SPOTLIGHT

Loss of nuclear envelope integrity? No probLEM—BAF has it covered

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Ruptures of the nuclear envelope can drive a catastrophic loss of nucleocytoplasmic compartmentalization. In this issue, Halfmann et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201901116) describe a mechanism for surveilling the integrity of the nuclear barrier and coupling the sensing of nuclear ruptures to the recruitment of the nuclear envelope repair machinery.

The intermixing of cytosolic and nuclear contents is normal in the context of open mitosis. By contrast, nuclear envelope ruptures leading to loss of nuclear compartmentalization during interphase are thought to be pathological. Sealing of the nuclear envelope at mitotic exit involves a highly orchestrated program to reestablish compartmentalization that could, in principle, be exclusively tied to cell cycle progression. During interphase, ruptures of the nuclear envelope can also be sealed. In this context, however, one must in addition invoke the existence of a surveillance mechanism capable of recognizing such breaches of the nuclear barrier and targeting them for subsequent repair. In this issue, Halfmann et al. (1) provide new evidence for a functional relationship between the factors that promote nuclear envelope reformation at mitotic exit and the repair of interphase ruptures.

Several recent studies have identified components of the machinery required for nuclear envelope sealing, including the endosomal sorting complexes required for transport (ESCRT; 2–5), integral membrane proteins of the LAP2-emerin-MAN1 (LEM) family (2, 5), and, as shown by Halfmann et al. (1), a small protein called barrier-to-autointegration factor (BAF; Fig. 1). BAF is necessary for the reformation of the nuclear envelope at the end of mitosis, as it coats chromatin in a network that provides a surface for membrane expansion (6). Halfmann et al. (1) investigate its role in surveilling and repairing mechanically induced nuclear envelope ruptures during interphase. In doing so, they not only demonstrate

a key role for BAF in nuclear envelope repair, but also increase the repertoire of relevant LEM proteins acting in the repair pathway(s). These new insights further support a close mechanistic relationship between how nuclear ruptures are repaired and how the nuclear envelope reassembles at the end of mitosis.

Using a variety of mechanical perturbations, Halfmann et al. (1) were able to induce nuclear envelope ruptures with high spatiotemporal control, which they find rapidly recruit BAF. The authors first addressed whether the pool of BAF that localized to rupture sites was derived from a nuclear or cytosolic pool. At steady state, BAF is present predominantly in the nucleus associated with the inner nuclear membrane. Interestingly, however, using photobleaching approaches, the authors discovered that it is the cytoplasmic pool of BAF that is specifically recruited to the rupture site. How is this controlled? Although DNA binding appears important for association of BAF with the rupture, it is also critical that BAF is in a dephosphorylated form; as nuclear BAF is phosphorylated, this pinpoints the cytoplasmic pool as the key factor that senses the exposure of genomic DNA under conditions of nuclear rupture (Fig. 1).

With such a critical role in surveilling the nuclear envelope barrier, Halfmann et al. next turn to investigating the functional contribution of BAF to nuclear envelope repair. Recognizing that loss of BAF could predispose nuclei to more frequent and/or larger nuclear ruptures due to mechanical contributions of the BAF network to nuclear integrity, the authors carefully

dissected the rupture and repair characteristics. Using loss of A-type lamins as a control for a predisposition toward larger nuclear ruptures independent of a repair defect, Halfmann et al. demonstrate that loss of BAF leads to a far stronger effect on repair kinetics, with essentially a complete failure to reconstitute the nuclear–cytoplasmic barrier even 3 h after rupture induced by cell migration through small constrictions. These observations support a critical role for BAF in nuclear envelope repair and imply that it likely acts upstream of LEMD2 and the subsequent recruitment of the ESCRT machinery to rupture sites.

Consistent with the idea that BAF acts upstream of LEMD2, a mutation that abrogates binding of BAF to (at least some) LEM domain proteins does not compromise its recruitment to nuclear ruptures. As BAF binds to the LEM domain, a repertoire of LEM domain proteins including LEMD2, emerin, MAN1, and Ankle2 (but surprisingly not LAP2β) were all recruited to sites of nuclear rupture. Perhaps most interestingly, despite the observation that LEMD2 was required for the specific recruitment of the ESCRT II–ESCRT III adapter CHMP7 to the rupture sites, the knockdown of LEMD2 and CHMP7 only delayed the kinetics of, but did not abolish, nuclear envelope repair. In contrast, knockdown of BAF drives a comparatively strong repair defect. These results evoke direct comparisons with prior studies in which the contribution of the ESCRT machinery to nuclear envelope repair is primarily kinetic, with the nuclear barrier ultimately being reestablished after rupture despite a delay (7, 8). Collectively,

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1. Surveillance of nuclear integrity
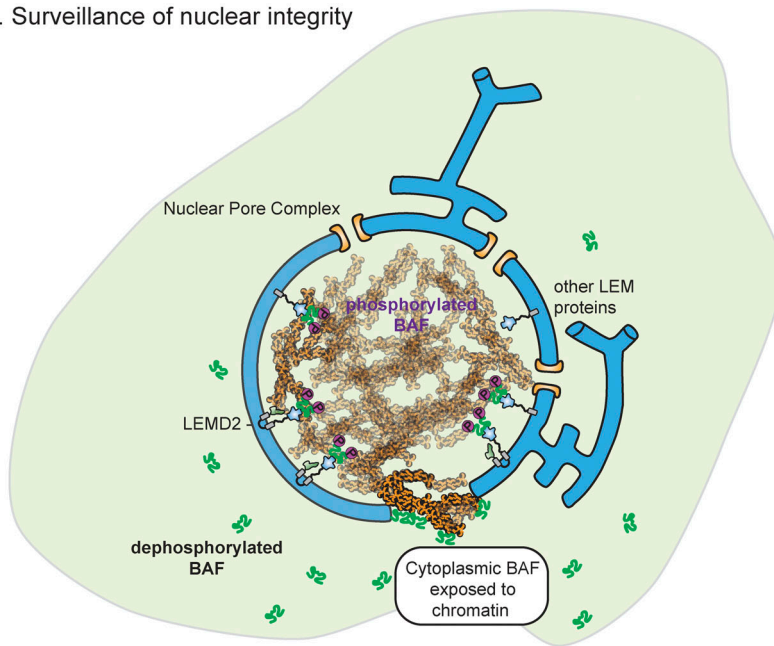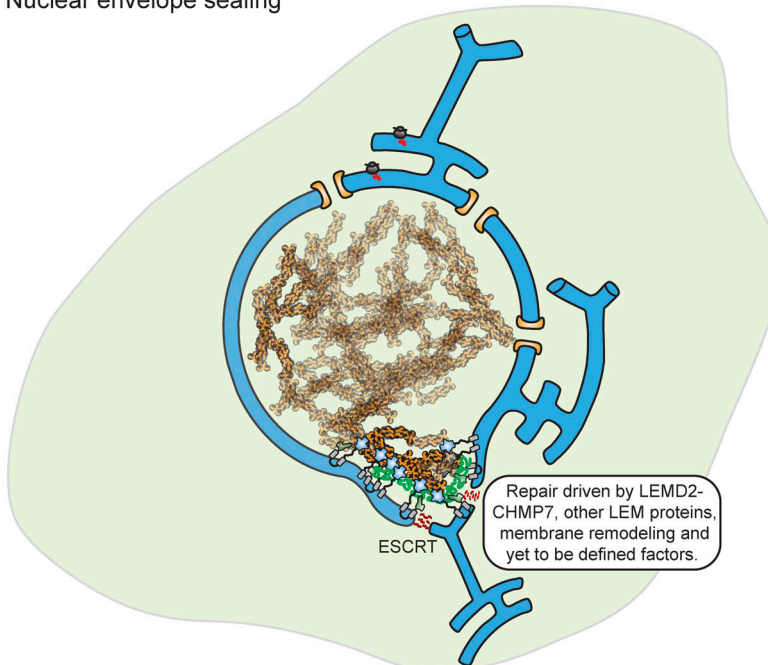

2. Nuclear envelope sealing

Figure 1. **Nuclear envelope repair requires two phases, surveillance and nuclear envelope sealing.** Loss of nuclear integrity is surveilled by BAF, which is only exposed to chromatin in its dephosphorylated form at sites of nuclear envelope rupture. BAF also promotes nuclear envelope sealing by locally recruiting LEM proteins, including LEMD2, which is an adapter for engaging CHMP7 and additional ESCRT components.

then, these studies support that nuclear envelope repair likely requires many additional factors that act in concert with the LEMD2-CHMP7 machinery. Consistent with this idea, Halfmann et al. ([1]) find that co-depletion of the LEM domain proteins emerin and Ankle2 along with LEMD2 leads to a dramatic repair defect that phenocopies BAF depletion. These results make

the exciting prediction that emerin and/or Ankle2 also contributes to nuclear envelope repair through a CHMP7-independent mechanism. Thus, the discovery that loss of BAF leads to essentially irreparable nuclear ruptures will provide new avenues for discovering the mechanisms involving these other LEM domain proteins and associated, yet to be defined, factors.

An emerging theme is that maintaining the nuclear envelope barrier involves two separable mechanistic steps in mammalian cells: surveillance and repair. This work clearly supports a model in which cytosolic, dephosphorylated BAF is a major factor that directly surveils the exposure of genomic DNA to the cytosol, which in turn recruits the nuclear envelope repair machinery

consisting of the LEM proteins, ESCRTs, and likely other molecules. Interestingly, budding yeast, which undergo closed mitosis, lack a BAF orthologue. Instead, both surveillance and repair are achieved by the combined activities of the LEMD2 orthologue (Heh1) and CHMP7 (Chm7), which are spatially segregated by a functional nuclear envelope barrier, preventing them from coming together to stimulate repair unless there is a failure of compartmentalization (9). In the open mitosis of human cells, surveillance and repair appear to have been uncoupled, with surveillance requiring BAF upstream of the role for LEMD2 and CHMP7 in repair.

Much work will be required to define how repair is orchestrated. It is important to consider that in this study (as in many others), nuclear repair is monitored by the reestablishment of the normal nuclear accumulation of an NLS-GFP reporter. Lacking ultrastructure of these repair sites, it is plausible that a protein-mediated mechanism establishes a diffusion barrier at the rupture site before the actual sealing of the inner and outer nuclear membranes. BAF, which forms a complex oligomeric structure that coats the chromosomes at mitotic exit

(6), could contribute to such a barrier to safeguard nuclear–cytoplasmic compartmentalization while the (perhaps slow) process of membrane repair takes place. The application of modern cryo-electron tomography approaches will likely be an enabling technology to help answer these questions. Another key question is whether the addition of new membrane to the nuclear envelope contributes to repair and, if so, whether this membrane flows from preexisting connections between the ER and the nuclear envelope (which, after all, is a single-membrane system), is derived from recruitment of cytoplasmic ER to the rupture site, and/or is synthesized de novo. Indeed, Halfmann et al. (1) provide evidence that ER recruitment likely contributes, but more studies will be required to fully define this aspect of the membrane remodeling pathway.

Lastly, what are the consequences if nuclear envelope repair fails? One theme that has reemerged is crosstalk between the innate immune surveillance pathways and nuclear integrity. Indeed, BAF was first characterized as a binding factor for cytoplasmic viral DNA (10), akin to the innate immune factor cGAS, which is also recruited

to nuclear rupture sites. It will be exciting to see how this new context for fundamental nuclear envelope biology brings about new discoveries in the future.

1. Halfmann, C.T., et al. 2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201901116
2. Gu, M., et al. 2017. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.1613916114
3. Olmos, Y., et al. 2015. *Nature.* https://doi.org/10.1038/nature14503
4. Vietri, M., et al. 2015. *Nature.* https://doi.org/10.1038/nature14408
5. Webster, B.M., et al. 2016. *EMBO J.* https://doi.org/10.15252/embj.201694574
6. Samwer, M., et al. 2017. *Cell.* https://doi.org/10.1016/j.cell.2017.07.038
7. Denais, C.M., et al. 2016. *Science.* https://doi.org/10.1126/science.aad7297
8. Raab, M., et al. 2016. *Science.* https://doi.org/10.1126/science.aad7611
9. Thaller, D.J., et al. 2019. *eLife.* https://doi.org/10.7554/eLife.45284
10. Lee, M.S., and R. Craigie. 1998. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.95.4.1528