© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 146-152 doi:10.1242/jeb.126383

REVIEW

Nebulin, a multi-functional giant

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ABSTRACT

Efficient muscle contraction in skeletal muscle is predicated on the regulation of actin filament lengths. In one long-standing model that was prominent for decades, the giant protein nebulin was proposed to function as a 'molecular ruler' to specify the lengths of the thin filaments. This theory was questioned by many observations, including experiments in which the length of nebulin was manipulated in skeletal myocytes; this approach revealed that nebulin functions to stabilize filamentous actin, allowing thin filaments to reach mature lengths. In addition, more recent data, mostly from *in vivo* models and identification of new interacting partners, have provided evidence that nebulin is not merely a structural protein. Nebulin plays a role in numerous cellular processes including regulation of muscle contraction, Z-disc formation, and myofibril organization and assembly.

KEY WORDS: Actin filament, Sarcomere, Skeletal muscle

Introduction

Actin is the prominent protein in most eukaryotic cells. Control of actin filament organization and architecture is crucial for numerous cellular functions. The arrangement of actin filaments in striated muscle is quite unique. Individual contractile units of striated muscle cells (sarcomeres) are composed of overlapping thin (actin) and thick (myosin) filaments forming a near-crystalline structure. Efficient contraction is dependent on the precise regulation of both thin and thick filament lengths. A few actin-binding proteins have been identified as playing important roles in maintaining thin filament length; however, the mechanisms that specify thin filament length have been more elusive. For more than two decades, the huge protein nebulin (600–900 kD), which is highly expressed in skeletal muscle, was thought to function as a molecular ruler specifying thin filament length. Because of the 'nebulous' nature of this protein – its susceptibility to proteolysis, enormous size and difficulties in purifying it in its native state – progress in testing the ruler hypothesis and determining the roles of nebulin in striated muscle has been slow.

The nebulin family comprises four additional members with diverse localization patterns and cellular roles: N-RAP, nebulette, lasp-1 and lasp-2. Although each family member interacts with actin via their distinguishing 'nebulin repeats', each is made up of distinct arrangements of protein motifs (e.g. SH3, LIM) resulting in very diverse molecular sizes (34 to 900 kDa). In particular, the other nebulin family members contain fewer nebulin repeats. Nebulette, lasp-1 and lasp-2 do not have super repeats, while N-RAP has fewer super repeats than nebulin. With the exception of N-RAP, the other family members contain an SH3 domain. Finally, N-RAP, lasp-1 and lasp-2 contain a LIM domain not found in nebulin and nebulette. As such, all members of the nebulin family are unique multi-domain proteins that bind actin filaments via their common nebulin repeats. Notably, all members seem to have roles as stabilizers and/or scaffolds for the highly specialized cytoskeletal assemblies with which they are united with (e.g. sarcomeres, intercalated discs and focal adhesions) (for review, see Pappas et al., 2011). Moreover, increasing evidence links members of the nebulin family to human disease.

Expression of nebulin outside of skeletal muscle is still debated. Several studies using RT-PCR and immunofluorescence staining have demonstrated that nebulin is found (albeit at reduced levels) in the heart and localizes in the sarcomere, similar to what is observed in skeletal muscle (Bang et al., 2006; Joo et al., 2004; Kazmierski et al., 2003). Via the cross-breeding of heterozygous nebulin mice (where the endogenous *nebulin* gene was replaced by Cre recombinase cDNA) with Rosa26 reporter mice, Bang and colleagues further showed that nebulin is predominantly expressed in atrial cardiomyocytes with only minimal expression in the ventricle (Bang et al., 2006). Reduction of nebulin expression in neonatal cardiac myocytes in culture results in elongation of thin filaments from their pointed ends (McElhinny et al., 2005). However, nebulin protein has never been reported in the heart by immunoblot analysis. Furthermore, global nebulin knockout mice do not exhibit cardiac dysfunction (Bang et al., 2006; Witt et al., 2006). Taken together, these results reveal that nebulin is present at low levels and could potentially be involved in thin filament length regulation by a mechanism in which a limited number of nebulin molecules control the length of many thin filaments in the heart (for how this may work, see Horowits, 2006). To help resolve whether there is a functional role for nebulin in the heart, further studies using cardiac-specific nebulin knockout mice need to be conducted. For a more detailed description of nebulin and its family members in the heart, see the recent review by Bang and Chen (2015).

Nebulin interacting partners

The N terminus of nebulin is located near the pointed end of the thin filament and contains a high-affinity interacting site for the actin filament capping protein tropomodulin (Tmod). Tmod has two tropomyosin binding sites, which contribute to the capping efficiency at actin filament pointed ends. It also contains two actin-binding domains that interact with two different sites on actin filament pointed ends (Boczkowska et al., 2015; Kostyukova et al., 2007). Because in mature muscle thin filament pointed ends extend past the N terminus of nebulin (see next section for discussion), it is possible that the interaction of nebulin and Tmod is transient, with both proteins working together only early in myofibril assembly to regulate actin filament assembly (McElhinny et al., 2001; Castillo et al., 2009). A compelling 'cap locator' model of thin filament length regulation was proposed by Fowler and coworkers (2006). In this model, nebulin recruits Tmod to a location specified by the length of nebulin. Tmod is then able to diffuse and transiently cap thin filaments that are either shorter or longer than nebulin's length. Because of the potential transient nature of the Tmod–nebulin interaction, further studies are required to test whether this model is

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Journal of Experimental Biology (2016) 219, 146-152 doi:10.1242/jeb.126383

feasible and, in fact, whether a functional association of Tmod and nebulin takes place *in vivo*.

The central super repeat region of nebulin has been demonstrated to interact with kelch-like family member 40 (KLHL40). In *klhl40* KO mice and KLHL40-deficient patients, nebulin protein expression is significantly reduced, indicating that KLHL40 stabilizes nebulin (Garg et al., 2014). Interestingly, the Garg study also demonstrated that KLHL40 interacts with leiomodin3 (Lmod3) and regulates its protein levels. The Lmods are structurally similar to the Tmods; however, they lack a second tropomyosin-binding domain and possess an extended C-terminal region containing an additional (third) actin-binding domain. Mutations in LMOD3 were recently discovered to underlie autosomal recessive nemaline myopathy, and Lmod3 is essential for myofibrillogenesis and/or organization of skeletal muscle thin filaments (Cenik et al., 2015; Tian et al., 2015; Nworu et al., 2015; Yuen et al., 2014; for review, see Sandaradura and North 2015). Furthermore, knockout of a different Lmod family member (Lmod2) reduces thin filament length in the heart and results in a rapid onset dilated cardiomyopathy (Pappas et al., 2015). Lmod2 and Tmod1 appear to have opposing functions at the pointed end, with Lmod2 promoting elongation, while Tmod1 shortens thin filaments (Sussman et al., 1998; Littlefield and Fowler, 2002; Tsukada et al., 2010). Further studies are required to investigate nebulin's interaction with pointed end proteins to determine how they cooperatively impact thin filament lengths.

Nebulin's C terminus is located within the highly specialized boundary of the sarcomere (Z-disc), and plays an important role in myofibril assembly, mechanosensing, signaling, force generation and transmission, and sarcolemmal resilience (reviewed by Clark et al., 2002; Frank et al., 2006; Luther, 2009; Sheikh et al., 2007; Fig. 1). The actin filament barbed end capping protein CapZ interacts with the linker repeats and SH3 domain of nebulin, providing structural stability to the sarcomere (Labeit et al., 2006; Pappas et al., 2008). The intermediate filament protein desmin also binds nebulin's linker repeats and this interaction plays a role in regulation of thin filament length, spacing of adjacent thin filaments within the Z-disc, and myofibril alignment (Conover and Gregorio, 2011; Conover et al., 2009; Bang et al., 2006; Witt et al., 2006). Based on the location of CapZ binding sites within nebulin, we proposed a unique model in which nebulin cross-links two adjacent actin filaments at the Z-disc periphery (Pappas et al., 2008) (Fig. 1). Testing this model would require high-resolution immunoelectron or super-resolution fluorescence microscopy.

The nebulin SH3 domain in the Z-disc interacts with multiple proteins that, based on their function, can be separated into three different groups. The first group is associated with actin cytoskeletal organization and includes α-actinin, myopalladin and its ubiquitously expressed homologue palladin, Ena/VASP and cysteine-rich protein (CSRP) family members, and zyxin (Bang et al., 2001; Chitose et al., 2010; Li et al., 2004; Louis et al., 1997; Nave et al., 1990; Ma and Wang., 2002; Moncman

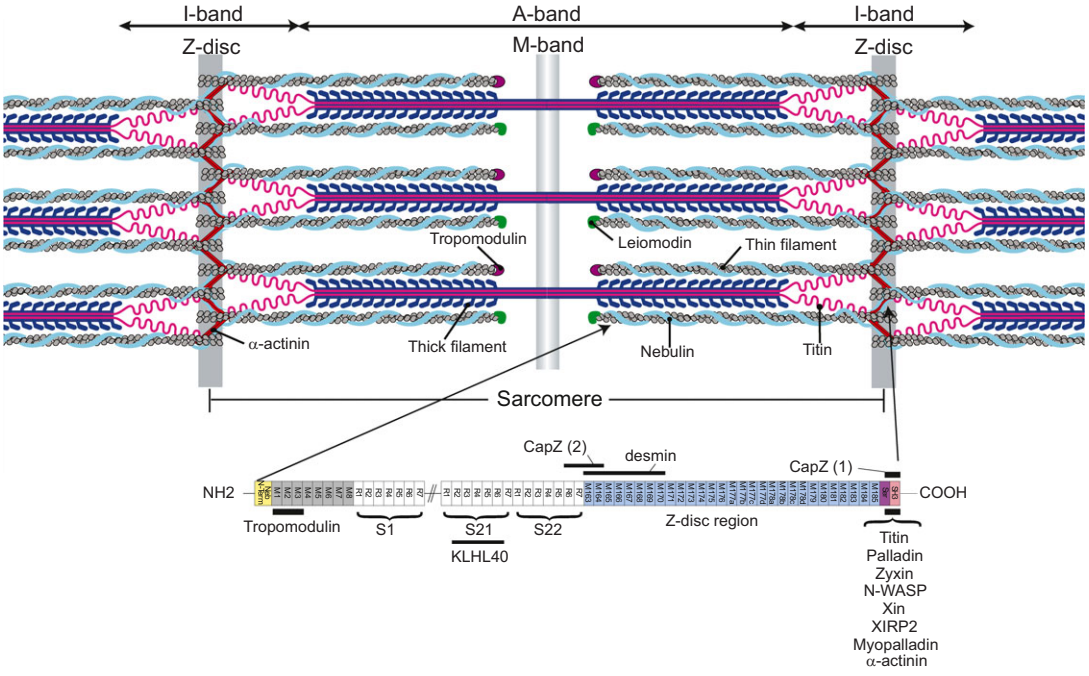

Fig. 1. The structure of nebulin and the location of its binding partners. The basic contractile units of muscle (sarcomeres) include actin (thin) filaments, myosin (thick) filaments, and giant molecules of nebulin and titin. The thin filaments insert in the Z-disc by their barbed ends, span the I-band, interdigitate with the thick filaments in the A-band, and extend toward the M-line with their pointed ends, where tropomodulin and leiomodin bind to regulate thin filament length (note that it is not known whether tropomodulin and leiomodin can bind to the same thin filament). Nebulin is associated with the thin filament; its N-terminal region extends near the pointed end of the thin filament, and the C-terminal region is anchored within the Z-disc. The nebulin modules 1–3 (M1–3) contain the binding site for the thin filament pointed end capping protein, tropomodulin. Modules 1 through 8 connect the acidic N-terminal domain to the central, super-repeat region. This central region encompasses M9–M162, where seven module repeats (R1–R7) form 22 super repeats (S1–S22). The super repeats are characterized by potential binding motifs SDXXYK (actin-binding motif found within each module) and WLKGIGW (tropomyosin/troponin-binding motif found once in every super repeat). S21 contains the binding site for KLHL40, which belongs to the BTB-BACK-kelch family of proteins. In nebulin's C-terminal region, modules 163 through 170 connect the super repeats to the Z-line region and contain the binding site for the intermediate filament protein desmin and the thin filament barbed end capping protein, CapZ. The C-terminal region contains unique serine-rich and SH3 domains, which bind to α-actinin, titin, myopalladin, palladin, zyxin, N-WASP, Xin and XIRP2.

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and Wang., 1999; Reinhard et al., 1995). The second group is associated with myofibrillogenesis, including Xin-repeat-containing protein family members (XIRP2 and Xin) and neuronal Wiscott–Aldrich syndrome protein (N-WASP). The interaction between Xin-repeat proteins and nebulin is transient, only occurring during myofibril assembly and remodeling (Eulitz et al., 2013). The nebulin–N-WASP complex is important for actin nucleation and elongation, which is crucial for IGF-induced muscle hypertrophy (Takano et al., 2010). The third group is associated with the giant sarcomeric protein titin (~3.7 MDa), a molecular spring that spans half the sarcomere. The SH3 domain of nebulin binds within two different regions of titin, one located in the Z-disc (Z2-Zis1) and the other in the I band (PEVK) (Ma and Wang, 2002; Ma et al., 2006; Witt et al., 2006). Both titin domains contain proline-rich regions with affinity for the nebulin SH3 domain. However, the function of the interaction between nebulin and titin is still unclear. For the molecular layout of nebulin-interacting proteins, see Fig. 1.

Role in thin filament architecture: nebulin stabilizes actin filaments, thereby regulating filament length

A plethora of evidence indicates that nebulin regulates thin filament length. Analysis of nebulin-deficient mice revealed the importance of nebulin in maintaining proper skeletal muscle function *in vivo*. Nebulin knockout mice die within approximately 2 weeks of birth because of skeletal muscle weakness. Investigation of skeletal muscle from these knockout mice revealed reduced thin filament lengths (Bang et al., 2006; Witt et al., 2006). Consistent with this observation, cultured chicken skeletal myocytes with a reduction in nebulin levels also present with shorter thin filaments (Pappas et al., 2008). The correct assembly of nebulin contributes to its actin regulatory functions because displacement of Z-disc (C-terminal) nebulin in cultured myocytes results in altered thin filament lengths (Conover and Gregorio, 2011). Clinically, specific mutations in human nebulin are linked directly to the skeletal muscle disorder nemaline myopathy (see below), which also involves muscle weakness (for recent reviews, see Labeit et al., 2011; Wallgren-Pettersson et al., 2011). Interestingly, it was observed that one patient expressing a nemaline myopathy-causing mutation in nebulin also presented with shorter thin filaments (Ottenheijm et al., 2009). Together, these reports demonstrate that nebulin is required for correct thin filament length regulation in skeletal muscle.

Prior to 2006, nebulin was hypothesized to be a 'molecular ruler' for thin filament assembly, although only correlative data supported this idea. Many unique properties of nebulin are consistent with it possessing a thin filament ruler function. For example, the length of nebulin (which varies significantly via alternative splicing) correlates with the lengths of thin filaments in different skeletal muscle types (Kruger et al., 1991; Labeit et al., 1991). Additionally, single molecules of nebulin are arranged along the length of the thin filament and interact with every major component of the thin filament (Wright et al., 1993). In particular, nebulin from humans contains 185 tandem copies of an ~35 aa repeat (with a conserved SDxxYK motif); each interacts weakly with a single actin monomer (Jin and Wang, 1991; Labeit and Kolmerer, 1995; Labeit et al., 1991; Pfuhl et al., 1994). Most of the repeats (M9–M162) are organized in 22 consecutive seven-module 'super repeats' (with a conserved WLKGIGW motif) that correspond to the arrangement of the Ca^{2+} regulatory proteins troponin and tropomyosin on the thin filament (Labeit and Kolmerer, 1995; Wang et al., 1996). The structure of nebulin's N terminus is unknown but is acidic in nature, whereas the C terminus comprises linker repeats (M163–M170) and simple repeats (M171–M183), followed by serine-rich and SRC homology 3 (SH3) domains (Labeit and Kolmerer, 1995; for reviews, see Horowits, 2006; McElhinny et al., 2003; Trinick, 1994).

Several current reports provide evidence that nebulin does not behave as a strict ruler in thin filament length specification (Fowler et al., 2006). In a survey of 21 different human leg muscles, it was discovered that nebulin transcripts with varying numbers (22–29) of super repeats are present within the same muscle that displays thin filament length uniformity (Laitila et al., 2012). This finding is not compatible with nebulin acting as a thin filament ruler (Laitila et al., 2012). Furthermore, in 2009, Littlefield and co-workers reported that the N-terminal end of nebulin does not co-localize with the thin filament pointed end (Castillo et al., 2009). Their investigations of skeletal muscles from numerous species revealed that although the distance of the N terminus of nebulin to the Z-disc is relatively constant (~0.95 µm), the location of Tmod (used as a marker for actin filament pointed ends) is observed at a variable distance from the Z-disc (ranging from ~1.00 to ~1.40 µm, but always further from the Z-disc than nebulin's N terminus) (Castillo et al., 2009; Gokhin et al., 2012). These data prompted Gokhin and Fowler to propose a compelling 'two-segment model' for the function of nebulin in thin filament length regulation (Gokhin and Fowler 2013). They propose that a mechanism exists whereby nebulin specifies the minimum thin filament length (i.e. proximal segment) beginning in the Z-disc and continuing to ~0.95 µm from the Z-disc. The second segment corresponds to a variable-length, nebulin-free thin filament extension (i.e. distal segment) that begins ~0.95 µm from the Z-line and continues to the pointed end of the thin filament. The consistent length of the proximal segment associated with nebulin eliminates a ruler function for nebulin, as varying thin filament lengths are found in different muscles (e.g. Ringkob et al., 2004). Additionally, the variable length of the distal segment would allow for nebulin-independent actin dynamics at the extreme thin filament pointed ends. In this model, members of the Tmod family could act as the primary driver in determining skeletal muscle thin filament length *in vivo* (for more discussion, see Gokhin and Fowler, 2013).

The hypothesis that nebulin acts as a molecular ruler was directly tested by the generation of a much smaller synthetic nebulin (i.e. 'mini-nebulin') (Pappas et al., 2010). Mini-nebulin consists of the distinctive N- and C-terminal ends of human nebulin with only four (out of 22) central super repeats. When expressed in primary cultures of skeletal myocytes, mini-nebulin extends out ~200 nm from the Z-disc as compared with ~1 µm for endogenous nebulin. When mini-nebulin was substituted for endogenous nebulin, actin filament lengths were not specified to the length of mini-nebulin; these data indicate that nebulin does not appear to have a strict ruler function. However, when latrunculin A was used to depolymerize actin thin filaments, the filaments that remained either matched or were longer than the length of mini-nebulin. This experiment revealed that mini-nebulin is capable of stabilizing (i.e. protecting) actin filaments at not only its own length, but also at lengths extending past mini-nebulin. Moreover, nebulin reduction in cardiomyocytes resulted in more dynamic populations of other key thin filament proteins including actin, tropomodulin and tropomyosin) – again indicating that nebulin provides thin filament stability.

Different mechanisms of thin filament stabilization by nebulin could be envisioned. In one model, thin filaments could be stabilized by nebulin directly by supplying actin monomers with

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additional molecular contacts and preventing their dissociation from the filament. Nebulin could also stabilize thin filaments indirectly by stabilizing tropomyosin, which itself is known to inhibit depolymerization of actin (Cooper, 2002). Another possibility is that thin filaments could be stabilized by nebulin via compressive forces. Atomic force microscopy revealed that nebulin is moderately compliant and, when extended, could provide substantive restorative forces to the thin filament (Yadavalli et al., 2009). This force could mechanically protect the filament from contractile-induced strain, preventing actin depolymerization. Additionally, nebulin interacts with the muscle-specific protein kelch-like family member KLHL40; this interaction could assist in providing stability to the thin filament. Specifically, it was proposed that KLHL40 functions to either block degradation and/or promote successful folding of nebulin, thereby enhancing stability of the thin filament. In muscles lacking KLHL40, nebulin protein levels are reduced, resulting in destabilization of thin filaments, sarcomere dysfunction, and subsequent nemaline myopathy (Garg et al., 2014).

The advent of more advanced molecular tools, mouse models and imaging techniques utilized in these current reports provide compelling evidence that nebulin regulates thin filament lengths by stabilizing the filaments, not by a traditional ruler mechanism. Numerous ideas (not all discussed here) have been proposed to describe how nebulin could provide a stabilizing role in the sarcomere. Although it is likely that these mechanisms are not mutually exclusive, more research is required to determine how nebulin stabilizes thin filaments.

Nebulin has diverse roles

Besides its obvious role in thin filament length regulation, the majority of recent publications on nebulin have revealed it to be a multi-functional protein implicated in a diverse range of cellular functions. Increasing evidence shows that nebulin regulates skeletal muscle contraction. Muscles from nebulin knockout mice produce significantly less force than their wild-type littermates (Bang et al., 2006; Gokhin et al., 2009; Witt et al., 2006). This is likely the result of loss of multiple nebulin-associated functions. Decreased actin filament lengths in knockout mice may lead to a decreased potential for actin–myosin cross-bridge formation. Analysis of nebulin knockout mice also revealed that: (1) nebulin is involved in calcium handling of the sarcoplasmic reticulum (Ottenheijm et al., 2008); and (2) nebulin augments the interaction of actin and myosin (Bang et al., 2009; Chandra et al., 2009; Ochala et al., 2011; Ottenheijm and Granzier, 2010).

The C terminus of nebulin interacts with, and appears to be regulated by, the abundant intermediate filament protein desmin. In nebulin knockout mice, loss of myofibril lateral alignment and a reduction in desmin assembly at the Z-disc is observed (Bang et al., 2006; Tonino et al., 2010). Thus, nebulin contributes to sustaining the lateral alignment of myofibrils, an essential component of coordinated contractile activity.

Nebulin is also necessary for proper Z-disc structure. Nebulin knockout mice have wider Z-discs and electron-dense structures reminiscent of nemaline rod bodies, which are aggregates of thin filament and Z-disc proteins found in the muscles of patients with nemaline myopathy (Bang et al., 2006; Tonino et al., 2010; Witt et al., 2006). Additionally, nebulin reduction in skeletal myocytes decreases the assembly of CapZ and results in non-uniform organization of actin filament barbed ends (Pappas et al., 2008). Nebulin not only has an important role in maintaining the Z-disc structure, but it could also have a role in specifying its width. In the mouse soleus muscle, the number of C-terminal nebulin exons expressed increases during development and correlates with an increase in Z-disc width (Buck et al., 2010). Conversely, although much data supports a role for nebulin in Z-disc function, mice without the C-terminal SH3 domain (NebΔSH3 mice) present with no detectable histological or structural skeletal muscle abnormalities and no significant effects on gene expression, passive muscle mechanics or binding partner localization (i.e. myopalladin, palladin, zyxin and N-WASP) (Bang et al., 2001; Chitose et al., 2010; Li et al., 2004; Louis et al., 1997; Nave et al., 1990; Ma and Wang, 2002; Moncman and Wang, 1999; Reinhard et al., 1995). This is very surprising based on all the integral sarcomeric binding partners that have been identified for this region (see above). However, NebΔSH3 muscle does have a slight deficiency in generating stress under isometric conditions at certain frequencies and is significantly more susceptible to eccentric contraction-induced injury (Yamamoto et al., 2013).

Recent studies also indicate that nebulin may be necessary for myofibrillogenesis. It was shown that nebulin interacts with N-WASP upon IGF-1 stimulation through activation of the PI3K-AKT signaling cascade (Takano et al., 2010). In non-muscle cells, N-WASP promotes actin nucleation by cooperating with other factors such as Arp2/3 (Campellone and Welch, 2010). Interestingly, in skeletal muscle, the nebulin–N-WASP interaction promotes actin nucleation without Arp2/3. This interaction has a synergistic effect on actin polymerization, barbed end formation and nucleation rates. Knocking down N-WASP in mice blocked both developmental and IGF-1-induced hypertrophy (Takano et al., 2010). Additional studies have demonstrated that the Xin repeat-containing proteins Xin and XIRP2 interact with nebulin during myofibril formation and remodeling (Eulitz et al., 2013). Xins are multi-adaptor proteins mainly expressed in cardiac and skeletal muscles, and are involved in muscle development, function, regeneration and disease (Wang et al., 2014). They contain several peptide motifs, which bind actin filaments by coiling around them, similar to nebulin repeats (Cherepanova et al., 2006; Pacholsky et al., 2004). Xin and XIRP interact temporally and spatially with nebulin in differentiating striated muscle cells and in areas of myofibrillar remodeling in adult muscle fibers (Eulitz et al., 2013). These data suggest that nebulin may initiate myofibrillar actin filament formation through complex formation with N-WASP and/or Xin repeat-containing proteins.

The role of nebulin in disease: nebulin mutations affect muscle function

Nemaline myopathy is the most common non-dystrophic, human skeletal muscle congenital disease, and mutations in the nebulin gene (*NEB*) account for ∼50% of all cases (Pelin and Wallgren-Pettersson, 2008). Nemaline myopathies encompass a set of genetically heterogeneous diseases that are defined pathologically by the presence of characteristic rod-like, cytoplasmic structures composed of thin filaments and thin-filament-associated proteins, called nemaline bodies (for review, see Wallgren-Pettersson et al., 2011). The underlying pathogenesis of nemaline myopathy remains unresolved; however, causative genes for nemaline myopathy include tropomyosin 3 (*TPM3*), *NEB*, α 1 actin (*ACTA1*), tropomyosin 2 (*TPM2*), troponin T type 1 (*TNNT1*) and cofilin 2 (*CFL2*), all encoding components of the thin filament. This discovery has led to the current hypothesis that nemaline

myopathy is a disease of the thin filament (for reviews, see Labeit et al., 2011; Wallgren-Pettersson et al., 2011). The majority of patients are compound heterozygous for two different *NEB* mutations (Lehtokari et al., 2006, 2014; Wallgren-Pettersson et al., 2011). Considering that *NEB* mutations are typically recessive and that two mutations are required for the development of nemaline myopathy, it was predicted that one functional nebulin allele would be sufficient to preserve normal nebulin levels, which would result in unaltered skeletal muscle function. Consistent with this prediction, Gineste and coworkers (2013) found that force-generating capacity of skeletal muscle in nebulin+/– mice (i.e. a single functional allele) was unperturbed (Gineste et al., 2013).

Nemaline myopathy is not the only disease thought to be caused by defects in nebulin. The nebulin gene has also been identified as a genetic cause of core-rod myopathy and distal myopathies (Romero et al., 2009; Wallgren-Pettersson et al., 2004). Therapeutic advances in the treatment of nemaline myopathy are predicted to occur rapidly, owing to recently obtained insights into the diverse functions of nebulin, the generation and availability of mouse models that mimic nemaline myopathy, and the widespread application of next-generation sequencing technology to facilitate faster determination of recessive missense mutations, compound heterozygote genotypes, deletions, stop codons or frame shifts that are causative for nemaline myopathy. Excitingly, initial reports focused on improving contractile strength of skeletal muscle in nemaline myopathy via a compound that activates fast skeletal muscle troponins are promising (de Winter et al., 2013; de Winter et al., 2015).

Conclusions

In conclusion, it is now well recognized that nebulin is not merely a structural protein, but contributes to proper muscle function through an assortment of other abilities. Since its discovery in 1980 (Wang and Williamson, 1980), many laboratories have contributed to identifying important roles for nebulin in the force-generating machinery of the muscle sarcomere. Clear evidence indicates that thin filament lengths are not static, and are not specified by nebulin as a strict molecular ruler. In other words, the length of nebulin does not correspond to the full length of the thin filament, and changing the length of nebulin does not change the length of the thin filament. As such, increasing evidence points to nebulin functioning as a thin filament 'stabilizer'. Whether this role as a stabilizer is the mechanism by which thin filaments lengths are specified and maintained still needs to be deciphered. It does appear, however, that thin filament length specification requires the orchestrated activity of many thin-filament-associated components, including members of the tropomodulin family of pointed-end-associated proteins. Accompanying nebulin's capacity to regulate thin filament architecture, mounting evidence has linked nebulin to sarcoplasmic Ca^{2+} handling, myofibrillar force generation, directing actomyosin interactions, and preservation of sarcomeric and Z-disc integrity. The giant molecule nebulin is clearly a multi-functional protein with additional functions likely to be discovered.

Acknowledgements

The authors thank Dr Alla Kostyukova (Washington State University) for insightful discussions.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was supported by the National Institutes of Health [grants R01HL108625 and R01HL123078]. Deposited in PMC for release after 12 months.

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