

Flexibility and interactions of titin

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Titin is the largest known protein (~4 MDa) and its single molecules span half striated muscle sarcomeres. In the myosin filament region of the sarcomere we have proposed that titin acts as a 'protein-ruler' to regulate exact myosin filament assembly. The remainder of the titin molecule forms an elastic connection between the end of the myosin filament and the Z-line. These connections are the main route of mechanical connectivity through relaxed muscle and they give muscle its passive elasticity. They also maintain myosin filaments midway between Z-lines during sarcomere length variations; this ensures an even development of force between the two halves of a myosin filament during active contraction.

Titin self-association

We are studying the properties of 'end-filaments', which are thought to be a hexameric aggregate of 22 conserved Ig domains in titin adjacent to the end of the thick filament. We have measured the mass/unit length of end-filaments, using scanning transmission electron microscopy (STEM). From this and the known mass/unit length of the titin molecule, we determined that there are 6 titin molecules in each end filament, and therefore there are 6 titin molecules associated with each half of the thick filament. From this result we can calculate that titin makes up 12% of sarcomere protein, which makes it the third most abundant sarcomere component after myosin and actin. We are also studying a 400 kDa peptide from this part of the molecule.

Elastic mechanism

Titin is a relatively flexible molecule, as evidenced by the fact that its radius of gyration is only about one tenth its contour length. The first phase of its elastic mechanism in muscle involves straightening from an initially coiled conformation, before unfolding of the polypeptide. Hitherto, it has been assumed that the coiled conformation *in situ* will be similar to the equilibrium random coil of the purified protein *in vitro*. However, consideration of the restricted space between the numerous actin filaments surrounding the elastic part of the molecule suggests that the conformation will be far from the solution equilibrium.

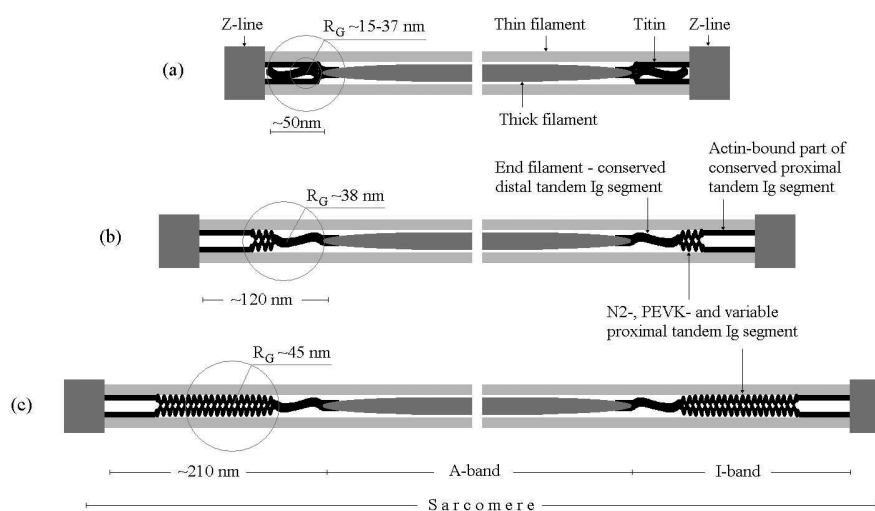


Fig. 1 Arrangement of titin molecules in three different muscles which differ in both slack sarcomere length and size of titin isoform: (a) cardiac; (b) psoas; (c) soleus muscle. The diagram shows how titin in the I-band may be constrained by thin filaments, compared with the expected radius of gyration of each isoform in free solution (circles on left side of diagram).

Super-repeats

The part of the titin molecule associated with the myosin filament consists almost entirely of immunoglobulin- and fibronectin-like domains. Throughout much of this region, these domains are arranged into two types of long-range patterns or 'super-repeats'. The large super-repeat, which occurs eleven times and spans 500 nm, contains eleven domains arranged as Ig-Fn-Fn-Fn-Ig-Fn-Fn-Fn-Ig-Fn-Fn. We have expressed seven overlapping constructs consisting of two or three of these domains and spanning 11 domains and studied their folding and myosin-binding characteristics.

Collaborators

L Brady, Bristol; D Holmes, Manchester; P Olmstead, Leeds.

Publications

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