Nuclear envelope rupture: Actin fibers are putting the squeeze on the nucleus

Jan Lammerding[1] and Katarina Wolf[2]

[1]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY 14853
[2]Department of Cell Biology, Radboud University Medical Center, 6525 GA Nijmegen, Netherlands

Cells exhibit transient nuclear envelope ruptures during interphase, but the responsible biophysical processes remain unclear. In this issue, Hatch and Hetzer (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201603053) show that actin fibers constrict the nucleus, causing chromatin protrusions and nuclear membrane ruptures at sites with nuclear lamina defects.

The nuclear envelope (NE) consists of the inner and outer nuclear membranes, nuclear pore complexes, and the nuclear lamina, a dense interdigitating meshwork of lamin proteins composed of A type (mostly lamin A and C) and B type (lamin B1 and B2) lamins that interface with the inner nuclear membrane, nuclear pores, and chromatin. The NE forms a selective barrier separating the nuclear content from the cell's cytoplasm, thereby creating an intranuclear environment that protects the genome and enables highly coordinated processes such as DNA duplication and transcription. Until recently, it had been assumed that NE integrity was essential for cell viability, and that the NE formed a stable structure during interphase. The NE would only disintegrate—in precisely regulated fashion—during mitosis, apoptosis, specialized export of some messenger ribonucleoproteins, or certain viral infections. This was until work by De Vos et al. (2011) and Vargas et al. (2012) showed that cultured cells from patients with lamin mutations and cells derived from tumors exhibit spontaneous and repeated transient loss of NE integrity (called NE ruptures) that could last from a few minutes to tens of minutes. These NE ruptures, which can be visualized by the rapid escape of fluorescent proteins containing a nuclear localization sequence from the nucleus into the cytoplasm, result in the uncontrolled exchange of nuclear and cytoplasmic content, including organelles and nuclear promyelocytic leukemia bodies (De Vos et al., 2011; Vargas et al., 2012). Intriguingly, cells remain viable, even after repeated NE rupture. Recent studies demonstrated that NE ruptures frequently occur during confined cell migration in vitro and in vivo, and that incidence of NE rupture dramatically increases with cell confinement (Denais et al., 2016; Raab et al., 2016). Along with previous work showing that mechanical compression of cells induces NE rupture (Broers et al., 2004; Le Berre et al., 2012), these studies suggest that physical forces acting on the nucleus are responsible for NE ruptures. Nonetheless, the precise biophysical mechanisms underlying the ruptures remain unclear, and, in particular, it is not known whether the nuclear membrane blebs and chromatin protrusions that precede NE rupture arise from cytoskeletal forces pulling on the NE or intranuclear pressure pushing against the NE. This is particularly true for cells in unconfined conditions, in which several cell types exhibit spontaneous NE rupture (De Vos et al., 2011; Vargas et al., 2012; Robijns et al., 2016). In this issue, Hatch and Hetzer address the mechanism underlying NE rupture in cultured cells and identify an important contribution of the actin cytoskeleton in this process.

Hatch and Hetzer (2016) used a previously developed cell line with reduced levels of lamin B1 that is prone to spontaneous NE rupture. They applied selective disruption of cytoskeletal structures and nucleo-cytoskeletal connections, while monitoring cells for NE rupture and chromatin protrusions. The authors show that, in cells cultured on flat, rigid substrates, contractile actin structures that span over and compress the nucleus (Fig. 1, A and B [top left]; Khatau et al., 2009) are sufficient to cause protrusion of chromatin through the nuclear lamina (chromatin hernias) and NE ruptures (Fig. 1 A, inset). Disruption of the actin cytoskeleton by cytochalasin D or latrunculin A or inhibition of myosin contractility with blebbistatin abolished spontaneous NE ruptures (Fig. 1 B, top right; Hatch and Hetzer, 2016). These results are consistent with previous studies that observed fewer NE ruptures after inhibition of myosin or reduction of cytoskeletal tension (Denais et al., 2016; Robijns et al., 2016). To confirm that the nuclear confinement imposed by the apical actin fibers, rather than other actin fiber–associated processes, is responsible for NE ruptures, Hatch and Hetzer (2016) used an innovative approach: they controlled nuclear confinement through a mechanical compression device that enabled them to modify nuclear height independently of the perinuclear actin cytoskeleton (Fig. 1 B, bottom). When cytochalasin D–treated cells were compressed so as to confine their nuclear height to match that of untreated cells, the cells showed NE rupture rates comparable to untreated cells, demonstrating that nuclear confinement alone is sufficient to cause NE rupture and does not require contractile actin fibers. Thus, actomyosin contractility resembles, and complements, external mechanical compression of the nucleus to induce NE rupture. Interestingly, rupture of micronuclei, which are intact or fragmented chromosomes lost from the main nucleus during mitosis, was not affected by actin depolymerization. These findings further support the concept that actomyosin-derived compression induces NE rupture of the large nucleus, whereas loss of NE integrity in the small

Correspondence to Jan Lammerding: jan.lammerding@cornell.edu

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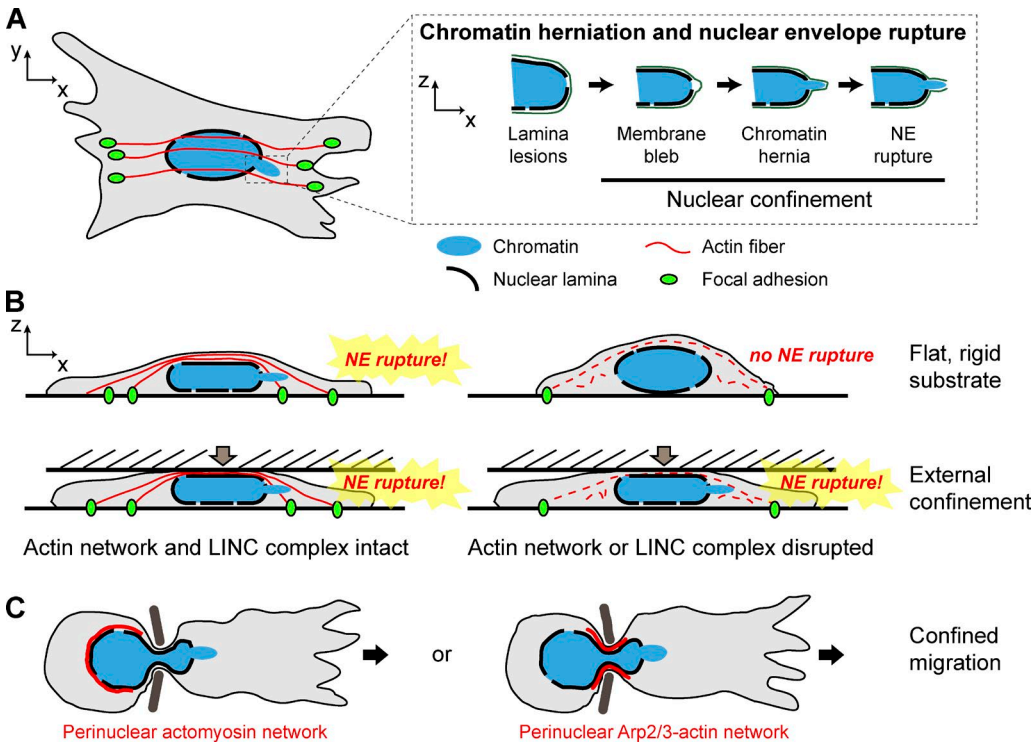

Figure 1. **Nuclear confinement by perinuclear actin network causes chromatin herniation and NE rupture.** (A) In cells cultured on rigid substrates, contractile actin fibers spanning the nucleus compress the nucleus (see also side view in B), causing chromatin herniation and NE rupture. (inset) Sequence of events leading to NE rupture upon nuclear confinement, starting with formation of nuclear membrane blebs at nuclear lamina lesions that progress to chromatin hernias and NE rupture. (B) Side views of untreated cells (left) and cells in which actin organization/contractility or LINC complex function are disrupted (right). In normal cell culture conditions (top), actin or LINC complex disruption releases nuclear confinement and prevents NE rupture. When external confinement is applied through a compression device (bottom), cells exhibit NE rupture regardless of treatment. (C) Nuclear confinement during cell migration through tight openings, as encountered in extracellular matrix networks, extravasation, and interstitial spaces. (left) Perinuclear actomyosin network pushing the nucleus through the pore. (right) Arp 2/3-mediated actin polymerization supports the deformation of the nucleus through the pore. For simplicity, both processes are shown separately, even though they can likely appear in parallel in migrating cells.

micronuclei, which are not confined by the perinuclear actin network, arises from gradual disorganization of the lamin network of the micronucleus (Hatch et al., 2013).

To further confirm that chromatin hernias and NE ruptures are caused by increased intranuclear pressure, and not by the actin cytoskeleton pulling on the NE, Hatch and Hetzer (2016) used elegant molecular approaches to visualize and disrupt nucleo-cytoskeletal connections. These connections are formed by LINC (Linker of nucleoskeleton and cytoskeleton) complex proteins embedded in the NE (Chang et al., 2015). The LINC complex consists of SUN domain–containing proteins that are anchored in the inner nuclear membrane by binding to the nuclear lamina, nuclear pores, and chromatin and that connect across the nuclear lumen to KASH domain proteins, which are located on the outer nuclear membrane. KASH domain proteins bind to actin filaments, microtubules, and intermediate filaments in the cytoplasm (Chang et al., 2015). Hatch and Hetzer (2016) showed that nuclear membrane blebs were devoid of LINC complex proteins, ruling out that these protrusions arose from the cytoskeleton pulling on the nuclear membranes. Nonetheless, disruption of LINC complex proteins by SUN protein knockdown and dominant-negative KASH domain expression reduced the incidence of NE rupture and chromatin protrusions. However, this effect could be explained by the disappearance of apical and perinuclear actin fibers in LINC complex–disrupted cells, which abolishes the normal confinement of the nucleus by the actin cytoskeleton. Accordingly, both LINC complex disruption and actin depolymerization resulted in significant increase in nuclear height (Fig. 1 B, top right). Surprisingly, although SUN1 and SUN2 are often thought of as being redundant in their ability to interact with KASH domain proteins, Hatch and Hetzer (2016) found that depletion of SUN1, but not SUN2, was sufficient to disrupt LINC complex function in U2OS cells, indicating an isoform-specific preference in these cells.

Collectively, the work by Hatch and Hetzer (2016) paints a picture in which actomyosin contractility of apical stress fibers in cells cultured on flat, rigid substrates generates nuclear confinement and increases intranuclear pressure that results in chromatin hernias and NE rupture. Thus, the actin network contractility in those cells mimics the situation of cells during confined migration, where actomyosin contractility or Arp2/3-mediated actin polymerization around the nucleus facilitates squeezing of the nucleus through tight spaces (Fig. 1 C; Thomas et al., 2015; Denais et al., 2016; Thiam et al., 2016). In both scenarios, cytoskeletal forces exerted on the nucleus may increase intranuclear pressure and cause NE ruptures (Denais et al., 2016; Hatch and Hetzer, 2016; Raab et al., 2016; Robijns et al., 2016). Given the abundant presence of nuclear pores, it remains unclear how pressure gradients are established across the NE and persist for tens of minutes. Nonetheless, intracellular pressure measurements provide compelling evidence for pressure build-up across the NE (Petrie et al., 2014), and collapse of nuclear membrane blebs after NE rupture indicates that rupture releases intranuclear pressure (Denais et al., 2016; Raab et al., 2016).

Previous studies of either spontaneous NE rupture (De Vos et al., 2011; Vargas et al., 2012) or NE rupture induced by external cell compression (Broers et al., 2004; Le Berre et al., 2012) or by confined migration (Denais et al., 2016) indicated that nuclear blebs, chromatin protrusions, and ultimately NE rupture occur at preexisting lesions in the nuclear lamina, where the nuclear membrane is insufficiently supported to withstand increasing intranuclear pressure (Fig. 1 A, inset). Hatch and Hetzer (2016) similarly observed that preexisting lesions in the lamin network give rise to chromatin hernias and NE rupture, confirming the importance of the lamin network in stabilizing nuclear membranes. Although their study does not address how nuclear lamina defects arise, this work demonstrates that neither LINC complex disruption nor actomyosin inhibition alter the number of nuclear lamina defects, further supporting the idea that the decreased incidence of NE rupture arises from the reduced nuclear confinement under these conditions. However, these data do not exclude the possibility that, in migrating cells and under external compression, severe nuclear deformation results in the formation of new defects in the nuclear lamina, which could further increase the likelihood of NE rupture.

One small caveat of the current study is that most experiments were performed in a previously published U2OS reporter cell line (Hatch et al., 2013) in which levels of lamin B1 are depleted by 60% to increase the incidence of spontaneous NE rupture. Lamin B1 depletion may not only affect NE organization, including that of other lamins, but may also impact other cellular functions. However, Hatch and Hetzer (2016) confirmed several of their key findings in HeLa cells with unmodified lamin expression, and their observations are consistent with those by other groups using fibroblasts, dendritic cells, and various cancer cells (Le Berre et al., 2012; Denais et al., 2016; Raab et al., 2016; Robijns et al., 2016). Therefore, it is likely that the conclusions reached by Hatch and Hetzer (2016) apply to a broad range of cell types.

Although the work by Hatch and Hetzer (2016) illustrates that intranuclear forces, resulting from nuclear confinement and compression through the actin cytoskeleton, are responsible for chromatin hernias and NE ruptures, their current work does not address the consequences of NE rupture. In micronuclei, NE breakdown causes DNA double strand breaks, which can contribute to extensive genomic rearrangements known as chromothripsis (Hatch et al., 2013). Supporting a role for NE rupture in genomic instability, migration-induced NE rupture can result in DNA damage (Irianto et al., 2015 *Preprint*; Denais et al., 2016; Raab et al., 2016) and formation of nuclear fragments that resemble micronuclei (Denais et al., 2016). Nonetheless, the long-term effects of NE rupture on genomic integrity await to be evaluated, as well as potential effects on chromatin organization and gene expression. Furthermore, the transient nature of NE ruptures indicates that cells are able to rapidly restore NE integrity during interphase. Recent studies identified that NE repair is facilitated through members of the ESCRT (endosomal sorting complexes required for transport) protein family (Denais et al., 2016; Raab et al., 2016; Robijns et al., 2016), but several important questions remain, including how cells detect NE rupture and recruit ESCRT proteins to sites of NE rupture. Similarly, it remains to be tested whether cells that are particularly prone to NE rupture, such as metastatic cancer cells or fast moving dendritic cells (Vargas et al., 2012; Denais et al., 2016; Raab et al., 2016; Robijns et al., 2016), have evolved specific molecular mechanisms to tolerate or overcome NE rupture. Identification of such mechanisms could inform new therapeutic approaches to specifically target metastatic cancer cells. The recent years have seen a rapidly increasing number of publications on NE rupture, demonstrating the prevalence of NE ruptures in numerous cell types and settings, both in vitro and in vivo. Mechanistic studies, such as the current work by Hatch and Hetzer (2016), are crucial to address the cause and consequences of NE ruptures and their relevance in physiological and pathological processes. Ultimately, such studies should be expanded in vivo, where cells and their nuclei may face unique mechanical challenges.

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