Unraveling the mysteries of the titin–N2A signalosome

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The muscle protein titin is best known for its mechanical role in myocytes. Titin filaments span one half of the sarcomere, the contractile unit of striated muscles, and are extensible within the I-band region, where they link myosin-based thick filaments and actin-based thin filaments as viscoelastic springs (Fig. 1, A and B). These titin springs provide nearly all stiffness and tension in resting myofibrils (reviewed by Linke, 2018). The composition of these molecular springs depends on alternative splicing of I-band titin exons, giving rise to different titin isoforms known as “N2A” in different skeletal muscles that differ in stiffness (Prado et al., 2005). The I-band region of the N2A isoforms is comprised of distinct in-series spring elements, tandem Ig-domain segments located near the Z-disk (proximal) and A-band (distal), which straighten out at low stretch forces and also unfold/refold individual domains (Rivas-Pardo et al., 2016), and the proline-glutamate-valine-lysine-rich (PEVK) segment, which extends at higher stretch forces (Linke et al., 1996). The proximal-Ig and PEVK segments flank the N2A region, which consists of four Ig domains and a unique insertion sequence called UN2A (Fig. 1 B; Labeit and Kolmerer, 1995). In an earlier issue of JGP, van der Pijl et al. (2021) address a novel, intriguing mechanical function of the titin–N2A region.

Apart from being a viscoelastic element, I-band titin is also thought to be a signaler of myofibrillar stress in healthy and diseased muscle, with a critical sensor region at the N2A region, thought to be a signaler of myofibrillar stress in healthy and intriguing mechanical function of the titin

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titin fragments (notably PEVK fragments) have been found to bind thin filament proteins in vitro (Dutta et al., 2018; Nagy et al., 2004; Zhou et al., 2021; Linke et al., 2002), this does not guarantee the same in vivo. We recently presented evidence of a titin–thin filament interaction in permeabilized fibers, where I-band titins were controllably cleaved close to the A-band, and titins got stuck on thin filaments while recoiling toward the Z-disc, showing that titin–thin filament binding is possible per se (Li et al., 2020). However, numerous experiments have been conducted on passive fibers and single myofibrils, where I-band titin extension during sarcomere stretch was not obviously impeded by an interaction with the thin filament, although no specific experiments have been designed to assess low-level titin–thin filament interactions. A single result suggesting the opposite, based on experiments with actively contracting myofibrils, was inconclusive (DuVall et al., 2017) because antibodies were used to track titin extension, which likely caused cross-linking of titin with other (including contractile) proteins. Therefore, the van der Pijl et al. (2021) finding that mechanically ventilated mouse and human diaphragm muscles produce MARP1, subsequently linking titin to the thin filament, is extraordinary. Unfortunately, it should be noted that this seemingly strong and permanent binding does not provide a mechanism for an activation-dependent titin–thin filament interaction, as MARP1 is sparse in healthy muscle and binds well in resting and contracting muscle. However, a pathway for titin–thin filament anchoring is now demonstrated through the N2A signalosome in myopathy—so why not also in healthy muscle?

Second, do the other proteins at the N2A signalosome play a role in titin–thin filament tethering? Among these proteins, myopalladin is an actin-associated scaffold that binds directly to MARPs (1 and 2; Miller et al., 2003), and so may stabilize the MARP1/titin–thin filament interaction, as MARP1 is sparse in healthy muscle and binds well in resting and contracting muscle. However, a pathway for titin–thin filament anchoring is now demonstrated through the N2A signalosome in myopathy—so why not also in healthy muscle?

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proteins localized to the N2A region, such as Smyd2-HSP90, HSP27, or αβ-C, appear to have little, if any, effect on the titin extension–sarcomere length relationships in resting muscle (Unger et al., 2017), and so do not seem to play a role in the titin–thin filament interaction; however, just a few data are available, and none measured during contraction. In summary, apart from MARP1 and myopalladin, the other identified N2A signalosome proteins either show no prevalence to thin filament binding in vivo or have not yet been evaluated.

Third, is there enough MARP1 in vivo, during disease, to make a meaningful change to passive force and protect sarcomere overstretch? The reported ~76-fold increase in MARP1 in mechanically ventilated human diaphragms is impressive (van der Pijl et al., 2021), but it is important to remember that MARP1 is present in trace amounts in resting skeletal muscle, and the increase in MARP1 may not saturate all available titins. A quick estimation is tricky because the amount of MARP1 seems to vary between muscles and is not necessarily localized to the sarcomeres only. Wette et al. (2017) showed that, in healthy human vastus lateralis, MARP2 is 150-fold more abundant than MARP1 (only trace amounts), and of the available MARP2, only ~15% are bound within the cytoskeleton, which Wette et al. estimated to cover ~25% of total titins. If we assume the MARP1-titin binding distribution is similar to MARP2 (Wette et al., 2017), then MARP1 would cover ~0.2% of titins in healthy muscle (i.e., functionally inconsequential). With the measured 76.1-fold increase in diaphragms of mechanically ventilated humans by van der Pijl et al. (2021), this would cover ~15% of total titins, and so we predict only 15% of titins are linked to the thin filament, and thus change their extension characteristics. However, the confocal images of van der Pijl et al. (2021) do not support the idea that a large fraction of titins are unbound, producing a “normal” titin extension pattern. This discrepancy could be because confocal imaging, even at the super-resolution level, may not resolve these subpopulations, and/or MARP1 has a larger binding distribution to titins than reported for MARP2 (Wette et al., 2017), potentially increasing the fraction of bound titins to a maximum of 85% if all MARPs bind. It would be worthwhile to repeat the experiments of Wette et al. (2017), with control and mechanically ventilated human diaphragms, to get a clearer picture of MARP1 levels and binding patterns. Furthermore, immunoelectron micrographs may provide a clearer visual of any titin-extension subpopulations.

Regardless of how many MARPs bind titin in critically ill patients, an increase in myofibril passive tension is measured (Fig. 5 in van der Pijl et al., 2021), but it is not yet clear whether these increases in passive tension (5–10 kPa) are important for sarcomere stability or over-stretch protection during contraction (active tension >100 kPa). Why would increased titin-based force and stiffness be advantageous? The first theory is to stimulate hypertrophy through protein sensors associated with (full-length) titin, as discussed in van der Pijl et al. (2021, 2019, 2018). Only passive overstretch is needed to up-regulate N2A signalosome proteins, leading to their titin localization (van der Pijl et al., 2021, 2018), and subsequently sparking a hidden signal cascade for muscle hypertrophy. MARP1 linking the N2A region to the thin filament leads to relatively more extension of PEVK, and enhanced titin-based forces, with sarcomere stretch, compared with healthy tissue. These changes could impact the proteins bound to mechanosensory titin regions, triggering a pro-hypertrophic signal cascade. It is also possible that MARP1’s impact on titin-based force is not meant to be a sensor at all; instead, the purpose of it is to increase [Ca²⁺] sensitivity. Titin-based forces strain the thick filament, which has been shown to
reorient myosin heads into a more active (“on”) state, enhancing Ca\(^{2+}\) sensitivity and improving active force production (Ait-Mou et al., 2016). This mechanism could be advantageous for a muscle: if the muscle is being overstretched by the applied load, why not improve force production through the MARP1 pathway to protect overstretch in the short term, while also increasing muscle size in the long term? These types of ideas are already generating experiments that should be completed over the next few years.

In conclusion, van der Pijl et al. (2021) provide concrete evidence that skeletal muscle can react to muscle disease through an up-regulation of the N2A signalosome protein MARP1, which links titin to the thin filament, functionally increasing titin-based stiffness and force. Although the purpose of this is not yet clear, it is likely related to (1) an “override” of the normal hypertrophic signaling pathway, as well as (2) a short-term measure that both protects against overstretch and enhances active force generation until muscle turnover is complete. Clearing up these questions, as well as others (such as how MARP1 is removed), will keep the field busy for many years; we are only observing the tip of the iceberg for this important topic.

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N2A–titin as a mechanosensor

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