Ruprecht-Karls-Universität Heidelberg

Medizinische Fakultät Mannheim

Dissertations-Kurzfassung

Molecular Studies on the Titin Filament System and its Role in Cardiovascular Physiology

Autor: Bryan D. Hudson
Institut / Klinik: Klinik für Anästhesiologie und Operative Intensivmedizin
Doktorvater: Prof. Dr. S. Labeit

Titin is the largest known protein in the human body and has multiple important functions. This dissertation focuses on titin's actions as a molecular spring and its ability to be rapidly modified by large proteins, known as kinases (which are activated or deactivated depending on the activity of specific upstream pathways); these kinases work by adding phosphate groups to localized amino acids, thus altering their local mechanical and binding properties. Specifically I studied a kinase known to be active in the heart, called Protein Kinase C (PKC), and its affect on the elasticity of titin. Previously, PKC has been shown to change the properties of multiple proteins known to be involved in systolic function (the contraction of the heart). However, it was not known whether PKC could modify the spring-like function of titin. The experiments in this dissertation showed that PKC does in fact phosphorylate titin and that this phosphorylation occurs specifically within a portion of the elastic region of titin, called the PEVK spring element. Using a wide range of experiments it was discovered that two specific amino acids (out of a total of ~40,000 residues) can be phosphorylated by PKC and when these sites are phosphorylated the PEVK molecule doesn't extend as well (and therefore produces an increase in passive force). This was shown by using a combination of radio-labeled phosphate groups, mass spectrometry, and a novel approach called atomic force microscopy (AFM), which can measure the level of force produced at the single molecular level. In addition, I found that cardiac titin in cardiac cells is phosphorylated by PKC, resulting in an increase in the peak force that can be produced by the cell when stretched. Furthermore, I took advantage of an innovative mouse knockout model in which the titin gene has been modified to delete a small group of residues, which includes the two sites that I identified as the sites phosphorylated by PKC. This model further established what was found above and showed that heart muscle lacking in these phosphorylation sites does not alter its passive tension following PKC phosphorylation. Another exciting discovery was that these specific phosphorylated amino acid residues are highly conserved and are found in all known titin isoforms in striated muscle in addition to being found in all mammalian species in which the titin sequence is known, thus indicating the importance of these exons in a variety of physiological adaptations. In conclusion, this thesis indicates that phosphorylation of the PEVK element of titin by PKC is an important mechanism for altering passive muscle stiffness and that this might play an important role during both physiological (such as during exercise) and pathophysiological (heart failure) adaptations of the heart.