Mechanical Nuclear Integrity as a Regulator of PKA/Phosphatase Signalling Homeostasis

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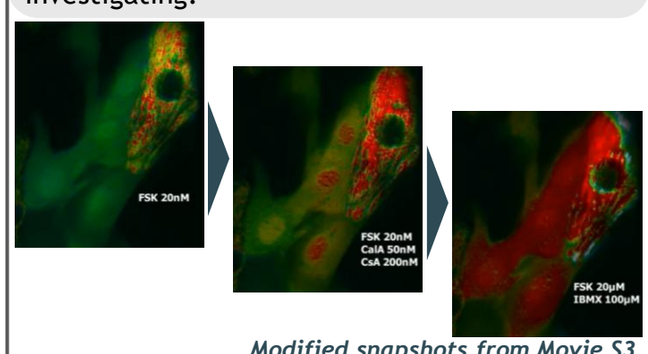
Abstract

Compartmentalization is a fundamental feature of cell organization that allows biochemical processes to occur in distinct spatial domains and temporal scales. The nuclear envelope (NE), by acting as a gatekeeper for molecules and reactions between the cytosol and the nucleoplasm, is a key determinant of cell compartmentalization. Among the plethora of functions, the nucleus also spatiotemporally regulates signalling machineries, thanks to the synergistic actions of the nuclear pore complex (NPC), the Lamin scaffold, and the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. However, nuclear integrity is reported to be altered in pathophysiological conditions, such as ageing and nuclear envelopathies and laminopathies. In this context, despite many studies revealing how NE alterations affect chromatin organization and mechanotransduction, how structural NE defects are translated into dynamic nuclear dysfunction remains unclear. Here, we propose that nuclear PKA/phosphatase signalling homeostasis represents an additional, underappreciated molecular mechanism in the pathogenesis of disorders related to the alteration of nuclear integrity. By combining biochemical approaches with advanced live-cell imaging performed with novel FRET-based biosensors, we identified a unique nuclear signalling domain in which Protein Kinase A activity is strongly restrained by local phosphatases. Interestingly, we observed that nuclear phosphatase pressure is conserved in electroporated *ex vivo* mouse skeletal muscle fibers but is lost in aged muscles. An artificial disruption of nuclear PKA/phosphatase equilibrium in favour of the kinase dramatically altered nuclear shape and homeostasis. Therefore, given the central role of Lamin A/C in nuclear mechanics, we next overexpressed LMNA mutants and observed signalling signatures resembling those of old mouse skeletal muscle, suggesting that the nuclear kinase/phosphatase balance may be an underappreciated disease contributor.

Background

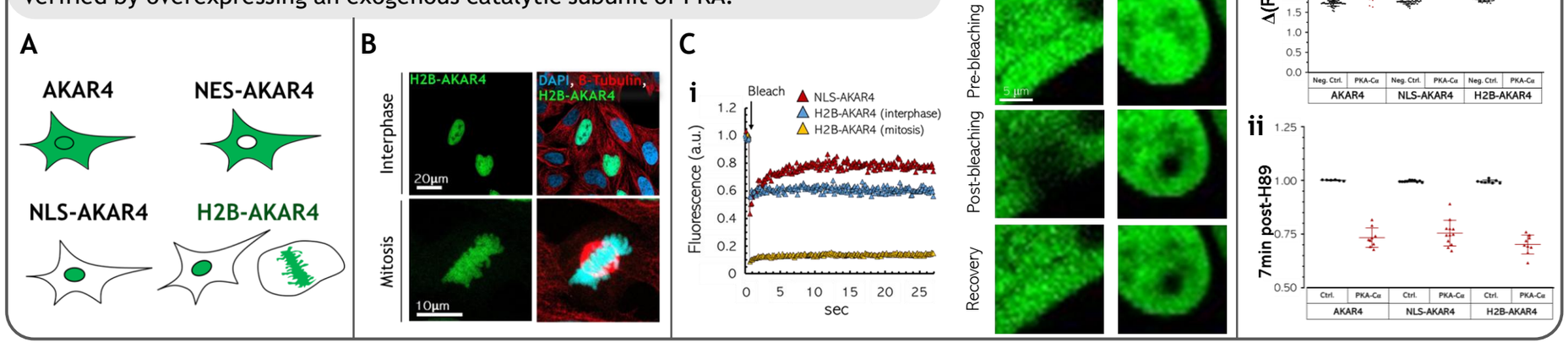
The use of the PKA-dependent FRET sensor AKAR4, previously used to monitor subcellular PKA-dependent phosphorylation, highlighted the nucleus as a compartment worth investigating.



Modified snapshots from Movie S3 (Burdyga, A. et al., 2018)

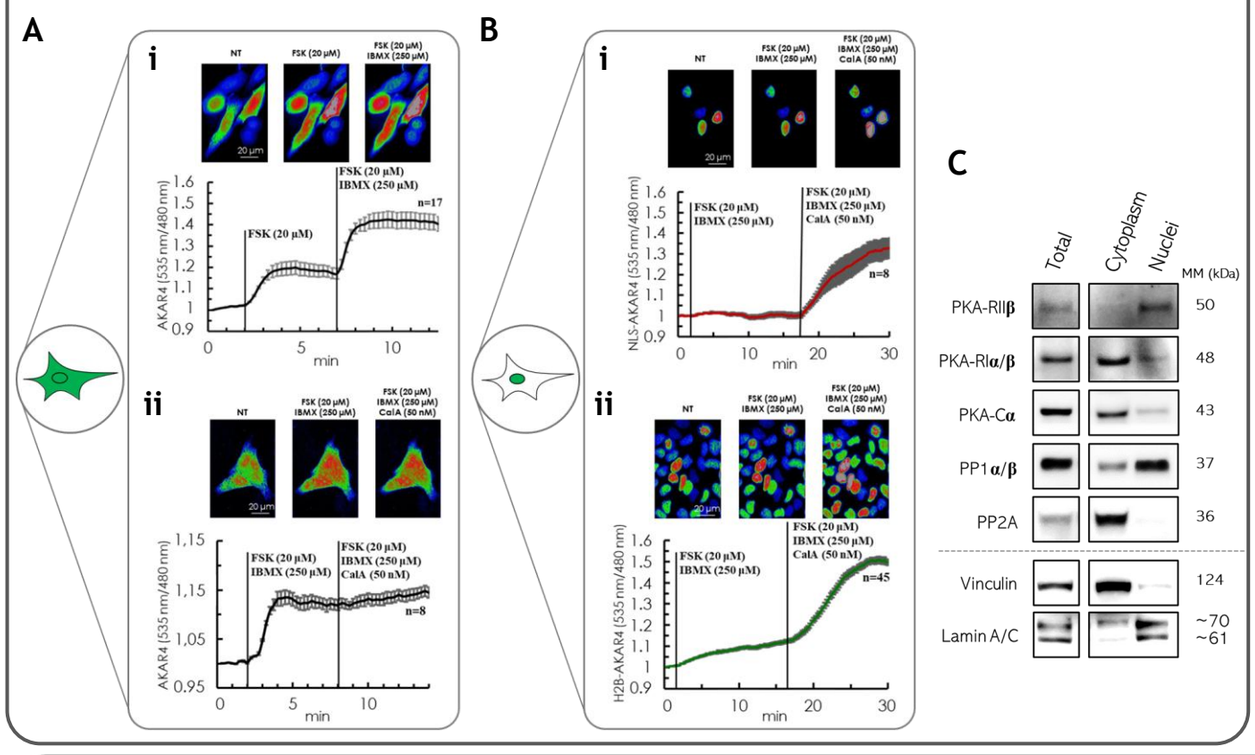
Validation of a novel nuclear FRET sensor: H2B-AKAR4

(**A**) H2B-AKAR4 was developed for this study by merging the Histone Protein H2B with the soluble AKAR4 sensor. (**B**) Confocal microscopy confirmed the correct localization of H2B-AKAR4. (**C**) FRAP experiments confirmed that H2B-AKAR4 diffusion was impeded by its targeting to histones. (**D**) Proper sensor function was verified by overexpressing an exogenous catalytic subunit of PKA.

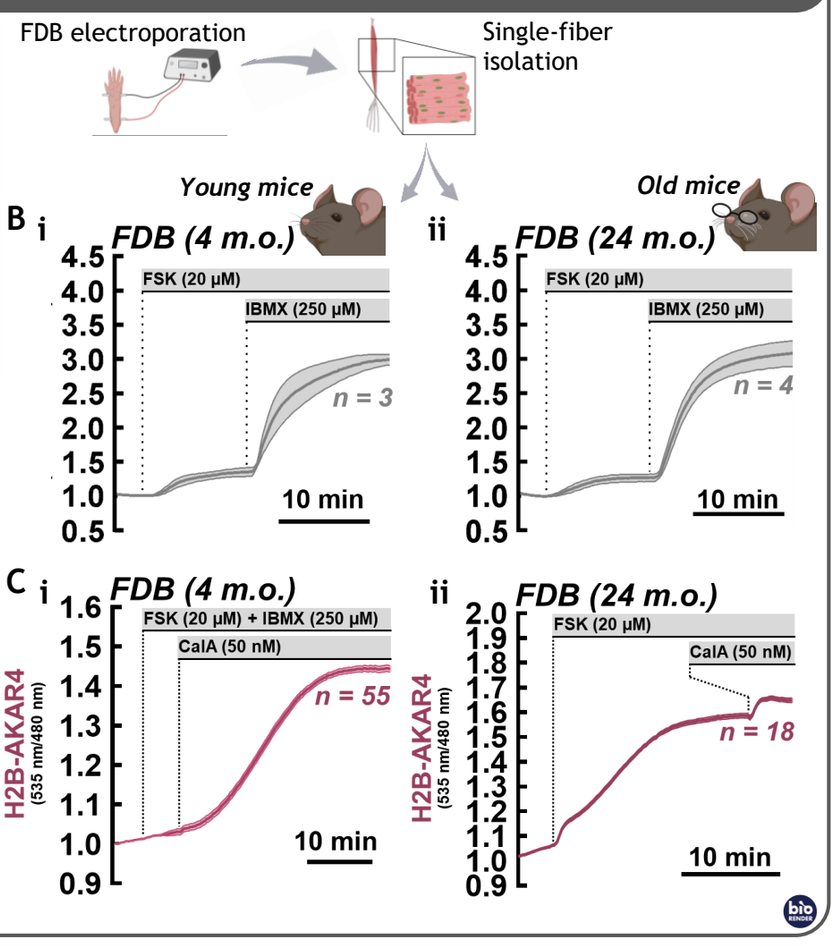


Phosphatases restrain nuclear PKA activity

(A_i) An increase in cAMP levels determines FRET responses in the cytosol, measured by AKAR4 (A_{ii}-B_{ii}) Administration of a PPs inhibitor results in a strong nuclear FRET response, suggesting that phosphatases are major regulators of the nuclear PKA signals. **(C)** Preliminary biochemical analysis confirmed the importance of PPs compartmentalisation.



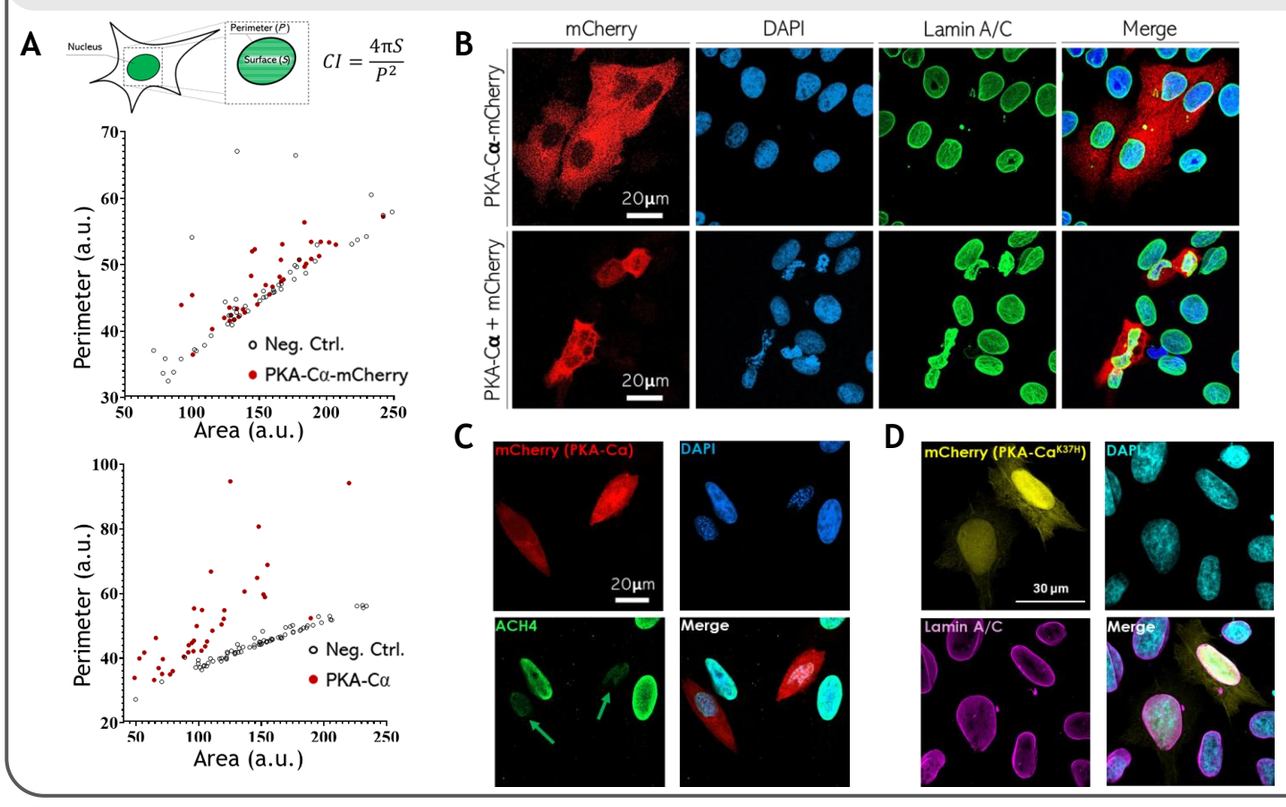
Nuclear PKA in skeletal muscle fibers



Ex vivo skeletal muscle fibers (*FDB*) were isolated and imaged from mice electroporated with the cAMP-sensitive sensor H187 (**B**) or with (**C**) H2B-AKAR4. H2B myonuclear localization was confirmed with confocal imaging (**A**). FRET experiments on living *ex vivo* FDB fibers reproduced the preliminary results obtained in immortalized cell lines in young animals but evidenced differences in the old ones.

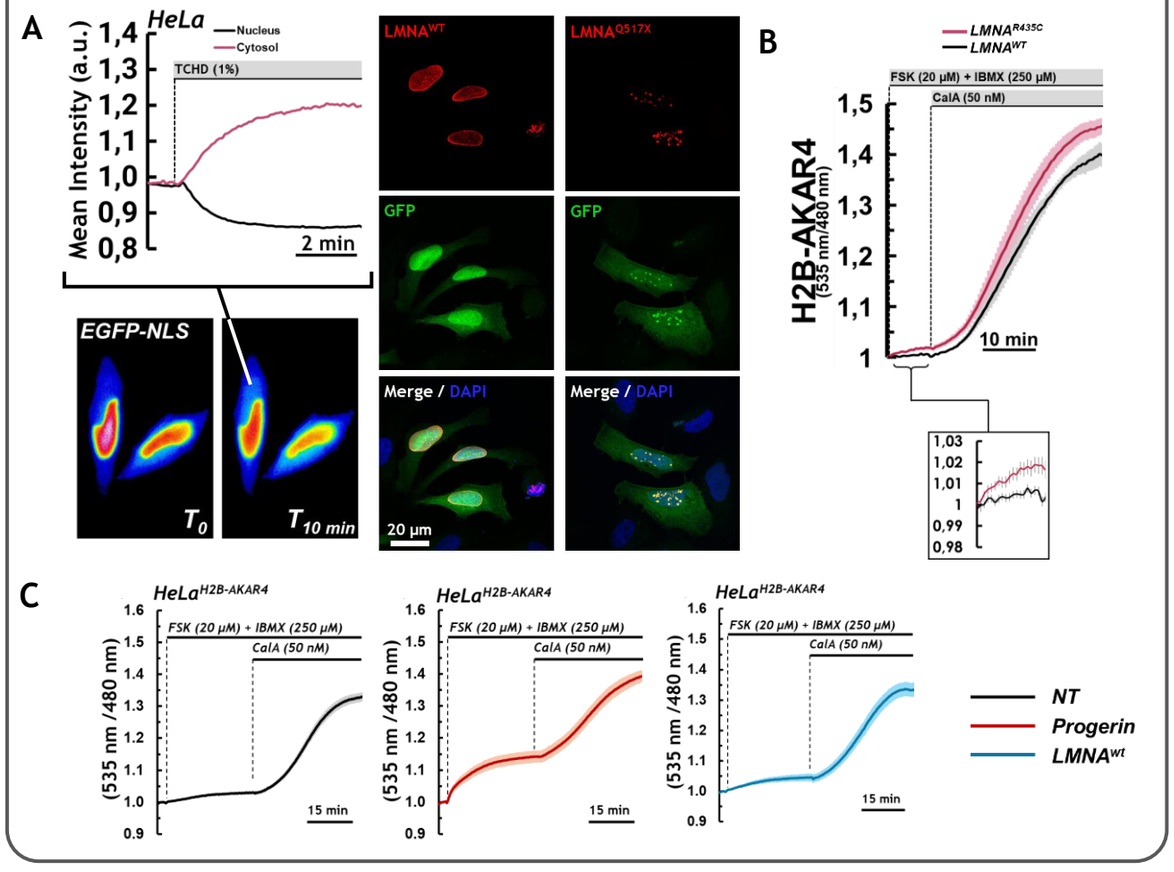
PKA-Cα overexpression affects nuclear morphology

To investigate the importance of nuclear PKA inhibition we compared the nuclear morphology in cells overexpressing two constitutively active PKA constructs, one that was able to reach the nucleus and one that was unable to enter this compartment due to the addition of an mCherry tag. Interestingly, only when PKA entered the nucleus, the nuclear shape was strongly affected. (**A&B**) This data was numerically defined by the circularity index (CI). (**C**) Additional preliminary studies of the altered nuclei highlighted a decreased Histone 4 acetylation. (**D**) Nuclei of HeLa cells transfected with catalytically inactive mutants of PKA were not morphologically altered.



LMNA mutants and nuclear signalling

The combination of the NLS-GFP-based nuclear permeability sensor with mutated Lamins can be used to understand how alterations of nuclear permeability affect the equilibrium between PPs and PKA. Some pathogenic LMNA mutants alter the PKA/phosphatase equilibrium defining a signalling signature that resembles that of old animals.



Conclusions

- Phosphatases restrain nuclear PKA activity in cell lines and *ex vivo* skeletal muscle fibers. Moreover, uncontrolled nuclear PKA activity alters nuclear morphology.
- Nuclear Phosphatase pressure is lost with skeletal muscle ageing. Additionally, we observed signalling signatures of LMNA mutants that resemble those of old mouse skeletal muscle.
- ***We propose that nuclear PKA/phosphatase signalling homeostasis represents an additional, underappreciated molecular mechanism in the pathogenesis of disorders related to the alteration of nuclear integrity.***

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