# A-type lamins have perturbed targeting to nuclear ruptures in nuclear envelope-associated progerias

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## Abstract

Mutations in three genes encoding nuclear envelope (NE) proteins, *LMNA, BANF1*, and *LEMD2*, result in accelerated aging syndromes known as progerias. These proteins interact with each other to accomplish various cellular processes. One of these processes is the repair of interphase nuclear ruptures to ensure the efficient reconstitution of the nuclear-cytoplasmic barrier. Previously, we have shown that barrier-to-autointegration factor (BAF) localizes to sites of nuclear rupture and is required for recruiting NE-repair machinery, including the LEM-domain proteins, ESCRT-III complex, and membranes. Here, we show that a mobile, nucleoplasmic population of A-type lamins is recruited to ruptures in a BAF-dependent manner via BAF's association with the Ig-like domain.. Multiple progeria-associated lamin A (LaA) mutations inhibit its recruitment to nuclear ruptures. The permanently farnesylated progeric LaA-Δ50 does not accumulate at ruptures, likely due to decreased mobility. The progeric LaA-K542N mutation, structurally predicted to disrupt its association with BAF, also did not localize to nuclear ruptures despite appearing to have normally mobility. Furthermore, a progeria-associated BAF mutant targets to nuclear ruptures; however, it is unable to recruit A-type lamins Together, these data support a model where defective localization of A-type lamins at nuclear ruptures could be a shared mechanism of NE-associated progeria, perhaps by enhancing the propensity for nuclear rupture and/or by compromising nuclear rupture repair which contribute to progeria phenotypes, including increased DNA damage and cellular senescence. Ongoing studies are assessing the functional consequences of progeria mutations in BAF and A-type lamins during the nuclear rupture and repair process.

## Nuclear Envelope and Nuclear Rupture

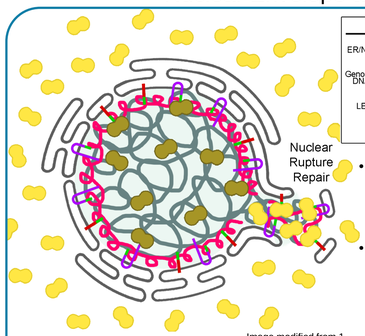
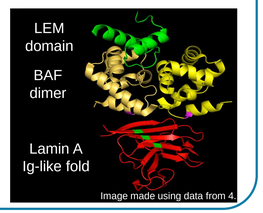



Image modified from 1.

- Nuclear rupture occurs when the nuclear envelope integrity and nucleocytoplasmic compartmentalization are compromised.
- During rupture, BAF binds to exposed genomic DNA in the cytoplasm and is responsible for recruitment of several rupture-responsive proteins.[1,2]
- Under normal conditions, the rupture is resealed in order to stop the unrestricted exchange of nuclear and cytoplasmic constituents.

- BAF dimers can bridge LEM-domain proteins and A-type lamins at the nuclear envelope[4].
- Interestedly, each of these proteins localize at nuclear ruptures[2,3,5].
- Furthermore, mutations in these proteins can cause NE-associated progeria[6-8].
  - Recessive mutations in Lamin A (LaA) (green) and BAF (purple) exist in the BAF-Lamin binding interface.

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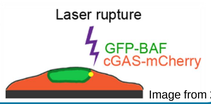
## Experimental Methods

- BJ-5ta hTERT-immortalized human fibroblast cells were utilized for GFP-tagged exogenous protein experiments as well as for BAF depletion experiments.
  - Also used for progeric BAF experiments
- Use of NIH3T3 mouse fibroblasts allowed for endogenous mouse LaA to be depleted in cells expressing exogenous human LaA.
- For depletion of proteins of interest, small interfering RNA (siRNA) was added to cells for 72-120 hours.
- To produce concise ruptures, we exposed a small ROI on the nuclear envelope of live cells to a 405 nm laser for 8-10 sec
  - Secondary rupture markers used for each experiment
- All scale bars are 10 μm.

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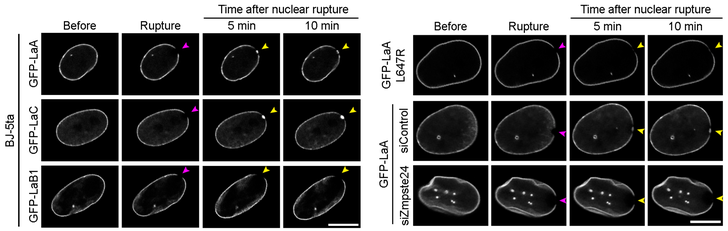
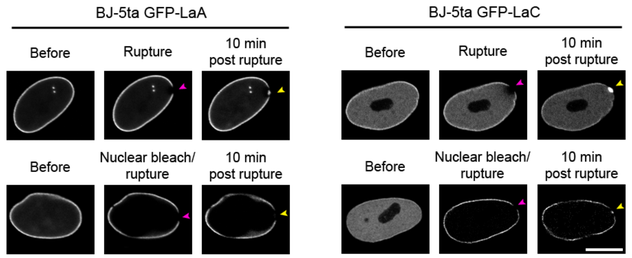
## Lamin Targeting to Nuclear Ruptures

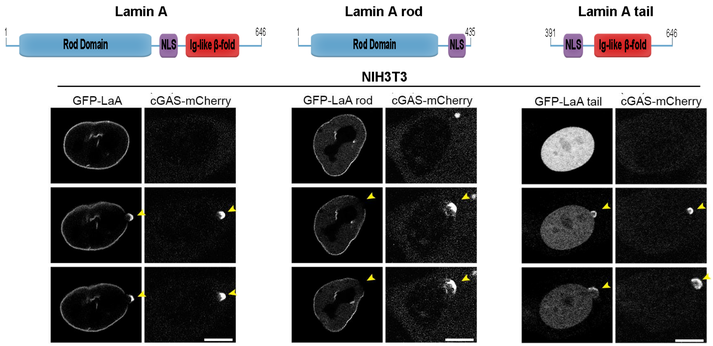
- **Mature A-type lamins accumulate at nuclear ruptures, but not farnesylated lamins**



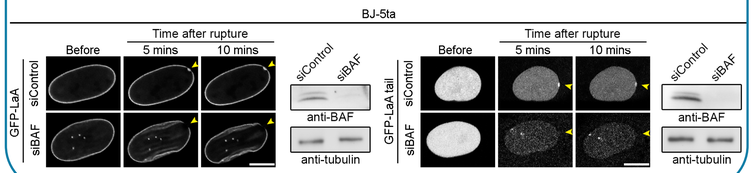

- **A mobile population of A-type lamins accumulates at nuclear ruptures**




- **The tail domain of LaA is responsible for its recruitment to nuclear ruptures**

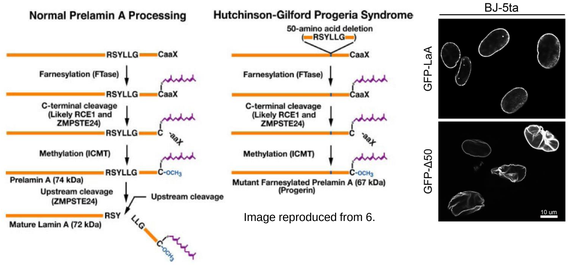



- **Lamin A tail domain recruitment to nuclear ruptures is BAF-dependent**




## Hutchinson-Gilford Progeria Syndrome (HGPS)

- Symptoms appear ~18 months of age
  - Common aging defects, subcutaneous fat loss, skeletal defects, atherosclerosis
  - Abnormal shaped nuclei, DNA damage, senescence
- Average age at death ~15 yr
  - No cognitive defects
  - Usually succumb to atherosclerosis
- Caused by mutations in *LMNA* encoding the A-type lamins[6]
  - ~90% of cases caused by autosomal dominant splicing defect
- More rare recessive mutations of residues within the Ig-fold of LaA exist at the predicted BAF-Lamin binding interface
  - K542N is a homozygous recessive HGPS mutation

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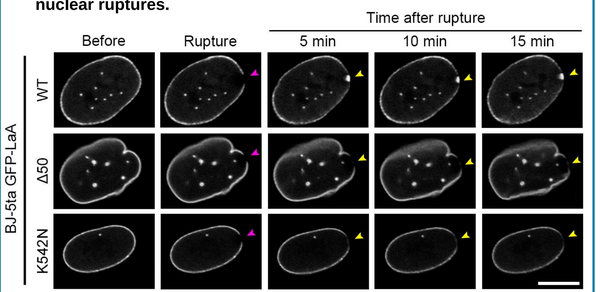
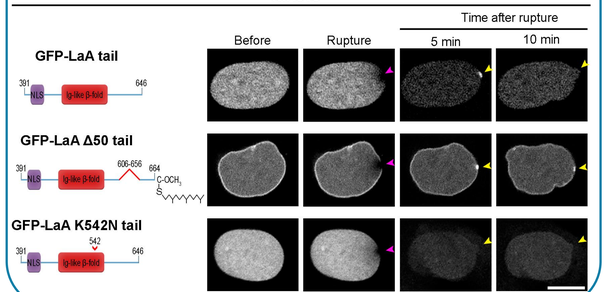
- **Progeric LaA (LaA-Δ50 and LaA-K542N) fails to properly localize to nuclear ruptures.**

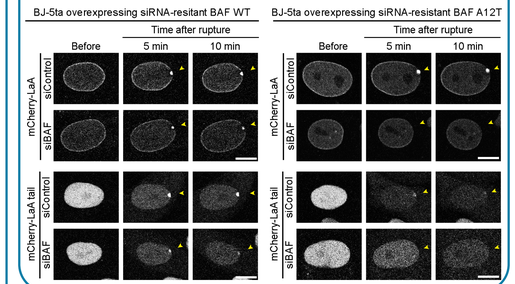



- **The LaA-Δ50 tail is more envelope-associated than the WT tail but is recruited to nuclear rupture sites.**
- **The LaA-K542N tail, however, is not recruited to nuclear ruptures.**



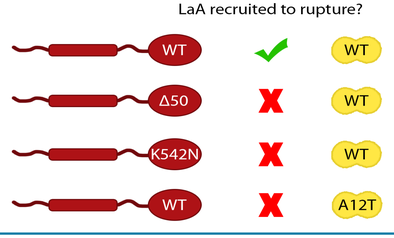

## Progeric BAF and Lamin Recruitment

- A homozygous recessive BAF A12T point mutation results in Nestor-Guillermo Progeria Syndrome (NGPS)[7].
  - Mutation located in the predicted BAF-Lamin binding interface
- **Progeric BAF is unable to recruit full-length LaA or the LaA tail to nuclear ruptures**




## Progeric Proteins During Nuclear Rupture

- **A-type lamins are recruited to nuclear ruptures via the tail domain in a BAF-dependent manner**
- **Progeric mutations in LaA and BAF disrupt LaA recruitment to nuclear ruptures**




## Future Directions

- Evaluate nuclear leakage and rupture repair of cells expressing progeric NE proteins
- Determine how presence of progeric NE proteins effect other nuclear rupture repair proteins during rupture

## References

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8. Marbach et al., 2019, *Am J of Human Gent.* "The Discovery of a LEMD2-Associated Nuclear Envelopathy with Early Progeroid Appearance Suggests Advanced Applications for AI-Driven Facial Phenotyping."

## Acknowledgments

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