Project title: Investigating the role of nuclear phosphatase control in aging-related skeletal muscle dysfunction

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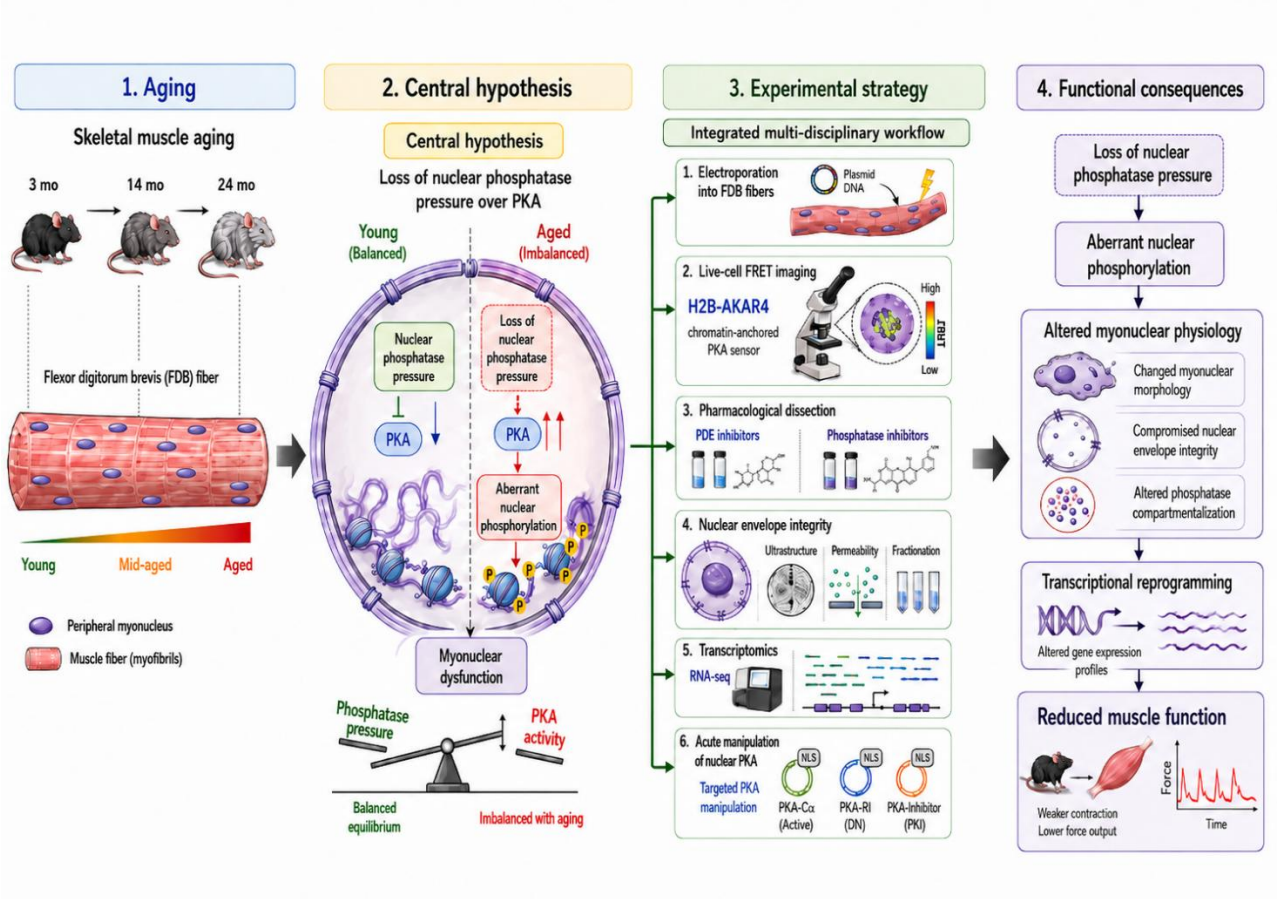
Background. Aging is associated with progressive skeletal muscle frailty, reduced adaptive capacity and loss of muscle plasticity [1]. Although age-dependent alterations in myonuclear morphology, nuclear positioning, post-translational modification patterns and nuclear envelope integrity have been described [2,3], the upstream signalling mechanisms responsible for these changes remain poorly understood. In skeletal muscle fibres, which contain hundreds of peripheral myonuclei, the nucleus is not merely a repository of genetic information but a dynamic signalling hub in which transcriptional competence, chromatin organization and nuclear mechanics are shaped by local kinase–phosphatase equilibria [3–5]. A central unresolved question is whether disruption of nuclear phosphatase-dependent control of kinase activity contributes causally to the decline in muscle performance during aging. Preliminary evidence indicates that nuclear phosphorylation is tightly constrained by phosphatase activity and that this phosphatase pressure may be progressively lost in aged skeletal muscle fibres [4,5]. This project will therefore investigate whether aging alters the nuclear PKA/phosphatase balance, leading to aberrant nuclear phosphorylation, impaired myonuclear physiology, transcriptional remodelling and reduced muscle function.



Aims. The overall aim of the project is to define the role of nuclear phosphatase control in skeletal muscle aging and to determine whether loss of phosphatase pressure over PKA represents a mechanistic driver of age-related myonuclear and muscle dysfunction. The specific aims are:

1. To define the temporal profile of nuclear PKA/phosphatase signalling alterations across skeletal muscle aging, comparing young, middle-aged and old mice.
2. To determine whether age-dependent changes in nuclear envelope integrity and phosphatase compartmentalization contribute to the loss of nuclear phosphatase pressure.
3. To identify the transcriptional consequences of nuclear phosphorylation imbalance in aging skeletal muscle fibres.
4. To assess the functional relevance of nuclear PKA/phosphatase imbalance by linking altered nuclear signalling to changes in muscle performance and by evaluating the translational relevance of the identified mechanisms in human skeletal muscle samples.

Techniques. The project will combine live-cell imaging, molecular biology, biochemistry, transcriptomics and muscle physiology in an integrated experimental workflow. Flexor digitorum brevis and other leg muscles from 3-, 14- and 24-month-old mice will be electroporated with FRET-based biosensors, including the chromatin-anchored PKA sensor H2B-AKAR4, to monitor nuclear PKA/phosphatase dynamics in individual myonuclei of isolated skeletal muscle fibres [4,6]. Pharmacological dissection will be used to resolve the contribution of specific components of the nuclear cAMP/PKA/phosphatase axis, including PDE-specific inhibitors and phosphatase inhibitors. Nuclear envelope integrity and phosphatase compartmentalization will be assessed through ultrastructural analysis, live-cell permeability assays and subcellular biochemical fractionation [3,5]. RNA-seq analysis will be performed to identify transcriptional signatures associated with aging and with acute manipulation of nuclear PKA activity. Targeted PKA catalytic subunit constructs and nuclear PKA inhibitors will be used to test the causal relationship between nuclear phosphorylation imbalance and myonuclear dysfunction [4]. Finally, muscle physiology assays will assess the impact of nuclear signalling alterations on force generation and contractile performance, while selected molecular signatures will be validated in human skeletal muscle biopsies.

Bibliography

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