© 2022. Published by The Company of Biologists Ltd | Journal of Cell Science (2022) 135, jcs260179. doi:10.1242/jcs.260179

REVIEW

The insect perspective on Z-disc structure and biology

Frieder Schöck[1] and Nicanor González-Morales[2,*]

ABSTRACT

Myofibrils are the intracellular structures formed by actin and myosin filaments. They are paracrystalline contractile cables with unusually well-defined dimensions. The sliding of actin past myosin filaments powers contractions, and the entire system is held in place by a structure called the Z-disc, which anchors the actin filaments. Myosin filaments, in turn, are anchored to another structure called the M-line. Most of the complex architecture of myofibrils can be reduced to studying the Z-disc, and recently, important advances regarding the arrangement and function of Z-discs in insects have been published. On a very small scale, we have detailed protein structure information. At the medium scale, we have cryo-electron microscopy maps, super-resolution microscopy and protein–protein interaction networks, while at the functional scale, phenotypic data are available from precise genetic manipulations. All these data aim to answer how the Z-disc works and how it is assembled. Here, we summarize recent data from insects and explore how it fits into our view of the Z-disc, myofibrils and, ultimately, muscles.

KEY WORDS: Insects, Muscle, Sarcomere, Z-disc

Introduction

Together with a nervous system, muscles evolved in multicellular animals to provide the capacity to move freely. Muscles appeared in eumetazoans, which comprise bilateral animals (e.g. mammals and insects) and cnidarians (e.g. jellyfishes). Both bilateral animals and cnidarians have muscles (Burton, 2008; Seipel and Schmid, 2005). The muscles power movement by bridging two rigid skeleton structures and pulling them together (Sparrow and Schöck, 2009; Lemke and Schnorrer, 2017). Vertebrate muscles are linked to the bones, whereas insect muscles are attached to the exoskeleton (Gunage et al., 2017). A good example of muscle function in insects is the indirect flight muscle (IFM), which is attached to the thoracic exoskeleton and powers flight in several large insect groups, including Coleoptera, Hymenoptera and Diptera (Vigoreaux, 2001; Deora et al., 2017). Muscle contraction deforms the exoskeleton, indirectly causing the wings to move (Vigoreaux, 2001).

Muscle fibers are composed of long myofibrils, which are a tandem array of cylinder-shaped structures called sarcomeres. The sarcomere is the basic contractile unit in the muscles and is mainly composed of two antiparallel filament systems. The myosin thick filaments are composed of myosin heavy chains and light chains (Wang et al., 2018), and the actin thin filaments are composed of actin, tropomyosin and troponin (Szikora et al., 2022). Actin sliding on myosin filaments shortens the sarcomere. Because sarcomeres contract synchronously, the myofibrils shorten, powering muscle contraction (Fig. 1).

[1]Department of Biology, McGill University, Montreal, Quebec, H3A 1B1, Canada. [2]Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4R2, Canada.

*Author for correspondence (nicanor.gonzalez@dal.ca)

F.S., 0000-0002-1351-0574; N.G., 0000-0003-1305-8992

Microscopy of sarcomere longitudinal sections has revealed important defined regions or bands based on the presence of myosin and actin filaments. The A-band is the region where myosin filaments are present; the remaining region is called the I-band. In the middle of the A-band lies a region where actin filaments are excluded; it is called the H-zone. At the center of the H-zone, myosin filaments are anchored to a structure called the M-line. Most importantly, at the center of the I-band, actin filaments are anchored to an electron-dense disc, termed the Z-disc (Shafiq, 1963; Reedy and Beall, 1993a; Gunage et al., 2017). The Z-disc is the structure responsible for anchoring and recruiting actin filaments (Fig. 1).

In this Review, we summarize the recent contributions of insect biology to our understanding of the structure, function and assembly of Z-discs. We will focus on the components that are conserved in insects and vertebrates, as they are likely essential components. We will occasionally contrast the mechanistic details in insects with those from vertebrate species to provide a view of the flexibility of the components of the Z-disc.

A brief history of sarcomere descriptions

In 1949, the first high-resolution electron microscopy images of striated muscle appeared (Draper and Hodge, 1949). A few years later, in 1953, the filamentous nature of the sarcomere was established using phase-contrast and electron microscopy (Hanson and Huxley, 1953), and one year later the sliding of actin and myosin filaments was proposed (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). Soon after, researchers became interested in insects, reporting phase contrast and electron microscopy of a blowfly in 1955 (Hodge, 1955). Similar images were then obtained for other insect species – dragonflies (Smith, 1961), fruit flies (Shafiq, 1963) and cockroaches (Hagopian, 1966). The consensus from this work was that the general structure of sarcomeres is well conserved.

The connecting filaments, the filaments that span the entire half sarcomere and provide passive elasticity, now known to be strings of the titin protein, were reported in a series of papers from 1962 to 1965 (reviewed in Dos Remedios and Gilmour, 2017). In 1963, the first reports of connecting filaments in flies appeared (Auber and Couteaux, 1963), and later, several groups reported titin homologs as the main protein of the connecting filaments (Dos Remedios and Gilmour, 2017; Hu et al., 1990; Nave and Weber, 1990; Lakey et al., 1990; reviewed in Dos Remedios and Gilmour, 2017). Since then, given that most components of the sarcomere are highly conserved among animals (Table 1), insect models have become an essential part of muscle research.

The Z-disc structure

The Z-disc is a massive protein complex. The Z-disc from the IFM of honeybees is ∼120 nm thick and has a diameter of ∼2.5 µm (Rusu et al., 2017; Saide and Ullrick, 1973). The Z-disc diameter from the same muscle in *Drosophila* is slightly smaller, ∼90 nm thick, and has a diameter of ∼1.5 µm (Reedy and Beall, 1993a). An adult IFM sarcomere contains ∼2430 actin filaments and 825

Journal of Cell Science (2022) 135, jcs260179. doi:10.1242/jcs.260179

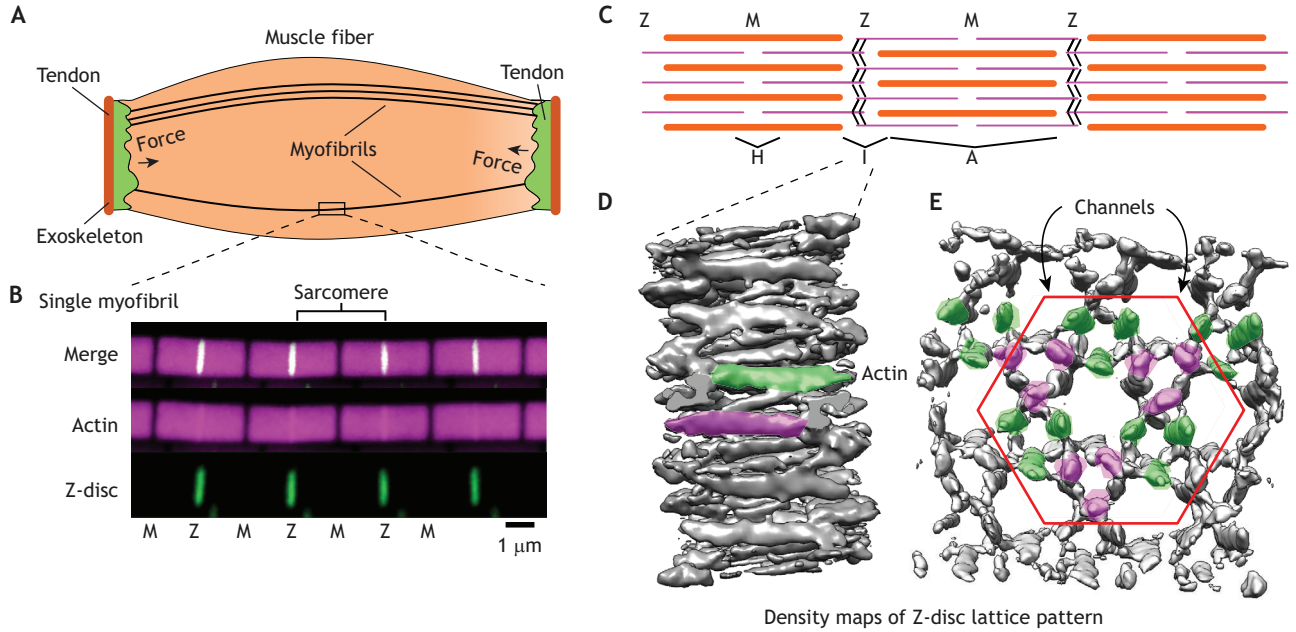

Fig. 1. The conserved anatomy of muscles, myofibrils, sarcomeres and Z-discs. (A) An IFM muscle fiber is connected to the rigid exoskeleton (brown) through the tendons (green) at both ends of the muscle. Fibrillar muscle types are composed of thousands of myofibrils each attached to the membrane at the tendon attachment site. (B) High magnification confocal microscopy image of a single myofibril, actin cables (magenta) and a Z-disc marker (green). Myofibrils are composed of tandemly arranged sarcomeres. Image taken in our laboratory. (C) Simplified cartoon of a myofibril showing myosin (orange) and actin (magenta) filaments forming an antiparallel cable of interconnected filaments. The black lines denote actin crosslinks. (D,E) Surface density maps of an isolated Z-disc of the honeybee (EMDB: EMD-8727; Rusu et al., 2017). Actin filaments from opposing sides are highlighted in green and magenta. (D) Side view of the Z-disc; note the crosslinking between the colored actin filaments. (E) The cross-sectional view of the lattice pattern from an isolated Z-disc from honeybee IFM, with large channels visible that form in between filaments. This repeated pattern extends through the whole disc.

myosin filaments, with each myosin filament surrounded by six actin filaments (Fernandes and Schöck, 2014). Other muscles have the same overall architecture but with slight structural variations. For example, the Z-disc from larval body muscles is smaller, less dense and less regular compared to the IFM Z-disc (Wojtowicz et al., 2015; Jani and Schöck, 2007). Additionally, larval body Z-discs are not entirely connected and do not form a straight band (Wojtowicz et al., 2015; Jani and Schöck, 2007). Because of the high structural regularity of the IFM, muscle research is strongly focused on the IFM.

Actin filaments from opposing sarcomeres interleave and anchor at the Z-disc (Reedy and Beall, 1993a; Shafiq, 1963). The main protein responsible for anchoring actin filaments is α-actinin, a dimeric protein that is a member of the spectrin superfamily (Lakey et al., 1990; Murphy and Young, 2015). α-Actinin dimers organize the thin filaments by joining them together and providing equal spacing between them (Figs 1D and 2). Single-molecule localization microscopy of the *Drosophila* IFM has shown that there are two α-actinin-organizing pools flanking the Z-disc from a side view (Szikora et al., 2020). Because each myofibril has ∼2430 actin filaments (Fernandes and Schöck, 2014), each α-actinin pool consists of ∼1215 α-actinin dimers.

Viewed from the front, the Z-disc is a remarkably ordered lattice structure formed by repeated units (Rusu et al., 2017; Cheng and Deatherage, 1989; Saide and Ullrick, 1973). Vertebrate Z-discs have a semi-flexible tetragonal lattice that changes upon muscle contraction (Burgoyne et al., 2015; Perz-Edwards and Reedy, 2011; Luther, 2009). Insect Z-discs have a hexagonal lattice structure (Rusu et al., 2017; Cheng and Deatherage, 1989; Saide and Ullrick, 1973). A cryo-electron tomography model from isolated honeybee Z-discs has been solved to ∼6 nm, providing details about their structure (Fig. 1D,E). Each repeated unit of the lattice anchors six actin filaments, three on each side of the sarcomere (Rusu et al., 2017). Additionally, a large triangular channel per repeat unit extends through the entire Z-disc (Rusu et al., 2017). Overall, the Z-disc is extremely large, has a highly organized repetitive pattern, and is responsible for organizing and anchoring actin filaments.

The Z-disc protein core and the first steps of Z-disc assembly

Myofibril assembly occurs very fast in insects, taking at most several hours depending on the specific muscles. Regardless of the assembly speed, the Z-disc assembly steps are the same. First, the Z-disc grows from small actin-organizing centers called Z-bodies (Reedy and Beall, 1993a; Katzemich et al., 2013; Loison et al., 2018). These bodies condense from the cytoplasmic pool of proteins into discrete insoluble bodies (Katzemich et al., 2013; Orfanos et al., 2015). The core Z-disc proteins Zasp and α-actinin are present as soon as the Z-bodies appear (Katzemich et al., 2013). Then, Z-bodies stably grow into mature Z-discs (Fig. 2).

Step 1 – α-actinin crosslinking

The core of the Z-disc comprises actin crosslinked by α-actinin, and this crosslinking is the first step of Z-disc formation. Most of our knowledge of crosslinking between α-actinin and actin comes from *in vitro* studies (Murphy and Young, 2015). α-actinin is a dimer; each monomer contains two actin-binding calponin homology (CH) domains, four spectrin domains and two EF-hand domains (Almeida Ribeiro et al., 2014). In *Drosophila*, muscles without α-actinin or actin cannot form Z-discs (Rui et al., 2010; González-Morales et al., 2019b; Karlik et al., 1984). Vertebrate α-actinin exists as muscular and non-muscular forms that are encoded by separate genes (reviewed in Murphy and Young, 2015). Both forms

Table 1. Overview over the main structural insect Z-disc proteins with their human homologs

Protein names	Human ortholog	Function	Reference(s)
Actn	Actinin	Crosslinks actin at the Z-disc	Fyrberg et al., 1990
cheerio	Filamin	Bridges actin filaments and Sls molecules from opposing sarcomeres, resists contractile forces	González-Morales et al., 2017; Green et al., 2018; Wojtowicz et al., 2015
Cpa, Cpb	CapZ (CAPZA1, CAPZA2, CAPZB)	Binds the actin filament plus-ends to prevent spontaneous turnover of actin monomers	Hopmann et al., 1996
CryAB/l(2)efl	αB-crystallin	Acts as a filamin chaperone at the Z-disc	Wojtowicz et al., 2015
DAAM	DAAM1	Promotes filament elongation by inhibiting tropomodulin and by recruiting actin polymers	Molnar et al., 2014
Fhos	FHOD1	Incorporates actin polymers into early sarcomeres and recruits the actin filaments to the Z-disc	Shwartz et al., 2016
Lasp	Nebulin and nebulette	Controls I-band architecture and interacts with actin and myosin setting filament spacing	Fernandes and Schöck, 2014
MLP84B/MLP60A	Cysteine-rich protein family of LIM domain proteins	Associates with Sls and maintains the integrity of the Z-disc	Clark et al., 2007; Stronach et al., 1999
Msp300	SYNE1 spectrin repeat-containing nuclear envelope protein 1	Anchors nuclei, mitochondria and ER to the Z-discs, possibly through oligomeric filaments	Elhanany-Tamir et al., 2012
Rhea	Talin 1 and 2	Concentrates at the muscle attachment sites to sustain sharp contraction forces	Lemke et al., 2019
Rols	TANC1 and TANC2	Ankyrin repeat-containing scaffolding protein	Kreiskother et al., 2006
Sallimus, Stretchin and Projectin	Titin	Serves as an elastic filament that provides the sarcomere passive elasticity and maintains sarcomere length. In insects, the function of titin is split among the three proteins listed.	Ayme-Southgate et al., 2004; Burkart et al., 2007
Sals	No orthologs found	Binds actin filament minus ends and promotes filament elongation.	Bai et al., 2007
Starvin, Hsc70, HspB8 and dCHIP	BAG-3, Hsc70, HspB8 and CHIP	Components of the chaperone machinery, which degrades damaged proteins.	Arndt et al., 2010
Tropomodulin	TMOD1	Binds actin filament minus ends to act as a transient elongation block.	Mardahl-Dumesnil and Fowler, 2001
Zasp52, Zasp66 and Zasp67	Zasp and Enigma	Scaffolding proteins recruited for Z-disc growth and stability	Jani and Schöck, 2007; Katzemich et al., 2013; Stronach, 2014; Liao et al., 2016; González-Morales et al., 2019a

are actin crosslinkers and their main difference is their ability to bind Ca^{2+} at their EF-hand domains. The muscle α-actinin is insensitive to Ca^{2+}, while actin-binding by the non-muscle form is inhibited by Ca^{2+} (Burridge and Feramisco, 1981; Waites et al., 1992). In contrast, *Drosophila* has only one α-actinin gene (*Actn*) (Fyrberg et al., 1990). It encodes at least two isoforms, one restricted to the ovaries and one enriched in muscles (Wahlström et al., 2004). Structurally, the two forms differ in just five amino acids in the EF-hand domain (Wahlström et al., 2004).

In muscles, α-actinin has several binding partners, which preferably bind the two EF-hand domains. Their association is tightly regulated and dynamic (Young and Gautel, 2000). Structural data from vertebrate α-actinin has revealed that the EF-hand domains of α-actinin naturally exist in a closed conformation, where protein-binding sites are masked (Almeida Ribeiro et al., 2014). Protein-binding sites are revealed when phospholipids bind to the actin-binding domain (Almeida Ribeiro et al., 2014). The *Drosophila* Z-disc contains a large number of phospholipids (Bullard et al., 1990), suggesting this regulatory mechanism of α-actinin could be conserved.

In non-muscle cells, α-actinin crosslinks parallel and antiparallel actin filaments, and is found along the entire length of stress fibers, which contain actin in a mix of both parallel and antiparallel orientations (Hampton et al., 2007; Courson and Rock, 2010). In contrast, in muscles, α-actinin is restricted to the Z-disc where it crosslinks antiparallel actin filaments (Fig. 2E), suggesting that additional proteins or molecules are required to restrict α-actinin to the Z-disc and its crosslinking activity to antiparallel filaments. The IFM has two crosslinking sites per actin filament pair (Szikora et al., 2020), whereas other muscles have more than two (Luther, 2009). It is unclear which factor restricts α-actinin to the Z-disc. A good candidate are Zasp proteins, scaffolding proteins that organize Z-disc assembly; these have been shown to bind actin filaments and α-actinin directly, and localize in between two actinin dimers at the Z-disc (Szikora et al., 2020; Liao et al., 2020, 2016).

Step 2 – setting up a scaffolding center, the Zasp proteins

Drosophila has three Zasp-encoding genes – *Zasp52*, *Zasp66* and *Zasp67*. Zasp52 and Zasp66 are present in all muscle types, whereas Zasp67 is specific to the IFM (González-Morales et al., 2019a). Zasp proteins have a conserved function in developing and maintaining the Z-disc by acting as a scaffold for recruiting proteins. Structurally, the Zasp proteins typically contain an N-terminal PDZ domain, a Zasp-motif (ZM) domain and one to four C-terminal LIM domains, as well as large unstructured flexible linkers in between (Zheng et al., 2010).

The complex arrangement between actin, α-actinin and Zasp proteins provides the structural repetitive unit that ultimately forms the Z-disc. In this context, a combination of structural studies using vertebrate proteins and functional studies using *Drosophila* have provided important insights into how the ordering of these proteins occurs. α-Actinin binds actin through its CH domains (Borrego-Diaz et al., 2006; Liem, 2016), leaving the spectrin and the EF-hand domains free (Gautel and Djinovic-Carugo, 2016). Then, a Zasp

Journal of Cell Science (2022) 135, jcs260179. doi:10.1242/jcs.260179

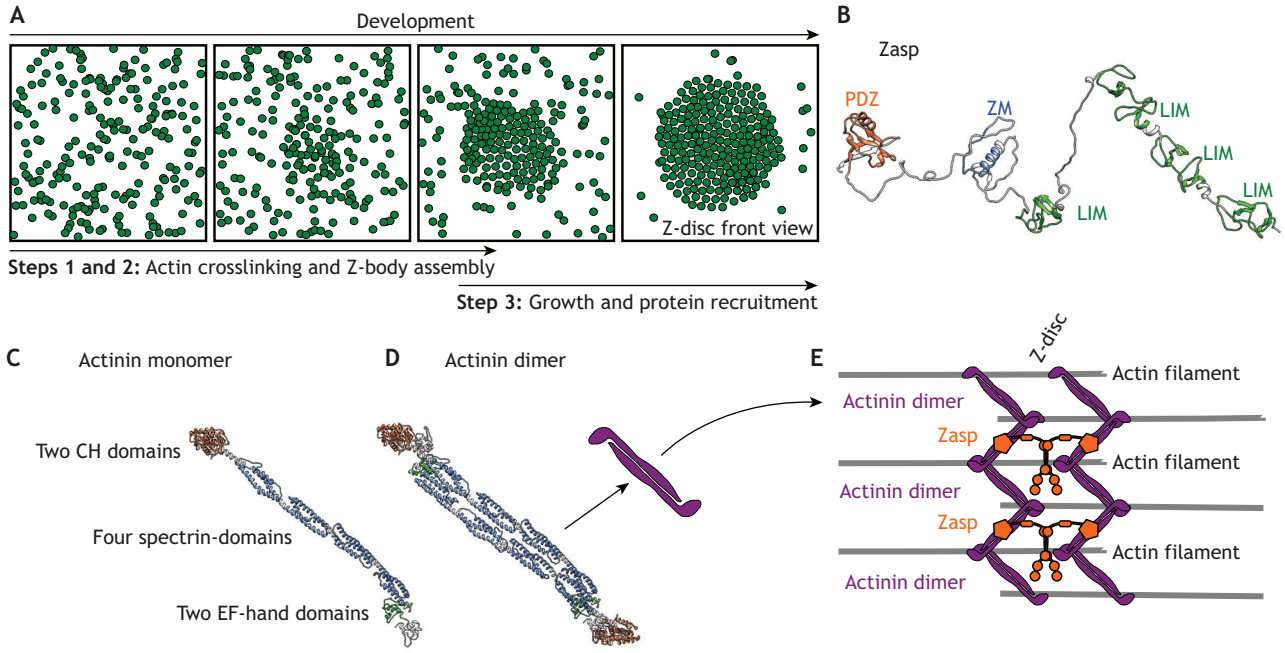

Fig. 2. Zasp and α-actinin assemble and mediate growth of the Z-disc. (A) Z-disc assembly and growth. First, Z-bodies are formed by condensation of cytoplasmic pools of α-actinin, Zasp, and crosslinked actin filaments (steps 1 and 2). The stable recruitment and crosslinking of these cytoplasmic proteins then grow the Z-disc to its final size and shape (step 3). (B) Homology model of a Zasp growing isoform. Its PDZ domain is shown in orange, the ZM domain in blue, and the four LIM domains in green. (C) Structure model of the α-actinin monomer. Actin binding occurs at the two CH-actin-binding domains (orange), and the four spectrin domains are required for dimerization. From PDB ID: 4D1E (Almeida Ribeiro et al., 2014). (D) Actinin dimer from PDB ID: 4D1E (Almeida Ribeiro et al., 2014). The CH domain is shown in orange, spectrin domains in blue and EF-hand domains in green. (E) Model of α-actinin-actin crosslinking at the Z-disc. Zasp binds to α-actinin dimers through its PDZ domain. The free LIM domains recruit either other Zasp molecules or other proteins into the Z-disc.

protein binds to α-actinin through an extended PDZ domain, which contains a conserved PWGFRL motif in between two β-sheets on the outside of the PDZ core (Liao et al., 2016; Au et al., 2004). Independently of α-actinin, Zasp can also join the Z-bodies through direct binding to actin filaments (Liao et al., 2020). LIM domains, like those in Zasp, are known protein–protein interaction domains (Kadrmas and Beckerle, 2004), and the flexible unstructured linkers between the LIM and the PDZ domains of Zasp might provide the spacing for simultaneous binding of multiple targets. Importantly, Zasp LIM domains are not involved in binding to actin or α-actinin and are located at the center of the Z-disc (González-Morales and Schöck, 2020; Szikora et al., 2020; Liao et al., 2020, 2016), making them an ideal scaffold for subsequent protein recruitment.

Step 3 – Z-disc growth

Once stable Z-bodies are formed and aligned, they grow to their final size recruiting additional proteins that form a mature Z-disc. The Z-disc grows in diameter by the coordinated oligomerization of Zasp proteins mediated by direct binding of their ZM and LIM domains (González-Morales et al., 2019b). In the IFM, the Z-bodies are 0.45 µm in diameter and grow to 1.2 µm (Orfanos et al., 2015).

The oligomerization process is tightly controlled, and the Z-disc diameter is equal for all sarcomeres in a muscle (Reedy and Beall, 1993a; Spletter et al., 2018). There are two types of Zasp isoforms, ‘growing’ isoforms with LIM domains and ‘blocking’ isoforms without LIM domains. Later during myofibril development, the blocking Zasp isoforms are expressed and bind to full-length Zasp52, thereby halting any further recruitment of Z-disc proteins and blocking Z-disc growth (González-Morales et al., 2019b). Indeed, depletion of blocking isoforms results in the Z-disc growing larger than normal, whereas the depletion of growing isoforms makes it smaller than normal (González-Morales et al., 2019b). Although Zasp oligomerization has been described in *Drosophila*, mice with mutations in their two Zasp homologs, Zasp and Enigma (also known as PDLIM3 and PDLIM7), also have smaller sarcomeres than normal, suggesting that a similar oligomerization mechanism occurs in vertebrates (Cheng et al., 2010; Zhou et al., 2001).

How is the switch in the Zasp isoform expression regulated? Differential isoform usage is typically controlled by splicing factors or by distinct transcription start sites and is particularly abundant in muscles (Spletter and Schnorrer, 2014; Venables et al., 2012; Nikonova et al., 2020). Bruno (also known as Bruno 1), a conserved RNA-binding protein is a major regulator of alternative splicing in flies and has been shown to control the splicing of many proteins during muscle development, including that of Zasp proteins (Oas et al., 2014; Spletter et al., 2015). Bruno is under transcriptional control of Salm, the major transcription factor that specifies muscle identities (Schönbauer et al., 2011; Bryantsev et al., 2012). Bruno, as well as possibly other yet unidentified splicing factors, regulates a global change in isoform usage that is required during muscle development, including that of switching the growing Zasp isoform to the blocking one (Spletter et al., 2018).

During the Z-disc growth phase, protein recruitment to the Z-disc is likely achieved in several ways. Proteins might join by binding to α-actinin, the LIM domains of Zasp, the actin filaments or other additional proteins. The *Drosophila* interaction database (http://www.droidb.org/) lists 67 distinct actinin-binding proteins, including Z-disc proteins, such as Lasp, MSP300, MLP84B and Rols (Yu et al., 2008; Kreiskother et al., 2006; Fernandes and

Journal of Cell Science (2022) 135, jcs260179. doi:10.1242/jcs.260179

Schöck, 2014). Overexpression of a Zasp isoform containing four LIM domains causes huge protein aggregates, suggesting that this is an important recruitment mechanism (González-Morales et al., 2019b).

Roles of the Z-disc

Anchoring actin filaments to resist contractile forces

The Z-disc provides strong anchoring support for actin filaments to resist the pulling forces of myosin filaments. Actin attachment to the Z-disc starts with α-actinin crosslinking, but α-actinin crosslinks are not sufficient to hold actin filaments in place during muscle contraction. To resist the contraction forces while maintaining a rigid structure, the Z-disc incorporates several spring-like proteins (Lemke and Schnorrer, 2017; Bullard et al., 2006; Kulke et al., 2001), with titin and filamin as two notable examples (Fig. 3).

Vertebrate titin is a large modular protein that spans half of the sarcomere from the Z-disc to the H-zone; it establishes a direct connection between the Z-disc and myosin filaments and has a role in setting the length of the sarcomere (Tskhovrebova and Trinick, 2017; Tonino et al., 2017; Linke, 2018). In the insect IFM, the function of titin is provided by at least two proteins – Sallimus (Sls), which connects the Z-disc to the beginning of the A-band, and Bent (also called Projectin), which overlaps with Sls at the interface between the I-band and the A-band and extends into the A-band along the myosin filaments until ∼350 nm from the Z-disc, covering ∼20% of the A-band (Schueder et al., 2022 preprint; Ayme-Southgate et al., 2004; Bullard et al., 2006; Ayme-Southgate et al., 2005). In other muscles, Bent extends further into the A-band where it almost reaches the M-line (Loreau et al., 2022 preprint). Thus, vertebrate titin reaches the M-line and covers the entire A-band, whereas in insects, neither Sls nor Bent reaches the M-line. In the IFM, targeted removal of Sls by calpain digestion severely decreases sarcomere stiffness (Kulke et al., 2001). Like vertebrate titin, Sls is composed of a variable number of immunoglobulin (Ig) and fibronectin (FN) domains, from 59 to 82 repeated domains, and one C-terminal SH3 domain (Bullard et al., 2002; Gramates et al., 2017). The repeated domains constitute a force-sensing spring that folds and unfolds during the contraction cycles (Bullard et al., 2006). In addition, vertebrate titin is also responsible for setting up the number of α-actinin layers in muscles (Gautel et al., 1996). Titin splice variants differ in their number of 'Z-repeats'. Each of these repeats binds a single α-actinin dimer directly, thereby setting the number of actinin layers. Multiple layers are required since individual interactions are weak (Grison et al., 2017). Z-repeats (Pfam: PF09042) are unique to chordates and not present in insects (El-Gebali et al., 2019), suggesting a different binding site exists between Sls and α-actinin.

Filamin is a much shorter protein; it contains two CH actin-binding domains and 22 to 24 Ig domains. Filamin is a conserved homodimer linked by the final C-terminal Ig domain (Razinia et al., 2012). Ig domains 14 to 19 are the mechano-sensing region of filamin (Huelsmann et al., 2016). They typically exist in a closed conformation that is maintained by direct interaction between Ig domains; masking protein-binding sites at the mechano-sensing region. However, upon a pulling force of 2–5 pN, the mechano-sensing region unfolds into an open conformation, unmasking those binding sites (Ehrlicher et al., 2011; Huelsmann et al., 2016). The C-terminal region of filamin localizes to the center of the Z-disc where it binds Sls. The N-terminal actin-binding region of filamin localizes to the edge of the Z-disc where it binds actin filaments from opposing sarcomeres, serving as an inter-sarcomere bridge (González-Morales et al., 2017; Szikora et al., 2020). Accordingly, *Drosophila* filamin mutants have unstable Z-discs that break easily (González-Morales et al., 2017). Filamin function at the Z-disc is likely conserved. In humans, mutations in filamin-c constitute a common type of myopathy that affects the heart and the skeletal muscles (Verdonschot et al., 2020). Accumulation of filamin-c at damaged Z-discs has been observed in mouse muscles after prolonged exercise, in cultured muscle cells after electrical stimulation and after laser-induced general damage (Leber et al., 2016; Orfanos et al., 2016).

Coordinating sarcomere maintenance

Elastic proteins at the Z-disc that resist contractile forces inevitably become damaged, so that the Z-disc also incorporates a multicomponent chaperone system that selectively degrades them. This system is called the chaperone-assisted selective autophagy (CASA) pathway and is composed of Starvin (BAG-3 in

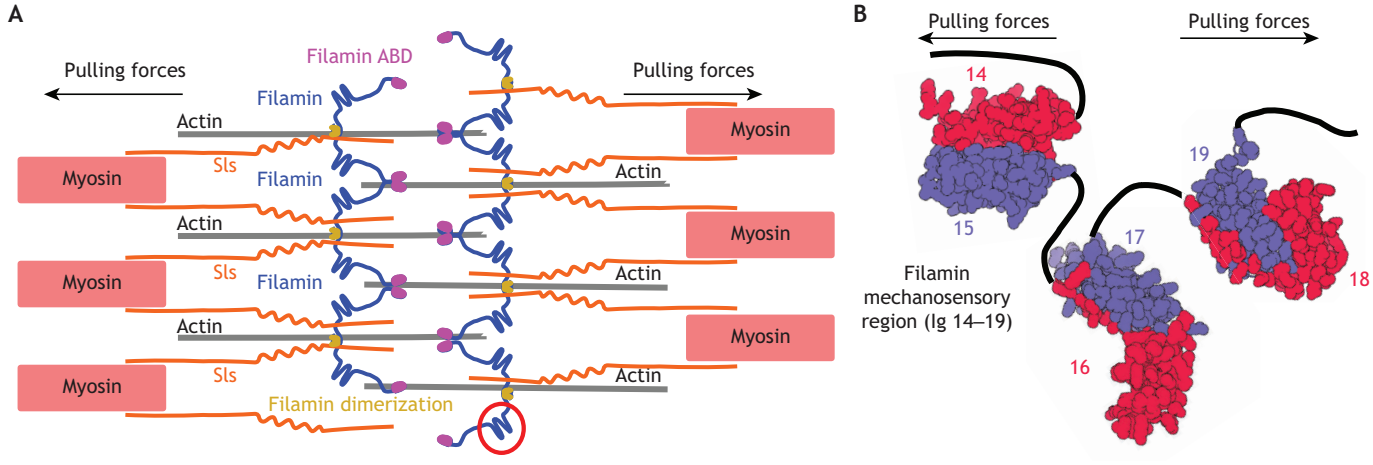

Fig. 3. Titin/Sls and filamin anchor actin filaments to the Z-disc. (A) Model of Titin/Sls and filamin at the I-band. The N-terminal tip of Sls is anchored to the center of the disc, with Sls extending until the start of myosin filaments where it physically interacts with myosin. Filamin is oriented with its dimerization domains outside of the disc and the actin-binding domains (ABD) directed towards the center. The mechanosensitive region of filamin is highlighted by a red circle. (B) Alphafold model of the six Ig domains (Ig 14–19) of the mechanosensitive region of filamin (AF Q9VEN1). Presumably, these domains adopt an open configuration upon contraction, and a closed conformation in relaxed muscles.

vertebrates), HSC70 (also known as HSPA8) and HSPB8, as well as CHIP (also known as STUB1), a ubiquitin ligase (Arndt et al., 2010). The degradation of filamin by the co-chaperone Starvin is particularly well studied in insects. The cochaperone Starvin initiates the formation of the chaperone complex at the Z-disc, which consists of the chaperones Hsc70 and HspB8 and the ubiquitin ligase CHIP (Arndt et al., 2010). Filamin is phosphorylated by the NUAK kinase (Brooks et al., 2020), possibly upon damage or mechanical unfolding, and this phosphorylation serves as a signal for the Starvin chaperone complex to mediate the selective autophagy of filamin (Arndt et al., 2010). It is unclear how the damaged unfolded state of filamin that is recognized by NUAK is different from the open state constantly occurring during contractions.

The CASA pathway is not the only sarcomere maintenance system. The CryAB chaperone localizes to the Z-discs, binds filamin and maintains myofibril integrity in insects (Wojtowicz et al., 2015). The mechanistic details of CryAB function are not entirely understood, but one hypothesis is that it stabilizes filamin at the Z-disc. Besides the CASA pathway and CryAB, other chaperones might be involved; indeed, the *Drosophila* genome encodes more than 100 different predicted chaperone proteins and some might be Z-disc components (Gramates et al., 2017). We do not know the exact number of proteins with continuous turnover at the Z-disc, but there are some clues. By knocking down genes in differentiated muscles and not during development, a recent study showed that there is a continuous turnover of some myofibril proteins, such as filamin, Sls, Bent, actin and myosin, in addition to that of endocytosis-related proteins and cell adhesion proteins (Perkins and Tanentzapf, 2014). Future studies will undoubtedly shed light on the extent of myofibril repair systems.

Serving as an enzymatic hub

Insects adapt their IFM metabolism to sustain long flights. The tephritid fruit fly can fly for more than 1 h (Chen et al., 2015), and *Drosophila* can fly up to 12 km (Leitch et al., 2021). Specialized mitochondria, variations of classic metabolic pathways and the localization of metabolic enzymes to the Z-disc are some of the adaptations required for insect flight (Teulier et al., 2016; Wojtas et al., 1997; Rai et al., 2014; Wang et al., 2016). As most of the cytoplasm in muscle cells is occupied by bulky myofibrils, cytosolic enzymes concentrate in discrete regions that function as catalytic hubs. For example, some enzymes localize to the Z-disc, which provides a condensed environment that promotes the assembly of metabolic complexes that require multiple subunits and thus favors enzymatic reactions (González-Morales et al., 2021 preprint; Sullivan et al., 2003). Concentrating these reactions in one place keeps metabolites away from undesired locations (Menard et al., 2014; Sweetlove and Fernie, 2018). For instance, Zasp proteins recruit the tricarboxylic acid cycle enzyme oxoglutarate dehydrogenase (OGDH), which together with other tricarboxylic acid cycle enzymes coordinates amino acid cataplerotic metabolism to sustain myofibril growth (González-Morales et al., 2021 preprint). Vertebrate myofibril proteins are translated at the Z-disc (Denes et al., 2021). A pool of amino acids at the Z-disc might be required for local translation.

Another well-documented example of enzymatic usage of the Z-disc backbone are glycolytic enzymes. At least five out of ten glycolytic enzymes localize to the Z-disc (Wojtas et al., 1997; Sullivan et al., 2003). Glycerol-3-phosphate dehydrogenase, which links carbohydrate and lipid metabolism, also localizes to the Z-disc, where it coordinates the recruitment of glycolytic enzymes (Sullivan et al., 2003). Flies that only have the mitochondrial glycerol-3-phosphate dehydrogenase are flightless, demonstrating a functional requirement for its Z-disc localization (Sullivan et al., 2003; Wojtas et al., 1997).

The Z-disc localization of metabolic pathway components is conserved. Vertebrates have pools of glycogen particles associated with the Z-disc, and their metabolism is different from that of the other glycogen pools (Ørtenblad et al., 2011; Nielsen et al., 2022), suggesting compartmentalization of glycolytic metabolism. Furthermore, the phosphoglycerate mutase enzyme localizes to the Z-disc in mammalian skeletal muscles (Kowalski et al., 2009), and some glycolytic enzymes directly bind actin filaments (Menard et al., 2014). In addition, a recent proximity labeling method revealed many metabolic enzymes that are associated with myofibrils in mice (Rudolph et al., 2020).

Organizing actin filaments

Actin filaments polymerize *in vitro* in a two-step process. First, a complex comprising three actin monomers nucleates, which then grows by recruiting additional actin monomers into a forming filament (Szikora et al., 2022). In contrast to what has been observed *in vitro*, in muscles, actin filament elongation mainly occurs at the minus end and filament elongation proceeds through the incorporation of short actin polymers into the growing filament, as well as by the addition of individual actin monomers (reviewed in Szikora et al., 2022). Filament length is controlled by capping proteins both at the minus ends located at the M-line and the plus ends located at the Z-disc (Shwartz et al., 2016; Bai et al., 2007; Littlefield et al., 2001).

At the M-line, the recruitment of actin polymers is regulated by the minus-end-capping protein tropomodulin (Fig. 4A). In *Drosophila*, tropomodulin binds to the minus ends and blocks actin elongation (Mardahl-Dumesnil and Fowler, 2001). In contrast, the WH2-domain actin regulator Sals and the DAAM formin family homolog dDAAM promote filament elongation by inhibiting tropomodulin and by recruiting actin polymers into the growing filament (Molnar et al., 2014; Bai et al., 2007). The balance between these proteins sets the final actin filament length.

At the Z-disc, several parallel mechanisms finetune actin filament organization. α-Actinin determines filament spacing and recruitment of actin filaments into the Z-disc. In insects and vertebrates, CapZ, a heterodimeric actin-binding protein, prevents the spontaneous turnover of actin monomers at the plus ends and localizes to the Z-disc (Cooper and Sept, 2008; Hopmann and Miller, 2003; Szikora et al., 2020). Fhos, a homolog of the FHOD sub-family of formins, mediates the incorporation of actin polymers into early sarcomere units and coordinates the recruitment of actin filaments at the Z-disc, thus coordinating Z-disc growth with actin filament incorporation (Shwartz et al., 2016). Lasp, the only member of the nebulin family in insects (Lee et al., 2008; Suyama et al., 2009), is an actin-binding scaffolding protein that stabilizes muscle actin filaments and sets proper filament spacing (Fernandes and Schöck, 2014).

Finally, not all actins are alike. *Drosophila* has six actin genes, and the small sequence variations in them result in different functions and localization patterns within the sarcomere (Fyrberg et al., 1983; Röper et al., 2005). Actin88F is the main actin present in IFM sarcomeres, but overexpression studies suggest that it is excluded from the Z-disc; instead, Actin5C is present only at the Z-disc (Röper et al., 2005; Shwartz et al., 2016). Interestingly, Fhos mediates the elongation of filaments containing Actin88F but not Actin5C monomers (Shwartz et al., 2016). However, the exact

Journal of Cell Science (2022) 135, jcs260179. doi:10.1242/jcs.260179

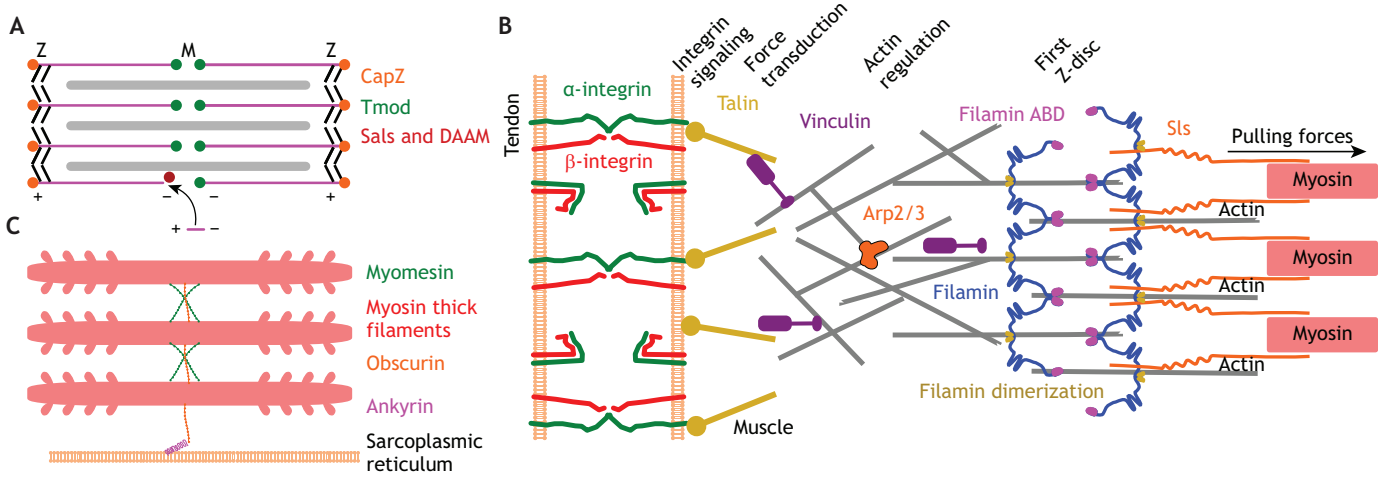

Fig. 4. Actin incorporation, the MTZ and the M-line. (A) Simplified model of the control of actin filament incorporation in the sarcomere. CapZ binds to the plus ends of the actin filaments bordering the Z-disc. On the opposite end, tropomodulin (Tmod) binds to the minus ends and blocks actin incorporation. Sals (Sarcomere length short) also binds the minus ends and promotes actin incorporation by antagonizing tropomodulin. Small actin filaments incorporate at the M-line. (B) Model of the cellular processes that mediate the linkage between integrins and myofibrils. The first layer is found at the membrane and involves integrin signaling. The second layer involves a force-transduction mechanism that is mediated by talin and vinculin. The third layer concentrates actin regulators such as the Arp2/3 complex. The fourth layer is the first Z-disc. (C) Simplified model of the M-line. The bipolar myosin filaments are shown in red; they are held together by myosin binding proteins. In vertebrates, myomesin bridges parallel myosin filaments to prevent them from sliding out, and obscurin links the outer myosin filaments to the sarcoplasmic reticulum via ankyrin. Insects do not have a clear myomesin ortholog but have an obscurin ortholog, which likely covers the role of myomesin.

contribution of the different actin isoforms to sarcomere architecture is still largely unresolved and requires further studies. Overall, regulating acting filament growth and organizing filaments is an incredibly complex feat that requires the simultaneous action of several processes to guarantee a stereotypical pattern.

The evolution of the Z-disc

Much of what we know about Z-discs comes from bilateral animals. The overall shape of sarcomeres from vertebrates and insects is mostly identical; however, there are some differences in the myofibril components. Some important vertebrate muscle proteins are not present in insects at all, and conversely, insects have their own set of specific proteins (Box 1). Therefore, muscles can build almost identical structures even when some components are different. Looking beyond Bilateria holds more surprises – jellyfishes and ctenophores are non-bilateral animals, and their sarcomeres appear almost identical to their bilateral counterparts by electron microscopy (Tanaka et al., 2018; Blanquet and Riordan, 1981; Mackie et al., 1988). But intriguingly, their muscles lack titin entirely and have only one Zasp family member without the oligomerization ZM domain (Steinmetz et al., 2012; González-Morales et al., 2019b; Koch et al., 2012). Therefore, either a disc-like structure that is molecularly distinct from a bilateral Z-disc holds actin filaments in place, or a supportive disc is not entirely required for sarcomere assembly.

Some evidence suggests the existence of sarcomeres without Z-discs, even in bilateral animals. For instance, mathematical modeling of the contractile properties of actin and myosin suggests that some degree of striation spontaneously appears when accounting for filament sliding and the coalescence of actin filaments (Friedrich et al., 2012). In this case, sarcomere spacing is determined by the dynamics of actin filaments alone and does not involve a Z-disc-like structure (Friedrich et al., 2012). In *Drosophila*, depletion of α-actinin prevents Z-disc formation, but some degree of myosin filament spacing and striation is maintained (Rui et al., 2010). In *C. elegans*, α-actinin is also dispensable for muscle striation, whereas a homolog of titin is required (Ono et al., 2019; Moulder et al., 2010). Furthermore, the actin cytoskeleton in non-muscle cells exhibits structures that resemble miniature sarcomeres but without Z-discs (Coravos and Martin, 2016; Hu et al., 2017). Thus, Z-discs of bilateral animals might have evolved from striated muscles without canonical Z-disc proteins.

The myofibril attachment site – a modified Z-disc

Translation of myofibril contraction into muscle contraction requires the fibrils to be physically linked to the exoskeleton through the tendons (Fig. 4B). The modified terminal Z-disc (MTZ) anchors the extracellular matrix (ECM) to the myofibril (Reedy and Beall, 1993b). The MTZ shares components and functions with focal adhesions (FAs) and Z-discs. Like an FA, it relies on integrins to establish force-resistant attachments. Structurally, the MTZ is composed of four different layers: (1) the integrin signaling layer, (2) the force-transduction layer, (3) the actin-regulatory layer and (4) the first Z-disc (Green et al., 2018). A proper attachment also plays a developmental role by aligning and straightening myofibrils during pupal IFM assembly (Lemke et al., 2019; Lemke and Schnorrer, 2017). Finally, stochastic muscle contractions occur during the last steps of sarcomere maturation (Katzemich et al., 2013; Spletter et al., 2018; Weitkunat et al., 2017), and these appear to be important for proper muscle development given that genetic or physical disruption of the tension between tendons and muscles during development severely affects sarcomere maturation and the coordinated sarcomere assembly into myofibrils (Lemke et al., 2019; Weitkunat et al., 2014).

The M-line

The M-line (Fig. 4C), located at the center of the sarcomere, anchors and stabilizes myosin filaments (Manring et al., 2017; Lange et al., 2020). The Z-disc and M-line are connected by their interactions with actin and myosin filaments and, in vertebrates, through titin

Journal of Cell Science (2022) 135, jcs260179. doi:10.1242/jcs.260179

Box 1. Sarcomere proteins found specifically in either vertebrates or insects

Vertebrate-specific sarcomere proteins

Telethonin: crosslinks the N-terminal end of two titin molecules at the core of the Z-disc (Gautel and Djinovic-Carugo, 2016).
FATZ: also called myozenin; a structural protein that connects Z-disc proteins, it binds to filamin, telethonin, and α-actinin (Faulkner et al., 2000; Gontier et al., 2005; Sponga et al., 2021).
Desmin: an intermediate filament protein that surrounds the Z-disc and provides support (Paulin and Li, 2004).
Myotilin: a Z-disc protein that interacts and stabilizes F-actin, coordinates Z-disc assembly and moves to the M-line upon exercise-induced damage (Carlsson et al., 2007; Kostan et al., 2021).
MyBP-C: accessory myosin-binding protein that coordinates the interactions between actin and myosin filaments (Heling et al., 2020).
Xin: binds and stabilizes actin filaments found almost exclusively at intercalated discs and myotendinous junctions (Pacholsky et al., 2004).
Myopodin: bundles actin filaments and is speculated to be involved in the very early steps of Z-disc assembly (Linnemann et al., 2013).
Chap: an essential actin-binding Z-disc protein that can translocate to the nucleus (Beqqali et al., 2010).
Myomesin: an M-line elastic protein that forms bridges between myosin filaments and serves to resist the deformation caused by sarcomere contractile forces (Tskhovrebova and Trinick, 2012).

Insect-sarcomere proteins

Flightin: a protein specific to the IFM that controls the assembly of myosin filaments and is required for correct contraction frequency (Vigoreaux et al., 1993; Chakravorty et al., 2017).
Myofilin: a protein of unknown function that binds and localizes to the core of myosin filaments (Qiu et al., 2005)
Paramyosin: a structural coiled-coiled dimer composed of a myosin-tail domain that occupies the interior of myosin filaments (Bullard et al., 1977) The small isoform of the *paramyosin* gene is called Miniparamyosin (Maroto et al., 1995).
Sals: a WH2 domain-containing protein that binds actin pointed ends and promotes filament elongation (Bai et al., 2007).

(Lange et al., 2020). Other connections through a series of protein interactions are also likely and revealed by the existence of proteins that localize to both, for example a splice variant of Sls called Zormin (Burkart et al., 2007). In addition, the M-line provides some of the filament regularity observed in the absence of the Z-disc. For example, without Sls or OGDH the Z-discs are small and do not span the entire myofibril width, but the actin filaments remain somehow stable and regular (González-Morales et al., 2021 preprint; Orfanos et al., 2015).

In vertebrates, myomesin, an antiparallel dimeric protein, crosslinks myosin filaments through its C-terminal Ig-like domain (Schoenauer et al., 2008; Pinotsis et al., 2012; Lange et al., 2005). Subsequently, the large multidomain protein obscurin further stabilizes the thick filaments by forming a ternary complex with myosin and myomesin (Fukuzawa et al., 2008; Pernigo et al., 2017). Finally, the C-terminus of titin is inserted into the M-line via the myomesin–obscurin–myosin complex (Fukuzawa et al., 2008). Insects do not have a clear myomesin homolog (Box 1), but they have an obscurin homolog. Obscurin was originally identified in *C. elegans* (Benian et al., 1996); it was later found in insects (Katzemich et al., 2012) and vertebrates (Sutter et al., 2004).

Human obscurin contains 56 Ig-like domains, three FN type-III domains, one IQ calmodulin-binding motif, a RhoGEF domain and an SH3 domain, as well as two serine/threonine kinase domains (Kontrogianni-Konstantopoulos et al., 2009). Vertebrates have at least two obscurin-encoding genes with partially redundant functions (Blondelle et al., 2019). In contrast, *Drosophila* has a single obscurin homolog gene, *unc-89*; it encodes several alternatively spliced isoforms that localize exclusively to the M-line in all muscles analyzed (Katzemich et al., 2012). The largest obscurin isoform is 475 kDa; it has fewer Ig domains than the vertebrate counterpart and lacks the calmodulin-binding motif (Katzemich et al., 2012). *Drosophila* obscurin forms a complex with Mask, a protein containing multiple ankyrin repeats, and with the serine/threonine kinase Ball to maintain the M-line structure (Katzemich et al., 2015).

A distant *Drosophila* protein called myomesin and myosin-binding protein (MnM) has a protein domain structure similar to myomesin and myosin-binding proteins H and C; it is highly expressed in the heart and muscles and might perform some of the functional roles of myomesin (Auxerre-Plantie et al., 2020; Gramates et al., 2017). However, given that muscles are only minimally affected in the absence of MnM, it is unlikely that MnM fulfills all myomesin functions in insects. Instead, it seems that, in *Drosophila*, the obscurin homolog fulfills the role of assembling and tethering myosin filaments at the M-line by physically interacting with myosin thick filaments (Katzemich et al., 2012). It is recruited into periodic sarcomere-like structures before other M-line components, including the myosin heavy chain (Katzemich et al., 2012). Consistent with this, in muscles that lack obscurin, the M-line is misplaced (Katzemich et al., 2012) suggesting that obscurin is the physical link between myosin filaments that maintains them aligned.

Conclusions

In this Review, we summarized recent advances in our understanding of the structure and the function of the Z-disc, mainly in insects, and contrasted them with their vertebrate counterparts. The Z-disc is a critical structure that controls many aspects of myofibril ultrastructure, size, metabolic state and contractility.

Muscle research is not new; indeed, the sarcomere ultrastructure from transmission electron microscopy has been around for more than 50 years. Although the majority of the abundant structural Z-disc proteins are known, the current challenge is to decipher their intricate interactions that assemble and maintain the Z-disc structure. Traditional loss-of-function analysis can only go so far in a highly interconnected complex, as if a piece is missing, the entire complex collapses. Here, technological advances in genomic engineering are highly valuable in separating discrete protein functions and interrogating the precise molecular mechanisms (Bier et al., 2018; González-Morales et al., 2021 preprint). Similarly, novel imaging methods, such as cryo-electron microscopy and super-resolution microscopy, will allow the observation of structural details that were previously inaccessible. Cryo-electron microscopy and cryo-electron tomography have already provided the structure of Z-discs from honeybees (Rusu et al., 2017), the myosin tails of native thick filaments from Lethocerus (Rahmani et al., 2021) and the entire mouse psoas muscle sarcomere (Wang et al., 2021)! Moreover, super-resolution microscopy has provided amazing details on protein localization within the Z-disc (Fernandes and Schöck, 2014; Szikora et al., 2020; González-Morales et al., 2021 preprint; Schueder et al., 2022 preprint). Now is the perfect time to study insect muscles.

Competing interests

The authors declare no competing or financial interests.

Funding

Our work in this area was supported by the Canadian Institutes of Health Research (MOP-142475 and PJT-155995 to F.S.) and the Natural Sciences and Engineering

Research Council of Canada (RGPIN-2022-02984 and DGECR-2022-00166 to N.G.-M.).

References

Almeida Ribeiro, E., Pinotsis, N., Ghisleni, A., Salmazo, A., Konarev, P. V., Kostan, J., Sjoblom, B., Schreiner, C., Polyansky, A. A., Gkougkoulia, E. A. et al. (2014). The structure and regulation of human muscle alpha-actinin. *Cell* **159**, 1447-1460. doi:10.1016/j.cell.2014.10.056

Arndt, V., Dick, N., Tawo, R., Dreiseidler, M., Wenzel, D., Hesse, M., Furst, D. O., Saftig, P., Saint, R., Fleischmann, B. K. et al. (2010). Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr. Biol.* **20**, 143-148. doi:10.1016/j.cub.2009.11.022

Au, Y., Atkinson, R. A., Guerrini, R., Kelly, G., Joseph, C., Martin, S. R., Muskett, F. W., Pallavicini, A., Faulkner, G. and Pastore, A. (2004). Solution structure of ZASP PDZ domain; implications for sarcomere ultrastructure and enigma family redundancy. *Structure* **12**, 611-622. doi:10.1016/j.str.2004.02.019

Auber, J. and Couteaux, R. (1963). Ultrastructure de la strie Z dans des muscles de Diptères. *J. Microsc.* **2**, 309-324.

Auxerre-Plantie, E., Nielsen, T., Grunert, M., Olejniczak, O., Perrot, A., Ozcelik, C., Harries, D., Matinmehr, F., Dos Remedios, C., Muhlfeld, C. et al. (2020). Identification of MYOM2 as a candidate gene in hypertrophic cardiomyopathy and Tetralogy of Fallot, and its functional evaluation in the Drosophila heart. *Dis. Model. Mech.* **13**,dmm045377. doi:10.1242/dmm.045377

Ayme-Southgate, A., Bounaix, C., Riebe, T. E. and Southgate, R. (2004). Assembly of the giant protein projectin during myofibrillogenesis in Drosophila indirect flight muscles. *BMC Cell Biol.* **5**, 17. doi:10.1186/1471-2121-5-17

Ayme-Southgate, A., Saide, J., Southgate, R., Bounaix, C., Cammarato, A., Patel, S. and Wussler, C. (2005). In indirect flight muscles Drosophila projectin has a short PEVK domain, and its NH2-terminus is embedded at the Z-band. *J. Muscle Res. Cell Motil.* **26**, 467-477. doi:10.1007/s10974-005-9031-8

Bai, J., Hartwig, J. H. and Perrimon, N. (2007). SALS, a WH2-domain-containing protein, promotes sarcomeric actin filament elongation from pointed ends during Drosophila muscle growth. *Dev. Cell* **13**, 828-842. doi:10.1016/j.devcel.2007.10.003

Benian, G. M., Tinley, T. L., Tang, X. and Borodovsky, M. (1996). The Caenorhabditis elegans gene unc-89, required fpr muscle M-line assembly, encodes a giant modular protein composed of Ig and signal transduction domains. *J. Cell Biol.* **132**, 835-848. doi:10.1083/jcb.132.5.835

Beqqali, A., Monshouwer-Kloots, J., Monteiro, R., Welling, M., Bakkers, J., Ehler, E., Verkleij, A., Mummery, C. and Passier, R. (2010). CHAP is a newly identified Z-disc protein essential for heart and skeletal muscle function. *J. Cell Sci.* **123**, 1141-1150. doi:10.1242/jcs.063859

Bier, E., Harrison, M. M., O'Connor-Giles, K. M. and Wildonger, J. (2018). Advances in Engineering the Fly Genome with the CRISPR-Cas System. *Genetics* **208**, 1-18. doi:10.1534/genetics.117.1113

Blanquet, R. S. and Riordan, G. P. (1981). An ultrastructural-study of the subumbrellar musculature and desmosomal complexes of Cassiopea-Xamachana (Cnidaria, Scyphozoa). *Trans. Am. Microsc. Soc.* **100**, 109-119. doi:10.2307/3225794

Blondelle, J., Marrocco, V., Clark, M., Desmond, P., Myers, S., Nguyen, J., Wright, M., Bremner, S., Pierantozzi, E., Ward, S. et al. (2019). Murine obscurin and Obsl1 have functionally redundant roles in sarcolemmal integrity, sarcoplasmic reticulum organization, and muscle metabolism. *Commun. Biol.* **2**, 178. doi:10.1038/s42003-019-0405-7

Borrego-Diaz, E., Kerff, F., Lee, S. H., Ferron, F., Li, Y. and Dominguez, R. (2006). Crystal structure of the actin-binding domain of alpha-actinin 1: evaluating two competing actin-binding models. *J. Struct. Biol.* **155**, 230-238. doi:10.1016/j.jsb.2006.01.013

Brooks, D., Naeem, F., Stetsiv, M., Goetting, S. C., Bawa, S., Green, N., Clark, C., Bashirullah, A. and Geisbrecht, E. R. (2020). Drosophila NUAK functions with Starvin/BAG3 in autophagic protein turnover. *PLoS Genet.* **16**, e1008700. doi:10.1371/journal.pgen.1008700

Bryantsev, A. L., Duong, S., Brunetti, T. M., Chechenova, M. B., Lovato, T. L., Nelson, C., Shaw, E., Uhl, J. D., Gebelein, B. and Cripps, R. M. (2012). Extradenticle and homothorax control adult muscle fiber identity in Drosophila. *Dev. Cell* **23**, 664-673. doi:10.1016/j.devcel.2012.08.004

Bullard, B., Hammond, K. S. and Luke, B. M. (1977). The site of paramyosin in insect flight muscle and the presence of an unidentified protein between myosin filaments and Z-line. *J. Mol. Biol.* **115**, 417-440. doi:10.1016/0022-2836(77)90163-2

Bullard, B., Sainsbury, G. and Miller, N. (1990). Digestion of proteins associated with the Z-disc by calpain. *J. Muscle Res. Cell Motil.* **11**, 271-279. doi:10.1007/BF01843580

Bullard, B., Linke, W. A. and Leonard, K. (2002). Varieties of elastic protein in invertebrate muscles. *J. Muscle Res. Cell Motil.* **23**, 435-447. doi:10.1023/A:1023454305437

Bullard, B., Garcia, T., Benes, V., Leake, M. C., Linke, W. A. and Oberhauser, A. F. (2006). The molecular elasticity of the insect flight muscle proteins projectin and kettin. *Proc. Natl. Acad. Sci. USA* **103**, 4451-4456. doi:10.1073/pnas.0509016103

Burgoyne, T., Morris, E. P. and Luther, P. K. (2015). Three-dimensional structure of vertebrate muscle Z-band: the small-square lattice Z-band in rat cardiac muscle. *J. Mol. Biol.* **427**, 3527-3537. doi:10.1016/j.jmb.2015.08.018

Burkart, C., Qiu, F., Brendel, S., Benes, V., Håag, P., Labeit, S., Leonard, K. and Bullard, B. (2007). Modular proteins from the Drosophila sallimus (sls) gene and their expression in muscles with different extensibility. *J. Mol. Biol.* **367**, 953-969. doi:10.1016/j.jmb.2007.01.059

Burridge, K. and Feramisco, J. R. (1981). Non-muscle alpha actinins are calcium-sensitive actin-binding proteins. *Nature* **294**, 565-567. doi:10.1038/294565a0

Burton, P. M. (2008). Insights from diploblasts; the evolution of mesoderm and muscle. *J. Exp. Zool. B Mol. Dev. Evol.* **310**, 5-14. doi:10.1002/jez.b.21150

Carlsson, L., Yu, J. G., Moza, M., Carpén, O. and Thornell, L. E. (2007). Myotilin: a prominent marker of myofibrillar remodelling. *Neuromuscul. Disord.* **17**, 61-68. doi:10.1016/j.nmd.2006.09.007

Chakravorty, S., Tanner, B. C. W., Foelber, V. L., Vu, H., Rosenthal, M., Ruiz, T. and Vigoreaux, J. O. (2017). Flightin maintains myofilament lattice organization required for optimal flight power and courtship song quality in Drosophila. *Proc. Biol. Sci.* **284**, 20170431. doi:10.1098/rspb.2017.0431

Chen, M., Chen, P., Ye, H., Yuan, R., Wang, X. and Xu, J. (2015). Flight capacity of Bactrocera dorsalis (Diptera: Tephritidae) adult females based on flight mill studies and flight muscle ultrastructure. *J. Insect. Sci.* **15**, 141. doi:10.1093/jisesa/iev124

Cheng, N. Q. and Deatherage, J. F. (1989). Three-dimensional reconstruction of the Z disk of sectioned bee flight muscle. *J. Cell Biol.* **108**, 1761-1774. doi:10.1083/jcb.108.5.1761

Cheng, H., Kimura, K., Peter, A. K., Cui, L., Ouyang, K., Shen, T., Liu, Y., Gu, Y., Dalton, N. D., Evans, S. M. et al. (2010). Loss of enigma homolog protein results in dilated cardiomyopathy. *Circ. Res.* **107**, 348-356. doi:10.1161/CIRCRESAHA.110.218735

Clark, K. A., Bland, J. M. and Beckerle, M. C. (2007). The Drosophila muscle LIM protein, Mlp84B, cooperates with D-titin to maintain muscle structural integrity. *J. Cell Sci.* **120**, 2066-2077. doi:10.1242/jcs.000695

Cooper, J. A. and Sept, D. (2008). New insights into mechanism and regulation of actin capping protein. *Int. Rev. Cell Mol. Biol.* **267**, 183-206. doi:10.1016/S1937-6448(08)00604-7

Coravos, J. S. and Martin, A. C. (2016). Apical sarcomere-like actomyosin contracts nonmuscle Drosophila epithelial cells. *Dev. Cell* **39**, 346-358. doi:10.1016/j.devcel.2016.09.023

Courson, D. S. and Rock, R. S. (2010). Actin cross-link assembly and disassembly mechanics for alpha-Actinin and fascin. *J. Biol. Chem.* **285**, 26350-26357. doi:10.1074/jbc.M110.123117

Denes, L. T., Kelley, C. P. and Wang, E. T. (2021). Microtubule-based transport is essential to distribute RNA and nascent protein in skeletal muscle. *Nat. Commun.* **12**, 6079. doi:10.1038/s41467-021-26383-9

Deora, T., Gundiah, N. and Sane, S. P. (2017). Mechanics of the thorax in flies. *J. Exp. Biol.* **220**, 1382-1395. doi:10.1242/jeb.128363

Dos Remedios, C. and Gilmour, D. (2017). An historical perspective of the discovery of titin filaments. *Biophys. Rev.* **9**, 179-188. doi:10.1007/s12551-017-0269-3

Draper, M. H. and Hodge, A. J. (1949). Sub-microscopic localization of minerals in skeletal muscle by internal micro-incineration within the electron microscope. *Nature* **163**, 576. doi:10.1038/163576a0

Ehrlicher, A. J., Nakamura, F., Hartwig, J. H., Weitz, D. A. and Stossel, T. P. (2011). Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature* **478**, 260-263. doi:10.1038/nature10430

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., Qureshi, M., Richardson, L. J., Salazar, G. A., Smart, A. et al. (2019). The Pfam protein families database in 2019. *Nucleic Acids Res.* **47**, D427-D432. doi:10.1093/nar/gky995

Elhanany-Tamir, H., Yu, Y. V., Shnayder, M., Jain, A., Welte, M. and Volk, T. (2012). Organelle positioning in muscles requires cooperation between two KASH proteins and microtubules. *J. Cell Biol.* **198**, 833-846. doi:10.1083/jcb.201204102

Faulkner, G., Pallavicini, A., Comelli, A., Salamon, M., Bortoletto, G., Ievolella, C., Trevisan, S., Kojic, S., Dalla Vecchia, F., Laveder, P. et al. (2000). FATZ, a filamin-, actinin-, and telethonin-binding protein of the Z-disc of skeletal muscle. *J. Biol. Chem.* **275**, 41234-41242. doi:10.1074/jbc.M007493200

Fernandes, I. and Schöck, F. (2014). The nebulin repeat protein Lasp regulates I-band architecture and filament spacing in myofibrils. *J. Cell Biol.* **206**, 559-572. doi:10.1083/jcb.201401094

Friedrich, B. M., Fischer-Friedrich, E., Gov, N. S. and Safran, S. A. (2012). Sarcomeric pattern formation by actin cluster coalescence. *PLoS Comput. Biol.* **8**, e1002544. doi:10.1371/journal.pcbi.1002544

Fukuzawa, A., Lange, S., Holt, M., Vihola, A., Carmignac, V., Ferreiro, A., Udd, B. and Gautel, M. (2008). Interactions with titin and myomesin target obscurin and obscurin-like 1 to the M-band: implications for hereditary myopathies. *J. Cell Sci.* **121**, 1841-1851. doi:10.1242/jcs.028019

Fyrberg, E. A., Mahaffey, J. W., Bond, B. J. and Davidson, N. (1983). Transcripts of the six Drosophila actin genes accumulate in a stage- and tissue-specific manner. *Cell* **33**, 115-123. doi:10.1016/0092-8674(83)90340-9

Fyrberg, E., Kelly, M., Ball, E., Fyrberg, C. and Reedy, M. C. (1990). Molecular genetics of Drosophila alpha-actinin: mutant alleles disrupt Z disc integrity and muscle insertions. *J. Cell Biol.* **110**, 1999-2011. doi:10.1083/jcb.110.6.1999

Gautel, M. and Djinovic-Carugo, K. (2016). The sarcomeric cytoskeleton: from molecules to motion. *J. Exp. Biol.* **219**, 135-145. doi:10.1242/jeb.124941

Gautel, M., Goulding, D., Bullard, B., Weber, K. and Furst, D. O. (1996). The central Z-disk region of titin is assembled from a novel repeat in variable copy numbers. *J. Cell Sci.* **109**, 2747-2754. doi:10.1242/jcs.109.11.2747

Gontier, Y., Taivainen, A., Fontao, L., Sonnenberg, A., Van Der Flier, A., Carpen, O., Faulkner, G. and Borradori, L. (2005). The Z-disc proteins myotilin and FATZ-1 interact with each other and are connected to the sarcolemma via muscle-specific filamins. *J. Cell Sci.* **118**, 3739-3749. doi:10.1242/jcs.02484

González-Morales, N. and Schöck, F. (2020). Commentary: nanoscopy reveals the layered organization of the sarcomeric H-zone and I-band complexes. *Front. Cell Dev. Biol.* **8**, 74. doi:10.3389/fcell.2020.00074

González-Morales, N., Holenka, T. K. and Schöck, F. (2017). Filamin actin-binding and titin-binding fulfill distinct functions in Z-disc cohesion. *PLoS Genet.* **13**, e1006880. doi:10.1371/journal.pgen.1006880

González-Morales, N., Marsh, T. W., Katzemich, A., Marescal, O., Xiao, Y. S. and Schöck, F. (2019a). Different Evolutionary Trajectories of Two Insect-Specific Paralogous Proteins Involved in Stabilizing Muscle Myofibrils. *Genetics* **212**, 743-755. doi:10.1534/genetics.119.302217

González-Morales, N., Xiao, Y. S., Schilling, M. A., Marescal, O., Liao, K. A. and Schöck, F. (2019b). Myofibril diameter is set by a finely tuned mechanism of protein oligomerization in Drosophila. *Elife* **8**, e50496. doi:10.7554/eLife.50496

González-Morales, N., Marescal, O., Szikora, S., Erdelyi, M., Bíró, P., Mesquita, T., Mihály, J. and Schöck, F. (2021). Oxoglutarate dehydrogenase coordinates myofibril growth by maintaining amino acid homeostasis. *bioRxiv* 10.1101/2021.12.13.472149. doi:10.1101/2021.12.13.472149

Gramates, L. S., Marygold, S. J., Santos, G. D., Urbano, J. M., Antonazzo, G., Matthews, B. B., Rey, A. J., Tabone, C. J., Crosby, M. A., Emmert, D. B. et al. (2017). FlyBase at 25: looking to the future. *Nucleic Acids Res.* **45**, D663-D671. doi:10.1093/nar/gkw1016

Green, H. J., Griffiths, A. G., Ylänne, J. and Brown, N. H. (2018). Novel functions for integrin-associated proteins revealed by analysis of myofibril attachment in Drosophila. *Elife* **7**, e35783. doi:10.7554/eLife.35783

Grison, M., Merkel, U., Kostan, J., Djinovic-Carugo, K. and Rief, M. (2017). α-Actinin/titin interaction: a dynamic and mechanically stable cluster of bonds in the muscle Z-disk. *Proc. Natl. Acad. Sci. USA* **114**, 1015-1020. doi:10.1073/pnas.1612681114

Gunage, R. D., Dhanyasi, N., Reichert, H. and Vijayraghavan, K. (2017). Drosophila adult muscle development and regeneration. *Semin. Cell Dev. Biol.* **72**, 56-66. doi:10.1016/j.semcdb.2017.11.017

Hagopian, M. (1966). The myofilament arrangement in the femoral muscle of the cockroach, Leucophaea maderae fabricius. *J. Cell Biol.* **28**, 545-562. doi:10.1083/jcb.28.3.545

Hampton, C. M., Taylor, D. W. and Taylor, K. A. (2007). Novel structures for alpha-actinin:F-actin interactions and their implications for actin-membrane attachment and tension sensing in the cytoskeleton. *J. Mol. Biol.* **368**, 92-104. doi:10.1016/j.jmb.2007.01.071

Hanson, J. and Huxley, H. E. (1953). Structural basis of the cross-striations in muscle. *Nature* **172**, 530-532. doi:10.1038/172530b0

Heling, L., Geeves, M. A. and Kad, N. M. (2020). MyBP-C: one protein to govern them all. *J. Muscle Res. Cell Motil.* **41**, 91-101. doi:10.1007/s10974-019-09567-1

Hodge, A. J. (1955). Studies on the structure of muscle. III. Phase contrast and electron microscopy of dipteran flight muscle. *J. Biophys. Biochem. Cytol.* **1**, 361-380. doi:10.1083/jcb.1.4.361

Hopmann, R. and Miller, K. G. (2003). A balance of capping protein and profilin functions is required to regulate actin polymerization in Drosophila bristle. *Mol. Biol. Cell* **14**, 118-128. doi:10.1091/mbc.e02-05-0300

Hopmann, R., Cooper, J. A. and Miller, K. G. (1996). Actin organization, bristle morphology, and viability are affected by actin capping protein mutations in Drosophila. *J. Cell Biol.* **133**, 1293-1305. doi:10.1083/jcb.133.6.1293

Hu, D. H., Matsuno, A., Terakado, K., Matsuura, T., Kimura, S. and Maruyama, K. (1990). Projectin is an invertebrate connectin (titin): isolation from crayfish claw muscle and localization in crayfish claw muscle and insect flight muscle. *J. Muscle Res. Cell Motil.* **11**, 497-511. doi:10.1007/BF01745217

Hu, S., Dasbiswas, K., Guo, Z., Tee, Y. H., Thiagarajan, V., Hersen, P., Chew, T. L., Safran, S. A., Zaidel-Bar, R. and Bershadsky, A. D. (2017). Long-range self-organization of cytoskeletal myosin II filament stacks. *Nat. Cell Biol.* **19**, 133-141. doi:10.1038/ncb3466

Huelsmann, S., Rintanen, N., Sethi, R., Brown, N. H. and Ylanne, J. (2016). Evidence for the mechanosensor function of filamin in tissue development. *Sci. Rep.* **6**, 32798. doi:10.1038/srep32798

Huxley, H. and Hanson, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973-976. doi:10.1038/173973a0

Huxley, A. F. and Niedergerke, R. (1954). Structural changes in muscle during contraction; interference microscopy of living muscle fibres. *Nature* **173**, 971-973. doi:10.1038/173971a0

Jani, K. and Schöck, F. (2007). Zasp is required for the assembly of functional integrin adhesion sites. *J. Cell Biol.* **179**, 1583-1597. doi:10.1083/jcb.200707045

Kadrmas, J. L. and Beckerle, M. C. (2004). The LIM domain: from the cytoskeleton to the nucleus. *Nat. Rev. Mol. Cell Biol.* **5**, 920-931. doi:10.1038/nrm1499

Karlik, C. C., Coutu, M. D. and Fyrberg, E. A. (1984). A nonsense mutation within the act88F actin gene disrupts myofibril formation in Drosophila indirect flight muscles. *Cell* **38**, 711-719. doi:10.1016/0092-8674(84)90266-6

Katzemich, A., Kreiskother, N., Alexandrovich, A., Elliott, C., Schock, F., Leonard, K., Sparrow, J. and Bullard, B. (2012). The function of the M-line protein obscurin in controlling the symmetry of the sarcomere in the flight muscle of Drosophila. *J. Cell Sci.* **125**, 3367-3379. doi:10.1242/jcs.097345

Katzemich, A., Liao, K. A., Czerniecki, S. and Schöck, F. (2013). Alp/Enigma family proteins cooperate in Z-disc formation and myofibril assembly. *PLoS Genet.* **9**, e1003342. doi:10.1371/journal.pgen.1003342

Katzemich, A., West, R. J., Fukuzawa, A., Sweeney, S. T., Gautel, M., Sparrow, J. and Bullard, B. (2015). Binding partners of the kinase domains in Drosophila obscurin and their effect on the structure of the flight muscle. *J. Cell Sci.* **128**, 3386-3397. doi:10.1242/jcs.170639

Koch, B. J., Ryan, J. F. and Baxevanis, A. D. (2012). The diversification of the LIM superclass at the base of the metazoa increased subcellular complexity and promoted multicellular specialization. *PLoS One* **7**, e33261. doi:10.1371/journal.pone.0033261

Kontrogianni-Konstantopoulos, A., Ackermann, M. A., Bowman, A. L., Yap, S. V. and Bloch, R. J. (2009). Muscle giants: molecular scaffolds in sarcomerogenesis. *Physiol. Rev.* **89**, 1217-1267. doi:10.1152/physrev.00017.2009

Kostan, J., Pavsic, M., Puž, V., Schwarz, T. C., Drepper, F., Molt, S., Graewert, M. A., Schreiner, C., Sajko, S., Van Der Ven, P. F. M. et al. (2021). Molecular basis of F-actin regulation and sarcomere assembly via myotilin. *PLoS Biol.* **19**, e3001148. doi:10.1371/journal.pbio.3001148

Kowalski, W., Gizak, A. and Rakus, D. (2009). Phosphoglycerate mutase in mammalian striated muscles: subcellular localization and binding partners. *FEBS Lett.* **583**, 1841-1845. doi:10.1016/j.febslet.2009.05.004

Kreiskother, N., Reichert, N., Buttgereit, D., Hertenstein, A., Fischbach, K. F. and Renkawitz-Pohl, R. (2006). Drosophila rolling pebbles colocalises and putatively interacts with alpha-Actinin and the Sls isoform Zormin in the Z-discs of the sarcomere and with Dumbfounded/Kirre, alpha-Actinin and Zormin in the terminal Z-discs. *J. Muscle Res. Cell Motil.* **27**, 93-106. doi:10.1007/s10974-006-9060-y

Kulke, M., Neagoe, C., Kolmerer, B., Minajeva, A., Hinssen, H., Bullard, B. and Linke, W. A. (2001). Kettin, a major source of myofibrillar stiffness in Drosophila indirect flight muscle. *J. Cell Biol.* **154**, 1045-1057. doi:10.1083/jcb.200104016

Lakey, A., Ferguson, C., Labeit, S., Reedy, M., Larkins, A., Butcher, G., Leonard, K. and Bullard, B. (1990). Identification and localization of high molecular weight proteins in insect flight and leg muscle. *EMBO J.* **9**, 3459-3467. doi:10.1002/j.1460-2075.1990.tb07554.x

Lange, S., Himmel, M., Auerbach, D., Agarkova, I., Hayess, K., Fürst, D. O., Perriard, J. C. and Ehler, E. (2005). Dimerisation of myomesin: implications for the structure of the sarcomeric M-band. *J. Mol. Biol.* **345**, 289-298. doi:10.1016/j.jmb.2004.10.040

Lange, S., Pinotsis, N., Agarkova, I. and Ehler, E. (2020). The M-band: The underestimated part of the sarcomere. *Biochim. Biophys. Acta Mol. Cell Res.* **1867**, 118440. doi:10.1016/j.bbamcr.2019.02.003

Leber, Y., Ruparelia, A. A., Kirfel, G., Van Der Ven, P. F., Hoffmann, B., Merkel, R., Bryson-Richardson, R. J. and Furst, D. O. (2016). Filamin C is a highly dynamic protein associated with fast repair of myofibrillar microdamage. *Hum. Mol. Genet.* **25**, 2776-2788. doi:10.1093/hmg/ddw135

Lee, S., Zhou, L., Kim, J., Kalbfleisch, S. and Schock, F. (2008). Lasp anchors the Drosophila male stem cell niche and mediates spermatid individualization. *Mech. Dev.* **125**, 768-776. doi:10.1016/j.mod.2008.06.012

Leitch, K. J., Ponce, F. V., Dickson, W. B., Van Breugel, F. and Dickinson, M. H. (2021). The long-distance flight behavior of Drosophila supports an agent-based model for wind-assisted dispersal in insects. *Proc. Natl. Acad. Sci. USA* **118**, e2013342118. doi:10.1073/pnas.2013342118

Lemke, S. B. and Schnorrer, F. (2017). Mechanical forces during muscle development. *Mech. Dev.* **144**, 92-101. doi:10.1016/j.mod.2016.11.003

Lemke, S. B., Weidemann, T., Cost, A. L., Grashoff, C. and Schnorrer, F. (2019). A small proportion of Talin molecules transmit forces at developing muscle attachments in vivo. *PLoS Biol.* **17**, e3000057. doi:10.1371/journal.pbio.3000057

Liao, K. A., González-Morales, N. and Schöck, F. (2016). Zasp52, a Core Z-disc Protein in Drosophila Indirect Flight Muscles, Interacts with alpha-Actinin via an Extended PDZ Domain. *PLoS Genet.* **12**, e1006400. doi:10.1371/journal.pgen.1006400

Liao, K. A., González-Morales, N. and Schock, F. (2020). Characterizing the actin-binding ability of Zasp52 and its contribution to myofibril assembly. *PLoS One* **15**, e0232137.

Liem, R. K. (2016). Cytoskeletal integrators: the spectrin superfamily. *Cold Spring Harb. Perspect. Biol.* **8**, a018259. doi:10.1101/cshperspect.a018259
Linke, W. A. (2018). Titin gene and protein functions in passive and active muscle. *Annu. Rev. Physiol.* **80**, 389-411. doi:10.1146/annurev-physiol-021317-121234
Linnemann, A., Vakeel, P., Bezerra, E., Orfanos, Z., Djinovic-Carugo, K., Van Der Ven, P. F., Kirfel, G. and Furst, D. O. (2013). Myopodin is an F-actin bundling protein with multiple independent actin-binding regions. *J. Muscle Res. Cell Motil.* **34**, 61-69. doi:10.1007/s10974-012-9334-5
Littlefield, R., Almenar-Queralt, A. and Fowler, V. M. (2001). Actin dynamics at pointed ends regulates thin filament length in striated muscle. *Nat. Cell Biol.* **3**, 544-551. doi:10.1038/35078517
Loison, O., Weitkunat, M., Kaya-Çopur, A., Nascimento Alves, C., Matzat, T., Spletter, M. L., Luschnig, S., Brasselet, S., Lenne, P. F. and Schnorrer, F. (2018). Polarization-resolved microscopy reveals a muscle myosin motor-independent mechanism of molecular actin ordering during sarcomere maturation. *PLoS Biol.* **16**, e2004718. doi:10.1371/journal.pbio.2004718
Loreau, V., Rees, R., Chan, E. H., Taxer, W., Gregor, K., Mußil, B., Pitaval, C., Luis, N. M., Mangeol, P., Schnorrer, F. et al. (2022). A nanobody toolbox to investigate localisation and dynamics of *Drosophila* titins. *bioRxiv* 10.1101/2022.04.13.488177. doi:10.1101/2022.04.13.488177
Luther, P. K. (2009). The vertebrate muscle Z-disc: sarcomere anchor for structure and signalling. *J. Muscle Res. Cell Motil.* **30**, 171-185. doi:10.1007/s10974-009-9189-6
Mackie, G., Mills, C. and Singla, C. (1988). Structure and function of the prehensile tentilla of Euplokamis (Ctenophora, Cydippida). *Zoomorphology* **107**, 319-337. doi:10.1007/BF00312216
Manring, H. R., Carter, O. A. and Ackermann, M. A. (2017). Obscure functions: the location-function relationship of obscurins. *Biophys. Rev.* **9**, 245-258. doi:10.1007/s12551-017-0254-x
Mardahl-Dumesnil, M. and Fowler, V. M. (2001). Thin filaments elongate from their pointed ends during myofibril assembly in Drosophila indirect flight muscle. *J. Cell Biol.* **155**, 1043-1053. doi:10.1083/jcb.200108026
Maroto, M., Arredondo, J. J., San Román, M., Marco, R. and Cervera, M. (1995). Analysis of the paramyosin/miniparamyosin gene. Miniparamyosin is an independently transcribed, distinct paramyosin isoform, widely distributed in invertebrates. *J. Biol. Chem.* **270**, 4375-4382. doi:10.1074/jbc.270.9.4375
Menard, L., Maughan, D. and Vigoreaux, J. (2014). The structural and functional coordination of glycolytic enzymes in muscle: evidence of a metabolon? *Biology* **3**, 623-644. doi:10.3390/biology3030623
Molnar, I., Migh, E., Szikora, S., Kalmár, T., Végh, A. G., Deák, F., Barkó, S., Bugyi, B., Orfanos, Z., Kovács, J. et al. (2014). DAAM is required for thin filament formation and Sarcomerogenesis during muscle development in Drosophila. *PLoS Genet.* **10**, e1004166. doi:10.1371/journal.pgen.1004166
Moulder, G. L., Cremona, G. H., Duerr, J., Stirman, J. N., Fields, S. D., Martin, W., Qadota, H., Benian, G. M., Lu, H. and Barstead, R. J. (2010). α-actinin is required for the proper assembly of Z-disk/focal-adhesion-like structures and for efficient locomotion in Caenorhabditis elegans. *J. Mol. Biol.* **403**, 516-528. doi:10.1016/j.jmb.2010.08.055
Murphy, A. C. and Young, P. W. (2015). The actinin family of actin cross-linking proteins - a genetic perspective. *Cell Biosci.* **5**, 49. doi:10.1186/s13578-015-0029-7
Nave, R. and Weber, K. (1990). A myofibrillar protein of insect muscle related to vertebrate titin connects Z band and A band: purification and molecular characterization of invertebrate mini-titin. *J. Cell Sci.* **95**, 535-544. doi:10.1242/jcs.95.4.535
Nielsen, J., Dubillot, P., Stausholm, M.-L. H. and Ørtenblad, N. (2022). Specific ATPases drive compartmentalized glycogen utilization in rat skeletal muscle. *J. Gen. Physiol.* **154**, e202113071. doi:10.1085/jgp.202113071
Nikonova, E., Kao, S. Y. and Spletter, M. L. (2020). Contributions of alternative splicing to muscle type development and function. *Semin. Cell Dev. Biol.* **104**, 65-80. doi:10.1016/j.semcdb.2020.02.003
Oas, S. T., Bryantsev, A. L. and Cripps, R. M. (2014). Arrest is a regulator of fiber-specific alternative splicing in the indirect flight muscles of Drosophila. *J. Cell Biol.* **206**, 895-908. doi:10.1083/jcb.201405058
Ono, K., Qin, Z., Johnsen, R. C., Baillie, D. L. and Ono, S. (2019). Kettin, the large actin-binding protein with multiple immunoglobulin domains, is essential for sarcomeric actin assembly and larval development in Caenorhabditis elegans. *FEBS J.* **287**, 659-670. doi:10.1111/febs.1503
Orfanos, Z., Leonard, K., Elliott, C., Katzemich, A., Bullard, B. and Sparrow, J. (2015). Sallimus and the dynamics of sarcomere assembly in Drosophila flight muscles. *J. Mol. Biol.* **427**, 2151-2158. doi:10.1016/j.jmb.2015.04.003
Orfanos, Z., Gödderz, M. P., Soroka, E., Gödderz, T., Rumyantseva, A., Van Der Ven, P. F., Hawke, T. J. and Fürst, D. O. (2016). Breaking sarcomeres by in vitro exercise. *Sci. Rep.* **6**, 19614. doi:10.1038/srep19614
Ørtenblad, N., Nielsen, J., Saltin, B. and Holmberg, H. C. (2011). Role of glycogen availability in sarcoplasmic reticulum Ca^{2+} kinetics in human skeletal muscle. *J. Physiol.* **589**, 711-725. doi:10.1113/jphysiol.2010.195982
Pacholsky, D., Vakeel, P., Himmel, M., Lowe, T., Stradal, T., Rottner, K., Furst, D. O. and Van Der Ven, P. F. (2004). Xin repeats define a novel actin-binding motif. *J. Cell Sci.* **117**, 5257-5268. doi:10.1242/jcs.01406
Paulin, D. and Li, Z. (2004). Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. *Exp. Cell Res.* **301**, 1-7. doi:10.1016/j.yexcr.2004.08.004
Perkins, A. D. and Tanentzapf, G. (2014). An ongoing role for structural sarcomeric components in maintaining Drosophila melanogaster muscle function and structure. *PLoS One* **9**, e99362. doi:10.1371/journal.pone.0099362
Pernigo, S., Fukuzawa, A., Beedle, A. E. M., Holt, M., Round, A., Pandini, A., Garcia-Manyes, S., Gautel, M. and Steiner, R. A. (2017). Binding of Myomesin to Obscurin-Like-1 at the Muscle M-Band Provides a Strategy for Isoform-Specific Mechanical Protection. *Structure* **25**, 107-120. doi:10.1016/j.str.2016.11.015
Perz-Edwards, R. J. and Reedy, M. K. (2011). Electron microscopy and x-ray diffraction evidence for two Z-band structural states. *Biophys. J.* **101**, 709-717. doi:10.1016/j.bpj.2011.06.024
Pinotsis, N., Chatziefthimiou, S. D., Berkemeier, F., Beuron, F., Mavridis, I. M., Konarev, P. V., Svergun, D. I., Morris, E., Rief, M. and Wilmanns, M. (2012). Superhelical architecture of the myosin filament-linking protein myomesin with unusual elastic properties. *PLoS Biol.* **10**, e1001261. doi:10.1371/journal.pbio.1001261
Qiu, F., Brendel, S., Cunha, P. M., Astola, N., Song, B., Furlong, E. E., Leonard, K. R. and Bullard, B. (2005). Myofilin, a protein in the thick filaments of insect muscle. *J. Cell Sci.* **118**, 1527-1536. doi:10.1242/jcs.02281
Rahmani, H., Ma, W., Hu, Z., Daneshparvar, N., Taylor, D. W., Mccammon, J. A., Irving, T. C., Edwards, R. J. and Taylor, K. A. (2021). The myosin II coiled-coil domain atomic structure in its native environment. *Proc. Natl. Acad. Sci. USA* **118**, e2024151118. doi:10.1073/pnas.2024151118
Rai, M., Katti, P. and Nongthomba, U. (2014). Drosophila Erect wing (Ewg) controls mitochondrial fusion during muscle growth and maintenance by regulation of the Opa1-like gene. *J. Cell Sci.* **127**, 191-203. doi:10.1242/jcs.135525
Razinia, Z., Makela, T., Ylänne, J. and Calderwood, D. A. (2012). Filamins in mechanosensing and signaling. *Annu. Rev. Biophys.* **41**, 227-246. doi:10.1146/annurev-biophys-050511-102252
Reedy, M. C. and Beall, C. (1993a). Ultrastructure of developing flight muscle in Drosophila. I. Assembly of myofibrils. *Dev. Biol.* **160**, 443-465. doi:10.1006/dbio.1993.1320
Reedy, M. C. and Beall, C. (1993b). Ultrastructure of developing flight muscle in Drosophila. II. Formation of the myotendon junction. *Dev. Biol.* **160**, 466-479. doi:10.1006/dbio.1993.1321
Röper, K., Mao, Y. and Brown, N. H. (2005). Contribution of sequence variation in Drosophila actins to their incorporation into actin-based structures in vivo. *J. Cell Sci.* **118**, 3937-3948. doi:10.1242/jcs.02517
Rudolph, F., Fink, C., Hüttemeister, J., Kirchner, M., Radke, M. H., Lopez Carballo, J., Wagner, E., Kohl, T., Lehnart, S. E., Mertins, P. et al. (2020). Deconstructing sarcomeric structure-function relations in titin-BioID knock-in mice. *Nat. Commun.* **11**, 3133. doi:10.1038/s41467-020-16929-8
Rui, Y., Bai, J. and Perrimon, N. (2010). Sarcomere formation occurs by the assembly of multiple latent protein complexes. *PLoS Genet.* **6**, e1001208. doi:10.1371/journal.pgen.1001208
Rusu, M., Hu, Z., Taylor, K. A. and Trinick, J. (2017). Structure of isolated Z-disks from honeybee flight muscle. *J. Muscle Res. Cell Motil.* **38**, 241-250. doi:10.1007/s10974-017-9477-5
Saide, J. D. and Ullrick, W. C. (1973). Fine structure of the honeybee Z-disc. *J. Mol. Biol.* **79**, 329-337. doi:10.1016/0022-2836(73)90009-0
Schoenauer, R., Lange, S., Hirschy, A., Ehler, E., Perriard, J.-C. and Agarkova, I. (2008). Myomesin 3, a novel structural component of the M-band in striated muscle. *J. Mol. Biol.* **376**, 338-351. doi:10.1016/j.jmb.2007.11.048
Schönbauer, C., Distler, J., Jährling, N., Radolf, M., Dodt, H. U., Frasch, M. and Schnorrer, F. (2011). Spalt mediates an evolutionarily conserved switch to fibrillar muscle fate in insects. *Nature* **479**, 406-409. doi:10.1038/nature10559
Schueder, F., Mangeol, P., Chan, E. H., Rees, R., Schünemann, J., Jungmann, R., Görlich, D. and Schnorrer, F. (2022). Nanobodies combined with DNA-PAINT super-resolution reveal a staggered titin nano-architecture in flight muscles. *bioRxiv* 10.1101/2022.04.14.488306. doi:10.1101/2022.04.14.488306
Seipel, K. and Schmid, V. (2005). Evolution of striated muscle: jellyfish and the origin of triploblasty. *Dev. Biol.* **282**, 14-26. doi:10.1016/j.ydbio.2005.03.032
Shafiq, S. A. (1963). Electron microscopic studies on the indirect flight muscles of Drosophila melanogaster. I. Structure of the myofibrils. *J. Cell Biol.* **17**, 351-362. doi:10.1083/jcb.17.2.351
Shwartz, A., Dhanyasi, N., Schejter, E. D. and Shilo, B.-Z. (2016). The Drosophila formin Fhos is a primary mediator of sarcomeric thin-filament array assembly. *eLife* **5**, e16540. doi:10.7554/eLife.16540
Smith, D. S. (1961). The organization of the flight muscle in a dragonfly, Aeshna sp. (Odonata). *J. Biophys. Biochem. Cytol.* **11**, 119-145. doi:10.1083/jcb.11.1.119
Sparrow, J. C. and Schöck, F. (2009). The initial steps of myofibril assembly: integrins pave the way. *Nat. Rev. Mol. Cell Biol.* **10**, 293-298. doi:10.1038/nrm2634
Spletter, M. L. and Schnorrer, F. (2014). Transcriptional regulation and alternative splicing cooperate in muscle fiber-type specification in flies and mammals. *Exp. Cell Res.* **321**, 90-98. doi:10.1016/j.yexcr.2013.10.007

Spletter, M. L., Barz, C., Yeroslaviz, A., Schonbauer, C., Ferreira, I. R., Sarov, M., Gerlach, D., Stark, A., Habermann, B. H. and Schnorrer, F. (2015). The RNA-binding protein Arrest (Bruno) regulates alternative splicing to enable myofibril maturation in Drosophila flight muscle. *EMBO Rep.* **16**, 178-191. doi:10.15252/embr.201439791

Spletter, M. L., Barz, C., Yeroslaviz, A., Zhang, X., Lemke, S. B., Bonnard, A., Brunner, E., Cardone, G., Basler, K., Habermann, B. H. et al. (2018). A transcriptomics resource reveals a transcriptional transition during ordered sarcomere morphogenesis in flight muscle. *Elife* **7**, e34058. doi:10.7554/eLife.34058

Sponga, A., Arolas, J. L., Schwarz, T. C., Jeffries, C. M., Rodriguez Chamorro, A., Kostan, J., Ghisleni, A., Drepper, F., Polyansky, A., De Almeida Ribeiro, E. et al. (2021). Order from disorder in the sarcomere: FATZ forms a fuzzy but tight complex and phase-separated condensates with α-actinin. *Sci. Adv.* **7**, eabg7653. doi:10.1126/sciadv.abg7653

Steinmetz, P. R., Kraus, J. E., Larroux, C., Hammel, J. U., Amon-Hassenzahl, A., Houliston, E., Wörheide, G., Nickel, M., Degnan, B. M. and Technau, U. (2012). Independent evolution of striated muscles in cnidarians and bilaterians. *Nature* **487**, 231-234. doi:10.1038/nature11180

Stronach, B. (2014). Extensive nonmuscle expression and epithelial apicobasal localization of the Drosophila ALP/Enigma family protein, Zasp52. *Gene Expr. Patterns* **15**, 67-79. doi:10.1016/j.gep.2014.05.002

Stronach, B. E., Renfranz, P. J., Lilly, B. and Beckerle, M. C. (1999). Muscle LIM proteins are associated with muscle sarcomeres and require dMEF2 for their expression during Drosophila myogenesis. *Mol. Biol. Cell* **10**, 2329-2342. doi:10.1091/mbc.10.7.2329

Sullivan, D. T., Macintyre, R., Fuda, N., Fiori, J., Barrilla, J. and Ramizel, L. (2003). Analysis of glycolytic enzyme co-localization in Drosophila flight muscle. *J. Exp. Biol.* **206**, 2031-2038. doi:10.1242/jeb.00367

Sutter, S. B., Raeker, M. O., Borisov, A. B. and Russell, M. W. (2004). Orthologous relationship of obscurin and Unc-89: phylogeny of a novel family of tandem myosin light chain kinases. *Dev. Genes Evol.* **214**, 352-359. doi:10.1007/s00427-004-0413-5

Suyama, R., Jenny, A., Curado, S., Pellis-Van Berkel, W. and Ephrussi, A. (2009). The actin-binding protein Lasp promotes Oskar accumulation at the posterior pole of the Drosophila embryo. *Development* **136**, 95-105. doi:10.1242/dev.027698

Sweetlove, L. J. and Fernie, A. R. (2018). The role of dynamic enzyme assemblies and substrate channelling in metabolic regulation. *Nat. Commun.* **9**, 2136. doi:10.1038/s41467-018-04543-8

Szikora, S., Gajdos, T., Novák, T., Farkas, D., Földi, I., Lenart, P., Erdélyi, M. and Mihály, J. (2020). Nanoscopy reveals the layered organization of the sarcomeric H-zone and I-band complexes. *J. Cell Biol.* **219**, e201907026. doi:10.1083/jcb.201907026

Szikora, S., Görög, P. and Mihály, J. (2022). The Mechanisms of Thin Filament Assembly and Length Regulation in Muscles. *Int. J. Mol. Sci.* **23**, 5306. doi:10.3390/ijms23105306

Tanaka, H., Ishimaru, S., Nagatsuka, Y. and Ohashi, K. (2018). Smooth muscle-like Ca(2+)-regulation of actin-myosin interaction in adult jellyfish striated muscle. *Sci. Rep.* **8**, 7776. doi:10.1038/s41598-018-24817-x

Teulier, L., Weber, J. M., Crevier, J. and Darveau, C. A. (2016). Proline as a fuel for insect flight: enhancing carbohydrate oxidation in hymenopterans. *Proc. Biol. Sci.* **283**, 20160333. doi:10.1098/rspb.2016.0333

Tonino, P., Kiss, B., Strom, J., Methawasin, M., Smith, J. E., 3rd, Kolb, J., Labeit, S. and Granzier, H. (2017). The giant protein titin regulates the length of the striated muscle thick filament. *Nat. Commun.* **8**, 1041. doi:10.1038/s41467-017-01144-9

Tskhovrebova, L. and Trinick, J. (2012). Making muscle elastic: the structural basis of myomesin stretching. *PLoS Biol.* **10**, e1001264. doi:10.1371/journal.pbio.1001264

Tskhovrebova, L. and Trinick, J. (2017). Titin and Nebulin in thick and thin filament length regulation. *Subcell. Biochem.* **82**, 285-318. doi:10.1007/978-3-319-49674-0_10

Venables, J. P., Tazi, J. and Juge, F. (2012). Regulated functional alternative splicing in Drosophila. *Nucleic Acids Res.* **40**, 1-10. doi:10.1093/nar/gkr648

Verdonschot, J. A. J., Vanhoutte, E. K., Claes, G. R. F., Helderman-Van Den Enden, A., Hoeijmakers, J. G. J., Hellebrekers, D., De Haan, A., Christiaans, I., Lekanne Deprez, R. H., Boen, H. M. et al. (2020). A mutation update for the FLNC gene in myopathies and cardiomyopathies. *Hum. Mutat.* **41**, 1091-1111. doi:10.1002/humu.24004

Vigoreaux, J. O. (2001). Genetics of the Drosophila flight muscle myofibril: a window into the biology of complex systems. *BioEssays* **23**, 1047-1063. doi:10.1002/bies.1150

Vigoreaux, J. O., Saide, J. D., Valgeirsdottir, K. and Pardue, M. L. (1993). Flightin, a novel myofibrillar protein of Drosophila stretch-activated muscles. *J. Cell Biol.* **121**, 587-598. doi:10.1083/jcb.121.3.587

Wahlström, G., Lahti, V. P., Pispa, J., Roos, C. and Heino, T. I. (2004). Drosophila non-muscle alpha-actinin is localized in nurse cell actin bundles and ring canals, but is not required for fertility. *Mech. Dev.* **121**, 1377-1391. doi:10.1016/j.mod.2004.06.004

Waites, G. T., Graham, I. R., Jackson, P., Millake, D. B., Patel, B., Blanchard, A. D., Weller, P. A., Eperon, I. C. and Critchley, D. R. (1992). Mutually exclusive splicing of calcium-binding domain exons in chick alpha-actinin. *J. Biol. Chem.* **267**, 6263-6271. doi:10.1016/S0021-9258(18)42690-7

Wang, Z.-H., Clark, C. and Geisbrecht, E. R. (2016). Analysis of mitochondrial structure and function in the Drosophila larval musculature. *Mitochondrion* **26**, 33-42. doi:10.1016/j.mito.2015.11.005

Wang, L., Geist, J., Grogan, A., Hu, L. R. and Kontrogianni-Konstantopoulos, A. (2018). Thick filament protein network, functions, and disease association. *Compr. Physiol.* **8**, 631-709. doi:10.1002/cphy.c170023

Wang, Z., Grange, M., Wagner, T., Kho, A. L., Gautel, M. and Raunser, S. (2021). The molecular basis for sarcomere organization in vertebrate skeletal muscle. *Cell* **184**, 2135-2150.e13. doi:10.1016/j.cell.2021.02.047

Weitkunat, M., Kaya-Çopur, A., Grill, S. W. and Schnorrer, F. (2014). Tension and force-resistant attachment are essential for myofibrillogenesis in Drosophila flight muscle. *Curr. Biol.* **24**, 705-716. doi:10.1016/j.cub.2014.02.032

Weitkunat, M., Brasse, M., Bausch, A. R. and Schnorrer, F. (2017). Mechanical tension and spontaneous muscle twitching precede the formation of cross-striated muscle in vivo. *Development* **144**, 1261-1272. doi:10.1242/dev.140723

Wojtas, K., Slepecky, N., Von Kalm, L. and Sullivan, D. (1997). Flight muscle function in Drosophila requires colocalization of glycolytic enzymes. *Mol. Biol. Cell* **8**, 1665-1675. doi:10.1091/mbc.8.9.1665

Wojtowicz, I., Jablonska, J., Zmojdzian, M., Taghli-Lamallem, O., Renaud, Y., Junion, G., Daczewska, M., Huelsmann, S., Jagla, K. and Jagla, T. (2015). Drosophila small heat shock protein CryAB ensures structural integrity of developing muscles, and proper muscle and heart performance. *Development* **142**, 994-1005. doi:10.1242/dev.115352

Young, P. and Gautel, M. (2000). The interaction of titin and alpha-actinin is controlled by a phospholipid-regulated intramolecular pseudoligand mechanism. *EMBO J.* **19**, 6331-6340. doi:10.1093/emboj/19.23.6331

Yu, J., Pacifico, S., Liu, G. and Finley, R. L.Jr. (2008). DroID: the Drosophila interactions database, a comprehensive resource for annotated gene and protein interactions. *BMC Genomics* **9**, 461. doi:10.1186/1471-2164-9-461

Zheng, M., Cheng, H., Banerjee, I. and Chen, J. (2010). ALP/Enigma PDZ-LIM domain proteins in the heart. *J. Mol. Cell Biol.* **2**, 96-102. doi:10.1093/jmcb/mjp038

Zhou, Q., Chu, P. H., Huang, C., Cheng, C. F., Martone, M. E., Knoll, G., Shelton, G. D., Evans, S. and Chen, J. (2001). Ablation of Cypher, a PDZ-LIM domain Z-line protein, causes a severe form of congenital myopathy. *J. Cell Biol.* **155**, 605-612. doi:10.1083/jcb.200107092