

Regulation of titin, the cell's bungee cord

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Muscles are elastic structures - when you pull on them they stretch. This is due largely to the elastic properties of the muscle protein filaments themselves. But just like a piece of elastic, one end of the proteins must be held firm before the filaments can stretch. In skeletal muscle, structures called Z-disks anchor the filaments. Researchers are now discovering how a phospholipid contributes to anchoring filaments in the Z-disk.

The sarcomere is the smallest contractile unit of the skeletal muscle cell. The entire structure self-assembles through multiple molecular associations to form an ordered array of thick (myosin-containing) filaments and thin (actin-containing) filaments that work together to produce contraction. Two proteins, titin and α -actinin, are pivotally involved in setting up bilateral symmetry about the Z-disk, the structure that connects each sarcomere. In a paper published in *The EMBO Journal* in December¹, Paul Young and Mathias Gautel working at the EMBL in Heidelberg and the Max Planck Institute for Molecular Physiology in Dortmund (Germany) report their surprising discovery that a phospholipid regulates molecular recognition between α -actinin and titin during sarcomere growth and development. This finding also throws a new light on the regulation of α -actinin in nonmuscle cells

Titin (sometimes also called connectin) is the largest protein yet discovered at ~3MDa! Two titin molecules, aligned end to end, reach across the entire width of the sarcomere, a distance of about 2.5 μ m. Up to 166 immunoglobulin (Ig)-like domains of about 100 amino acid residues each occupy the N-terminal portion of this huge protein, and 132 similarly sized fibronectin type III domains lie towards the C-terminus (Figure 1). A unique sequence called the 'PEVK region' affords the whole molecule much of its elastic properties. As the sarcomere expands, folds within the PEVK region unfold like coils of the bungee cord being paid out as the jumper descends. Unlike the bungee cord, however, these titin folds

spontaneously reform as the sarcomere contracts guiding the thick filament back to its correct position^{2,3}.

The C- terminus of titin binds the myosin thick filament at the A-band towards the centre of the sarcomere, whereas an N-terminal region of titin tethers the protein to the Z-disk at the edge of the sarcomere⁴. This N-terminal region is composed of so-called Z-repeats that bind directly to α -actinin^{4,5}. The number of Z-repeats in the particular titin isoform that is expressed determines the thickness of the Z-disk^{6,7}.

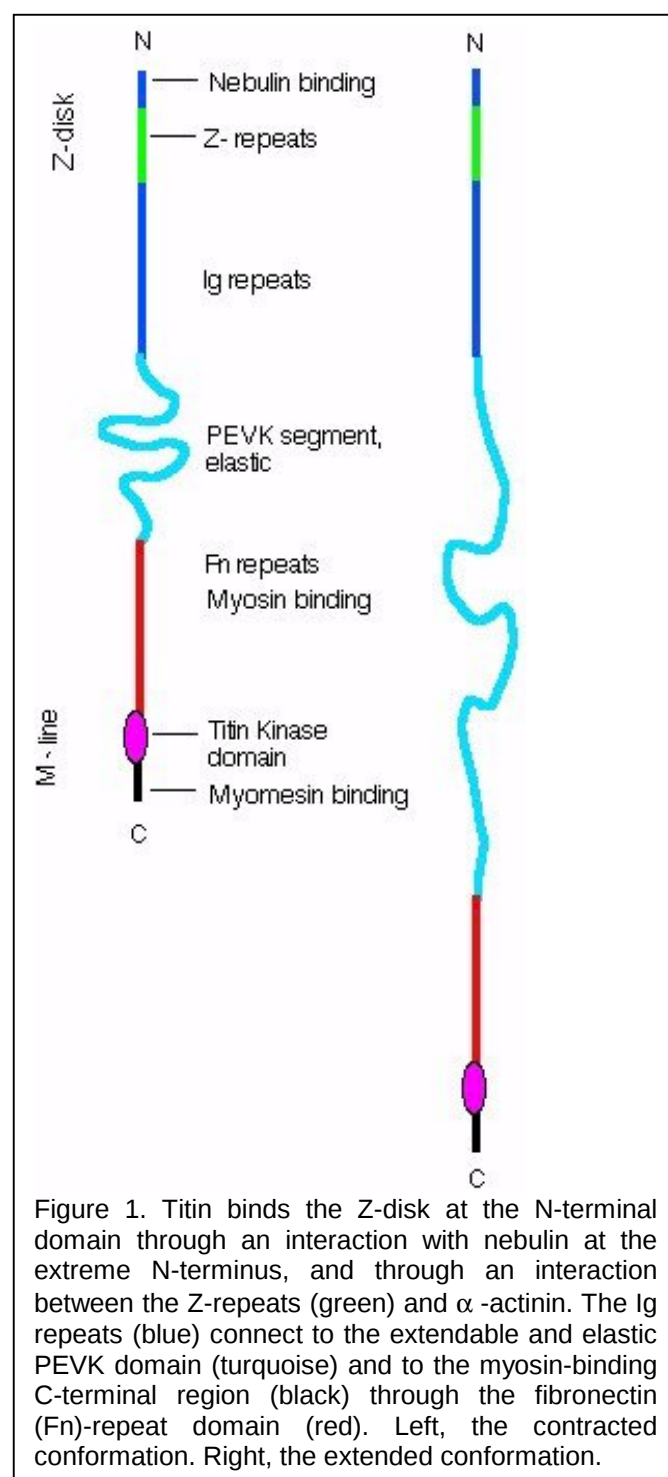


Figure 1. Titin binds the Z-disk at the N-terminal domain through an interaction with nebulin at the extreme N-terminus, and through an interaction between the Z-repeats (green) and α -actinin. The Ig repeats (blue) connect to the extendable and elastic PEVK domain (turquoise) and to the myosin-binding C-terminal region (black) through the fibronectin (Fn)-repeat domain (red). Left, the contracted conformation. Right, the extended conformation.

Titin interacts with α -actinin in two different ways⁸, and the interaction of both these proteins (and others) with actin filaments leads to the formation of an array of filaments with alternating polarity (Figure 2). The peptide comprising residues 760–826 of titin (close to Ig-like domain Z4, within the Ig-like repeat region) binds to spectrin-like repeats numbers 2 and 3 in α -actinin, and the Z-repeats of titin bind the C-terminal calcium-binding EF domain of α -actinin⁸. The first site makes a high affinity ($K_d=240\text{nM}$) interaction (as determined by a BiosensorTM assay) between a single titin and a single α -actinin dimer, whereas it is argued that the second site is multimeric with every Z-repeat binding to an α -actinin dimer^{6,8}. The interaction between the C-terminus of α -actinin and the Z-repeats of titin has been likened to that between two other regulatory muscle proteins troponin C and troponin I⁵.

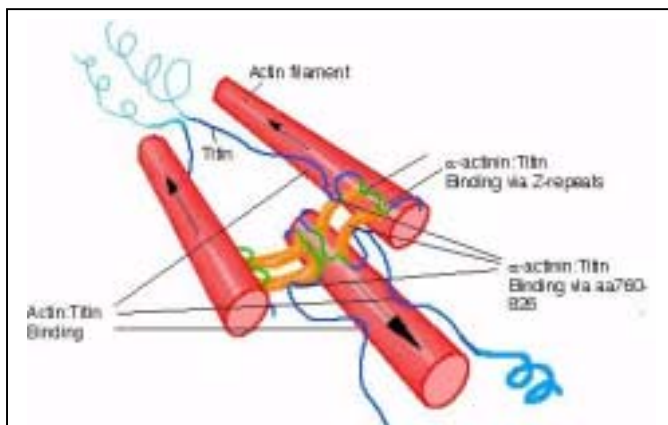


Figure 2. The arrangement of actin filaments (pink cylinders), α -actinin dimers (orange bananas), and titin (blue and green strings) at the Z-disk. The black arrows on the actin filaments indicate their polarity. Note that the amount of α -actinin associated with each actin filament depends on how many Z-repeats (green) are present in the titin isoform. Each titin makes two different types of interactions with α -actinin, the titin Z-repeats bind the EF-domain of α -actinin, and a second region towards the C-terminus of titin (residues 760–826) binds the 'spectrin-like' repeats of α -actinin. These interactions force an antiparallel association between the actin filaments at the Z-disk with the actin fibres extending in opposite directions from it. (Modified from Ref. 1 and Ref. 17.)

Many cytoskeletal proteins bind to and are modulated by a phospholipid called phosphatidylinositol 4,5-bisphosphate (PIP_2), which is a normal component of the plasma membrane but also acts as a second messenger, binding to specific regions of particular proteins to modify their activity. The affinity of α -actinin for actin increases in the presence of PIP_2 ⁹ and as PIP_2 appears to be present in the Z-disk⁹ (see below) this

may provide a mechanism whereby the α -actinin molecules end up in the Z-disk and not throughout the length of the thin filament.

Also in the Z-disks, CapZ, a protein that caps the barbed end of actin filaments, forms a complex with α -actinin that is weakened by the presence of PIP_2 ¹⁰. CapZ is located in the developing Z-disks during skeletal muscle myofibrillogenesis prior to the appearance of thin filaments in sarcomeres¹⁰, increasing the likelihood that α -actinin binds the thin filament at the barbed end. The interaction of CapZ with actin is itself PIP_2 -dependant¹¹, and it is not quite clear what effect the presence or absence of PIP_2 would have on the α -actinin–CapZ complex binding to actin filaments.

The influence of PIP_2 on sarcomere structure is further complicated by the fact that the interaction of titin with actin itself appears to be PIP_2 -sensitive¹² despite the fact that the site in titin that interacts with actin is well away from the Z-disk where the PIP_2 is located. On balance, it seems likely that PIP_2 plays a role in the Z-disk, and this conclusion is consistent with the finding that removal of PIP_2 from the Z-disk by the addition of calcium weakens the structure¹³.

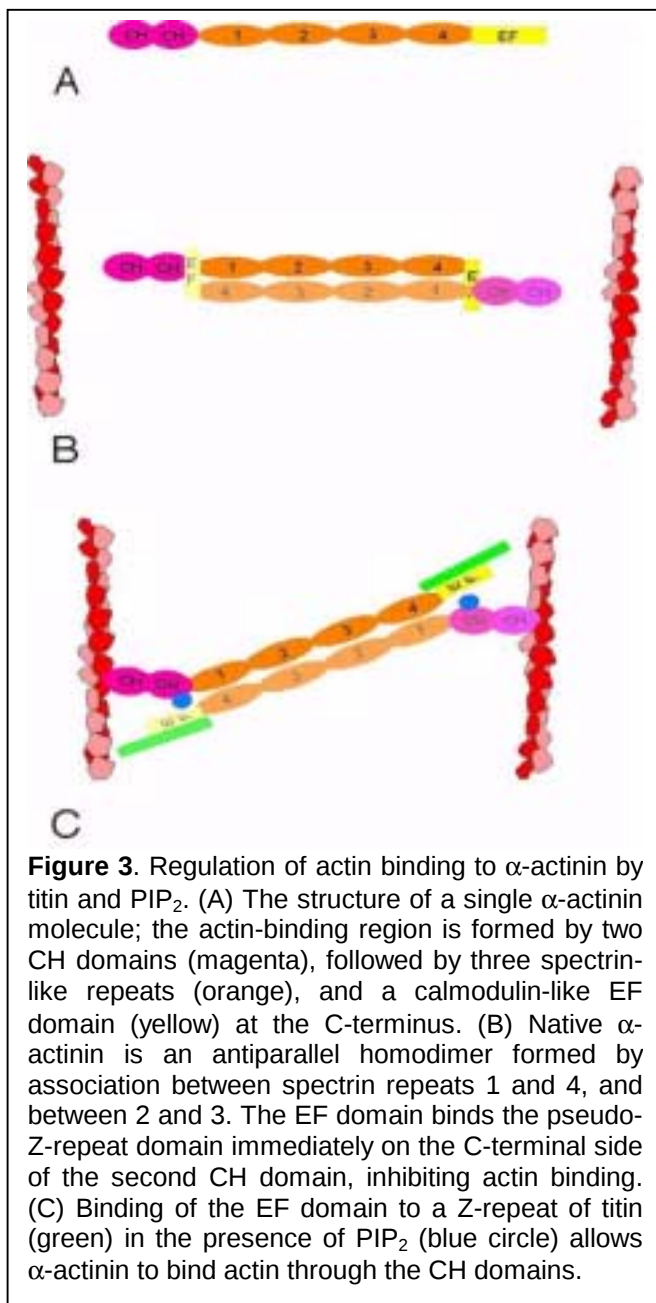
New clues to the regulation of α -actinin's binding to actin

Young and Gautel's new work¹ offers a solution to a long-standing puzzle in the regulation of α -actinin. Although it has long been known that nonmuscle α -actinins are regulated by calcium through EF domains, the molecular mechanism by which this regulation was mediated was a mystery. This new work reveals how the EF domain binds a newly identified region in α -actinin that is similar to the Z-repeats of titin to inhibit α -actinin binding to actin.

In nonmuscle cells in organisms as diverse as *Dictyostelium* and human, α -actinin is an abundant protein, regulated by calcium binding to EF domains at the C-terminus of the protein. In the presence of calcium, α -actinin does not bind to actin. The EF domains are calmodulin-like and influence the actin-binding domain within the antiparallel arrangement of the α -actinin dimer (Figure 3). The consensus finding of several structural studies of the α -actinin dimer^{1,14} is that repeat 1 binds repeat 4 of its partner in the dimer, leaving the EF domain close to the actin-binding domain composed of two CH domains¹⁵. When calcium binds to an EF domain the domain, in turn, binds the Z-repeat-like region in α -actinin between the actin-binding region and the first spectrin-like repeat.

Young and Gautel solved the puzzle when they noticed a similarity in sequence between the titin Z-repeats and a region in skeletal muscle α -actinin between the actin-binding domain and the first spectrin-like repeat¹. They propose that the EF domain of each α -actinin molecule in the antiparallel dimer binds to this Z-repeat-like region in its partner. When PIP₂ binds to the skeletal muscle α -actinin, however, they envisage that the Z-repeat region in titin competes with its homologue in α -actinin and the EF-domain now binds to titin allowing α -actinin to interact with actin.

Presumably, the structures involved in the interaction of the EF domain with the Z-repeat-like region of α -actinin are similar to those involved in the interaction of the EF domain with the titin Z-repeats⁵.



How is PIP₂ arranged in the Z-disk?

How PIP₂ is organized in the Z-disk is not clear. No lipid bodies, or membranes are seen by electron microscopy in preparations of Z-disks¹⁷ but these often include detergent washes that might remove them. By immunofluorescence microscopy, however, PIP₂ is present throughout the Z-disk. A PIP₂ micelle would be in the region of 5nm in diameter, probably too large for each α -actinin to be bound by a single micelle, and PIP₂ binds so tightly to α -actinin that it withstands even electrophoresis in SDS polyacrylamide gels. Taken together it seems most likely that PIP₂ binds directly to protein, possibly as a single molecule⁹.

Enzymes that synthesise PIP₂, such as PIP 4p-5 kinase, are present in the Z-disk¹ whereas others such as phosphatidylinositol 3-kinase are known to bind α -actinin¹⁸, indicating that PIP₂ and other polyphosphoinositides might be synthesised within the Z-disk itself.

Why use PIP₂ as a molecular glue to stick the Z-disk together? Many other cytoskeletal structures are disassembled by calcium. But in muscle, calcium flux controls the contractile interaction of the thick and thin filaments therefore the Z-disk must be regulated by other means. Muscle cells grow by the addition of new filaments to the Z-disk and so the structure must allow very localised deconstruction to permit remodelling, even as the majority of the same structure retains its ability to contract. Perhaps hydrolysis of PIP₂ at the Z-disk controlled by signalling molecules allows this very localised flexibility.

Further work is of course necessary to understand the regulation of α -actinin and titin, and their role in Z-disk assembly. Is there a role for phosphatidylinositol transfer proteins, phospholipase C and the other usual players in Z-disk remodelling? It is always wise to check how the bungee cord is secured before jumping!

References:

1. Young P, Gautel M (2000) *EMBO J.* 19, 6361–6340
2. Trinick J, Tskhovrebova L (1999) *Trends Cell Biol.* 9, 377–380
3. Tskhovrebova L, Trinick J, Sleep JA, Simmons RM (1997) *Nature* 387, 308–312
4. Maruyama K (1997) *FASEB J.* 11, 341–345

5. Atkinson RA, Joseph C, Dal Piaz F, Birolo L, Stier G, Pucci P, Pastore A (2000) *Biochemistry* 39, 5255–5264
6. Gautel M, Goulding D, Bullard B, Weber K, Furst DO (1996) *J. Cell Sci.* 109, 2747–2754
7. Peckham M, Young P, Gautel M (1997) *Cell Struct. Funct.* 22, 95–101
8. Young P, Ferguson C, Banuelos S, Gautel M (1998) *EMBO J.* 17, 1614–1624
9. Fukami K, Furuhashi K, Inagaki M, Endo T, Hatano S, Takenawa T (1992) *Nature* 359, 150–152
10. Papa I, Astier C, Kwiatek O, Raynaud F, Bonnal C, Lebart MC, Roustan C, Benyamin Y (1999) *J. Muscle Res. Cell Motil.* 20, 187–197
11. Heiss SG, Cooper JA (1991) *Biochemistry* 30, 8753–8758
12. Astier C, Raynaud F, Lebart MC, Roustan C, Benyamin Y (1998) *FEBS Lett.* 429, 95–98
13. Shimada K, Adhni DH, Takahashi K (1998) *Biosci. Biotech. Biochem.* 62, 919–926
14. Flood G, Kahana E, Gilmore AP, Rowe AJ, Gratzner WB, Critchley DR (1995) *J. Mol. Biol.* 252, 227–234
15. Djinnovic-Carugo K, Young P, Gautel M, Saraste M (1999) *Cell* 98, 537–546
16. Blanchard A, Ohanian V, Critchley D (1989) *J. Muscle Res. Cell Motil.* 10, 280–289
17. Luther PK (2000) *J. Struct. Biol.* 129, 1–16
18. Shibasaki F, Fukami K, Fukui Y, Takenawa T (1994) *Biochem. J.* 302, 551–557.