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Review

The muscle ultrastructure: a structural perspective of the sarcomere

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Abstract. Muscle ultrastructure is characterised by a complex arrangement of many protein-protein interactions. The sarcomere is the basic repeating unit of muscle, formed by two transverse filament systems: the thick and thin filaments. While actin and myosin are the main contractile elements of the sarcomere, other proteins act as scaffolds, control ultrastructure composition, regulate muscle contraction, and transmit tension between sarcomeres and hence to the whole myofibril. Elucidation of the structures of muscle proteins by X-ray crystallography and nuclear magnetic resonance spectroscopy has been essential in understanding muscle contraction, enabling us to relate biological to structural information. These structures reveal how components of the muscle interact, how different factors influence conformational changes within these proteins, and how mutant muscle proteins may interfere with the regulatory fine-tuning of the contractile machinery, hence leading to disease in some cases. Here, structures solved within the sarcomere have been reviewed in order to put the numerous components into context.

Key words. Muscle; sarcomere; thick filament; thin filament; Z-line; M-line.

Introduction

Movement plays a vital role in all living systems, from the transport of single molecules in a cell to the movement of the entire organism. In mammalian systems, skeletal muscle is essential for voluntary movements such as those effected by walking, running, swimming or flying. Involuntary movements performed by the body are just as important, for example, cardiac muscle for the beating heart or smooth muscle in peristalsis. Cardiac and skeletal muscle are both forms of striated muscle, which is formed by huge cells produced by the fusion of many separate cells. Contained within muscle cells are cylindrical structures known as myofibrils that are the contractile elements of the muscle cell. These in turn are made up of a repeated unit known as the sarcomere, which is a complex network of proteins (fig. 1). The mammalian sarcomere is ~2 µm in length, and can shorten to ~70% of its original length during contraction.

Electron micrographs of muscle give a low-resolution (up to 10–20 Å) view of muscle ultrastructure. Structural features of the sarcomere include the transverse thick and thin filaments and the longitudinal Z- and M-lines (Z- and M-lines are also referred to as disks or bands) (fig. 1). The region of the sarcomere containing the Z-disk and thin filaments is the I-band, while the remaining area is the A-band. The A-band comprises the area where the thick and thin filaments interdigitate, an area of thick filament alone and the M-line [1, 2]. The thin filament is composed primarily of actin; it is tethered at one end to the Z-disk, and it interdigitates with the thick filaments. The main constituent of the thick filament is myosin; the direction of the myosin heads changes polarity at the M-line, allowing interaction with the thin filaments anchored from the next adjacent Z-disk. Electron microscopy images of the cross-section of the A-band show that six thin filaments surround one thick filament in a hexagonally arranged lattice and the globular heads of

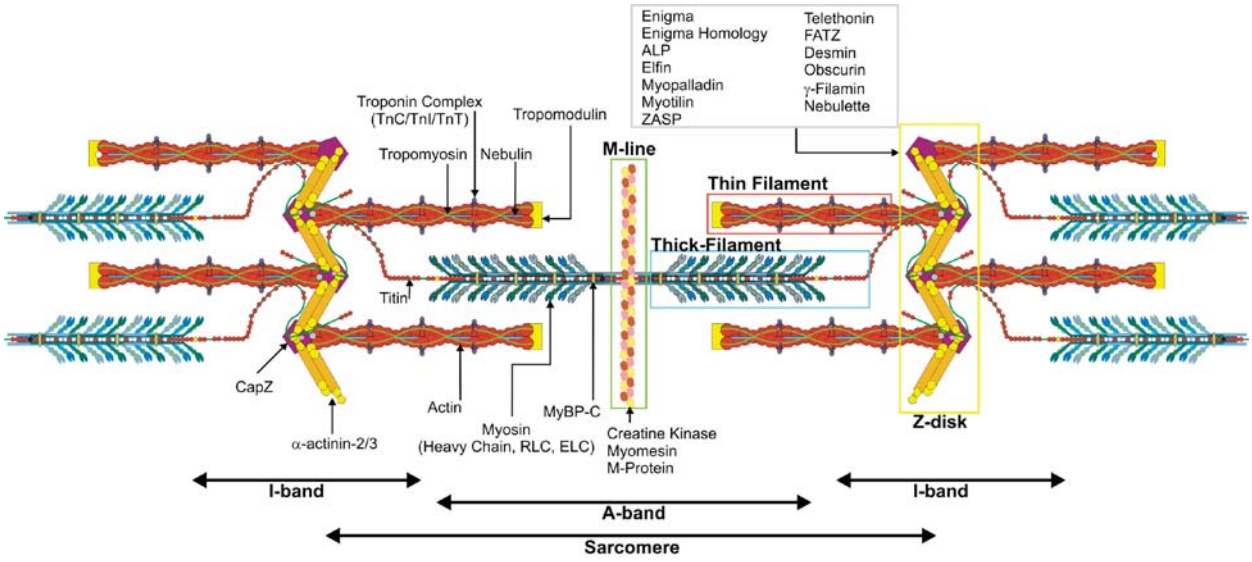

Figure 1. Schematic representation of the sarcomere. The thick filaments, thin filaments, Z- and M-lines are indicated by blue, red, yellow and green boxes, respectively. The protein components shown to localise in the sarcomere have been directly labelled on the appropriate area of the ultrastructure.

myosin extend to interact with actin, forming cross-bridges.

Muscle contraction is a multi-step cycle involving the globular heads of myosin, actin and ATP hydrolysis (for a detailed review on the mechanism of muscle contraction, see [3]). The original 'sliding filament' model describes muscle contraction occurring when the thick filaments slide past the thin filaments, thus shortening the entire sarcomere with no shortening of either filament [1]. This model for muscle contraction was developed to include a role for ATP hydrolysis in the 'Lymn-Taylor cycle' [4]. The model was further refined following structural and spectroscopic observations (reviewed in [5]), which showed that the relative movement of actin and myosin arise from the head of myosin acting as a lever (the 'lever-arm' hypothesis).

The myosin head undergoes a series of ordered conformational changes depending on the presence of ATP, ADP and inorganic phosphate (Pi). The start of the cycle begins with myosin bound to actin in the 'rigor' conformation. The binding of ATP to myosin reduces the affinity of myosin for actin, causing the two proteins to separate. Myosin then hydrolyses ATP, and this leads to a 'cocked' conformation, priming the conformational change required for the 'power stroke'. The weak association of the myosin-ADP-Pi complex with actin results in the concomitant release of Pi that increases the affinity of myosin for actin. This is immediately followed by a large conformational change in myosin (the power stroke), and the simultaneous release of ADP, re-establishing the initial rigor position. This series of reactions results in the force-generation required for filament movement, causing the sarcomere to shorten, and in turn the muscle to contract. Muscle contraction is controlled by the concentration of calcium (see section on tropomyosin and troponin).

Scope of the review

This review has been written to highlight the structural biology information available on the sarcomere, featuring structures solved by X-ray crystallography or by nuclear magnetic resonance (NMR) spectroscopy (table 1, fig. 2). Major advances in understanding muscle contraction came from the structures of the components involved, mainly actin and myosin. An atomic model of the actin filament [6] was derived from the crystal structure of globular actin solved in complex with DNase I [7], used in conjunction with X-ray fibre diagrams [8]. The structure of the scallop myosin S1 fragment was also determined [9], providing an atomic model of the rigor conformation of 'decorated actin' (i.e. the actin polymer bound with many S1 fragments) [10]. The structures of different conformational states of myosin have been critical for confirming details of the hypothesis of muscle contraction, and the mechanistic aspects of muscle have been well reviewed and debated [3, 11]. While an understanding of the basic mechanism of muscle contraction may be gained through studies of actin and myosin, the context in which the transformation of chemical to kinetic energy occurs can only be understood through the study of additional proteins of the sarcomere. These proteins regulate many aspects of the muscle: regulating muscle contraction, acting as scaffolds for actin and myosin, maintaining overall stability of the sarcomere and controlling myofibrillogenesis.

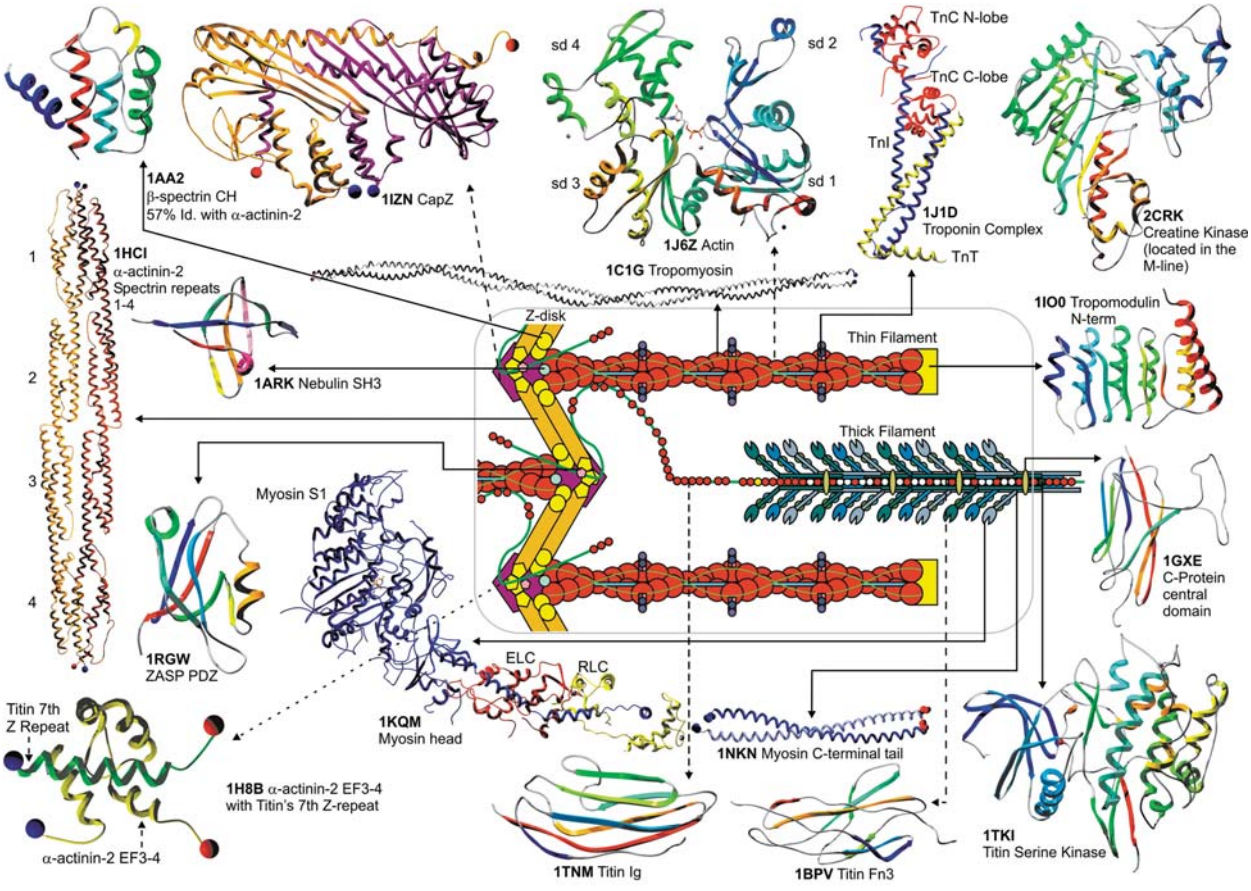

Figure 2. Diagram of representative structures of the components of the sarcomere. The localisation of the proteins has been indicated using the diagram of a section of the sarcomere (within the grey box), using black solid or dotted arrows. All structures are displayed using a ribbon representation and are labelled with their PDB accession codes, and subdomains or chain name. Monomeric structures are coloured in secondary structure succession from blue to red, from the N- to C-termini; a single colouring scheme per chain has been used for multimeric structures, with blue and red spheres indicating N- and C-termini (except myosin-S1 and the troponin complex).

This review is focussed on myofibrillar proteins, which have specific ultrastructural localizations within the sarcomere, using techniques such as electron microscopy or immuno-fluorescence. The focus of this review is on mammalian systems, but structures from non-mammalian systems have been included where appropriate. The interactions, dynamics, links to signalling pathways, functions and disorders in muscle have been reviewed fairly recently [12] and will not be covered here. In addition, an indication of the future work in muscle has also been highlighted.

Thin filament

Muscle contraction can be compared to a ratchet system where one static surface provides a regular series of 'anchoring points' for the opposing surface. In this analogy, the role of the thin filament is to provide the myosin anchors with an ordered series of binding sites to which it may attach. Globular actin (G-actin) polymerises to form fibres (F-actin), which have a double helical ultrastructure and a structurally polarised orientation in the thin filament. This super-helix has 13 actin monomers per six helical turns; the rotation per monomer is 166° [8]. The actin monomers are in a perpetual state of association and dissociation with the filament, in a dynamic process known as tread-milling. The fast growing 'barbed-end' of actin points toward the Z-disk, while the slow growing 'pointed-end' faces the M-line. CapZ is responsible for preventing the addition and loss of actin by capping the barbed-end, while tropomodulin has an analogous function at the pointed-end; both capping proteins have been shown to specifically bind F-actin, as opposed to actin monomers. The thin filament is constructed upon two molecules of the filamentous protein nebulin, thought to act as a 'molecular ruler' for actin filament assembly, regulating the length of the thin filament. Tropomyosin is a coiled-coil protein that also associates with actin, blocking its interaction with myosin. The position of tropomyosin, and hence the binding of myosin to actin, is controlled by the troponin complex in a calcium-dependent manner [13].

Table 1. Table of PDB files cited in this review.

Structure (with description)	Ligand(s)	Technique	Source	PDB Id.	Ref.
Thin filament					
Actin					
actin with DNase I	ADP, ATP	X-ray: 2.8 Å	rabbit skeletal *bos taurus* pancreas	1ATN	[7]
actin with gelsolin	ATP	X-ray: 2.30 Å	rabbit skeletal	1EQY	[15]
actin with gelsolin	ATP	X-ray: 3.4 Å	rabbit muscle actin human gelsolin	1DB0	[16]
actin with gelsolin	ATP, latrunculin A,	X-ray: 2.00 Å	rabbit muscle actin human gelsolin	1ESV	[23]
actin monomer	TMR, ADP	X-ray: 1.54 Å	rabbit skeletal	1J6Z	[21]
actin (dimer)	ATP, latrunculin A	X-ray: 2.85 Å	rabbit skeletal rabbit skeletal	1IJJ	[24]
actin with vitamin D binding protein	ATP	X-ray: 2.15 Å	human VDBP rabbit skeletal	1KXP	[19]
actin with vitamin D binding protein	ATP	X-ray: 2.5 Å	rabbit skeletal human VDBP	1LOT	[20]
actin with gelsolin	ATP	X-ray: 3.0 Å	rabbit muscle actin human gelsolin	1H1V	[17]
actin (trimer) with gelsolin	ATP	X-ray: 2.2 Å	chicken actin human gelsolin	1MDU	[25]
actin monomer	TMR, AMP-PNP	X-ray: 1.85 Å	rabbit skeletal	1NWK	[22]
actin with gelsolin	ATP	X-ray: 2.6 Å	rabbit muscle actin human gelsolin	1P8Z	[18]
Nebulin					
SH3 domain: 6610-6669 (60)	N/A	NMR: 15, Av.	human	1ARK 1NEB	[33]
CapZ					
heterodimer: α1 7-281 or 5-277 (286), β1 2-271 (277)	N/A	X-ray: 2.1 Å	chicken skeletal	1IZN	[38]
Tropomodulin					
C-terminus LRR domain, 160-344 (185)	N/A	X-ray: 1.45 Å	chicken	1IO0	[42]
Tropomyosin					
full-length (284 residues) – Cα trace only	N/A	X-ray: 15 Å	rabbit heart	2TMA	[43]
N-terminal: 14 res. Tm, 18 res. GCN4	N/A	NMR: 15	rabbit Tm yeast GCN4	1TMZ	[48]
full-length (284 residues)	N/A	X-ray: 9 Å	pig heart	1C1G	[47]
N-terminal: 81 residues 'Tm81'	N/A	X-ray: 2.00 Å	chicken skeletal	1IC2	[49]
C-terminal: 31-residues (263–284), preceded by 24 residues of GCN4, plus N-terminal Methionine – 56 total	N/A	X-ray: 2.70 Å	rat striated Tm yeast GCN4	1KQL	[50]
C-terminal: 258–284 (27-residues)	N/A	NMR: 10	rat striated	1MV4	[51]
Troponin C: full-length					
TnC full-length	2 Ca^{2+} (sites III and IV)	X-ray: 2.00 Å	chicken skeletal	4TNC	[55]
TnC full-length	2 Ca^{2+} (sites III and IV)	X-ray: 2.00 Å	turkey skeletal	5TNC	[56]
TnC full-length	2 Ca^{2+} (sites III and IV)	X-ray: 1.78 Å	chicken skeletal	1TOP	[57]
TnC full-length	4 Ca^{2+} (sites I, II, III, IV)	NMR: 23, Av.	chicken skeletal	1TNW 1TNX	[61]
TnC full-length	Mn^{2+}, Cd^{2+}, & Tb3+ (sites III and IV)	X-ray: 1.8 Å (Cd^{2+}), 2.1 Å (Mn^{2+}), 1.8 Å (Tb^{2+})	chicken skeletal	1NCX 1NCY 1NCZ	[58]

Table 1 (continued)

Structure (with description)	Ligand(s)	Technique	Source	PDB Id.	Ref.
TnC full-length: two crystal forms solved	4 Ca^{2+}	X-ray: 1.90 Å, 2.00 Å	rabbit skeletal	1TN4 2TN4	[59]
TnC: (2–89 and 90–161)	3 Ca^{2+} (sites II, III, IV)	NMR: 1 (model), 30 (N-TnC), 30 (C-TnC)	chicken cardiac	1AJ4 2CTN 3CTN	[62]
TnC full-length with TnI (1–47)	2 Ca^{2+} bound in C-TnC	X-ray: 2.3 Å	rabbit skeletal	1A2X	[80]
TnC full-length	4 Ca^{2+} (sites I, II, III, IV)	X-ray: 1.9 Å	rabbit skeletal	1TCF	[60]
TnC full-length with TnI full-length: TnI modelled onto TnC using a number of TnC/TnI structures	3 Ca^{2+} (sites II, III, IV)	NMR: 1	chicken cardiac	1LA0	[149]
Troponin C: N-terminal					
N-TnC (12–87)	N/A	NMR: 1	turkey skeletal	1TRF	[71]
N-TnC	2 Ca^{2+} (site I and II)	NMR: 40 (Apo), 40 (Ca^{2+})	rabbit skeletal	1TNP 1TNQ	[63]
N-TnC	1 Ca^{2+} (site II)	NMR: 40 (Ca^{2+}), 40 (Apo)	human cardiac	1AP4 1SPY	[67]
N-TnC	Ca^{2+} (site II)	NMR: 40	chicken skeletal	1SMG	[68]
N-TnC	2 Ca^{2+} (site I and II)	X-ray: 1.75 Å	chicken skeletal	1AVS	[64]
N-TnC with TnI (96–148) (TnI not displayed in the pdb)	2.8 mM Ca^{2+}, not shown	NMR: 29	chicken skeletal	1BLQ	[65]
N-TnC (1-89) with TnI (147–163)	1 Ca^{2+} (site II)	NMR: 40	human cardiac	1MXL	[69]
N-TnC: temperature conformational change	N/A	NMR: 40, Av.	chicken skeletal	1SKT 1ZAC	[72]
N-TnC with TnI (147–163)	Bepridil, 1 Ca^{2+} (site II)	NMR: 30	human cardiac	1LXF	[70]
N-TnC with TnI (115–131)	Bi-functional rhodamine, 2 Ca^{2+} (sites I and II)	NMR: 21	chicken skeletal	1NPQ	[66]
Troponin C: C-terminal					
C-TnC (81–161) C-terminal free and bound to cTnI (33–80). TnI not shown in the PDB	N/A	NMR: 20	chicken cardiac	1FI5	[73]
C-TnC in complex with and bepridil which increases the affinity between TnC to Ca^{2+}.	3 Ca^{2+}, bepridil	X-ray: 2.15 Å	chicken cardiac	1DTL	[77]
C-TnC, C-terminal Ca^{2+} saturated, in complex with EMD 57033, another Ca^{2+} sensitizer.	EMD 57033	NMR: 30	human cardiac	1IH0	[78]
C-TnC in complex with TnI (1–40) (TnI not shown)	2 Ca^{2+} (sites III and IV)	NMR: 30	chicken skeletal	1JC2	[74]
C-TnC in complex with TnI (128–147)	N/A	NMR: 30	human skeletal human cardiac	1OZS	[75]
Troponin Complex					
1J1D: TnC (1–161), TnI (31–163), TnT (183–288) 1JIE: TnC (1–161), TnI (31–210), TnT (183–288)	1J1D– 3 Ca^{2+} (1 in N-TnC, 2 in C-TnC) 1J1E – 3 Ca^{2+} (1 in N-TnC, 2 in C-TnC)	X-ray: 2.61 Å, 3.30 Å	human cardiac	1J1D 1J1E	[79]
Thick filament					
Myosin					
S1, α-carbon coordinates for the two light chains near-rigor (nucleotide-free) state	1 SO_4, 1 Mg^{2+}	X-ray: 2.8 Å	chicken skeletal	2MYS	[9]
regulatory domain: heavy chain (777–836), ELC, RLC	2 Ca^{2+}	X-ray: 2.80 Å	scallop	1SCM	[87]
regulatory domain: heavy chain (774–837), ELC, RLC	1 Mg^{2+}, 1 Ca^{2+}	X-ray: 2.0 Å	scallop	1WDC	[88]

Table 1 (continued)

Structure (with description)	Ligand(s)	Technique	Source	PDB Id.	Ref.
S1: heavy chain (a) – 5-835, RLC (y), ELC (z) detached state	1 ADP, 2 Mg^{2+}, 1 Ca^{2+}	X-ray: 2.5 Å	scallop	1B7T	[83]
S1: heavy chain (a), RLC (y), ELC(z) near-rigor (nucleotide-free) state (1DFK) pre-power stroke state (1DFL)	1 Ca^{2+}, MgADP·VO_4 (1DFL)	X-ray: 4.2 Å, 4.2 Å	scallop	1DFK 1DFL	[84]
S1: heavy chain (a), RLC(y), ELC(z) near-rigor (nucleotide-free) state (1KK7) detached state (1KK8, 1KQM, 1KWO, 1L2O)	1 SO_4, 2 Mg^{2+}, 1 Ca^{2+}, ADP·VeFx (1KK8), AMP-PNP (1KQM), ATP[γ-S] (1KWO), ADP (1L2O)	X-ray: 3.2 Å, 2.3 Å, 3.0 Å, 3.8 Å, 2.8 Å	scallop	1KK7 1KK8 1KQM 1KWO 1L2O	[85]
N-terminal segment of myosin rod: GSHM-heavy chain (835–885)-GCN4 (250–281)	N/A	X-ray: 2.50 Å	scallop/yeast chimera	1NKN	[89]
S1: heavy chain (a), RLC(y), ELC(z) pre-power stroke state	ADP·VO_4, 2 Mg^{2+}, 1 Ca^{2+}, 1 V^{3+}	X-ray: 2.54 Å	scallop	1QVI	[86]
Titin					
module M5, Ig domain	N/A	NMR: Av, 16	human cardiac	1TNM 1TNN	[101]
module I27, Ig domain	N/A	NMR: Av, 24	human cardiac	1TIT 1TIU	[103]
module M5, Ig domain – additional N terminal residues	N/A	NMR: Av, 16	human cardiac	1NCT 1NCU	[102]
serine kinase domain	N/A	X-ray: 2.0 Å	human cardiac	1TKI	[111]
module A71, Fn3 domain	N/A	NMR: 50	human cardiac	1BPV	[105]
module I1, Ig domain	N/A	X-ray: 2.1 Å	human cardiac	1G1C	[104]
MyBP-C					
Central domain Ig-like domain	N/A	NMR: 10	human cardiac	1GXE	[113]
Z-disk					
α-*Actinin-2*					
calponin homology domain (from β-spectrin not α-actinin-2)	N/A	X-ray: 2.00 Å	human	1AA2	[128]
2 central spectrin-like repeats	N/A	X-ray: 2.5 Å	human skeletal	1QUU	[122]
4 spectrin-like repeats (274 – 746)	N/A	X-ray: 2.8 Å	human skeletal	1HCI	[123]
EF-hands 3 and 4 (822–894) with titin Z-repeat 7 (648–698)	N/A	NMR: 30	human skel. actinin rabbit titin	1H8B	[100]
ZASP					
ZASP PDZ domain (1–85)	N/A	NMR: 20	human	1RGW	[138]
M-Line					
Creatine Kinase					
Full-length MCK	4 SO_4	X-ray: 2.35 Å	rabbit muscle	2CRK	[147]
Full-length MCK	N/A	X-ray: 3.5 Å	human muscle	1I0E	[148]

Fields include a short description of the protein chains contained in the structure, non-protein ligands, technique used, source of the construct protein, PDB accession code and the primary citation. Data in the technique field are followed by resolution for X-ray crystallography (in Å), number of representative structures for NMR, and details for additional ligands have also been included where applicable. N.B. 'Av.' denotes average NMR structure.

Actin

Actin is a ubiquitous cytoskeletal protein that functions in many cellular processes. There are three isoforms of actin (α, β and γ) – α-actin is found in muscle tissue, while most cell types express β- and γ-actin as part of the cytoskeleton (for a review of actin and its binding partners, see [14]). The formation of actin filaments involves ATP-actin associating at the barbed-end. ATP hydrolysis leads to ADP-actin that has a weaker affinity to the filament, leading to its dissociation at the pointed-end.

Human α-actin has a molecular mass of ~42 kDa and is a single polypeptide chain comprising four subdomains forming a globular protein (G-actin). Subdomains 1 and 2 have been named the small domain, while subdomains 3 and 4 form the 'large domain'. A cleft between these two domains in the centre of the molecule provides a site

for the binding of ATP or ADP nucleotide and a divalent cation, such as calcium (Ca^{2+}). In F-actin, subdomains 1 and 3 form the 'barbed' ends, while subdomains 2 and 4 comprise the 'pointed' end. The tendency of actin to polymerise has rendered the study of the monomeric state difficult; hence, structures of actin have been determined using cofactors that prevent polymerisation. The first structure of actin was reported in complex with DNase I [7]. While crystallised with ATP and Ca^{2+}, subsequent ATP hydrolysis in the crystal provided a structure of ADP-actin. Ensuing structures of actin have been solved in complex with gelsolin [15–18] and vitamin D binding protein [19, 20], all in the presence of ATP. Relatively small organic compounds have also been used to keep actin monomeric. The use of tetramethylrhodamine-5-maleimeide (TMR) allowed the 'uncomplexed' structure of ADP-actin to be solved [21], revealing a change in subdomain-2 when compared to the structure of actin bound to the non-hydrolysable ATP analogue, AMP-PNP [22]. As the change in subdomain-2 affects actin polymerisation, it is thought that this is the conformational change required for ADP-actin dissociation at the pointed-end. These observations are different from those made on the original ADP-actin structure [7], but it has been suggested that since ATP was hydrolysed in crystal in the earlier study, crystal contacts may have prevented the molecule re-arranging to the 'true' ADP-actin conformation [21]. Crystal structures of actin have also been solved in the presence of latrunculin A, a toxin produced by Red Sea sponge that prevents monomeric actin from forming polymers. A structure of actin has been solved in the presence of latrunculin A and gelsolin domain 1 [23]. Dimers solved with lantrulacin A [24] and trimers with gelsolin [25] of actin have been crystallised, providing insight into actin fibre nucleation.

Currently, there are no structures of actin without ATP or ADP as nucleotide-free actin denatures irreversibly [26], although it has been proposed that nucleotide-free actin has an 'open cleft' conformation [22]. There is clearly a lack of high-resolution structures of actin in complex with some of the more conventional sarcomeric proteins, but many of these proteins are specific to polymeric F-actin.

Nebulin

Along with the thin filament's caps (i.e. capZ and tropomodulin), the length of the thin filament is controlled by two molecules of the molecular ruler nebulin (for a review of nebulin see [27]). Nebulin is between 500 and 900 kDa, depending on the isoform, with a highly modular structure. Approximately 97% of the human nebulin sequence is composed of 185 ~35-residue modules, ending with a C-terminal SH3 domain. The orientation of nebulin is unambiguous, as its C-terminus is located in the Z-disk. Nebulin forms a fibrous structure that allows linear assembly of actin, with each repeated module interacting behind the large domain of a single actin monomer [28–30]. Alternatively, nebulin may bind to subdomain 1 of actin, placing it at the outer edge of the thin filament [31, 32]. The structure of the SH3 domain of nebulin has been determined by NMR [33]. It folds into a typical globular SH3 fold, with two triple-stranded β-sheets packed against each other at right angles. This SH3 domain has been suggested to bind the PEVK region of titin (a proline rich area near the N-terminus of titin), myopalladin [34, 35], and titin's N-terminus [36]. Myopalladin has also been shown to bind α-actinin, possibly acting as a linker between α-actinin and nebulin in the Z-disk [34]. Structures of the ~35-residue repeats of nebulin have been shown to be helical [37].

CapZ

CapZ localises in the Z-disk of the sarcomere where its main roles include nucleating actin assembly. It binds to the barbed ends of pre-existing actin filaments to regulate the addition and loss of actin monomers. CapZ may also interact with α-actinin-2, possibly helping to orientate the barbed-end of the actin filament in the Z-disk. CapZ is a heterodimer composed of two subunits, α and β. There are at least two isoforms of each of the subunits.

The crystal structure of the sarcomeric form (the α/β heterodimer) of capZ has been solved [38]. This was shown to be a stable heterodimer with α and β subunits of 286 and 277 residues, respectively. It is a mixed α-helix and β-sheet protein, for which no comparable structures were found by DALI, a computer algorithm which does an all-on-all shape three-dimensional comparison of protein structures [39]. The capZ dimer has a pseudo two-fold symmetry, with the monomers joining together to form a central 10-stranded antiparallel β-sheet. This creates an elongated molecule with the N- and C-terminus of each monomer on opposite faces of the central β-sheet. The C-termini of the subdomains are at opposite ends of the elongated molecule. The flexibility of the C-terminal sections has been postulated to allow for dynamic interaction with new G-actin added at the barbed-end [38]. One capZ heterodimer appears to be able to bind two actin molecules; this may explain why it is selective for the F-actin barbed end as opposed to monomeric G-actin. CapZ is thought to bind between actin subdomains 1 and 3 (the barbed-end), although there is no experimental evidence at present to locate the binding site.

Tropomodulin

Tropomodulin has a mass of ~40 kDa and is currently the only known F-actin pointed-end binding protein. It has a relatively low affinity for actin (0.3–0.4 μM) that is in-

creased in the presence of tropomyosin (≈50 pM), giving it selectivity for the thin filament over monomeric actin [40]. The N-terminal half of tropomodulin binds to the N-terminus of tropomyosin, while the C-terminal half binds to actin and nebulin, suggesting that tropomodulin may act as a flexible linker between these proteins [41].

The N-terminal part of tropomodulin has no tertiary structure and is thought to be fairly flexible, thus hampering the crystallisation of the full-length protein. The crystal structure of the ~20-kDa C-terminal half of tropomodulin (C20), excluding the last 15 C-terminal residues, was solved [42]. This structure revealed a typical leucine-rich repeat (LRR) motif, with five tandem α/β units and a non-homologous α-helix. These form an α/β sandwich, where a parallel β-sheet lies on one side, forming a positively charged surface, which is the putative site for actin binding.

Tropomyosin

Tropomyosin acts as an anchoring point for the troponin complex, binding to it in a 1:1 ratio to work together as a calcium-sensitive switch. Tropomyosin localises on the thin filament as two 284-residue tropomyosin polypeptides, forming an in-register and parallel α-helical coiled-coil [43]. Each tropomyosin dimer spans seven actin monomers, which bind end-to-end such as to run continuously along the thin filament. Tropomyosin binds the long pitch grooves of F-actin, by the large domain of actin, with its N-terminus orientated toward the pointed-end of the thin filament. The tropomyosin binding site on the actin filament is approximately the same site as that for myosin [44], hence tropomyosin interferes with the actin/myosin interaction via steric-blocking [13]. The sequence of tropomyosin shows a repeating heptad sequence (a-b-c-d-e-f-g), of which the 'a' and 'd' positions are hydrophobic residues required for coiled-coil formation, while salt bridges between residues in positions 'e' and 'g' in adjacent helices stabilise the coiled-coil. There is an additional 14-fold periodic distribution of charged and non-polar residues that have been associated with actin binding. A specific binding mechanism is unlikely as each of the seven actin monomers face different sequences of tropomyosin [44]. The tail-to-tail overlapping region between tropomyosin molecules has been suggested to form a globular domain [43], and both the N- and C-termini of tropomyosin are important for end-to-end dimer formation and high-affinity actin binding. Additionally, 20 seven C-terminal residues of tropomyosin are necessary for binding the troponin complex [45]. The PDZ-containing protein enigma has also been shown to bind skeletal tropomyosin [46].

The first reported structure of full-length tropomyosin, from rabbit cardiac muscle, was solved by X-ray crystallography at 15 Å resolution [43]. At this resolution, only the Cα trace of the troponin coiled-coil was visible. Later, a 9 Å crystal structure of tropomyosin from porcine cardiac muscle was solved [47], showing greater detail. Additional structural studies of tropomyosin at higher-resolution involved peptides derived from the N- and C-termini. A chimeric peptide was studied by NMR [48], composed of 14 N-terminal residues of rabbit striated muscle α-tropomyosin and 18 C-terminal residues of yeast GCN4, an archetypal coiled-coil protein used to prime coiled-coil formation. The 2.0 Å crystal structure of 81-residue N-terminal stretch of chicken skeletal α-tropomyosin [49] showed this part of the molecule not to be globular, as had been previously suggested [43]. C-terminal constructs of tropomyosin have also been studied by both crystallography [50] and NMR [51]. The crystal structure of a fusion protein with 24 residues from GCN4 followed by 31 C-terminal residues from rat striated muscle tropomyosin showed a 'splayed' conformation [50]. However, this was disputed by a NMR study of 27 C-terminal residues of tropomyosin, which showed a parallel arrangement of the two sets of C-terminal residues [51].

Troponin complex

The troponin complex couples sarcomeric calcium ion concentration to muscle contraction via the control of the availability of myosin binding sites on the actin filament. Troponin (Tn) is composed of three subunits and associates with actin in a 1:7 ratio and with tropomyosin in a 1:1 ratio. In the resting state, muscle contraction (i.e. the actin-myosin interaction) is inhibited by tropomyosin/troponin. The rise of cytosolic Ca^{2+} saturates the calcium-binding protein troponin C (TnC), revealing a hydrophobic patch that allows interaction with troponin I (TnI). This causes a conformational change in the troponin complex that subsequently displaces tropomyosin, thus allowing actin to interact with myosin. Troponin T (TnT) operates to anchor the troponin complex onto the thin filament by binding to tropomyosin.

Troponin C (TnC) is a 'muscle-specialised' form of calmodulin that requires the presence of calcium to bind to its target protein, TnI. The crystal structure of TnC resembles a dumbbell: two globular domains connected by a central helix. The globular domains are referred to as the N- and C-terminal lobes (N-TnC and C-TnC, respectively); each lobe is composed of two EF hands (or Ca^{2+} binding 'helix-loop-helix' motifs). TnC can bind up to four Ca^{2+} ions; calcium-binding sites I and II are present in the N-lobe, while C-TnC contains sites III and IV. The C-terminal lobe of TnC binds Ca^{2+} at high affinity ($K_a \approx 10^{-7}$ M) and is saturated under physiological conditions [52], while N-TnC ligates calcium at a low affinity ($K_a \approx 10^{-5}$ M), requiring a higher calcium concentration to become saturated [52, 53]. Due to this difference in calcium affinity, the N-TnC is known as the regulatory domain, as

this 'senses' the change in Ca^{2+} concentration, while the C-terminal lobe is named the structural domain, as the presence of calcium is thought to be a fixed feature in sites III and IV. Cardiac and slow muscle TnC (cTnC) differs from skeletal muscle TnC (sTnC) in its ability to bind Ca^{2+}. Site I in cTnC is inactivated by sequence mutations D29L and D31A and a V28 insertion [54], meaning that cTnC is primarily controlled by calcium binding to site II. The majority of structural studies of the troponin complex have focussed on TnC, but structures of TnC in complex with sections of TnI have also been studied. Due to the dynamically independent nature of the two lobes of TnC, structures of full-length TnC and its individual lobes have been determined. All X-ray structures of isolated full-length TnC show the two lobes to be connected by a rigid helical section [55–60]. This conformation does not reflect the degree of flexibility between the two lobes. However, NMR structures of chicken skeletal muscle full-length TnC [61] and chicken cardiac TnC [62] highlight the structural independence of the two lobes. This is confirmed by NMR relaxation analysis, a powerful measurement of flexibility [61]. It has been shown that cadmium (Cd^{2+}), manganese (Mn^{2+}) and terbium (Tb^{3+}) can occupy calcium-binding sites III and IV in TnC [58].

Along with the full-length structures of TnC, there are many structures of the separate N- and C-terminal lobes from chicken and turkey skeletal muscle or human cardiac muscle. Structures of the N-terminal lobes include those saturated with calcium at positions I and II [63–66], single calcium [67–70] and apo structures [63, 67, 71, 72]. N-TnC NMR structures have also been solved in complex with bepedril, a cardio-tonic drug which reduces the affinity of N-TnC for TnI [70], and bifunctional rhodamine [66]. Structures of the C-terminal lobe of TnC have been determined in complex with peptides derived from Troponin I: $TnI_{(33-80)}$ [73], $TnI_{(1-40)}$ [74] and also $TnI_{(128-147)}$ [75]. $TnI_{(128-147)}$ is the minimum sequence required to fully inhibit myosin ATPase activity on the thin filament [76]. The structures of cTnC without TnI have only been solved in the presence of calcium-sensitising compounds, including bepedril [77] and 'EMD 57033' [78].

The structure of the core of recombinant human cardiac muscle calcium-saturated troponin complex was solved by crystallography [79]. Modifications to TnI were made: residues 1–30 were removed making the core identical to the skeletal troponin complex. Also, it was found that deletion of the 46 C-terminal residues of TnI improved the crystal quality. The structure of the troponin core shows the structural orientation of TnT, TnC and TnI, with respect to each other, for the first time at atomic resolution. The sections of TnI and TnT resemble two parallel hairpins, interacting via an α-helical coiled-coil. The two lobes of TnC interact with two distinct parts of TnI; the N-lobe of TnC binds residues ~147–157 of TnI, while the C-lobe binds residues ~49–59, linking the ends of the TnI hairpin. The TnC interaction with TnI has been studied previously as a binary interaction. The complex of full-length skeletal muscle TnC and $TnI_{(1-47)}$ was crystallised as a dimer [80], comparing well with the troponin core, albeit featuring a change in orientation of the regulatory domain of TnC. Other structures of the TnC/TnI complex, such as structures of the N-terminal lobe in complex with various peptides deriving from TnI, ranging between residues 127 and 179 (cTnI number convention) [65, 66, 69, 70] also compare well with the troponin core.

Thick filament

Myosin

The main component of the thick filament is myosin, which forms a bundle of ~ 200–400 molecules for each filament. Additional components of the vertebrate thick filament include MyBP-C and MyBP-H (also known as C-protein and H-protein). The length of the thick filament is thought to be regulated by titin. The myosin heads on the thick filament have a polarity as they orientate in one direction, which reverses at the M-line. The complete myosin molecule is a complex of six polypeptide chains consisting of two 'heavy chains' and two pairs of different light chains – the essential and the regulatory light chains (ELC and RLC). The N-terminus of the heavy chain forms a globular head known as the motor domain, which associates with ELC and RLC, while the C-terminal half forms a fibrous α-helical tail, forming a coiled-coil with the second heavy chain (also known as the rod domain).

A single heavy chain of human cardiac muscle myosin-II has a total of 1935 residues. Proteolysis studies of myosin have lead to different nomenclature. Limited typsin digestion on myosin produces 'light mero-myosin' (LMM), which contains the rodlike domain, and also 'heavy meromyosin' (HMM), which is composed of the two globular heads of myosin with a section of rodlike domain [81]. Cleavage of myosin using papain gives the globular head of myosin, termed subfragment 1 (S1), and the rodlike domain (S2). Chicken skeletal muscle S1 consists of the first 843 residues of the heavy chain associated with ELC and RLC, while S2 consists of the remaining 1096 C-terminal residues. Further digestion of the S1 fragment using clostripain produces an intact regulatory domain, consisting of ELC, RLC and a section of heavy chain [82]. The structure of the full six-chain complex of myosin has not been solved; however, fragments of myosin have been studied. Structures of myosin include the myosin S1 head [9, 83–86], the regulatory domain [87, 88] and a section of the C-terminal rod domain

[89]. The interpretations of the different conformational states of myosin S1 fragment in the presence of ATP/ADP nucleotides, and analogues thereof, is still intensely debated and will not be covered here [3, 11]

The first structure of the myosin S1 head was from chicken skeletal muscle, solved in the absence of any nucleotide – the 'near-rigor' conformation [9]. Subsequently, structures have been solved for scallop myosin in this conformation [84, 85]. Structures of scallop myosin S1 have also been solved in the 'pre-power stroke' conformation using the ADP·Pi transition state analogue MgADP•VO4 [84, 86]. Also, structures of myosin have been solved in the presence of various compounds, representing the detached state, including ADP [83], ADP•BeFx, AMP-PNP, ATP[γ-S], and ADP again [85]. The myosin S1 motor domain consists of four subdomains containing binding sites for actin, nucleotide (ATP/ADP) and the two light chains, ELC and RLC. These subdomains have the following demarcations: N-terminal subdomain (residues 1–80), 50-kDa upper and lower subdomains (residues 81–486 and 487–600, respectively), and the converter domain (residues 707–774). The nucleotide binds between the N-terminal domain and the 50-kDa upper domain, while actin binds between the upper and lower 50-kDa subdomains. The function of the converter domain is to amplify the conformational changes of the motor domain. These subdomains are joined together via joints known as switch II (residues 461–470), 'relay' (residues 489–515) and the SH1 helix [residues 693–707, containing two reactive thiols SH1 (residue 707) and SH2 (residue 697)]. ELC binds to residues ~783–806, while RLC binds to ~808–842. A flexible region between the converter domain and the lever-arm is known as the 'pliant region' (residues ~775–780), which is thought to be important in positioning the myosin head. Hydrolysis of ATP causes conformational changes in the joints which then lead to rigid-body rearrangements of the subdomains. This translates into large conformational changes in the S1 head, resulting in the power stroke. In the 'near-rigor' conformation of S1 in the absence of a bound nucleotide, myosin is in a weak actin-binding state shortly after myosin has detached from actin. In the pre-power stroke conformation, S1 is bound to the ADP·Pi transition state. The lever arm is primed for the power stroke at a 90° angle to the actin filament. In the 'internally uncoupled state' (or 'detached from actin', or 'detached' state), S1 is bound to ATP (or ATP analogues).

The regulatory domain of myosin consists of a section of heavy chain (scallop 775–836) in complex with ELC and RLC. Only the Cα positions of the two light chains were defined in the first structure of S1 [9], and hence, a separate structure of the regulatory domain of myosin was solved later [87]. This 2.8 Å resolution structure was subsequently superseded by a 2.0 Å resolution structure [88], further clarifying details in the regulatory domain. The function of this section of myosin is mainly to stabilise a ~40 residue α-helix of the heavy chain, which creates a longer molecule designed to amplify conformational changes in the motor domain. Both available structures of the regulatory domain are from scallop myosin; the function of molluscan myosin is regulated by Ca^{2+} binding to ELC, as opposed to the troponin/tropomyosin system in the thin filament in mammals. Both light chains are composed of four EF-hands, and adopt a dumbbell shape analogous to that of calmodulin, while the heavy chain α-helix is bent 90° by RLC. Both light chains have a reverse polarity to the heavy chain, and bind via a specific 'IQ' motif on the heavy chain, causing a slight bend toward the C-terminal section. Houdusse et al. showed that Mg^{2+} is bound by the first EF-hand of RLC, while Ca^{2+} is bound via an unusual binding site on ELC [88]. These structures show a slight overlap between ELC and RLC, which is consistent with the observation that RLC is required for proper regulation of ATPase activity in scallop myosin.

The structure of the first 51 residues of scallop myosin S2 was solved by crystallography [89] by creating a hybrid with the archetypal coiled-coil GCN4 (see tropomyosin). The crystallised construct (S2N51) included the residues Gly-Ser-His-Met followed by scallop myosin (residues 836–885), Gly-Ser and finally yeast GCN4 (residues 250–281). The structure revealed an unstable coiled-coil structure, increasing in disorder in the C to N direction. This was attributed to the presence of polar and charged residues disrupting the 'a' and 'd' positions of the heptad sequence, conventionally occupied by non-polar residues. The coiled-coil also had fewer than usual salt-bridges between side-chains, further decreasing its stability. The disorder and apparent weakness of this section of S2 may be functionally required for correct myosin function, as this creates a mobility required for the myosin head to pivot with respect to the rod domain.

Titin

Titin (also known as connectin) is a giant elastic filamentous protein that is approximately 3 mega Daltons, and is the third most abundant protein in vertebrate striated muscle [90] (for review, see [91] and [92]). In vitro observations of single titin molecules show it to be ~2.0–2.5 µm in length. Titin spans half the sarcomere, with its N-terminus bound to the Z-disk and its C-terminus localising in the M-line, unambiguously orientating the titin molecule. The main functions of titin are to maintain sarcomeric alignment during muscle contraction [90, 93], to generate passive tension [94], and it is thought to act as a molecular ruler to regulate the assembly and length of the thick filament [95]. It is an essential component of the sarcomere's scaffold. Its sting-like appearance has been observed by EM, also revealing that it appears to have a globular head consisting of bound M-line proteins [93].

Various sections of titin appear to have specific functions: the I-band region of titin is believed to act as an elastic connection between the thick filaments and the Z-line, the A-band region of titin is associated with myosin and other constituents of the thick filament, and the M-line region of titin forms an integral part of an extensive protein network. The sequencing of human cardiac muscle titin [96] revealed that it is a modular protein, 90% of which is composed of 132 fibronectin type III domain-like (Fn3) and 168 immunoglobulin domain-like (Ig) sequences (named type I and type II modules, respectively). These repeats make up a complex modular architecture.

The N-terminal region of titin contains sequence elements known as Z-repeats, which localise between the third and fourth immunoglobulin domains of titin. Z-repeats are 45-residue motifs; their number varies depending on muscle type, and they have been suggested to determine Z-disk thickness [97]. The C-terminal two EF-hands of α-actinin-2 have been shown to bind to the Z-repeats of titin [98, 99]. The structure of this domain of α-actinin-2 bound to the seventh Z-repeat of titin (ZR7) has been solved by NMR [100] (see α-actinin-2).

Although the structure of full-length titin has not been solved, different sections of titin have been solved independently. The first solved structure from titin was that of an immunoglobulin (Ig) module from the M-line region of titin, known as M5 [101]. This structure shows that M5 adopts a typical Ig domain fold consisting of two β-sheets forming a β-sandwich, each sheet containing four β-strands. The structure of a modified construct of M5 with six additional residues in the N-terminus was solved later [102]. These additional residues improved the stability of the domain, attributed to additional packing of the residues between the BC and FG loops. A structure of an Ig domain from the I-band region of titin was also solved [103]. These are usually shorter than those from the M-band, and are assembled in tandem repeats, with no linker between consecutive domains. This structure also showed a typical Ig fold, but the BC and FG loops are shorter than those of M5. The first Ig domain in the sequence of titin (I1) was solved by crystallography [104]. The first structure of a type I module (Fn3-like), was solved by NMR [105]. Type I modules are found exclusively in the A-band, and this solution structure represents the 'A71' module. This folds into a typical Fn3 fold, consisting of two β-sheets forming a β-sandwich – strands A, B and E form one sheet, while C, C′, F and G form the other. Despite the relatively small number of structures of titin modules solved to date, exhaustive homology modelling of whole sets of type I (132) and type II (162) domains has been performed, giving a good indication of their general fold and surface features [106–108].

The serine kinase domain of titin (titin kinase) is located toward the M-line of the sarcomere, near the C-terminus, and is the only catalytic domain of titin. This MLCK-like kinase has the ability to phosphorylate telethonin [109] (also known as T-cap [110]); although telethonin does not locate in the M-line, it may interact with titin during myofibrillogenesis, when the locations of components of the sarcomere have not yet been established. The crystal structure of titin kinase was solved [111]. Titin kinase is a globular mixed α/β protein with two structural domains. The first domain is a two-layered mixed α/β sandwich (residues 18–101), consisting of five antiparallel β-strands, layered against an α-helix and an area of the second domain. The second domain is a 'mainly α orthogonal bundle' (residues 101–338), a distorted four-helix bundle, flanked by three helical sections and two β-hairpins, one of which forms a final β-sheet with the C-terminus. Activation of the kinase is achieved by two mechanisms. The first activation step is the phosphorylation of a tyrosine in its active site (referred to as Y170). This was demonstrated by a Y170E mutation, designed to mimic tyrosine phosphorylation, which allowed substrate binding to occur. Second, activation of titin kinase is achieved by the removal of a regulatory C-terminal tail (included in the structure) from the ATP binding site by the interaction of Ca^{2+}/calmodulin complex. These two inhibitory mechanisms result in tight control of catalytic activity of titin kinase.

MyBP-C

The thickness of the thick filament is regulated by the myosin binding protein C (MyBP-C). MyBP-C is expressed exclusively in striated muscle in three isoforms, slow skeletal, fast skeletal and cardiac. Slow skeletal MyBP-C was previously reported as X-protein (MyBP-X). MyBP-C is found in bands that occur at intervals of ~43 nm, 7 to 9 times along the C-zone, an area of the A-band which contains cross-bridges. MyBP-C is a multi-domain protein composed of Ig- and Fn3-like domains with unique sequences between the domains. The domain organisation of human cardiac MyBP-C is $(Ig)_6$-$(Fn3)_2$-Ig-Fn-Ig, labelled from C0 to C10. C6-C10 has been observed to bind to light mero-myosin sections of myosin and to titin in the C-zone, while the N-terminus binds the S2 region of myosin. There are also indications that MyBP-C may homodimerise through C5 and C8 Ig-like domains [112]. Hence, the main role of MyBP-C may be in myofibrillogenesis, regulating the thickness of the thick filament by interacting with titin and myosin.

The structure of the fifth domain of human cardiac MyBP-C (cC5) has been solved by NMR [113], revealing a β-sandwich Ig-like fold, of the IgI type [114]. Ten residues in the N-terminus of this construct, previously thought to be part of a linker between the fourth domain and cC5, are actually integrated as part of a β-sheet. An extended loop between the third and fourth β-strand

known as the 'CD-loop' is very dynamic. The CD-loop was seen to change the hydrodynamic properties of MyBP-C cC5, causing it to behave as a larger species than expected for Ig folds, from biophysical observations.

Z-disk

An essential component of the sarcomere is the multi-protein complex known as the Z-disk, which keeps the structure of the sarcomere in register and transmits tension during muscle contraction. The major component of the Z-disk is the muscle-specific α-actinin-2. α-Actinin-2 exists as a rod-shaped antiparallel homodimer [115], comprising two calponin homology domains followed by four spectrin-like repeats and a calmodulin-like domain. The calmodulin-like domain contains four potential EF-hands, which have divergently evolved and do not require Ca^{2+} to bind its target protein [116]. The main function of α-actinin-2 appears to be to act as a scaffold for assembly of the Z-disk, forming critical interactions between components of the sarcomere at various locations along its sequence. Different regions of α-actinin-2 are able to bind many different proteins: the calponin homology domains bind actin; the last two EF-hands bind Z-repeats of titin and also some members of the enigma family, including ZASP/Cypher/Oracle, elfin/CLP-36/hCLIM and ALP. Many other proteins have been shown to localise in the Z-disk, including calsarcin-1, 2 and 3 (calsarcin-2 is also known as FATZ and myozenin), myotilin, myopalladin, myopodin, enigma homology, calcineurin, γ-filamin, telethonin, desmin and obscurin (see Z-disk review [12, 117]). Currently, the only Z-disk proteins to be characterised structurally are α-actinin-2 and ZASP.

α-Actinin-2

α-Actinin belongs to the spectrin super-family, of which α-spectrin, β-spectrin, β_{heavy}-spectrin and dystrophin are members [118-120]. The superfamily is defined by the presence of a specific number of repeats first identified in spectrin, and the ability to bind actin. There are four variants of α-actinin that are expressed depending on the cell and muscle type: α-actinin-1 and α-actinin-4 are non-muscle proteins involved in cytoskeletal interactions, while α-actinin-2 and α-actinin-3 are muscle-specific proteins. Both α-actinin-2 and α-actinin-3 are present in striated muscle, but only α-actinin-2 is expressed in cardiac muscle. α-Actinin-3 may be functionally redundant, as its deficiency carries no obvious phenotype; α-actinin-2 may compensate for its function [121]. This redundancy may derive from the 81% sequence identity between the two isoforms.

The structure of full-length α-actinin-2 has not been elucidated. However, sections of α-actinin-2 solved using NMR or X-ray crystallography show it to be mostly composed of α-helical domains. These structures include that of two of the central spectrin-like repeats (R3-R4) [122], subsequently superceded by the structure of all four spectrin-like repeats (R1-R2-R3-R4) [123], and the structure of the C-terminal pair of EF-hands of α-actinin-2 in complex with the seventh Z-repeat of titin [100]. Spectrin-like motifs are 100–120-residue domains forming an elongated α-helical triple coiled-coil structure. Pairs of spectrin motifs are joined together by a continuous helical segment between the C-terminal helix of one spectrin domain, and the N-terminal helix of the next domain. The spectrin domains form antiparallel in-register homodimers, with a self-dissociation constant (K_d) of 2 µM and 10 pM for the two- and four-spectrin constructs, respectively [122, 124, 125]. The structures reveal a similar degree of surface burial (9.2 and 11% for R3-R4 and R1-R2-R3-R4, respectively) of the accessible surface of the monomer. These structures act as models for the dimerisation of α-actinin-2 in vivo; the R1-R4 structure shows a rigid-rod configuration, possibly caused by an end-to-end 90° twist.

The structure of the C-terminal pair of EF-hands of α-actinin-2 (Act-EF34) in complex with the seventh Z-repeat of titin (ZR7) was solved by NMR [100]. This complex shows an unconventional binding mechanism, as compared to calmodulin, since these EF-hands do not require the presence of Ca^{2+} for target binding. Act-EF34 has four α-helices forming two helix-loop-helix motifs joined by a linker. These EF-hands saddle titin-ZR7 in a 'semi-open' conformation, with an EF-hand either side of the target, which may be a general solution for calcium-independent target recognition. In the complex, titin-ZR7 adopts an α-helical configuration from a previously unstructured conformation [126, 127]. The Z-repeats of titin (ZR1 and ZR7) bind Act-EF34 at nanomolar affinities [127].

Although there is no structure of the calponin homology domains of α-actinin, the structure of a CH domain derived from β-spectrin has been solved [128]. This homologue shares 57% sequence identity with the second CH domain of α-actinin-2 (CH2), and is therefore expected to adopt a similar fold. The first CH of α-actinin-2 (CH1) domain has only 15% sequence identity with that of β-spectrin, so it may have significant structural differences. The CH domain is a compact globular domain of 109 residues with an unique fold. It has four main α-helices (A, C, E and G) between 11 and 18 residues long, connected by loops, and three short and less regular α-helices (B, D and F); helices C, E and G are arranged in a small three-helix bundle, while helix A lies at ~80º across helices C and G. The calponin homology domains are thought to work in tandem to bind actin, as the affinity of both domains for actin is tighter than that of a single CH domain [129]. Actin is thought to bind between the last helix of CH1 and the first helix of CH2.

ZASP and the Enigma Family Proteins

ZASP belongs to the enigma family defined by the presence of a combination of PDZ and LIM domains. The enigma family includes enigma [also known as LIM mineralization protein (LMP)] [46], enigma homology (ENH) [130], actinin-associated LIM protein (ALP) [131], RIL [132] and elfin [133]. All of these enigma proteins are expressed in heart or skeletal muscle tissue (or both) and localise in the Z-disk, with the exception of RIL, which is expressed in epithelial tissues. Enigma proteins usually have a cytoskeletal-related function: ZASP, ENH, ALP and elfin have been reported to interact with α-actinin-2 [130, 131, 133, 134], while enigma binds β-tropomyosin [46]. Depending on the isoform, ZASP is composed of either a PDZ domain alone or in conjunction with three carboxy-terminal LIM domains. PDZ and LIM domains are so named from the set of proteins first identified as containing these modules (for a review of PDZ domains see [135] and for LIM domains see [136]). PDZ domains are protein modules of 80–120 residues involved in targeting and clustering of membrane proteins or directing cellular proteins to multi-protein complexes. The interaction between ZASP and α-actinin-2 has been identified between the PDZ domain of ZASP (ZASP-PDZ) and the C-terminus of α-actinin-2 [134, 137].

Only the structure of ZASP-PDZ has been solved to date, showing a conventional PDZ domain containing an anti-parallel six-stranded β-sandwich, flanked by two α-helices [138]. Binding studies show that α-actinin-2 forms an intermolecular β-sheet with ZASP, between the second β-strand of ZASP-PDZ and the C-terminus of α-actinin-2. This study also showed that the α-actinin-2/ZASP-PDZ complex is able form a ternary complex with the seventh Z-repeat of titin. Sequence alignment of other enigma family PDZ domains against ZASP-PDZ shows sequence identity of between 54 and 61% and sequence similarity between 71 and 81%. Homology models of enigma, ENH, elfin, ALP and RIL are therefore expected to be fairly accurate. It was shown that many of the conserved residues in the enigma family PDZ domains were located in the α-actinin-2 binding pocket identified on ZASP. This suggests that there may be a degree of functional redundancy across the muscle-specific enigma proteins, as they may all bind α-actinin-2.

M-line

The M-line has a similar function to the Z-disk in that it confers stability to the sarcomere (for a review, see [139]). There are no myosin heads where the myosin tails overlap and where the polarity of the thick filament changes. This region is responsible for cross-linking the thick filaments in an ordered hexagonal lattice, via linkers called M-bridges. Many proteins have been found to associate in the M-line, including titin, myosin, myomesin, M-protein, skelemin and a muscle isoform of creatine kinase. The M-band region of titin consists of 10 Ig-like motifs (see above). Myomesin and M-protein may carry out an analogous function as α-actinin in the Z-disk as they appear to function to tether myosin and titin. Although myomesin and M-protein have been shown to be distinct proteins, they share ~ 50% sequence identity. The domain architecture of myomesin is two Ig domains, followed by five Fn3 domains and then a further four Ig domains, all joined by unique sequences. The N-terminal sequence of myomesin is also unique. M-protein has the same domain architecture, albeit with only three C-terminal Ig domains. The interaction of myomesin in the M-line has been localised to the first Ig domains in the N-terminus, while the myosin binding site has been localised to an N-terminal unique sequence of the protein [140]. Both myomesin and M-protein have been shown to bind to the LMM myosin fragment; the interaction with myomesin may possibly occur in the first 240 residues of the latter [141]. The bare-zone of the thick filament may only have binding sites here for myomesin, thus restricting myomesin to the M-line. The interaction between myomesin and titin has been localised to the central Fn3-like domains of myomesin. There are no reported structures of either myomesin or M-protein.

Muscle-specific creatine kinase

The main function of creatine kinase (CK) is to catalyse a reversible phosphotransferase reaction between creatine and ATP that produces creatine phosphate and ADP. Fast regeneration of ATP from ADP is necessary at times of high ATP demand, especially in situations of high metabolic requirement, such as prolonged muscle contraction. There are muscle- and brain-specific isoforms of creatine kinase (M-CK and B-CK), which function as three distinct cytosolic dimers: brain-type (BB-CK), muscle-type (MM-CK) and a heterodimer of the two forms (MB-CK) [142]. Mitochondrial isoforms also exist, expressed ubiquitously (MIa-CK) and sarcomerically (MIb-CK), both forming functional homo-octamers [143]. BB-CK and MB-CK cannot localise in the M-line, but a small proportion (5–10%) of cytosolic MM-CK does so, and has been shown to have a structural role in the sarcomere [144], and is hence considered part of the muscle ultrastructure. MM-CK contains two pairs of highly conserved lysine residues that are necessary for M-line interaction, forming high (residues K104 and K115) and low (residues K8 and K24) affinity sites of interaction [145]. Various structures of creatine kinase have been solved, notably that of mitochondrial creatine kinase [146]. However, only two structures of M-CK have been solved [147, 148]. The creatine kinase structure has a globular fold with two distinct domains, having an overall triangular appearance. The first domain consists of ~100 residues

forming a mainly α-helical bundle, consisting of one 3_{10} helix and five α-helices. The second domain is an α/β two-layered sandwich, consisting of a central eight-stranded β-sheet, flanked by seven α-helices, three of which pack against the central sheet. An additional β-hairpin is formed between the top of the sheet and the first domain.

Conclusion

From this review, it is interesting to see that many sarcomeric muscle proteins have been studied as a whole or in part, with the focus very much on soluble, well-structured, globular domains. For proteins or domains with high sequence similarity to solved folds, such as the calponin-homology domains of α-actinin-2 and the Ig and Fn3 domains of titin, it seems that homology modelling may be sufficient to get a general idea of their respective structures. Although the primary function of the sarcomere is muscle contraction, this network of proteins is essential for constructing, regulating and maintaining muscle architecture. The study of all sarcomeric proteins is critical in understanding the many molecular processes which govern the function of muscle as a whole.

We have seen a great diversity of the type of interaction between muscle proteins. Where some proteins do not require co-factors to bind, other interactions are regulated by cofactors such as calcium, ATP or ADP, and other proteins require phosphorylation or the presence of other proteins. These various factors confer upon these interactions a temporal dimension, necessary for dynamic phenomena to occur, such as regulation, inhibition and conformational change. The dynamic nature of protein-protein interactions renders in vivo localisation of binding essential, as in vitro methodology alone cannot determine whether interactions occur within the fully developed sarcomere, or only occur during myofibrillogenesis.

It is interesting to see different solutions to structural problems uncovered by studying these various proteins. At the level of the protein construct, these solutions include the use of sequences from different species to obtain high yields, the definition of smaller globular domains, the construction of chimeric proteins to mimic coiled-coils and the modification of constructs to allow easier handling of the protein (e.g. to improve crystal properties). Strategies to counteract problems arising in the study of certain proteins have also been interesting, including the use of proteins or small organic compounds to prevent actin polymerisation, and the use of ATP analogues to study different conformational states of actin and myosin.

This overview of muscle ultrastructure shows that there has been substantial structural characterisation of the proteins within the sarcomere; however, there remains considerable research if we are to refine our current knowledge of this complex biological system. In addition to proteins for which no atomic-resolution information is available, many structures describing interactions between binding partners have yet to be completed. Structures solved in complex, such as the myosin heavy chain in complex with RLC and ELC, the troponin complex (TnC, TnI and TnT), the capZ heterodimer, the tropomyosin homodimer, the α-actinin-2 EF-hands complex with a titin Z-repeat, and models of interaction based on biochemical evidence emphasise how knowledge of orientations between components enriches our understanding of how these components interact. The field of muscle protein is still developing, as new sarcomeric muscle proteins are still being discovered. Further characterisation of these proteins will provide novel insight into the mechanisms that exist to allow muscle to operate; hence, research into muscle ultrastructure continues.

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