Titin Isoforms in Heart Failure
Are There Benefits to Supersizing?
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Titin is a giant sarcomeric protein that functions as a complex, molecular spring. Its presence in the sarcomere of striated muscle was recognized in the 1980s, but its functions have been appreciated only over the past decade, in considerable measure because of the efforts of Granzier, Labeit, Linke, and coworkers. Earlier work by these investigators and others delineated the spring properties of titin and its role as the prime source of passive tension in the cardiomyocyte and, along with collagen, in myocardium. Titin also interacts and/or binds with a host of other proteins, including actin, a number of Z-disc proteins, obscurin, muscle LIM protein, and the group of muscle ankyrin repeat proteins. These interactions suggest additional functions, and recent evidence indicates that titin directly modifies sarcomere shortening and has intriguing and potentially diverse roles in mechanical sensing and signaling pathways.

Titin Gene and Protein
Titin is encoded by a single gene containing 363 exons. Differential splicing results in two major isoforms, the shorter and stiffer N2B and the longer, more compliant N2BA. N2BA titin has numerous fetal-neonatal variants, but most variation is lost in adult life. Titin is positioned within the sarcomere such that its N-terminal segments are anchored in the Z disc and its C-terminal segments are bound to the thick filament in the M-line region (see Figure 1 in Granzier and Labeit). The N-terminal segment penetrating the Z-disc is capped by telethonin (T-cap), a protein that may have a role in mechanical signaling and maintenance of important structural relationships—e.g., with the T-tubule system and sarcoplasmic reticulum. Adjacent to the Z disc is a nonextensible segment followed by titin’s complex, extensible I-band segments consisting of tandem immunoglobulin (Ig)– and PEVK-rich segments, and the N2B sequence (exon 49) present in both isoforms. Exons 102–109 encode the N2A sequence exclusive to N2BA titin, which also contains additional Ig and PEVK segments. The M-line region of titin bound to the thick filament is nonextensible. Titin also contains a kinase sequence the function of which is unknown.

Titin and Passive Cardiomyocyte and Myocardial Tension
The structural basis for titin as a source of cardiomyocyte passive tension is as follows (see Figure 2 in Granzier and Labeit). At slack sarcomere length (~1.85 μm), the extensible I-band segments are highly folded. As the sarcomere is elongated, extensible segments develop elastic tension as they are stretched between Z disc and thick filament. Extension of titin occurs sequentially, beginning with Ig segments and followed by PEVK and N2B/N2A unique segments. This results in distinctive portions of the cardiomyocyte passive length-tension relation as a function of sarcomere length. Treatments that detach titin from the thick filament result in virtual complete loss of cardiomyocyte passive tension over the physiological sarcomere length range. At short sarcomere lengths, titin is the major determinant of passive tension; at longer sarcomere lengths, collagen assumes increasing importance. The N2B sequence of titin is a substrate for protein kinase A. Phosphorylation results in reduced passive tension at short sarcomere lengths. With contraction below slack length, the thick filament drags titin’s extensible segments in the opposite direction (Figure 2, Granzier and Labeit), generating a restoring force that is the major source of elastic recoil of the cardiomyocyte. Thus, in addition to contributing to passive elastic stiffness, titin may also contribute to ventricular diastolic suction. Consistent with protein kinase A modulation of passive tension, we reported dobutamine-induced downward shifts of the fully relaxed left ventricular pressure–dimension relationship at distending pressures at and below 0 mm Hg, which facilitates diastolic suction.

Functional Consequences of Titin Isoforms and Alterations in Disease
The two titin isoforms are coexpressed within the sarcomere. In adult rodents, very little N2BA is present. In larger mammals, the proportion of N2BA increases, with the N2BA/N2B ratio averaging 0.56 in normal humans. Because the isoforms differ so markedly in stiffness, even modest shifts in their ratio can significantly modify passive tension. Thus, rodent myocardium is much stiffer than that of large mammals. This has been postulated to be important in utilization of the Frank-Starling relationship at rapid heart rates present in rodents. Stiffer titin should also result in greater elastic
recoil, which also may be useful for rapid filling at high heart rates.

Titin isoform variation offers the possibility of modulation of diastolic function in acquired disease. In their report in the present issue of Circulation, Nagueh et al15 document an increase in N2BA/N2B isoform ratio in hearts from patients with nonischemic dilated cardiomyopathy (DCM) compared with controls. The magnitude of shift to the larger isoform is such that a substantial decrease in passive myocardial stiffness would be expected, and this is exactly what was observed in myocardial strips. Loss of titin does not explain the stiffness change because normal stoichiometry in relation to myosin was maintained. Notably, several clinical parameters that reflect passive left ventricular chamber stiffness were strongly correlated with both the isoform shift and myocardial stiffness in strips. In this group of patients with nonischemic DCM, the collagen contribution to passive stiffness was not increased. Thus, this report constitutes the first example of a disease-associated titin isoform shift that causes predictable changes in both myocardial stiffness and ventricular diastolic function, without concomitant, potentially confounding alterations in collagen. It also suggests the possibility that isoform shifting contributes to changes in myocardial stiffness and ventricular diastolic function in other diseases.

In 1994, Morano et al16 reported decreased titin content in failing hearts, but it seems likely that this reflected unrecognized proteolysis, to which titin is exquisitely sensitive. More recently, Neagoe et al17 reported a similar increase in the N2BA/N2A ratio in ischemic but not nonischemic DCM. The reason for the apparent discrepancy between these two reports in nonischemic DCM is unclear. It is not likely related to techniques of isoform quantification because N2BA/N2B ratios were quite similar in controls in both studies. One potential contributing factor could be regional variation in isoform ratios. Transmural variations have been recognized18; however, Nagueh et al15 did not detect variation in different regions of the left ventricle, whereas Neagoe et al19 reported that such variation does exist.

An important question posed by Nagueh et al15 is whether the titin isoform shift is adaptive or maladaptive. A decrease in passive myocardial stiffness should reduce diastolic filling pressure and dyspnea and improve pump performance, which is consistent with the observed correlation between peak VO2 and the shift toward the N2BA isoform. However, the magnitude of the shift was directly correlated with end-diastolic and end-systolic volumes and inversely correlated with ejection fraction, evidence for a maladaptive change that may contribute in a permissive fashion to adverse remodeling and reduced shortening. N2B but not N2BA titin appears to interact directly with F-actin to retard filament sliding.4 Thus, if anything, the direct effects of the isoform shift would be expected to improve shortening. This question will not be resolved until more detailed information is available on how and when the isoform shift occurs and contributes to the progression of DCM.

Increases in the N2BA/N2B ratio observed in human DCM15-17 contrast with results in animal models. Canine myocardium normally displays an increasing N2BA/N2B ratio from subepicardium to subendocardium. After 2 weeks of rapid pacing, we reported an exaggeration of this normal transmural gradient.18 Thus, detectable isoform variation can occur over a time frame of weeks. An increase in the more compliant isoform would be predicted to reduce restoring forces in the subendocardial layer, and this was correlated with impaired development of ventricular restoring forces that cause diastolic suction. After 4 weeks of pacing in dogs, we detected a significant decrease in the overall N2BA/N2B ratio,10 consistent with an observed increase in passive myocardial stiffness. In this case, an increase in collagen-dependent stiffness also contributed. Most recently, Warren et al20 reported a decrease in the N2BA/N2B ratio in spontaneously hypertensive rats.

The reasons for these differences compared with human disease are entirely unknown at present. Pacing tachycardia is associated with rapid and dynamic matrix remodeling.10 Perhaps titin isoform changes in this condition result from mechanical stresses that are altered in an unusual, model-dependent fashion. However, spontaneously hypertensive rats undergo more gradual hypertrophy, with associated stresses that should be reasonably comparable to humans. Patients in the studies of both Neagoe et al17 and Nagueh et al15 had DCM severe enough to require transplantation. Thus, the isoform shift in these patients may represent an end-stage phenomenon that does not, in fact, play a key role (either adaptive or maladaptive) during earlier progression of disease. Clearly, this is an area where additional research should be most illuminating. Animal models may ultimately prove useful in understanding the role of titin in acquired disease by allowing a detailed examination of the time course of isoform patterns and how they relate to myocardial and ventricular function. Additionally, these questions should encourage studies in patients who do not have end-stage disease.

**Titin and Sarcomere Structure and Signaling**

One of the most intriguing results reported by Nagueh et al15 is the upregulation of several titin-binding proteins, specifically, obscurin and the ankyrin repeat proteins, cardiac ankyrin repeat protein, ankrd2, and diabetes ankyrin repeat protein. As reviewed by Granzier and Labeit,4 recent evidence indicates that these and a number of other titin-binding proteins are intimately involved in maintenance of sarcomeric structural integrity and relationships with adjacent structures and signal transduction pathways responsible for coordinated responses to mechanical stress. So-called signaling hotspots, where ligand interactions with these proteins occur, are located at multiple sites along titin. Obscurin binds to titin at a hotspot in the I band near the Z disc and may play a role in sarcomere assembly as well as interactions with the sarcoplasmic reticulum and T-tubule system. Also included in these hotspots are the N2B and N2A elements themselves within the I band. N2A interacts with a conserved motif common to the ankyrin repeat proteins, which are induced as a component of a variety of muscle stress responses. Of great interest is the fact that these same ankyrin ligands appear to function as nuclear transcription and cell cycle regulation factors. As pointed out by Nagueh et al,15 the mechanism underlying upregulation is unknown; however, the corresponding reduction in immunostaining for cardiac ankyrin
repeat protein in the nucleus suggests a possible titin ligand-mediated alteration in gene expression. Although the existence and significance of such an alteration remain to be demonstrated, these results exemplify new and novel aspects of titin and its function.

Conclusions

Nagueh et al\textsuperscript{15} provide convincing evidence of a significant role for titin isoform shifting in diastolic function in human DCM and suggestions that titin participates in more complex ways in the response to altered mechanical stress. Because of its size, multiple and distinct sequences, complex structure, and position within the myofilament, it is perhaps not surprising that previously unsuspected roles for titin are emerging. Indeed, the journey from newly discovered, "supersize" protein of unknown function to elucidation of diverse and critical roles for titin has been remarkably rapid. The article by Nagueh et al\textsuperscript{15} is an important chapter in this evolving story.

References


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