New and Notable

A Spring Tale: New Facts on Titin Elasticity

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INTRODUCTION

Since pioneering work two decades ago, a wealth of literature has been amassed demonstrating that vertebrate striated muscles contain, apart from thick and thin filaments, a third sarcomeric filament system made up of giant proteins, the titins (Maruyama, 1994; Wang, 1996, and references therein). A particularly important property of titins (also known as connectins) is their ability to act as molecular springs, providing nonactivated ("relaxed") myofibrils with elasticity. Although single titin polypeptides ($M_r > 3$ MDa) span the entire half of a sarcomere, only a molecular segment confined to the I-band is functionally elastic. This segment was shown by Labeit and Kolmerer (1995) to consist of two main structural elements, a unique sequence rich in proline, glutamate, valine, and lysine residues termed the PEVK segment, flanked by stretches of immunoglobulin (Ig)-like domains. Both elements are expressed in muscle-type specific length isoforms. The authors argued that the poly-Ig regions might represent stiff components, because the Ig modules fold into thermodynamically stable domains (Politou et al., 1995). Then, they reasoned, the PEVK region could be a compliant spring. This concept differed from earlier ones suggested before the discovery of the PEVK domain, which had proposed reversible unfolding of Ig modules as the molecular basis of elasticity (Erickson, 1994). Clearly, the concepts needed reinvestigation.

In studies of the elastic behavior of cardiac muscle titin, Trombitás et al. (1995) and Granzier et al. (1996) reported that in slack sarcomeres (i.e., at zero passive tension) the elastic portion of titin is not straight; rather, it is in a contracted state. Passive force developing in modestly stretched sarcomeres was proposed to be entropic in nature and to arise from straightening of titin's I-band region. After the full titin sequences became available, Gautel and Goulding (1996) and Linke et al. (1996) investigated I-band titin extensibility in skeletal muscle by following the location of sequence-assigned antibodies that flank the Ig-domain region of titin N-terminal of the PEVK segment. They found this region to lengthen predominantly at low sarcomere extension but much less at larger stretch—a fact seen most dramatically in the study by Linke et al. (1996). The results implied just the opposite of what had been hypothesized by Labeit and Kolmerer (1995): the poly-Ig regions apparently represent relatively compliant components providing extensibility at short sarcomere lengths. These regions straighten out at low stretch force, before elongation of the PEVK region and, on extreme stretch, unfolding of Ig repeats comes into play at higher forces.

State-of-the-art techniques have recently been used to explore titin elasticity at the single molecule level. Elegant laser trapping and AFM studies (Kellermayer et al., 1997; Rief et al., 1997; Tskhovrebova et al., 1997) revealed a nonlinear force response upon stretching titin, which was attributable to the entropic-chain characteristic of the molecule (also see the paper by Rief et al. (1998) in this issue). An entropic chain undergoes thermally induced bending movements that tend to shorten its end-to-end length. Stretching such a chain reduces its conformational entropy and thus requires an external force. In the mechanical studies on isolated filaments, titin's force-extension relation could be well fitted with a worm-like chain (WLC) model of entropic elasticity. A WLC is a deformable rod whose bending rigidity is expressed in terms of its persistence length, a distance within which the orientations of the chain are correlated. Rigid polymers have a large persistence length (low conformational entropy) and their straightening requires little external force, while the opposite is true for more flexible polymers. The experiments also indicated that titin contains a permanently unfolded segment which was proposed—considering that the PEVK domain has attributes of a denatured polypeptide (Labeit and Kolmerer, 1995)—to include the PEVK segment (Kellermayer et al., 1997). This conclusion is consistent with that of electron microscopic analysis of titin molecules stretched with meniscus force, thereby visualizing a thin molecular thread within the filament likely representing the unraveled PEVK segment (Tskhovrebova and Trinick, 1997).

The single molecule mechanical work revealed that the Ig domains are capable of unfolding when the applied stretch forces exceed a certain threshold of at least 20–40 pN—the higher the stretch velocity, the greater the unfolding forces. Above the force threshold, the native, ~4 nm-long modules unravel to a length of 20–25 nm. Upon release, however, the unfolded domains do not refold immediately, but do so only when the force drops to sufficiently low levels (~2.5 pN). Apparently, axial force increases the activation energy of folding intermediates, which stand as barriers to refolding. Differences between the unfolding and

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refolding kinetics under external force result in hysteresis (in which force is higher during stretch than during release), a well-known phenomenon of the passive tension response of muscle fibers. The results imply that, once titin's Ig domains unfold, large hysteresis should appear in the length-tension curve of resting muscle.

Fundamental questions about the nature of titin elasticity remained. For example, is the entropic spring concept valid in the skeletal-muscle sarcomere? Or is titin's elastic behavior constrained by the sarcomeric arrangement? Does the PEVK segment indeed behave as an entropic spring? Does Ig domain unfolding occur under physiological conditions? These questions are now addressed in new, independent reports that measure the in situ extensibility of both the poly-Ig regions and the PEVK domain from human soleus (Trombitás et al., 1998) and rat psoas muscle (Linke et al., 1998a,b) by immunoelectron microscopy and simulate the experimental results with the WLC model. The studies confirm, as the most likely scenario, that chains of folded Ig modules may behave as entropic springs. However, entropic elasticity of poly-Ig regions alone still cannot explain the unique passive length-tension relation of sarcomeres, because at higher force, PEVK extension prevails. The nature of PEVK elasticity is just beginning to be understood. Trombitás et al. (1998) suggest that a purely entropy-based mechanism accounts for the segment's elasticity in situ, as demonstrated by fitting the PEVK-extension data of human soleus muscle to a standard WLC model. They conclude that the region may behave as a relatively stiff spring with the characteristics of a permanently unfolded polypeptide. On the other hand, Linke et al. (1998a) use a modified WLC model to fit their extension data of rat psoas PEVK-titin. They find that entropic elasticity is likely to be relevant at short to moderate sarcomere stretch, but that enthalpic factors may dominate at high physiological extensions. Enthalpic contributions to PEVK elasticity are proposed to originate in electrostatic and perhaps hydrophobic interactions within the PEVK segment and/or result from elastic anisotropy. It will be interesting to follow up on these studies and test the hypotheses.

What appears to be clear is that Ig-domain unfolding is unlikely to take place throughout the working range of skeletal muscle. Therefore, the Ig unfolding mechanism, as attractive as it seems, cannot be generally responsible for titin elasticity. However, under pathophysiological conditions (overstretch of skeletal muscle) Ig unfolding may be relevant. Another possibility is that the unfolding concept still holds true for cardiac muscle (Granzier et al., 1997), which contains a significantly shorter I-band titin than skeletal muscles (Labeit and Kolmerer, 1995). Considering that different length isoforms of titin are co-expressed in a cardiac myofibril, with short isoforms in parallel with long isoforms (Linke et al., 1996), unfolding may allow the efficient working range of the different cardiac isoforms to be adjusted.

To sum up, latest results show that titin's Ig domains may not unfold in normally functioning skeletal muscle. It is the entropic-spring behavior of poly-Ig segments with folded domains that appears to underlie the passive force development at modest sarcomere stretch. The PEVK segment represents a relatively stiff spring that is principally responsible for the extensibility of skeletal myofibrils at higher physiological stretch.

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