Cytoskeletal-Nuclear Mechanotransduction Following Loss of Tension

Tina Njike, Madisyn Strange, Grey Hallstrom, PhD, and Nathaniel Dyment, PhD[1,2]

[1]McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[2]Pennsylvania Undergraduate Research Mentoring Program , University of Pennsylvania, Philadelphia, PA, USA

Introduction

Tendons are composed of organized bundles of collagen fibers that connect muscle to bone. Their function is to transmit forces generated by muscles to produce movements and stabilize joints.

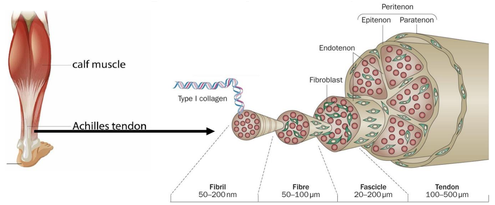


Figure 1: Hierarchical structure of the Achilles tendon. The Achilles tendon is composed of multiple fascicles (20-200µm), each made up of fibers (50-100µm), which in turn consist of collagen fibrils (50-200nm). The fascicles and the entire tendon are surrounded by loose connective tissues known as the endotenon and peritenon, respectively. Tendon fibroblasts (green) are present throughout. [1]

Tendon Homeostasis is critical for adapting to applied loading, with disruptions in tension resulting in a catabolic program and matrix remodeling.

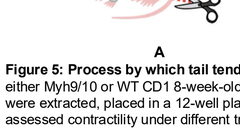


Figure 2: Effects of mechanical loading on tendon homeostasis. [A] Mechanical loading induces an anabolic response, increasing collagen synthesis and improving tendon mechanics. [B] De-tensioning triggers a catabolic response with elevated matrix-degrading enzymes and tissue breakdown. Proper balance is essential for tendon integrity and injury prevention. [3]

Working model of the tendinopathy disease pathogenesis

Three Main Categories of Tendinopathies:
Tendinitis: Inflammation of the tendon
Tenosynovitis: Inflammation of paratenon
Tendinosis: Degenerative changed from chronic overuse leading to collagen breakdown and structural damage.

Figure 3: Working model of tendinopathies and disease progression. Scheme showing the transition from a healthy tendon to tendinopathy. Repetitive overuse damages the matrix, reduces tension, and impairs remodeling, leading to structural disorganization and ultimately tendon degeneration or rupture. [4,5,6]

Mechanotransduction is the process by which cells convert mechanical stimuli into biochemical responses

Figure 4: Working model of the cytoskeletal–nuclear pathway. Scheme showing how mechanical forces on the tendon are transmitted through the cytoskeleton to the nucleus. Loading is sensed at focal adhesions, relayed via the actin cytoskeleton, and linked to the nucleus, where it alters gene expression. This mechanotransduction process regulates tendon adaptation and remodeling. [2,8]

Research Question: Which parts of the cytoskeletal–nuclear mechanotransduction pathway are important for tendon contraction?

Methods

Tail Tendon Extraction to Measure Contractility. This process was useful to see to which part of the cytoskeletal-nuclear pathway contributes the most to tendon contraction.

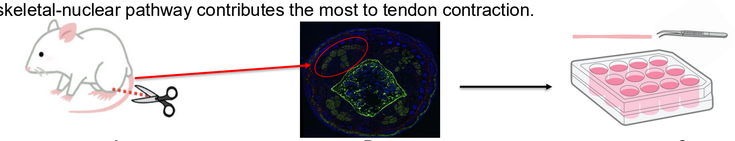


Figure 5: Process by which tail tendons were extracted to measure contractility. [A] Each tail was cut into two 1.5 cm pieces from either Myh9/10 or WT CD1 8-week-old mice. [B] The circled region in the histology image shows tendon structure. [C] Tendon bundles were extracted, placed in a 12-well plate, and cultured for 12 days with media changes every 2 days. Daily length measurements assessed contractility under different treatments targeting components of the cytoskeletal–nuclear pathway.

Myh9/10 genetic ablation (removing myosin from mice). To do this process the TAT-Cre system was used to excise Myh9 and Myh10, which encode non-muscle myosin IIA and IIB (NMIIA/B)

Figure 6: Working model of Myh9/10 genetic ablation. Scheme showing how non-muscle myosin is removed from mice. Floxed Myh9 and Myh10 genes (encoding NMIIA and NMIIB) are excised by TAT-Cre, eliminating myosin production. [7,8]

Methods

qPCR:Gene Expression Analysis in Treated Tendons Method used to identify the expression levels of specific genes under different tendon treatments. RNA was first isolated from the samples and then converted into cDNA, which was amplified to quantify gene expression.

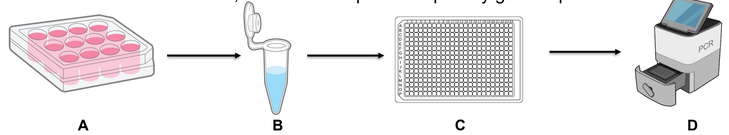


Figure 7: Process of qPCR. [A] After 12 days of culture, tendon samples were collected for RNA isolation. [B] Each epitude included 4–6 tendons; RNA was extracted and converted to cDNA. [C] cDNA was mixed with gene-specific primers and added to a 364-well plate. [D] The plate was run in a PCR machine to amplify the cDNA and measure gene expression for each sample.

Results

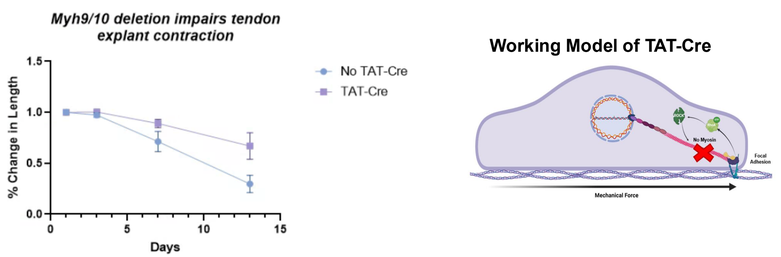


Figure 8: Myh9/10 deletion impairs tendon explant contraction. Myh9/10 f/f explants treated with TAT-Cre contract significantly less over time compared to untreated controls, showing that myosin IIA/IIB is essential for tendon contraction. Tat-Cre was used at 2.67µM.

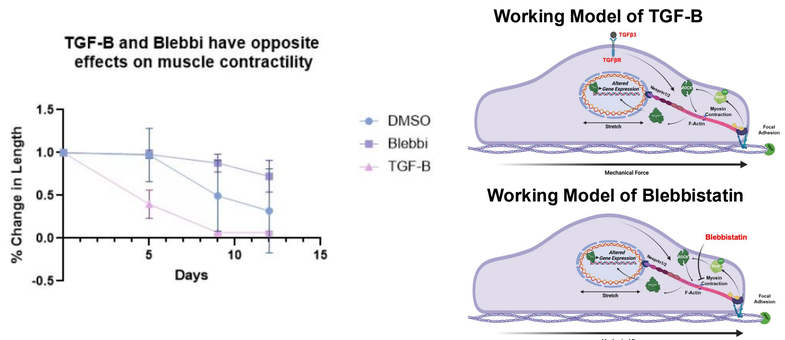


Figure 9: Opposing effects of TGF-β and blebbistatin on tendon contractility. TGF-β increases tendon explant contraction compared to DMSO control, while blebbistatin decreases contraction. This demonstrates that TGF-β promotes and blebbistatin inhibits contractility in the system. Blebbi was used at 10µM and TGF-β was used at 5ng/ml. DMSO was used as a vehicle control. n=3 tendons/mouse for 3 mice.

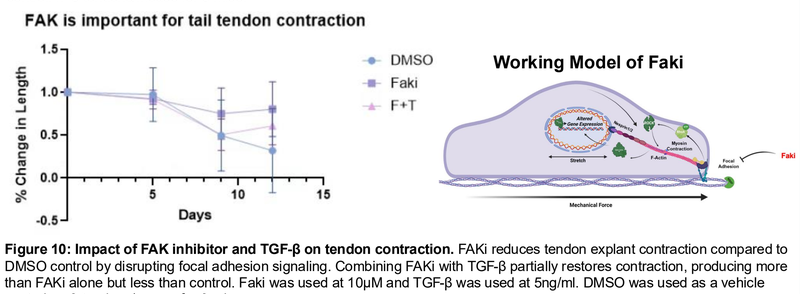
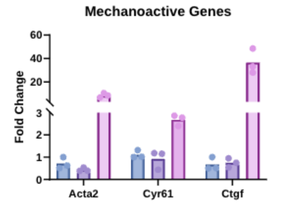


Figure 10: Impact of FAK inhibitor and TGF-β on tendon contraction. FAKi reduces tendon explant contraction compared to DMSO control by disrupting focal adhesion signaling. Combining FAKi with TGF-β partially restores contraction, producing more than FAKi alone but less than control. Faki was used at 10µM and TGF-β was used at 5ng/ml. DMSO was used as a vehicle control. n=3 tendons/mouse for 3 mice.

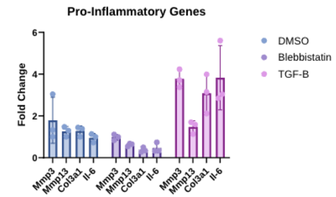
Results



- **Acta2** is essential for tendon cell contractility and force generation.
- **Cyr61** supports tendon cell adhesion and migration during mechanical stress.
- **CTGF** promotes extracellular matrix production, key for tendon strength and repair.

These Yap/Taz mechanoactive genes help tendon cells sense and respond to mechanical forces.

Figure 11: Effects of TGF-β and blebbistatin on mechanoactive gene expression. TGF-β significantly increased expression of Acta2, Cyr61, and Ctgf, while blebbistatin reduced their expression compared to DMSO control. These results demonstrate that contractility promotes, and inhibition of contractility suppresses, expression of Blebbi was used at 10µM and TGF-β was used at 5ng/ml. DMSO was used as a vehicle control. n=3 tendons/mouse for 3 mice. mechanoresponsive genes.



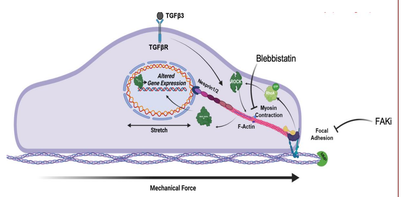
- **Mmp3** breaks down matrix to help tendon remodeling during inflammation.
- **Mmp13** degrades collagen to remove damaged tissue.
- **Col3a1** makes collagen for early tendon repair and scarring.
- **Il6** triggers inflammation and immune response.

These proinflammatory genes control tendon inflammation and healing.

Figure 12: TGF-β increases, and blebbistatin decreases, Col1a1 expression. Col1a1 expression was strongly upregulated by TGF-β and reduced by blebbistatin compared to DMSO control, showing that contractility promotes collagen expression in tendon explants. Blebbi was used at 10µM and TGF-β was used at 5ng/ml. DMSO was used as a vehicle control. n=3 tendons/mouse for 3 mice.

Conclusion

- Myosin II is required for tendon contraction and mechanotransduction.
- Blebbistatin (myosin inhibitor) and FAKi (FAK inhibitor) both block contractility.
- TGF-β promotes contractility.
- Mechanoactive genes are upregulated with TGF-β (high contractility) and downregulated with Blebbistatin (low contractility).
- Pro-inflammatory genes are elevated with TGF-β, as prolonged contractility triggers inflammation.
- Blebbistatin reduces pro-inflammatory gene expression compared to control due to blocked contraction.



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