombination and damage response (51). Thus, mislocalisation of these organelles to the cytoplasm may compromise proper DNA maintenance. Moreover, since PML bodies also function as oxidative stress sensors, their translocation and subsequent degradation could affect cellular redox balance (80). Of note, as lamin A/C depletion reduces general reactive oxygen species (ROS) buffering capacity (74, 88), lamin-deficient cells are already hypersensitive to changes in redox state. Similarly, the relocation of mitochondria to the nuclear interior represents a putative source of ROS that could inflict DNA damage (8).

NERs have also been shown to trigger DNA damage directly. Especially in cells that migrate through confining spaces, NERs were found to result in DNA double strand breaks (24, 77) as evidenced by the presence of ɣ-H2AX or 53BP1 foci (24, 45, 77). It should be noted that the severe nuclear deformation that cells experience during confined migration can already trigger the formation of 53BP1 foci as such (24); a feature that may be promoted by local depletion of DNA repair factors (45). Yet, it is clear that NERs entail a higher risk as the exposure of unprotected DNA to the cytoplasmic environment renders it susceptible to nucleolytic attack. In line with this, the cytoplasmic nuclease TREX1 has been shown to accumulate at cytoplasmically exposed chromatin bridges, and contributes to the generation of DNA damage after NER (62). Irrespective of the causative mechanism, the induction of DNA damage may increase the mutation rate and therefore prime cells for genome instability. After

severe ruptures, local contractions of the nucleus are observed, which are accompanied by chromatin condensation (79). It is uncertain whether this reflects an NER-induced DNA damage response (38) or, rather if it aids with the protection of chromatin from further damage accrual (92). Similarly, shortly after severe NER, lamin A accumulates at rupture sites. These patches of lamin, also referred to as "lamin scars", have been suggested to protect the NE from subsequent rupture, as successive ruptures of the NE take place at different sites (24).

A third potential consequence of NER is the activation of cytosolic DNA sensors (23) due to the sudden exposure of unprotected DNA. This may trigger inflammatory auto-immune responses (12). Supporting this notion, are the recent observations of AIM2 inflammasome activation in macrophages treated with the HIV protease inhibitor Nelfinavir (which blocks lamin A maturation) (26), and activation of the cytosolic DNA-sensing cGAS–STING (cyclic GMP–AMP synthase linked to stimulator of interferon genes) pathway in senescent cells that display cytoplasmic chromatin fragments (28).

Outstanding questions and future directions

a) Pinpointing alternative modulators of NER

Although many cancer cells are typified by altered lamin production (81), a variety of cancer cells without known lamin defects or mutations (according to the COSMIC database (32)) experience spontaneous NERs in the absence of external forces (97). This points to the presence of alternative pathways. Mounting evidence suggests that mechanical forces within the nucleus may have an equally profound effect on NE structure and thus promote NER (FIGURE 3a). One major putative source is chromatin. Chromatin is intimately connected with the nuclear lamina, and it has been shown that condensin II-mediated chromatin compaction can deform the NE during interphase (5). Moreover, trypsin-induced global

decondensation of chromatin results in expansion and even rupture of isolated nuclei (64). However, it remains to be determined if more subtle, local chromatin decondensation might exhibit forces large enough to induce NER as well. It has been found that chromatin forces co-modulate minute shape fluctuations (undulations) of the NE (13). *In vivo*, such forces are counteracted by the cytoskeleton and lamina (13) but it is conceivable that they become more dominant in the presence of a weakened lamina or upon changes in chromatin condensation status. The latter is a characteristic feature of laminopathy patient cells (63). An alternative source of intranuclear force may stem from nuclear actin polymers (3). Unlike cytoskeletal actin, nuclear actin does not generally polymerize into persistent filaments. However, upon cell spreading (76), in response to extracellular cues (*e.g.,* heat shock, serum stimulation) (3), and under certain pathological conditions (81), unconventional cofilin/actin rods and phalloidin-stainable actin filaments have been found to assemble in the nuclear interior of a variety of mammalian cells. Although chromosome and gene repositioning have been shown to be actin polymerization and myosin-dependent (14, 29), and lamins may directly bind polymerizable actin (89), whether actin truly exerts a mechanical force on the NE remains contested.

Conceptually, deregulation of proteins directly involved in NE remodeling (96), and by extension any membrane (de-)forming factor, could also have an influence on NER induction by raising NE curvature or altering its composition (66). For example, targeting torsin to the NE has been found to induce the formation of stacks and swirls of membrane inside the nucleus (36). Likewise, deregulated activity of Phosphocholine cytidylyltransferase (CCT) contributes to the expansion of the nucleoplasmic reticulum by altering lipid integration kinetics (35).

A very recent study revealed that the tumour suppressor genes p53 and Rb inhibit NER in Retinal Pigmented Epithelial (RPE-1) cells (107). Since downregulation of either gene did not

increase cell migration or alter the levels of NE components, these observations may point to yet another, but as of yet unknown, regulatory mechanism for NER (107). Finally, it is also possible that local disassembly of the lamina, *e.g.* by activation of dedicated kinases, can contribute to NE fragility. This notion is supported by the fact that nuclear entry of herpes viruses relies on targeted, local breakdown of the nuclear lamina, through viral protein-induced relocation and activation of protein kinase C (PKC) to the NE (FIGURE 3a) (70, 102).

b) Defining the exact NER repair mechanisms

The exact sequence of molecular events that mediate ESCRT-III assembly at the ruptured site is not yet fully understood. As ESCRT-III also operates at the level of the plasma membrane, there may be commonalities between lesion detection and recruitment mechanisms (83). Plasma membrane injury triggers a Ca^{2+} influx into the cell, which activates the calcium-binding protein apoptosis-linked gene ALG-2, that in turn facilitates the accumulation of the ESCRT-III recruitment factor ALIX to the site of injury (83). Since the perinuclear space (which is contiguous with the ER lumen) and nuclear interior both harbour significant calcium stores (30) that may become released upon NER, local Ca^{2+} sparks could represent a similar trigger for ESCRT-III assembly at the NE. *In extensu*, other calcium-binding molecules with known functions in plasma membrane resealing may become mobilized to the rupture site as well. One such candidate protein is Annexin 11, a calcium- and phospholipid binding protein that normally resides in the nucleoplasm, but relocates to the NE in response to increased intracellular [Ca^{2+}], and during late mitotic NE reformation (94). Moreover, ALG-2, the initiator for plasma membrane repair, has binding sites located in the N-terminus of Annexin 11 (82).

c) Assessing the long-term consequences of NER

Downstream of the NER event there are still many unknowns. For instance, it is not yet certain whether changes in transcription factor localization elicit measurable changes in gene expression and, by extension, cell fate decisions. This requires targeted reporter studies, *e.g.* using fluorescent proteins under control of transcription factor responsive elements. Similarly, the causal connection of NER with redox imbalance has yet to be explored and it has not yet been irrefutably proven that NER-induced DNA damage contributes to genome instability. Indeed, while a variety of factors could promote DNA damage during NER (mechanical damage, exposure to cytoplasmic nucleases and ROS, and depletion of repair factors, as recently compiled in (58, 85)), targeted experiments are required to determine the contribution of these individual factors to the long-term genetic markup of individual cells *in vitro* and *in vivo*.

d) Getting a grip on NER

The stochastic nature and variable frequency of spontaneously occurring NERs precludes investigation of the early downstream events, such as protein recruitment and adjoined protein/ion fluxes, with high spatiotemporal resolution. To bypass this problem, methods can be used to deliberately induce NERs. Several paradigms have been conceived to exert mechanical force onto cells and thereby promote NER. The most direct way is to subject cells grown on flat surfaces to compressive force (FIGURE 4a). A cellular stamp device has been conceived to apply pressure at the single cell level (6), but atomic force microscopy may serve equally well to squeeze the nucleus in a controllable manner (50). More recently, PDMS-based confinement devices have been developed to compress monolayers of cells (41, 56). An alternative approach could consist of stretching cells so as to increase the cytoskeletal tension on the nucleus (FIGURE 4b). This can be accomplished by growing cells on a silicone membrane which can be stretched with even force using a biaxial strain device (11, 53). Although, it should be noted that application of a stretching force has not yet been shown to

induce NER. Constrictive forces on the other hand, have (FIGURE 4c). Initially, NER was induced by constriction of the nucleus by micropipette aspiration (45). In more recent work, it was shown that cells experience NER, when stimulated to migrate through narrow pores, smaller than the diameter of the nucleus (24, 77) (FIGURE 4c). Although all the aforementioned methods offer more control, they still do not allow for the prediction of exactly when and where the rupture will occur. If one wants to investigate the dynamics that occur at the exact site of rupture, a targeted method should be used. Laser microbeam irradiation (or laser ablation) allows the induction of sub-micron pores into the NE in a targeted and non-contact dependent manner (24, 39, 75, 77) (FIGURE 4d). This was recently demonstrated using a two-photon laser (24). However, the downside of this technique is that throughput is very low, and the energy input is high thus creating potential artefacts. In order to increase throughput and decrease energy load, one could resort to nanoparticle-assisted photoporation, which has already proven to be successful at the level of the plasma membrane (104, 105).

Although artificially induced NER may not recapitulate all the features of spontaneous NER, it allows the investigation of the downstream molecular mechanisms independent of the disease context. Indeed, while lamin deficient cells can be used to determine NER kinetics (79), they do not allow for the discrimination between NER-specific effects from lamin-specific effects that are non-NER-dependent, including effects on gene regulation and chromatin organization (1, 37).

NER, a translational perspective

In the past decade, (transient) loss of nuclear compartmentalization has evolved as a defining feature for a variety of pathological conditions. Using NER-prone model cells and devices to mechanically induce NER, significant progress has been and is being made to shed light onto

the molecular mechanisms and functional consequences. As of yet, most available data originate from *in vitro* cell-based experiments. However, first lines of evidence now also point to their occurrence *in vivo*: fibrosarcoma cells that were injected in living mouse tumors experienced NER during migration (24), and NER as a consequence of lamin depletion has been shown to occur in developing *C. elegans* embryos (75). Furthermore, the tissues that are predominantly affected in laminopathies are specifically those that are under high mechanical strain, plausibly rendering their constituent cells more vulnerable to NER (18). In line with this, mitochondria have been detected inside the nucleus of cardiomyocytes from a laminopathy patient heart biopsy, which could represent a remnant of NER (31). These observations raise the clinical relevance of NERs. Moreover, given their pathological promiscuity, targeting NER might offer an avenue for developing novel therapeutic strategies. In the context of cancer, one option would be to exploit NER as a synthetic lethal target. Assuming that NER represents a means to reduce intranuclear pressure during confined migration, blocking this effect may reduce metastatic potential of migrating cancer cells. Alternatively, interfering with NE resealing by selectively blocking NE repair components could allow specific targeting of NER-prone cells. Inhibition of ESCRT-III mediated NE repair does not reduce cell viability as such, but simultaneous inhibition of DNA repair does substantially increase cell death after NER (24), thus providing a potential entry point for combination therapies. Conversely, in laminopathies, NER should be prevented and/or NE resealing promoted. For the latter, candidate compounds may be sought among those enhancing plasma membrane repair such as poloxamer 188 (68, 69) and Pluronic F-68 (86). Finally, the notion that NER transiently increases NE permeability, could also be exploited for selective delivery of large compounds and nucleotides, which would otherwise not be transported into the nucleus – *e.g.* in the context of gene therapy (10).

In conclusion, NER is rapidly emerging as a broad-spectrum hallmark of disease. Long-term follow-up studies in model organisms will allow elucidating the true pathophysiological impact of this phenomenon. Meanwhile, development of advanced *in vitro* techniques for targeted induction and investigation of NER, combined with high-throughput compound screening, will aid with the discovery of novel modulators that may have potent therapeutic value for a wide variety of disorders.

Figures

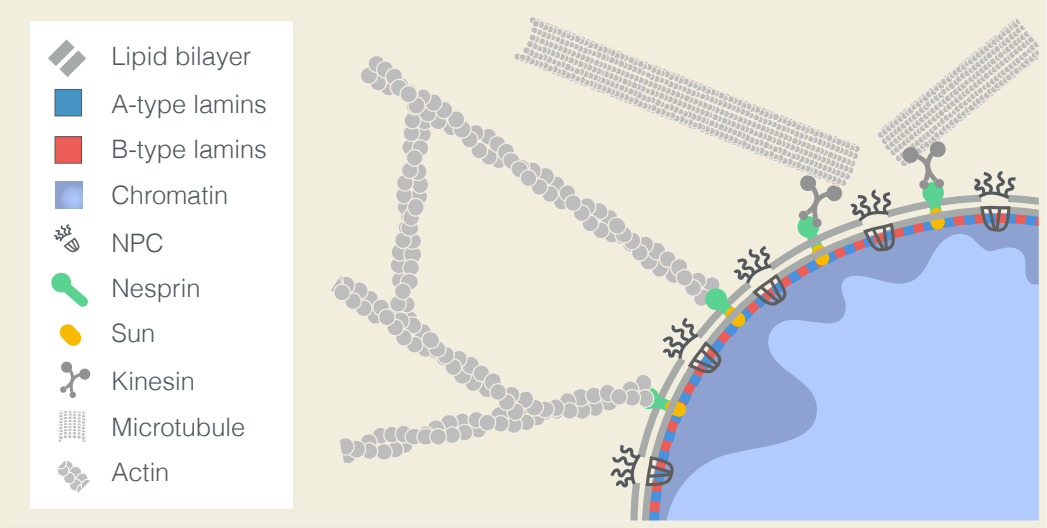

Figure 1. Schematic representation of the major building blocks of the nuclear envelope (Adapted from (78)). The mammalian interphase nucleus is filled with differentially condensed chromatin (light and dark blue) and surrounded by the bilayer nuclear envelope. This bilayer is supported by the nuclear lamina and harbors nuclear pore complexes (NPC). LINC complexes, consisting of interacting Nesprin and Sun proteins, connect the lamina with the cytoskeleton (actin filaments and microtubules).

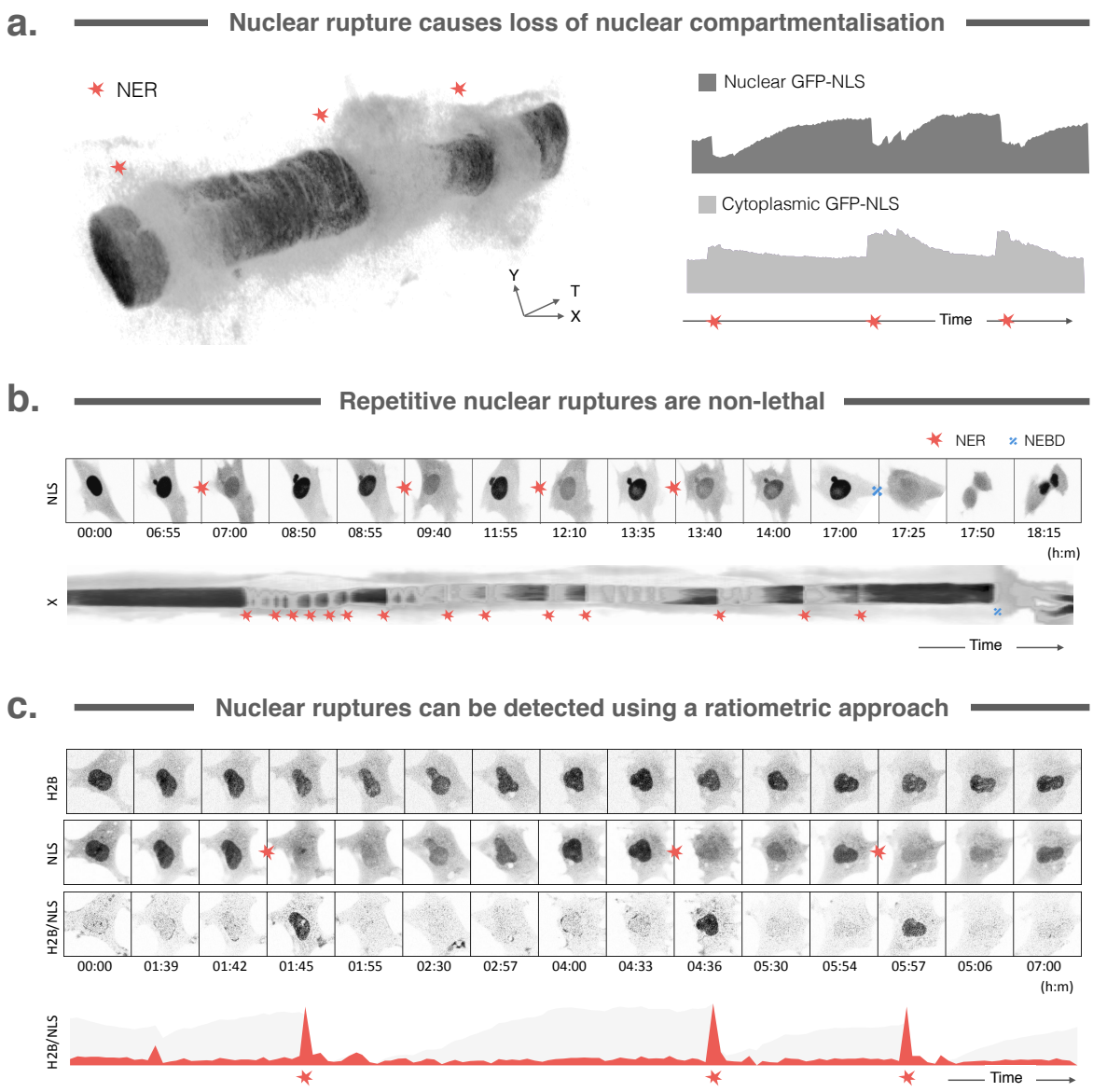

Figure 2. Nuclear Envelope Rupture (NER). a) Left: 3D (XYT) kymograph of an *LMNA* deficient mouse embryonic fibroblast nucleus, expressing a GFP-NLS reporter, showing three consecutive NERs. Right: The transient loss of nuclear compartmentalization can be quantified as a drop in nuclear signal intensity and concurrent increase in cytoplasmic intensity; b) Montage (top) and 2D (XT) kymograph (bottom) Compound progeroid patient cell, expressing a GFP-NLS reporter, undergoing mitosis after experiencing repetitive NER events; c) Robust detection of NER in *LMNA* knockout fibrosarcoma cells using the ratio of

two markers, one that leaks out of the nucleus during NER (NLS-mCherry) and one that does not (H2B-GFP) (Adapted from (79)).

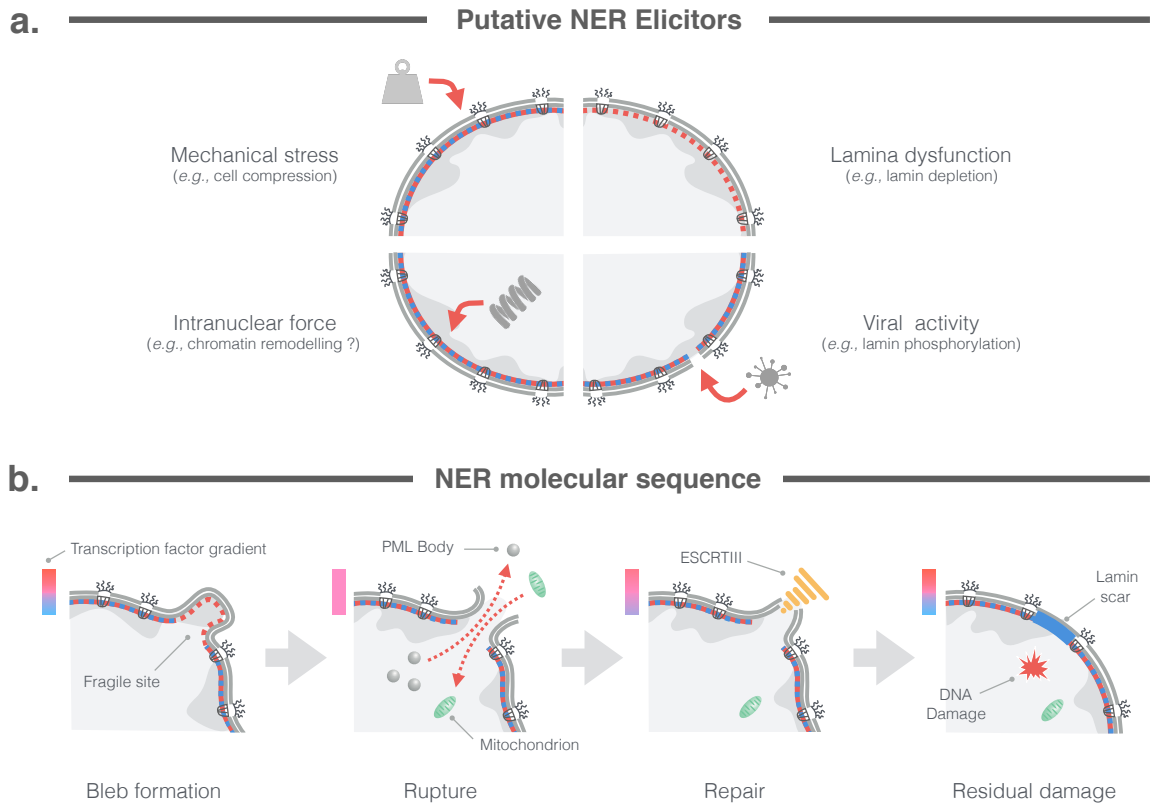

Figure 3. Molecular mechanisms of NER. a) NERs may be triggered by a variety of factors including compressive extra nuclear force, intranuclear force, (local) lamin depletion, or viral activity (causing local NE breakdown); b) remodeling of the NE triggers the formation of fragile sites (often seen as blebs), which rupture. This causes the uncoordinated exchange of soluble molecules (such as transcription factors) and larger complexes such as PML bodies and mitochondria. Rapidly after rupture, the NE is resealed by the ESCRT-III machinery and molecular fluxes are restored. However, NER also leaves more permanent tracks: bigger structures may remain relocated, DNA may be damaged and the site of rupture is often characterized by a lamin scar (Adapted from (78)).

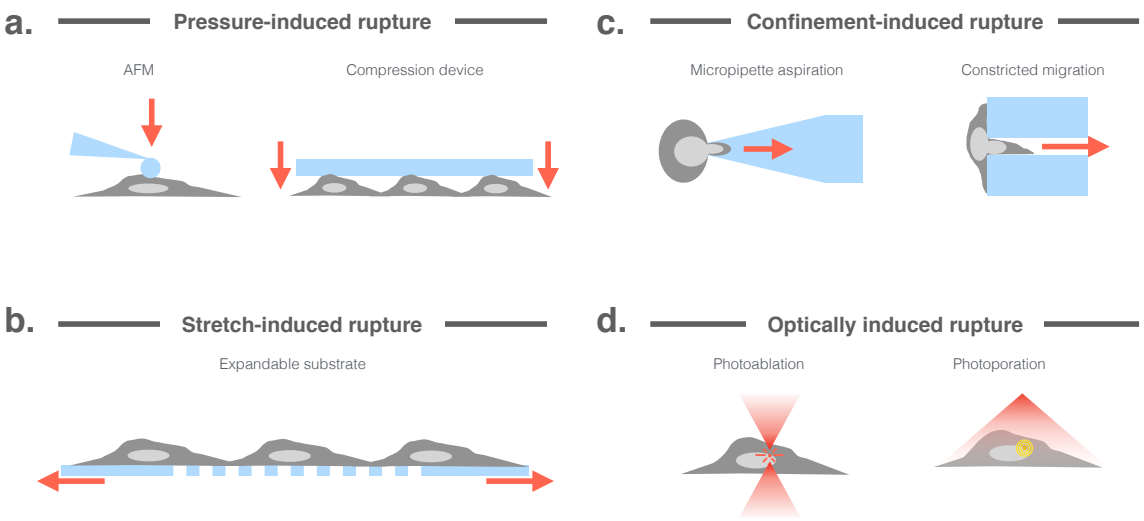

Figure 4. Paradigms for inducing NER through mechanical forces. a) Pressure can be exerted onto the nucleus via an atomic force microscopy probe or using dedicated compression devices; b) Cells can be stretched using expandable substrates; c) The nucleus can be locally confined using micropipette aspiration or by allowing cells to migrate through narrow microchannels; d) The nucleus can be locally perturbed using direct high-energy illumination (photo-ablation) or possibly by induction of vapor nanobubbles around NE-targeted gold nanoparticles.

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