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RESEARCH HIGHLIGHT

ESCRT machinery: Damage control at the nuclear membrane

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Rupture of the nuclear envelope (NE) during interphase is thought to be an infrequent event in healthy cells. Two papers recently published in *Science* describe the transient disruption of the NE continuity in cells migrating through confined spaces, and uncover an essential role for the Endosomal Sorting Complex Required for Transport (ESCRT) machinery in the resealing of these nuclear discontinuities.

The nuclear envelope (NE) provides the physical separation between nucleus and cytoplasm, maintaining a protective environment for the genetic material to avoid harmful cytoplasmic components, and a highly efficient barrier to control the exchange of materials between both compartments. An intact NE is fundamental to maintain genomic stability and cell viability, and loss of its integrity has been linked to several diseases including cancer and laminopathies [1].

The current dogma is that the NE is disrupted exclusively during mitosis, but increasing evidence indicates that transient NE ruptures can also occur during interphase [2]. In agreement with this notion, two exciting new studies by Denais *et al.* [3] and Raab *et al.* [4] reveal that the NE continuity is locally and transiently lost during the migration of cells through narrow spaces. The authors develop elegant experimental systems to study the impact on NE integrity of different confining microenvironments like microfluidic devices, collagen-based matrix, tissue explants or living mouse dermis. These experiments reveal crucial details of the sequence of events that result in nuclear rupture, showing that migration through constricted spaces induces the formation and rapid dissipation of blebs at the leading edge of the NE to release the increased hydrostatic pressure in the nucleus as a consequence of its compression. This blebbing is followed by the opening and resealing of the NE. The transient rupture of the NE was confirmed by the presence of gaps in the nuclear lamina accompanied by the influx of fluorescently labeled cytoplasmic proteins into the nucleus and by the accumulation of GFP fused to a nuclear localization signal (NLS) in the cytoplasm. Denais *et al.* [3] also demonstrate the restoration of the NE by the rapid accumulation of GFP-lamin A at the sites of rupture. Interestingly, these patches of lamin or "lamin scars" seem to increase the local resistance of the nuclear lamin, as successive ruptures of the NE occur at different places.

A key finding in both studies is the identification of the mechanism that repairs the damaged NE. This critical role is played by the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, an evolutionarily conserved pathway that resolves topologically unique membrane fission events such as multivesicular body formation, cytokinetic abscission and budding of enveloped viruses [5, 6]. NE rupture by laser ablation or confined migration is followed by the recruitment of ESCRT-III [3, 4], a filament-forming complex that promotes membrane remodeling [6]. In particular, the ESCRT-III subunit CHMP4B is rapidly and transiently recruited to discontinuities in the NE, and the silencing of other ESCRT-III subunits such as CHMP2A and CHMP3 increases the recovery time for nucleocytoplasmic re-compartmentalization after NE rupture. These results are entirely consistent with the role of ESCRT-III in the repair of wounds in the plasma membrane [7]. Moreover, recent work has established the critical role of the ESCRT machinery in the post-mitotic resealing of the NE [8, 9], thus implying a general role of the ESCRT complexes in maintaining the nuclear integrity. These unexpected findings open the question whether the ESCRT machinery could play a role in the progression of pathologies in which the propensity of spontaneous rupture of the NE is increased, such as cancer and laminopathies [2, 10]. For example, the results from Denais *et al.* and Raab *et al.* suggest that defective ESCRT components could affect the capacity of cancer cells to invade new tissues. It is also possible that ESCRT defects could result in the damage of immune cells that migrate through epithelial barriers, perhaps preventing the establishment of robust immune responses (Figure 1).

Critically, the diffusion of cytoplasmic factors into the nucleus during its transient rupture is accompanied by the induction of DNA damage, as cells that had passed through a constriction showed an increased number of 53BP1 foci. Raab *et al.* [4] show that the endogenous DNA-repairing machinery efficiently corrects the constriction-induced DNA damage, as 53BP1 foci disappear shortly after cells exit the

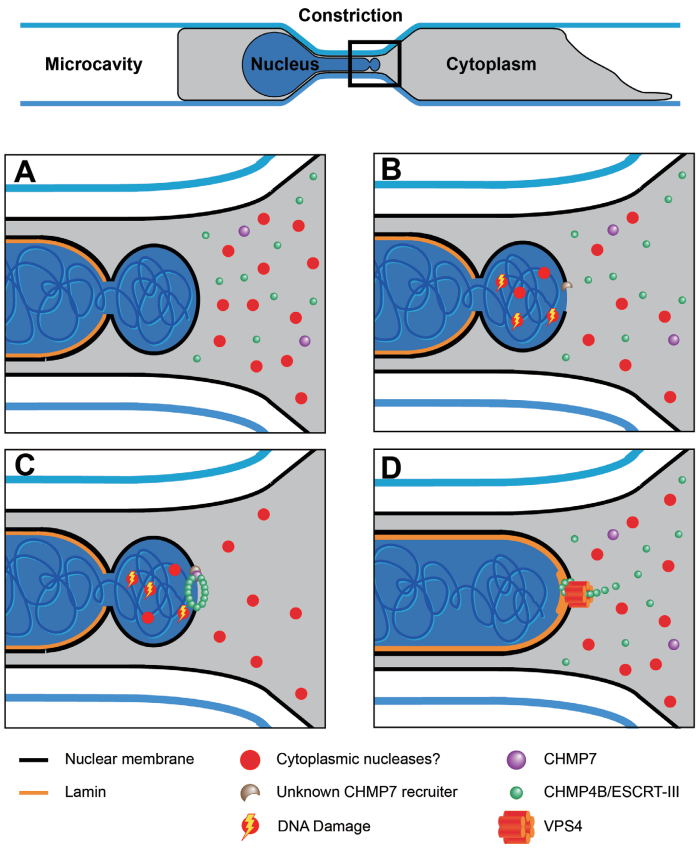

Figure 1 The ESCRT machinery repairs the constriction-dependent ruptures of the NE. **(A)** The compression of the nucleus induces the formation of blebs at the leading edge of the NE. **(B**, **C)** The rupture of the NE allows the entry of cytoplasmic nucleases into the nucleus, inducing DNA damage **(B)**, and triggers the recruitment of the ESCRT machinery by an as yet unidentified adapter **(C)**. **(D)**The DNA is repaired by the endogenous DNA-repairing machinery and finally, VPS4 promotes the cytoplasmic recycling of the ESCRT subunits, sealing the discontinuities in the NE.

constrictions. These observations raise the key question of how DNA damage occurs after NE rupture, and how the DNA repair machinery senses this damage. Although Raab *et al.* consider a role for the mechanical constrains exerted on the DNA in the generation of genetic damage, the accumulation of 53BP1 foci at nuclear openings suggests that the double-strand breaks in the DNA are a consequence of the entry of cytoplasmic factors into the nucleus. Intriguingly, the transient rupture of the NE in cells connected by chromatin bridges allows the entry of the cytoplasmic exonuclease TREX1 which subsequently damages the genome [11], suggesting that similar DNA damage mechanisms might operate in cells that migrate through confined spaces.

Interestingly, interfering with either the ESCRT-dependent NE repair or the DNA damage repair machinery separately has no effect on cell viability, although the simultaneous inhibition of both pathways significantly increases cell death after NE rupture [3, 4]. These results indicate that cells can cope with prolonged openings in the NE and suggest potential connections between ESCRT and DNA repair machineries. It is tempting to speculate that ESCRT-associated factors might recognize damaged DNA at the ruptured NE as one of the events that triggers ESCRT-III recruitment and the subsequent membrane repair process.

Lastly, the ESCRT machinery operates as a modular system that is recruited to different cellular locations by specific adaptor proteins [5, 6]. For example, ESCRT-0 recruits downstream components to the limiting membrane of multivesicular endosomes in order to sort endosomal cargo into intraluminal vesicles, whereas centrosome-associated protein 55 (CEP55) recruits the ESCRT components Tsg101 and Alix to the midbody in the cytokinesis context. However, the adaptors involved in the recruitment of the ESCRT machinery to the nuclear membrane are currently unknown. The ESCRT component CHMP7 may play a role in this process as one of the main recruiters of ESCRT-III polymers on the NE [8, 9], although other adaptor complexes are likely to be involved. Thus, future work will be required to uncover the cascade of events that promote rapid assembly of ESCRT polymers on the ruptured NE.

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