known as the skyrmion Hall effect. This effect is not predicted for hopfions[11], making them potentially ideal for applications. Finally, there is a current push towards 3D electronics, which re-evaluates the idea that information needs to be encoded on a 2D chip. A material that hosts 3D topological solitons, such as those demonstrated by Zheng and colleagues, could have a key role in this endeavour.

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Structural biology

Getting to the heart of thick-filament structure

Peter J. Knight

Thick filaments contain the protein myosin that generates the force of every heartbeat. Two studies report how these myosin molecules pack together in thick filaments with other proteins to form a surprisingly complex structure. **See p.853 & p.863**

As you read these words, your beating heart is keeping you alive. No surprise, then, that researchers have sought for decades to understand the structures and mechanisms that underlie how the human heart functions, and to discover what changes in heart disease. The central players that make the heart a pump are the proteins actin and myosin, and these assemble into what are called thin and thick filaments, respectively. Thin filaments have a fairly simple structure that is well understood. Thick filaments are much more complex, and their structure has been difficult to determine. On pages 853 and 863, respectively, Dutta *et al.*[1] and Tamborrini *et al.*[2] report their use of the technique cryo-electron microscopy (cryo-EM) to reveal most of the structure of thick filaments in exquisite detail. These results reveal a structure that no one had predicted and that opens the door to a new world of experiment and understanding.

The structure of thick filaments of vertebrate cardiac and skeletal muscle was outlined 60 years ago[3]. Myosin comprises two pear-shaped 'heads' attached to a tail that measures approximately 1,600 by 20 ångströms. The tails of many myosin molecules bundle together like a sheaf of sticks to form the backbone of each filament — with the heads protruding from the surface, where they can reach out to make force-generating interactions with neighbouring thin filaments. All the tails point towards the midpoint of the filament, and the heads are present all the way from both tips of the filament to a bare zone in the centre, where tails of opposite polarity overlap (Fig. 1).

Thick filaments from the cardiac and skeletal muscle of all vertebrates have indistinguishable dimensions and minor variations in composition. Each is assembled with great precision, and has 294 myosin molecules[4,5]. These are precisely positioned in the filament: groups, termed crowns, consisting of the six heads of three myosin molecules, lie on the filament surface at intervals of approximately 143 Å along each half of the filament and are arranged in a quasi-helical way that repeats every third crown (corresponding to 430 Å). One consequence of this is that 33 tails pack together at most points along the filament backbone. Visualizing the paths taken by individual tails in this array has been an insurmountable task.

Hopes for solving thick-filament structure were first raised in 2005, when cryo-EM was used to determine the structure and arrangement of the myosin heads for thick filaments from a tarantula spider[6]. Since then, there has been a revolution in the capabilities of the associated hardware and software. This enabled researchers in 2016 to determine the arrangement of tails in thick filaments from a giant water bug, *Lethocerus*[7]. It shows that the tails are packed together in a crystalline way — an arrangement similar to one that had been proposed in 1973 (ref. 8). Crucially, however, these invertebrate filaments are perfectly helical rather than quasi-helical, with an exact repeat every 143 Å. The reason that thick filaments in vertebrates are not absolutely helical could be that two proteins found in vertebrates — titin and MyBP-C — are absent in invertebrates. But how these proteins might affect the arrangement of myosin was unknown.

Each titin molecule is a long, thin thread. In the contractile organelle of muscle formed by arrays of overlapping thin and thick filaments (called a myofibril), part of a titin molecule lies along one half of a thick filament, and the rest elastically anchors the thick filament in the myofibril to prevent disorder of the arrays during contraction (Fig. 1). The thick-filament region of titin consists mostly of units that each comprise roughly 100 amino-acid residues; these are predicted to fold into two kinds of globular domain, each 40 Å wide. In much of the filament, these two types of domain create a pattern that repeats every 11 domains, forming a 'super-repeat' that could correspond to the repeat in the filament that occurs every 430 Å. Titin has therefore long been suggested to act as a 'molecular ruler' that modulates the arrangement of myosin, and is thereby responsible for the remarkable precision of thick-filament structure[9].

Cardiac MyBP-C (cMyBP-C) comprises a string of 11 of the same two kinds of 40 Å domains as titin, and is located at intervals of 430 Å along the filament in the region where titin has its 11-domain super-repeat. As well as binding to the thick-filament backbone, cMyBP-C's amino-terminal end binds to thin filaments, and is subject to modification through a process termed phosphorylation that correlates with altered contraction in the heart. Also, cMyBP-C is the site of approximately 40% of a type of genetic change (point mutations) that is known to cause the heart condition hypertrophic cardiomyopathy[10]. There is thus intense interest in cMyBP-C's role in thick-filament structure and activity.

The two groups of researchers used complementary cryo-EM methods to arrive at satisfyingly consistent reports about filament structure. Dutta *et al.* purified thick filaments from human hearts, examining the specimens under conditions that correspond to the relaxation phase of the cardiac cycle, and determined the structure of the 430 Å repeat, reaching an overall resolution level of 6.0 Å. Tamborrini *et al.* used myofibrils from mouse hearts to prepare arrays of thin and thick filaments in a near-natural relaxed state. They then used tomographic methods[11] to determine the structure, reaching a resolution of 8 Å for the thin filaments and 18 Å for the thick filaments. Although this is a lower resolution than that reported by Dutta *et al.*, the tomographic

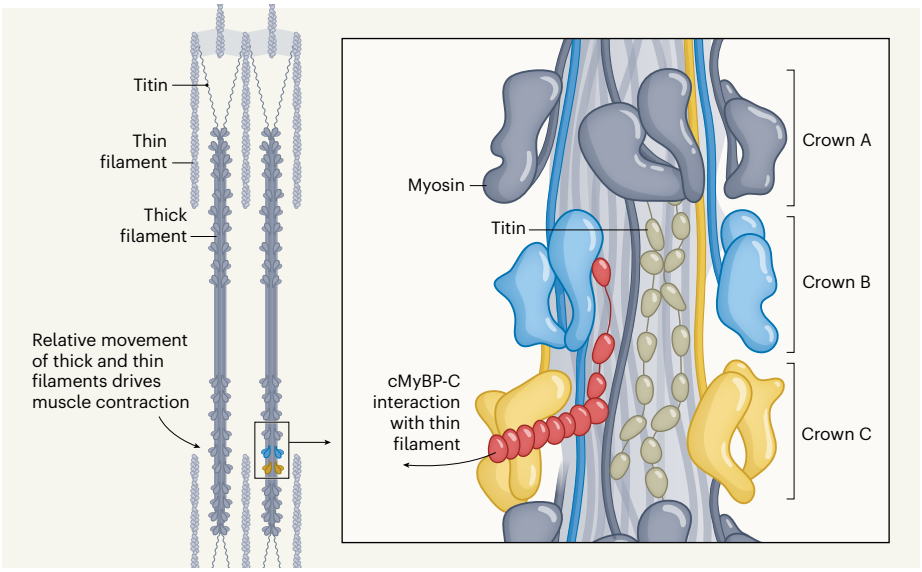

Figure 1 | The structure of thick filaments in the heart. Dutta *et al.*[1] and Tamborrini *et al.*[2] report the structure of cardiac thick filaments obtained using cryo-electron microscopy. These data reveal the relationship between three thick-filament components — the proteins myosin, titin and cMyBP-C. The interactions between thick and thin filaments produce the vertebrate heartbeat. The structure that repeats along the thick filament every 430 Å, boxed on the left, is shown in more detail on the right, as set out in the two latest papers. Titin and cMyBP-C both bind to myosin, but not to each other. The heads of myosin form structures called crowns, and the crowns form three repeating arrangements (labelled here as crowns A, B and C). The myosin tail associated with each type of crown follows a distinctive path that differs for each type, and this affects whether and how interactions with titin and cMyBP-C occur.

method makes it easier to analyse variation along the filament, and allows interactions to be seen with the neighbouring thin filaments.

Both studies used the artificial-intelligence tool AlphaFold2 (ref. 12) to predict the structures of the myosin tail, titin and cMyBP-C. These models show an excellent fit with the observed structures, confirming the utility of AlphaFold2 to enable atomic models to be generated for the interpretation of low-resolution structural data.

The biggest surprise in these results is how different the cardiac thick-filament structure is in vertebrates from that of invertebrates. No previous guess at the structure had come anywhere near the truth. Interactions between the myosin tails and titin produce a radically different structure in vertebrates compared with that of invertebrates. The tails of the three crowns in each 430 Å repeat follow different paths. Those of one crown run into the centre of the backbone; those of the neighbouring crown run alongside the first, but then break away to run peripherally; and those of the third crown are entirely peripheral. Correspondingly, the interactions with titin are different for each of the three vertebrate crowns.

The six titin molecules associated with each half of the filament, arranged as three pairs, run straight along the filament surface rather than winding around it. As predicted, a group of 11 titin domains fits within each 430 Å repeat, and the registration with myosin is unambiguous, so that interactions of each domain with myosin can be enumerated. Although the two molecules of a titin pair have identical sequence and structure, they interact differently with the three crowns to produce the unexpected diversity of myosin tail paths.

A second surprise is that cMyBP-C does not interact with titin. The correlation of cMyBP-C with the 430 Å repeat of titin rather than the 143 Å repeat of myosin led to the expectation that cMyBP-C would form a bridge between titin and myosin molecules. Instead, titin's impact on myosin packing limits suitable sites on myosin to those at intervals of 430 Å. Also unexpected is that cMyBP-C interacts with the inactive myosin heads of two of the crown types, suggesting a further role for the protein in modulating myosin activity.

> **"No previous guess at the structure had come anywhere near the truth."**

Because these samples were examined in a state of relaxation, it is not surprising to find that the two heads of each myosin molecule are paired to form the interacting head motif (IHM) that characterizes the inactive state of this class of myosin. Indeed, both groups used a drug (mavacamten) that is licensed to control symptoms of hypertrophic cardiomyopathy, to favour IHM formation. In the tomogram data, all the IHMs are seen, albeit with some variation in their orientation on the filament surface, but in isolated filaments they are poorly ordered in one of the three crown types. This suggests that the relaxed structure is unstable — which is probably a good thing, because the filament needs to cycle rapidly between active and inactive states with each heartbeat. The IHMs lie farther from the filament axis than did those in earlier studies of stained, dried filaments. Indeed, the freezing method that underlies cryo-EM reveals that the whole structure is more open and water-filled than was previously thought, indicating that the earlier methods of fixation and staining altered both the structure of thick filaments and our perceptions of that structure.

The vertebrate thick-filament structure that has been revealed by these studies is complex and almost bewildering. These papers mark the start of an exciting era. At last, a structural framework is available on which to design new experiments, both to understand how muscle works and to try to find treatments for disease.

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