Protein Engineering of Alpha-actinin Mutant Proteins to Test Biological Role in the Heart Muscle

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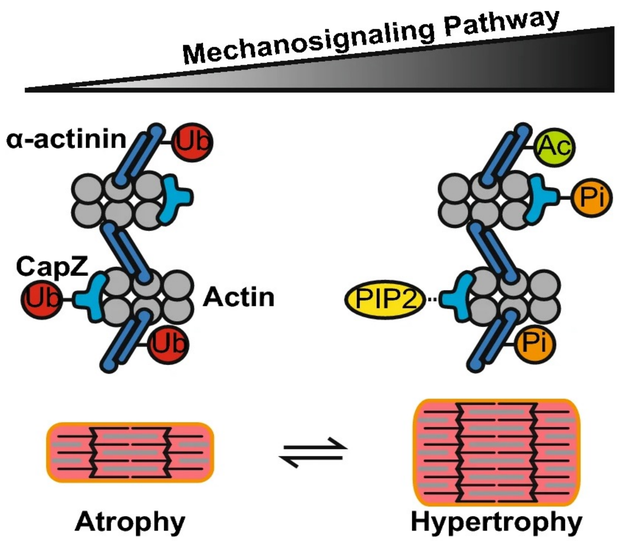
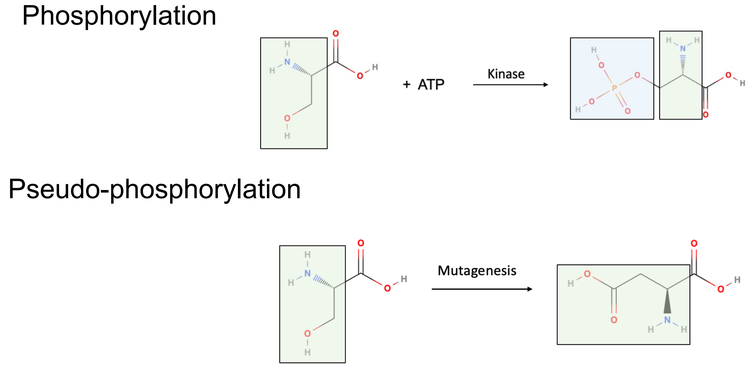
Abstract

The heart has the ability to grow in size to a healthy caliber, similarly to the growth seen in many athletes and gestating woman. However, many different cardiovascular diseases and pathologic causation's such as Hypertrophic Cardiomyopathy (HCM), prolonged bed rest, and heart failure with reduced ejection fraction (HFrEF) have the potential to cause an inimical physiological discrepancy between the mechanical capacity of the heart, and the human body's hemodynamic demand. We ultimately seek the understanding as to how cardiac muscle cells recognize, process, and respond to internal and externally-driven stimuli fluctuations produced by mechanical stimulation for short, as well-as prolonged terms. Specifically, we will be studying the role of post-translational modification of the structural protein α-Actinin within cardiac muscle cells in response to mechanical unloading. The approach is to introduce pseudo-phosphorylation sites in an α-Actinin plasmid to subsequently express these constructs into cardiomyocytes. We have identified that the plasmid pAcGFP-N1 is the most suitable for our work. We will continue with identifying the primers needed to induce mutagenesis to introduce the following pseudo-phosphorylation's: Ser50, Ser147, Thr43, and Thr237 for Aspartic Acid. The primers to induce the listed pseudo-phosphorylation sites were identified. Our results will help understand the mechanisms of sarcomere assembly driven by phosphorylation of α-Actinin.

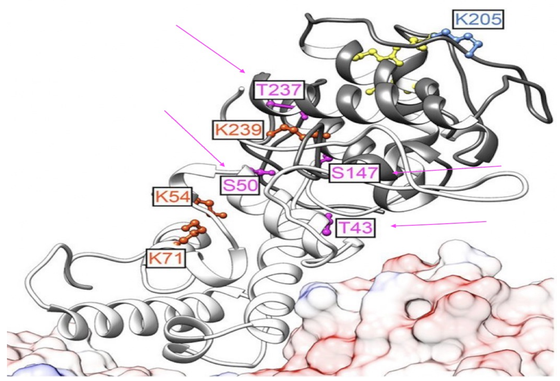
Working hypothesis: Alpha-actin2 Pseudo phosphorylation



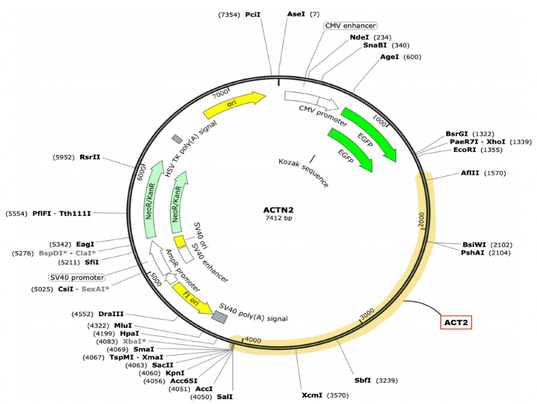
Pseudo-phosphorylation as a protein engineering strategy to model phosphorylation in proteins



Alpha-actinin phosphorylation sites



Ca^{2+} kinetics curve fitting parameters



Primers for Sequencing and Mutagenesis

Sequencing Primers:

CATGGTCCTGCTGGAGTTCGTG (BD Biosciences)
3' end of EGFP, forward primer

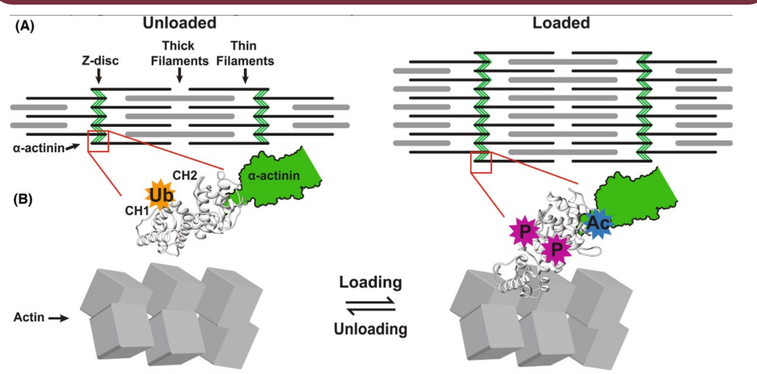
SV40pA-R GAAATTTGTGATGCTATTGC
SV40 polyA, reverse primer

Thr43 primer:
Q R K *T* F T A
cag agg aag acc ttc act gcc 5'
gtc tcc ttc tgg aag tga cgg 3'

Mutagenesis step #1
ag agg aag aAc ttc act gc 5' (Tm 59.1ºC, GC 47.4%)
tc tcc ttc tTg aag tga cg 3' (Tm 58.7ºC, GC 47.4%)

Mutagenesis step #2
ag agg aag GAc ttc act gc 5' (Tm 60.9ºC, GC 52.6%)
tc tcc ttc CTg aag tga cg 3' (Tm 60.5ºC, GC 52.6%)

Proposed mechanism of alpha-actinin phosphorylation regulation of sarcomere assembly



Conclusion

1.The plasmid actn2-pEGFP is the best suited plasmid for this study.
2. The designed primers will support the introduction of pseudo-phosphorylation sites in the alpha-actinin c-DNA.
3. Further our understanding of the mechanisms of heart growth and how the derailment of growth factors makes way for cardiovascular disease

References