2 Aim of the study

Titin is a giant protein found in the vertebrate muscle, where it extends over the half-sarcomere and integrates into the Z-disc and M-band through its N- and C-terminus. The Titin polypeptide is composed of subdomains that perform distinct functions. The N-terminal region of Titin contains multiple binding sites for Z-disc proteins and they appear to regulate the Z-disc assembly in concert. Titin's I-band region contains spring elements responsible for the elastic response of the stretched sarcomere. Titin's A-band provides multiple binding sites for thick filament-based proteins and serves as a molecular scaffold in charge of assembly and length control of the thick filament. The C-terminal region of Titin anchors within the sarcomeric M-band. This region contains a kinase domain with serine/threonine specificity and is highly conserved in vertebrates. So far, there are only *in vitro* data on the activation of Titin's kinase and its implication in signal transduction available. Crystal structure analysis showed that the active site is inhibited by a tyrosine within the kinase domain. A dual mechanism of activation consists of phosphorylation of this tyrosine and binding of Calcium/Calmodulin to the regulatory tail. Moreover, it was shown that the active Titin kinase phosphorylates the muscle protein T-cap in early differentiating myocytes, indicating that the kinase acts in myofibrillogenesis (Mayans et al., 1998). Different data showed that Titin's kinase functions as a stress sensor due to the conversion of mechanical stress into biochemical signal in muscle cells (Grater et al., 2005). This was supported by a study published recently. A two-hybrid screen identified Nbr1 and Sqstm1 to bind to Titin's kinase domain. They in turn interact with MuRF-2 that was shown to localize from the sarcomere to the nucleus upon mechanical arrest. In the nucleus it interacts with the transcription factor SRF and controls muscle gene expression and protein turnover (Lange et al., 2005b). Another yeast two-hybrid screen led to the identification of MuRF-1 that binds N-terminally of the Titin kinase domain. It has been shown to be associated with the periphery of the M-band lattice and may be involved in regulating the Titin kinase domain. Experiments with cultured myoblasts expressing a truncated Titin

protein revealed impaired myofibrillogenesis accompanied by a disturbed organization of the sarcomere. This suggest that the activity of the Titin kinase domain and downstream sequence are important in organizing myofibrils (Miller et al., 2003a). However, the physiological role of Titin's kinase in cardiac muscle cells during sarcomere assembly is not well known.
In summary, although several studies have focused on Titin's kinase domain, the understanding of its role *in vivo* is still unclear. Generating a constitutive Titin M-line knockout will help to learn more about the role of Titin's kinase in early cardiac development and might provide information about Titin's role in non-muscle cells.