Characterizing single molecules of the giant muscle protein titin with atomic force microscopy.

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Titin is a giant polypeptide that spans half of the striated-muscle sarcomere and generates passive force upon sarcomere stretch. The force-generating segment of the molecule is constructed of serially-linked immunoglobulin (Ig)-like domains interspersed with unique sequences.

The mechanical properties of titin have been explored by us and others with single-molecule techniques and this revealed that the molecule behaves as an entropic spring in which unfolding of the Ig domains occurs at high force during stretch and refolding at low force during release. Whether reversible unfolding of Ig domains contributes to titin's extensibility under physiological conditions remains a controversial issue.

Titin contains up to 92 Ig domains within its extensible region and recent work has indicated that the mechanical stability of different domains varies. Understanding Ig domain stability is important as mutations that affect the folding of titin's Ig domains have been reported recently in patients with dilated cardiomyopathy. Here we explored the mechanical properties of two Ig fragments, one 8-domain fragment that is constitutively expressed and another 6-domain fragment that is differentially expressed in some isoforms only.

We used an atomic force microscope specialized for stretching single molecules and characterized the unfolding of Ig domains at a wide range of stretch velocities. Our results indicate that the mechanical stability of domains varies, with domains from the differential expressed region having the lowest stability. Thus splicing in of these low stability domains creates titin isoforms with domains that may unfold at relatively low force.