6 Summary

Titin is the largest protein in mammals with a molecular weight of up to 3.7 MDa. It is considered the third filament in skeletal muscle and heart, along with actin and myosin. The titin molecule spans the half sarcomere from the Z-disc to the M-band. The C-terminus contains a serine/threonine kinase domain that is highly conserved in vertebrates. The aim of this study was to investigate the role of titin's kinase region in signal transduction using a striated muscle specific and an inducible cardiac specific titin kinase KO.

In striated muscle, the truncated titin was integrated properly into the Z-disc and I-band, but failed to integrate into the M-band. The deletion of titin's kinase region caused disassembly of the sarcomere starting between day 5 and 10 after birth and mislocalization and accumulation of proteins of the cytoskeleton. This indicates a role of titin's M-band in maintaining sarcomere structure.

The proteome analysis showed a stress response in titin kinase region KO animals, which includes the upregulation of heat shock proteins and components of the 26S-proteasome in skeletal muscle as well as in heart. Differences between titin M-band deficient skeletal and cardiac muscle related to metabolism and oxidative stress. The accumulation of enzymes involved in detoxification and a cystein modified cardiac protein indicated oxidative stress and increased mechanical strain in the skeletal muscle and hearts of titin kinase region-KO animals. This could either be due to sarcomere disassembly or related to the postulated stretch sensor function of titin. The postulated titin kinase substrate Tcap could mediate this function. Nevertheless, the protein level of Tcap was increased only in skeletal muscle but not in heart, indicating differences in titin kinase signaling in cardiac and skeletal muscle.

To investigate the cardiac phenotype in the adult mouse, we used an inducible titin kinase KO model. The KO animals developed a cardiac hypertrophy and enlarged lungs (pulmonary edema) as a sign of severe heart failure. The hypertrophy involved increased PKCδ signal transduction in knockout hearts.

Prior to cardiac hypertrophy a contractile phenotype with a reduced response to adrenergic stimulation of KO hearts could be detected in isolated heart experiments. This reduced adrenergic response was accompanied by altered Ca^{2+} homeostasis with decreased levels of calmodulin 1, the sarcoplasmic ATPase Serca2 as well as its regulator phospholamban. Here, we show for the first time that titin's M-band does not only determine the diastolic but also the systolic function of the heart.