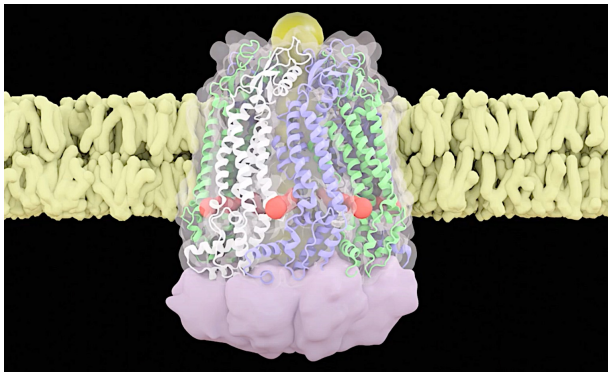
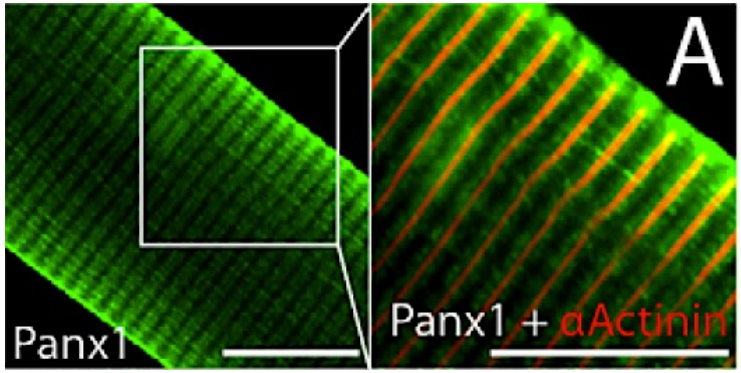
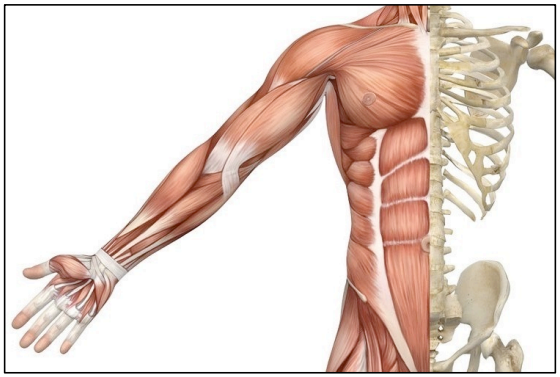
Pannexins and their potential contributions to musculoskeletal resilience

Bill Gittings, PhD, RKin



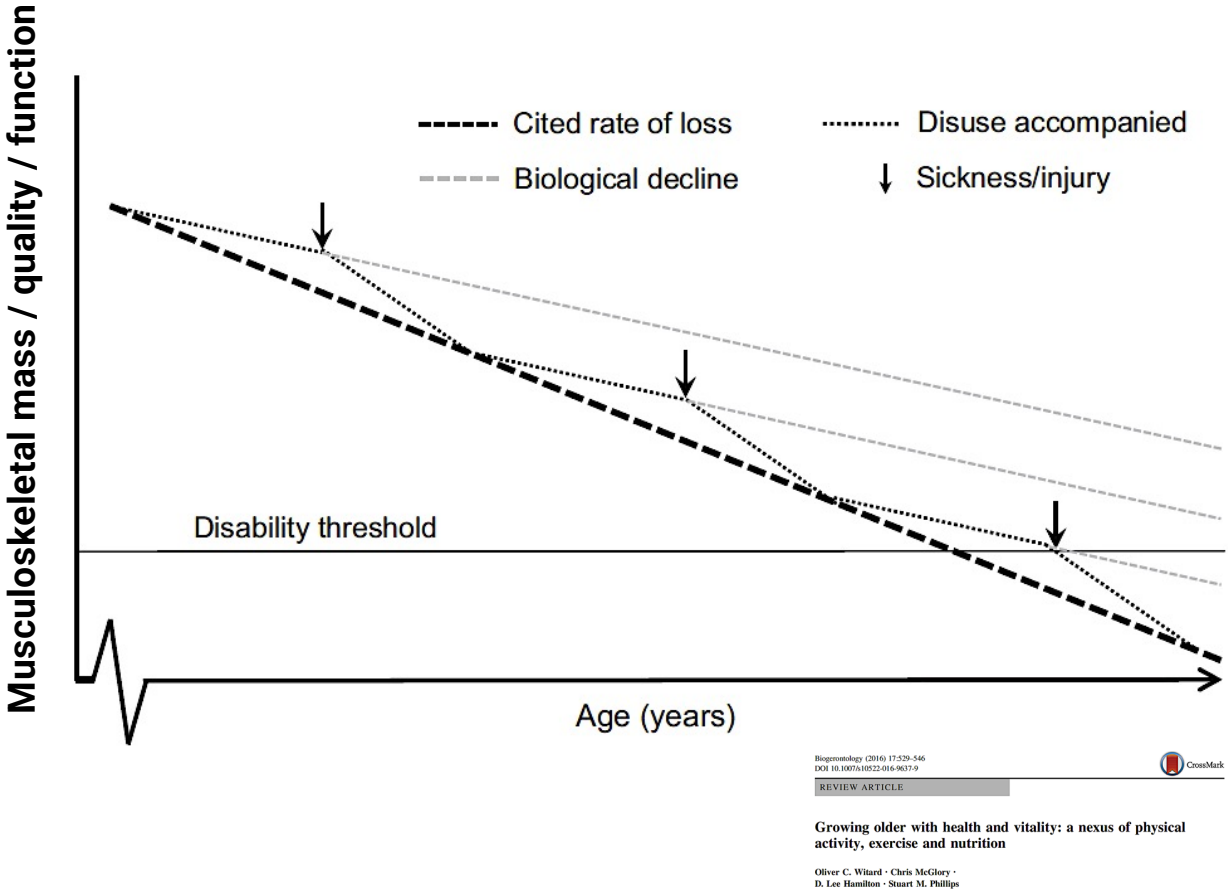




Musculoskeletal Health Education Forum (MHEF)
September 30, 2023

What is musculoskeletal resilience?

resilience: **a measure of the persistence of a system, its ability to tolerate and adapt to stressors, resist deterioration, and recover from damage or injury**



Biogerontology (2016) 17:529–546
DOI 10.1007/s10522-016-9637-9

REVIEW ARTICLE

Growing older with health and vitality: a nexus of physical activity, exercise and nutrition

Oliver C. Witard · Chris McGlory · D. Lee Hamilton · Stuart M. Phillips

Building MSK resilience

- Mitigation (coping)
- Vulnerability (risk)
- Adaptive capacity (preventative fortification)

Brock University

Building musculoskeletal resiliency: Objectives and priorities

Applied research and clinical interventions

- Efficacious treatments that are acceptable and can be adhered to
- Integrate professionals to tackle the challenges comprehensively

Education and knowledge translation

- Promote the rationale and importance of preventative interventions
- Ensure theory and guidelines are paired with implementation strategy

Basic / fundamental research

- Advance our understanding of how the MSK systems are regulated
- Identify key targets for intervention and validate relevant biomarkers
- Inform the development and design of applied research

1. Understanding the tissue dysregulation that occurs with hypokinesia
2. How is the 'coupling' between anabolic and catabolic processes regulated?
   (i.e., tissue turnover)
3. What processes are essential for regulating adaptive responses to mechanical loading?
   (i.e., mechanotransduction)
4. How do musculoskeletal tissues interact at the local level and communicate to the rest of the body
   (i.e., crosstalk, systemic signalling)

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Pannexins and their potential contributions to musculoskeletal resilience

- Overview of new NSERC Discovery research program

- What are pannexin channels? (Role in cell communication and signalling)
- Investigating the physiological role of pannexin 1 in skeletal muscle
- Overview of research model and experimental approach

- Diverse role of pannexins in other body systems

- Potential as a therapeutic target?

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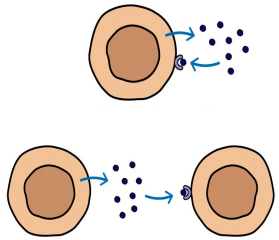
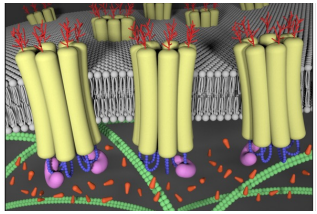
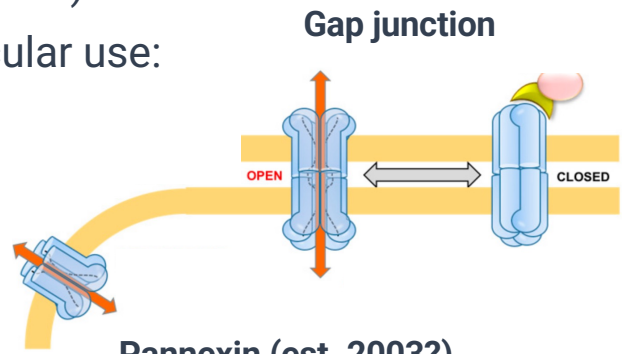
Gap junctions – classification and cellular function



- **Specialized glycoprotein complexes that span cell membranes**
  - Connexins (vertebrates), Innexins (invertebrates)
  - Pannexin single-membrane channels facilitate *autocrine* and *paracrine* signalling



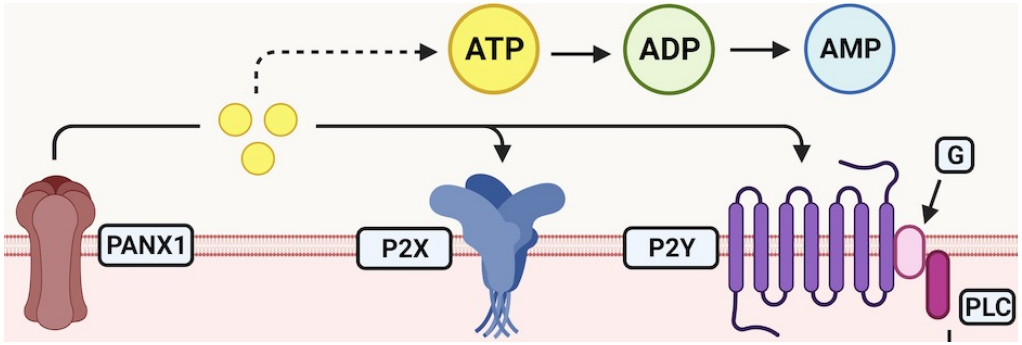
- **Pannexin isoforms 1, 2, 3 expressed in a wide range of body tissues**
  - Regulated by expression (abundance) and biophysical properties (activity)
  - When active, channels increase permeability to molecules up to ~1.5 kDa (ions, nucleotides, amino acids, substrates, small signaling molecules)
  - Pannexin activity (expression, permeability) is modulated by muscular use:
    - Fluctuations of intracellular Ca^{2+} (muscle activation)
    - Mechanical stimulation (membrane stress / strain)
    - Extracellular pH, sarcolemmal ion charge, metabolic activity

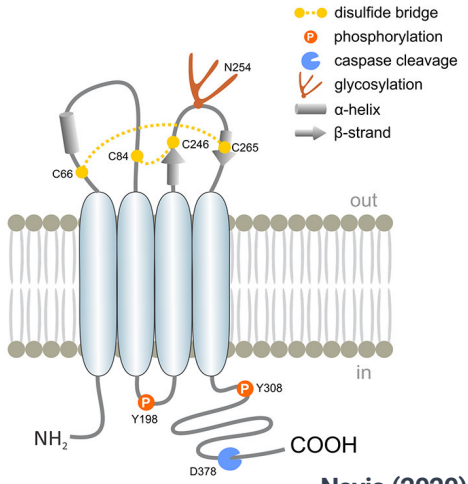


Pannexin (est. 2003?)
(single membrane channels)

Pannexin 1 (PANX1) in skeletal muscle – the key players

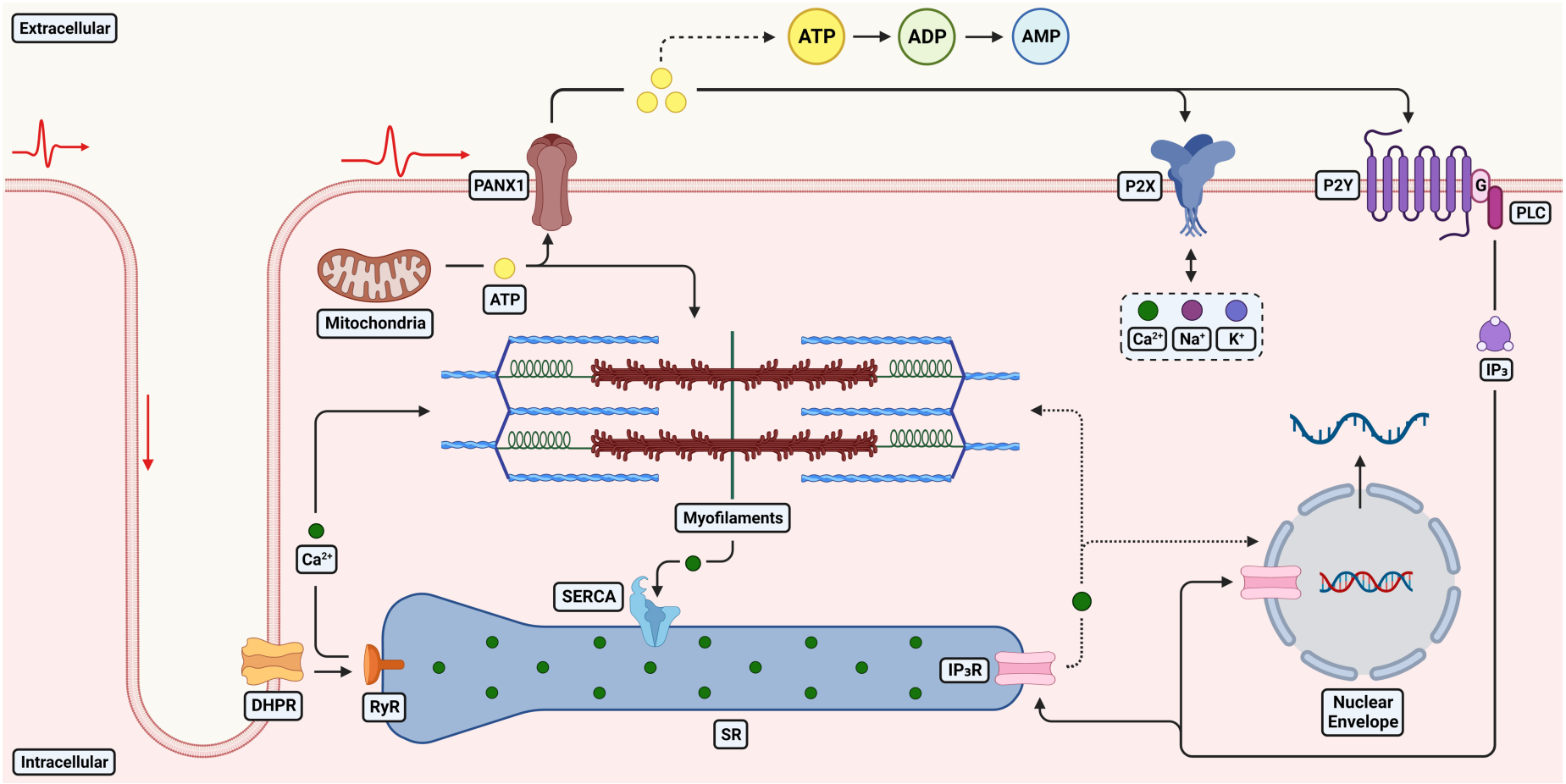
- **PANX1 regulated by a variety of posttranslational modifications (Penuela, 2014)**
  - Regulate activity of the complex, including permeability and selectivity(?)
  - Facilitation of extracellular ATP transport is of primary interest
- **Ecto-nucleotidases break down ATP and supply the availability of nucleotides**
  - Cell responds to these *autocrine* signals via Purinergic receptors (P2X, P2Y family)
  - Ionotropic receptors (P2X) control membrane excitability, ion transport (Ca^{2+}, K^{+}, Na^{+})
  - Metabotropic receptors (P2Y) initiate many intracellular signalling processes





Navis (2020)

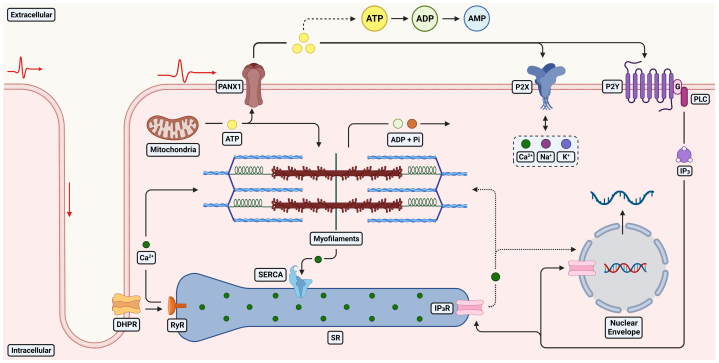
Modulation of excitation-contraction and excitation-transcription coupling by PANX1

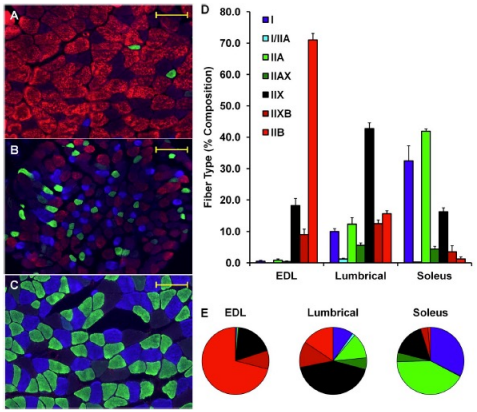


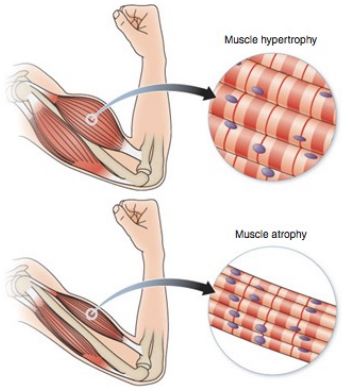
Buvinic (2009), Riquelme (2013), Jorquera (2013), Casas (2014), Arias-Calderon (2016), Jaque-Fernandez (2021), among others.

What are the direct implications of this mechanism to muscular physiology?

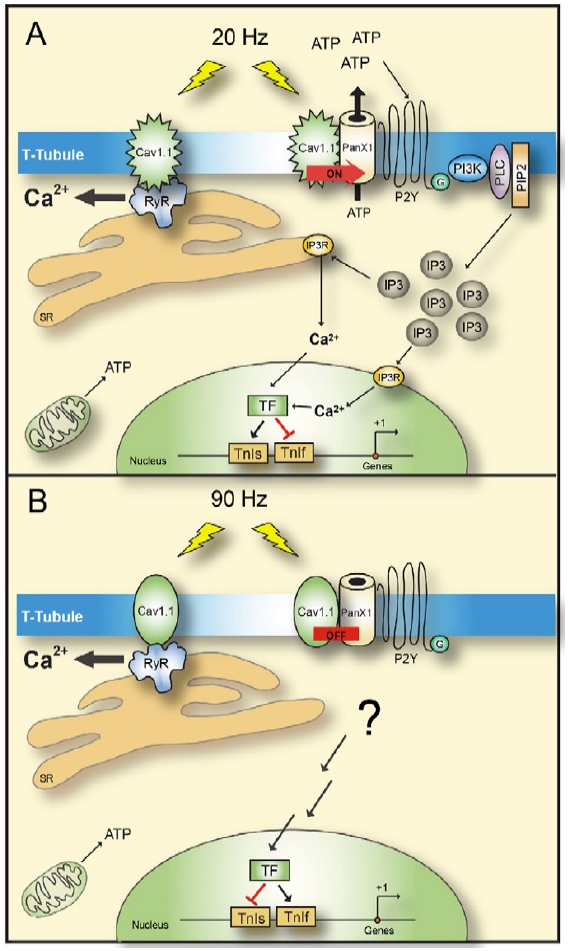
- **PANX1 modulates intracellular Ca^{2+} homeostasis during muscular activation (acute effects)**
  - Modulation of contractile performance , mechanical function, and fatigability
  - Participation in Ca^{2+}-dependent metabolic regulation and fuel utilization
- **PANX1 contributes to adaptive responses that result from patterns of muscle use (plasticity)**
  - Transcriptional control of fibre type (i.e., slow-oxidative vs. fast-glycolytic fiber expression)
  - Morphological responses to mechanical loading (i.e., hypertrophy, atrophy)



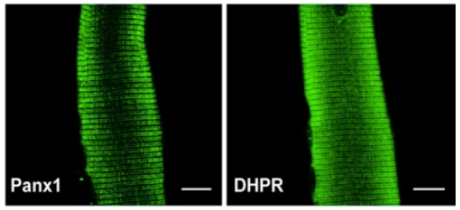


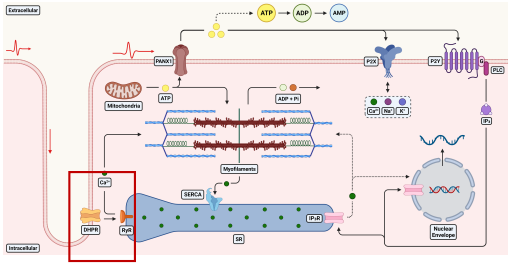


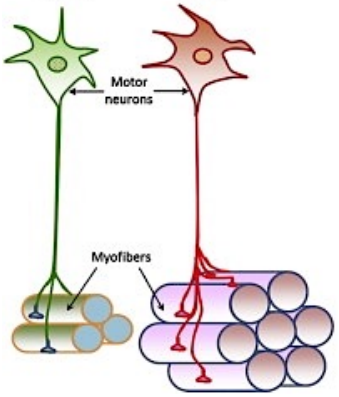
Experimental Target: Role of PANX1 in skeletal muscle phenotype expression



- PANX1 and CaV1.1 may co-localize in the t-tubule membrane
- PANX1 activation and transcriptional signals are freq. dependent
- Independent of the Ca^{2+} release mechanism, this may control the specific responses to muscle activation patterns (plasticity)
- **<u>Implication</u>: PANX1 may participate in the encoding and translation of motor unit firing patterns to determine muscle phenotype**

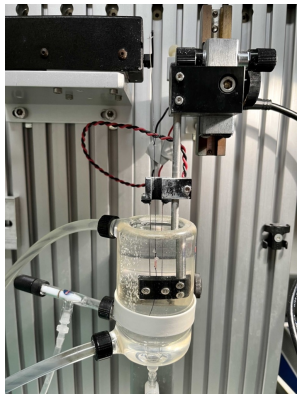




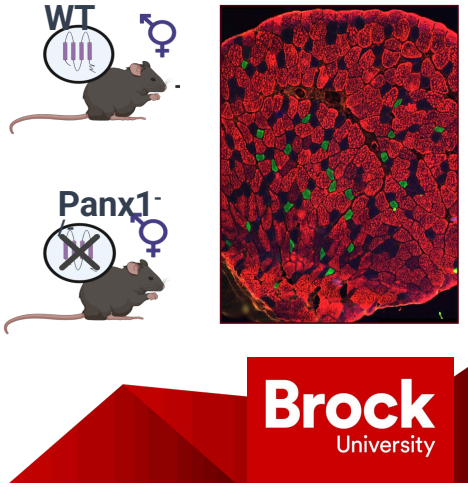


Jorquera et al. (2013)

Experimental approach: Intact mouse skeletal muscles *in vitro*



- Intact Ca^{2+} release mechanism and force responses
- Analysis of whole muscle morphometrics and phenotype
- Test pharmacological interventions
  - PANX1 and purinergic receptor agonists / antagonists
- *In vivo* interventions to elicit adaptive responses
  - Endurance training (treadmill running)
  - Atrophy (hind-limb suspension)
  - Mechanical loading/unloading (hindlimb tenotomy)
- Transgenic mouse models
  - $Panx1^{-/-}$ (global removal of PANX1 expression)
  - Tissue-specific conditional PANX1 knockout (skeletal muscle)



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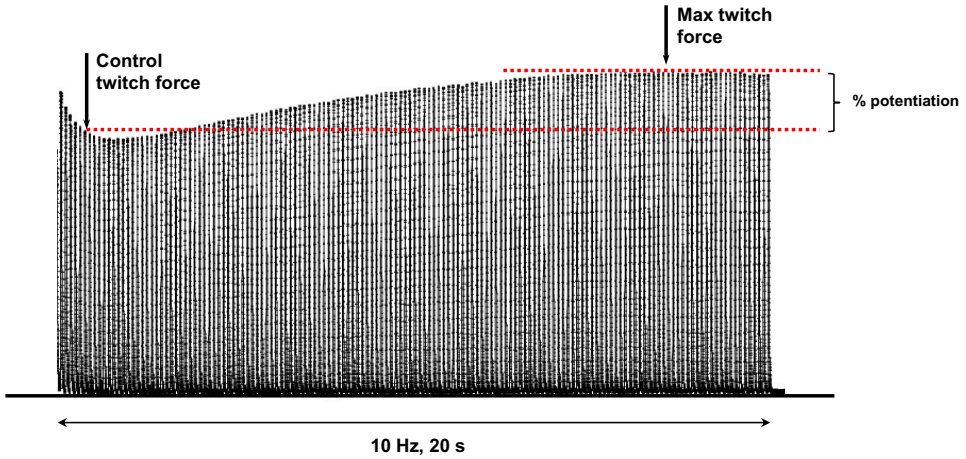
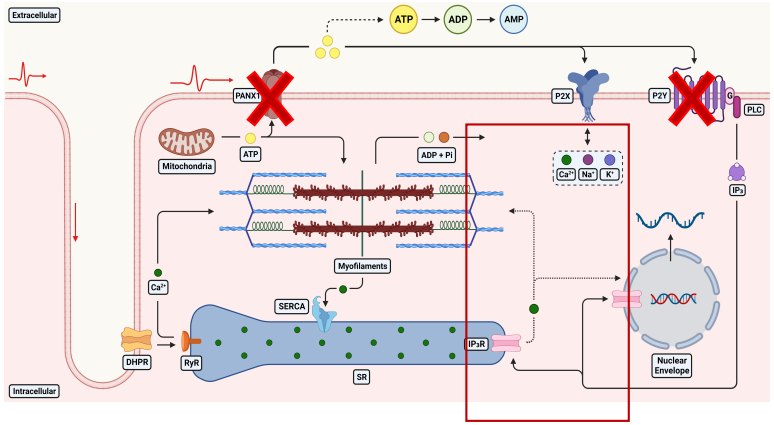
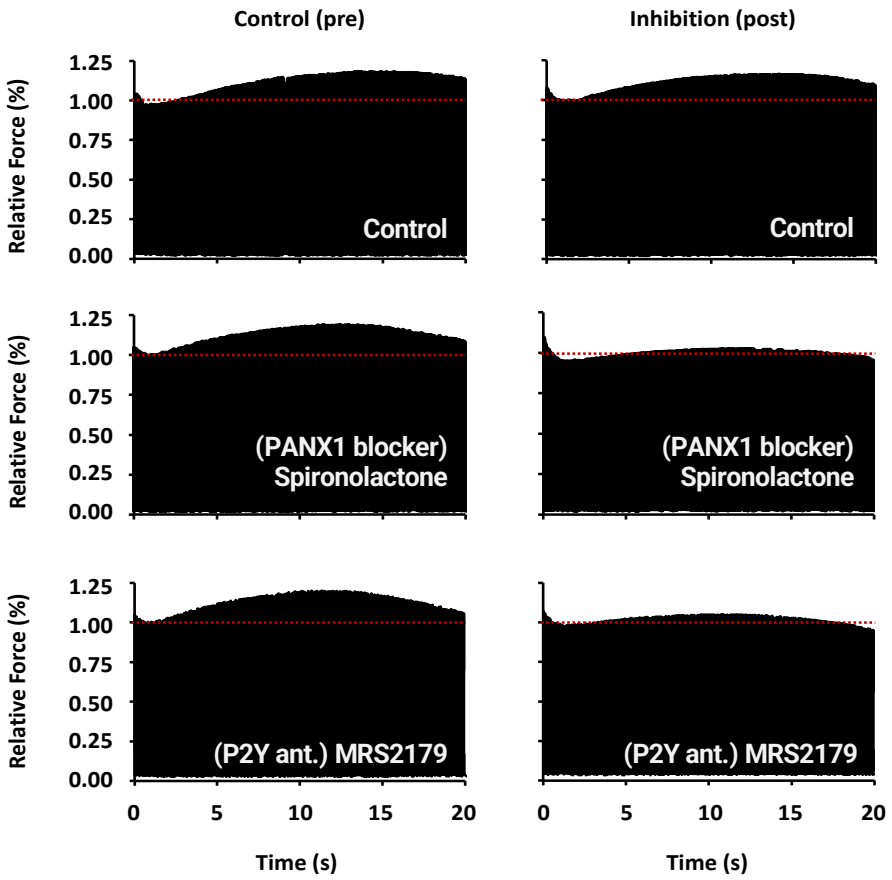
Preliminary findings: Staircase potentiation as an indicator of $[Ca^{2+}_i]$ homeostasis



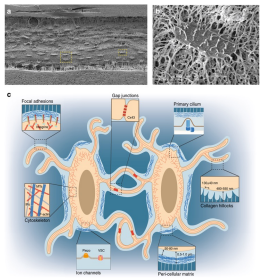




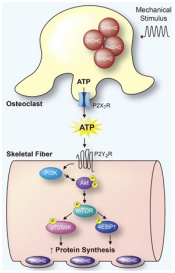
Ahad et al. (unpublished)

Pannexins and purinergic signalling are broadly implicated in many determinants of MSK resilience and overall health

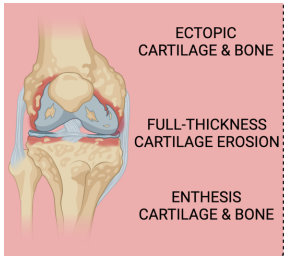
- Exciting opportunities for inter-disciplinary collaboration and application of knowledge
- Growing interest in identifying opportunities to target these pathways therapeutically



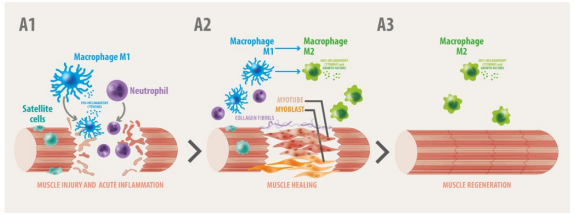
Osteocyte mechanosensing
Propagation of bone resorption signals



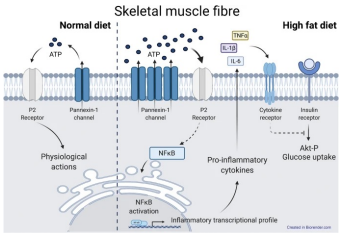
Skeletal responses to exercise
(bone-muscle crosstalk)



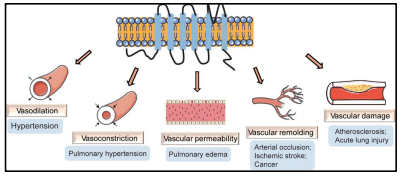
Degenerative joint disease
(Osteoarthritis)



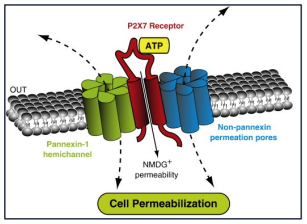
Muscle damage and regenerative processes
(dystrophies, injury and rehabilitation)



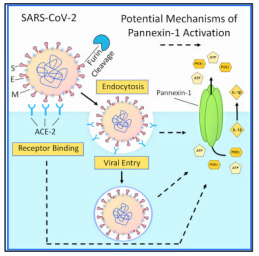
Diet-induced metabolic
adaptations in muscle



Vascular control / hypertension

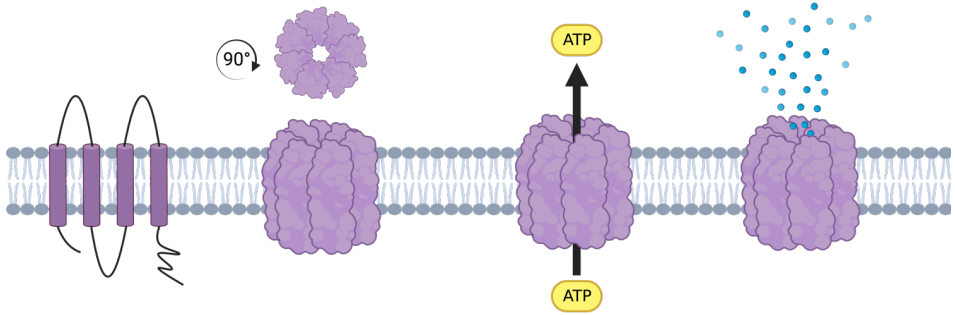


Propagating cellular inflammasome
and immune responses



Viral pathogenesis
(SARS-CoV-2)

Acknowledgements



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- Afra Nehchiri, MSc(c)
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- Dr. Silvia Penuela
- Dr. Brent Wakefield

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