Chapter 6
The Molecular Basis of the Frank-Starling Law of the Heart: A Possible Role for PIEZO1?

C. G. dos Remedios[1] (✉), K. Y. C. Law[1], J. W. McNamara[2], T. Kraft[3], M. Peckham[4], J. van der Velden[5], W. A. Linke[6], M. Ackerman[7], V. Sequeira[8], S. Lal[9], R. Cooke[10], M. Grosser[11], K. S. Campbell[12], B. Martinac[1], and A. Li[13, 14, 15]

[1] Victor Chang Cardiac Research Institute, Sydney, NSW, Australia
c.dosremedios@victorchang.edu.au
[2] Murdoch Children's Research Institute, The Royal Children's Hospital, Parkville, VIC, Australia
[3] Department of Molecular and Cell Physiology, Hannover Medical School, Hannover, Germany
[4] School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK
[5] Laboratory for Physiology, Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, The Netherlands
[6] Department of Cardiology I, Coronary, Peripheral Vascular Disease and Heart Failure, University Hospital Münster, Münster, Germany
[7] Department of Pediatric and Adolescent Medicine, Division of Pediatric Cardiology (M.J.A.), Mayo Clinic, Rochester, MN, USA
[8] Department of Translational Research, Comprehensive Heart Failure Center (CHFC), University Clinic Würzburg, Würzburg, Germany
[9] Sydney Medical School, University of Sydney, Camperdown, Australia
[10] Department of Biochemistry, University of California, San Francisco, CA, USA
[11] 23Strands, Unit 107, Pyrmont, Australia
[12] Department of Physiology, University of Kentacky, Lexington, KY, USA
[13] Department of Rural Clinical Sciences, La Trobe University, Bendigo, VIC, Australia
[14] Centre for Healthy Futures, Torrens University, Australia, AdelaideAustralia
[15] School of Medical Sciences, The University of Sydney, Sydney, Australia

Abstract. The Frank-Starling Law was proposed over 100 years ago, but it remains to be fully explained at the molecular level. The Law states that when the volume of blood returning to the heart suddenly increases, it stretches cardiomyocytes that instantly respond by increasing ejection pressure, thereby ensuring that the heart deals efficiently with beat-to-beat changes in the circulation. Here we review: The history of the Otto Frank and Ernest Starling role in formulating the Law; The key contractile proteins involved (Myosin ATPase, Titin, cMyBP-C); The role of Super Relaxation; and the contribution of computational analyses. We then propose two pathways by which **PIEZO1** (a mechanosensitive Ca^{2+} permeable channel that

B. Martinac et al. (eds.), *Mechanobiology*, Springer Series in Biophysics 25,
https://doi.org/10.1007/978-3-031-45379-3_6

recently was the subject of the 2021 Nobel Prize) may play a role in the Frank-Starling Law. In **Mechanism 1, PIEZO1** is a non-specific Ca^{2+} channel expressed in the T tubules adjacent to **TRPM4** which together activate the sarcoplasmic reticulum to release more Ca^{2+} causing a cascade of events resulting in contraction. In **Mechanism 2**, stretch of the heart chamber wall is sensed by **PIEZO1** in the **cardiomyocyte intercalated discs**, which is directly connected to **titin** molecules that activate **myosin crossbridges** at their S1-S2 via **myosin binding protein C (cMyBP-C)**.

Keywords: Frank-Starling law · Super relaxation · Intercalated discs · PIEZO1 · T-tubules

What Is the Frank-Starling Law?

The Frank-Starling Law is also known as the Starling Law of the Heart or the Frank-Starling Mechanism. It describes the pressure-volume relationship between the volume of blood that flows into the heart during diastole followed by the rapid and enhanced development of stroke volume. But how does the heart meet a rapid change in venous return by generating an enhanced ejection fraction? The Law is not restricted to the heart but applies to the stretching of skeletal or cardiac (i.e., striated) muscles, but there are some important differences. For instance, in skeletal muscle contractile force is regulated by recruiting more "motor units" (the number of muscle fibers innervated by a single motor axon), or by working synergistically with other muscle groups. Heart muscle does not function by responding to motor innervation. Furthermore, skeletal muscle cells are highly linear motors usually attached at each end to a bony skeleton. Cardiomyocytes have no origins or insertions but are interconnected by intercalated discs not only at their ends but also by lateral intercalated discs. Thus, the heart chamber effectively acts as a single "motor unit". Compared to skeletal muscle, cardiomyocytes are usually binucleated and are shorter (about 120 μm long) than highly elongated multi-nucleated skeletal muscles. Cardiac sarcomeres have shorter I band, and a shorter working range, typically 1.8–2.2 μm. Kobirumaki-Shimozawa [40] demonstrated using nano-imaging that the beating heart operates within much lower working ranges, with diastolic and systolic lengths of 1.95 μm and 1.68 μm, respectively. This strongly suggests the presence of a Frank-Starling reserve *in vivo*. Cardiomyocytes also branch, forming a syncytium enabling the heart chamber to contract synchronously. Furthermore, ejection of the blood from the heart is fast (about 200 msec). Accordingly, the heart exhibits a length-dependent activation that depends less on the degree of overlap of thick and thin filaments and more on the total number of participating myosin crossbridges [92, 51] (Fig. 6.1).

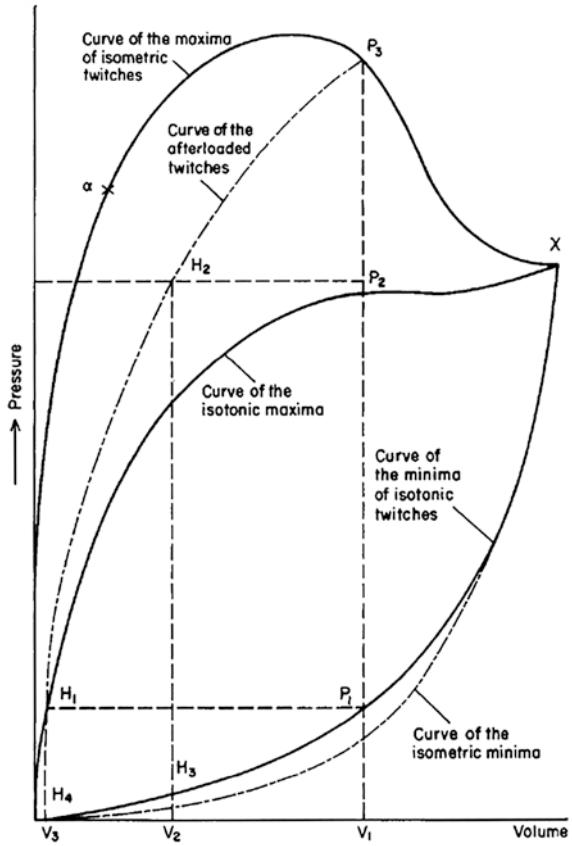

Fig. 6.1 Pressure-volume relationship in the frog ventricle. The top and bottom solid lines show the maxima and minima of isometric twitches, while the middle solid curve shows the isotonic maxima. This figure, originally published by Otto Frank, is reproduced from Sagawa et al. [74] with permission from Elsevier.

The Historical Background for the Frank-Starling Law

There is an extensive history on this topic that makes unexpectedly interesting reading. It was recently reviewed in considerable detail by Han et al. [28]. The authors examined essentially all the relevant papers from 1869 to 2021. Despite the existence of several earlier reports, today the Frank-Starling Law has its origins in a seminal paper by Otto Frank [24]. Frank was a medical graduate who studied mathematics, chemistry and physics in Germany but also spent some time in Edinburgh. He published hundreds of papers, the most important of which showed that two pressure-volume relationships exist as the heart goes from diastole (relaxed) to the end of systole (contracted) (Fig. 6.2).

Before we discuss the molecular basis for the Frank-Starling Law, it is worth recalling the scientific and cultural differences that existed at the time between Germany

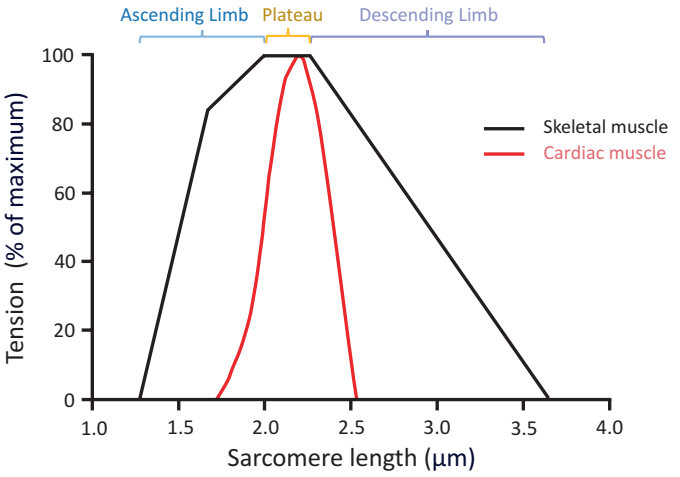

Fig. 6.2 The relationship between sarcomere length and tension development for skeletal (black) and cardiac (red) muscle. Note the short range of sarcomere length charcteristic of cardiac muscle. (This figure is modified from Sequeira and van der Velden with permission from Springer-Nature).

(Frank) and Great Britain (Starling) around the time the Law was first formulated [43]. Frank's seminal publication [24] was in German and according to the translators [38], the paper was "much too formidable to be approached at all except by accomplished linguists". Importantly, it contained the definitive pressure/volume diagram. Frank studied in Heidelberg, Munich, Glasgow and Strasbourg, and later moved to Munich where he completed his habilitation thesis (the equivalent to a post-doctorate). He remained there as professor until 1934, when he was compelled by the Nazi regime to accept emeritus status. He died in Munich on November 12, 1944. Today he would be immediately recognized as a renown biophysicist, but in the 1890s it was understandable he was not, although Frank has worked in Glasgow, Ernest Starling may not have heard of him. At the time, unlike in the UK, German medical schools were predominantly taught by medical graduates who were commonly expert scientists. This ensured that medical graduates had a working grasp of the basic sciences.

About 20 years later Ernest Starling, also an MD, adopted a more physiological approach using mammalian hearts with coronary arteries rather than amphibian hearts [60]. While this paper did not cite Frank's [24] publication, the pressure-volume data using a dog heart did agree with Frank's figures. However, they did not include data on isotonic maxima. Nevertheless, Starling was an excellent teacher, communicator, and physiologist. He recognized that British medical students were focused almost exclusively on clinical medicine [38], and strongly advocated for the inclusion of basic sciences in the first two years of their curriculum. He was also politically active, urging the UK Government of the day to overhaul its approach to medical practices during World War I [38]. In his celebrated Linacre Lecture presented in 1915 and published three years later [79], he proposed the Starling Law of the Heart which later became the Frank-Starling Law.

Han et al. [28] concluded that "despite being fully conversant with Frank's publications, Starling neither remarked on, nor tested Frank's finding of "contraction-mode-dependent performance of the heart". In his Linacer lecture he insightfully states that the pressure developed by the heart is a function of the length of the muscle fibre. However, this failed to reflect the different end-systolic pressure-volume relationships for isometric and after-loaded twitches. Instead, this left the field open for a study that eventuated a century later to explain both Frank's and Starling's findings. Kenneth Tran and his colleagues performed a systematic investigation of the force-length relationship across a wide spectrum of physiologically relevant preloads, afterloads, working range, temperature, and stimulus frequencies [28]. Widening the range of both preloads (blood flowing into the heart) and afterloads (blood flowing out of the aorta), rather than maintaining low preload or high afterloads, reveals the true contraction-mode-dependent nature of the pressure-volume relations as first observed by Frank [24, 28].

The Anrep Effect

A sudden stretch applied to heart muscle triggers a biphasic increase in force. The first rapid phase (1 s) is an immediate rise in contractility, described above as the Frank-Starling Law. A second phase, often known as the Anrep effect, is a force response that occurs slowly over the 10–15 min following the stretch. It was named after Gleb Vasilievich von Anrep, a Russian-born physiologist who worked with Starling. The mechanistic explanations of the Anrep effect involve autocrine events involving endothelin, several receptors, several enzymes, reactive oxygen species (ROS), and Na^+/H^+ activation [13, 71]. The increase in force development in the Anrep effect involves oxidative stress and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity. It works on a much longer time scale than the significantly faster Frank-Starling Law. A recent review by Sequeira et al. [76] proposes that the Anrep effect involves a two-phase activation of contraction involving a transition from the super-relaxed state to disordered-relaxed state [76].

Molecular Basis of the Frank-Starling Law

Starling predicted that the power of the heart lies "in molecular changes occurring at the surface of longitudinal fibrillae" [38]. Today these "fibrillae" are known as myofibrils which are simply a long series of sarcomeres. Precisely how does a rapid stretch of cardiac stretch instantly enhanced contraction? Decades later, Andrew F. Huxley and Rolf Niedergerke [33] and Hugh E. Huxley (not related to AF Huxley) and Jean Hanson [34] proposed the sliding filament hypothesis based on myosin thick filaments with their crossbridges, and actin thin filaments that slide relative to the thick filaments but do not change their lengths. Earlier Hanson and Huxley [30] had suggested the

presence of connecting "S filaments" that joined the ends of the thin filaments to the ends of the thick filaments. They were nearly right, but the field had to wait until 1976 when Koscak Maruyama and his colleagues reported a new elastic protein, which they called connectin [55]. Three years later Kuan Wang and his colleagues reported a "new" elastic thin filament protein they named titin, and the name stuck [90] (Fig. 6.3).

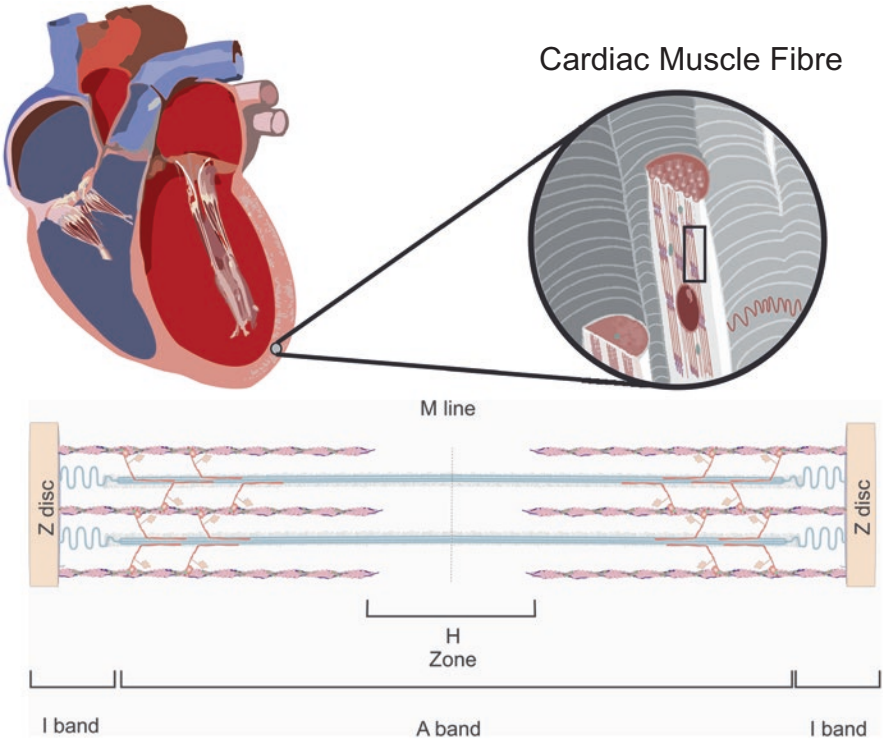

Fig. 6.3 Cellular and molecular architecture of the heart. The heart (upper left) is a muscular organ predominately comprising cardiac muscle fibers (cardiomyocytes) interconnected by (red) intercalated discs (upper right). Force is generated by myofibrils consisting of a series of sarcomeres (lower figure). A single myofibril (inset rectangle) is magnified (bottom figure) to show its components. The sarcomere is bounded by Z discs, has a large (anisotropic) A band and characteristically short I bands. Sarcomeres have three types of filaments: (1) Thin filaments (composed of F-actin and relaxing proteins) that extend from a Z discs towards the middle of the sarcomere, leaving a thin-filament-free H zone; (2) Thick filaments (predominantly myosin and titin) have a central M line that crosslinks the thick filaments; and (3) Titin filaments that run from the Z disc through the I band (curvy blue line) and along the surface of the thick filaments to reach the M line. Force is produced by myosin crossbridges shown projecting from the thick filaments that transiently attach to the thin filaments and move them towards the H zone. Top panel was adapted from Peng et al. [61], the lower panel from Dutta et al. [20].

Sarcomeric Proteins

Since the heartbeat refers to its contractile performance, our review of the Frank-Starling Law begins with the role of sarcomeric proteins in cardiomyocytes.

Myosin ATPase

In the early days training of scientists required that they be familiar with a classic language, e.g. German (e.g. [42]), and this persisted into the 1940s [21]. Subsequently, English was adopted as the language of science. One of the earliest papers to discuss the structure and function of muscle myosin in English came from John Gergely [26], a charming Hungarian who migrated to the US after the end of World War II and was mentored by the Nobel Prize winning Albert Szent-Gyorgyi (for his discovery of Vitamin C in 1937). Gergely ultimately settled in Boston where he headed the Boston Biomedical Research Institute (BBRI). Szent-Gyorgyi gravitated to nearby Woods Hole Massachusetts where he established the Institute of Muscle Research. Gergely is well known for his contributions to the relaxing proteins, but he will be remembered by many in the muscle field because he developed the Information Exchange Group (IEG) which compiled and circulated unreviewed advanced details of muscle research before it had been accepted by the journals. In the 1960–1970s he avoided the ire of the publishing companies by claiming the IEG reports were "personal communications" because they were communications between scientists and therefore, not a breach of their copyright. Today BioRxiv does a similar job.

Myosin is the motor molecule that produces systolic force in the Frank-Starling Law. In a healthy human heart, the ventricles mainly express a slow isoform of myosin (MYH7), while the atria mainly express the faster isoform, MYH6 [52]. Both isoforms are hexameric proteins consisting of two heavy chains, two essential light chains (ELCs) and two regulatory light chains (RLCs) (Fig. 6.4). Prior to the discovery of titin (about 3 MDa), the myosin molecule (~400 kDa) was the largest known protein in cardiomyocytes. It has two named components, the long light meromyosin (LMM) tails forming the thick filaments and the heavy meromyosin (HMM) composed of subfragment 1 (S1) and subfragment 2 (S2). Today the S1 is synonymous with crossbridges. Thick filaments are mainly composed of myosin LMM "rods". In the middle of the A band the LMMs are arranged tail-to-tail forming the central "bare" zone. The myosin "heads", pointing away from the centre M line with an axial periodicity of 43 nm. The two heavy chains separate to form separate S1 crossbridges. A total of 49 layers of myosin crossbridges project from the surface of the thick filaments. A myosin schematic is illustrated in Fig. 6.4. Its S1 component is rigidly attached to actin and does not appreciably change its orientation relative to the actin filament. Rotation of the crossbridge (the Power Stroke) occurs in the "converter" domain (or hinge) which is located just below the attachment of the myosin light chains, close to the S1–S2 junction. The original idea that the myosin crossbridge is a rigid ("oar-like") structure that rotates at its junction with actin was challenged based on fluorescence polarisation data [17] and later confirmed by Cooke et al. using EPR spin-labelled myosin crossbridges who showed that probes located at the ATPase sit do not change their

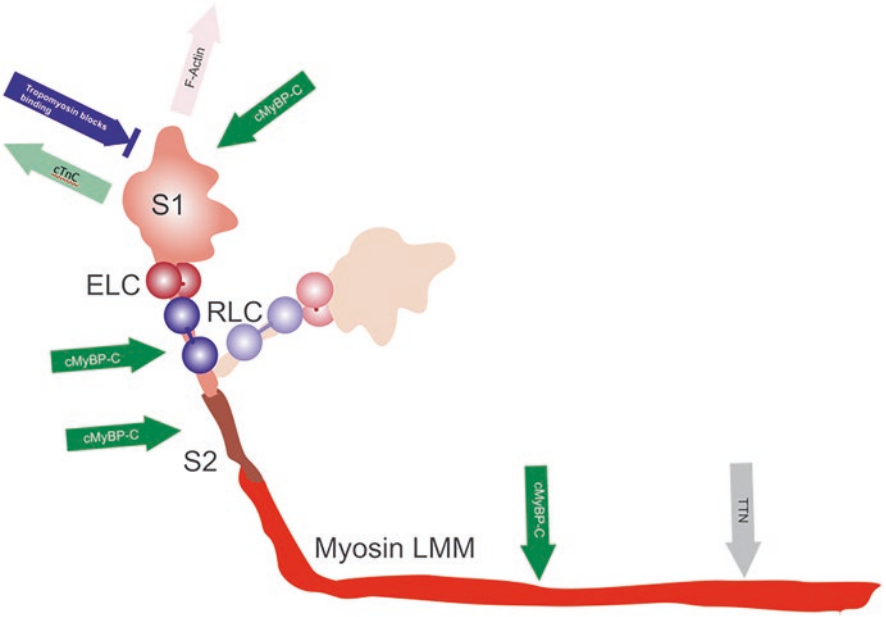

Fig. 6.4 The components of cardiac myosin and its binding partners. Myosin contains two heavy chains, and two regulatory light chains (RLCs, purple dumbbells) and two essential light chains (ELCs, red dumbbells). The N-terminal Light Meromyosin (LMM) component forms the bulk of the thick filaments and interacts with titin (TTN) and cardiac myosin binding protein C (cMyBP-C). LMM (red) is shown projecting into the interfilament space as heavy meromyosin (HMM, not labelled) a two-headed tryptic fragment consisting of one myosin subfragment 2 (S2, brown) and two S1 subfragments or crossbridges (pink). HMM can be further cleaved by papain into two S1 subfragments, each carrying one ELC, one RLC, and the ATPase catalytic site. S-1 is the only part of myosin that binds to the thin filaments and is sufficient to account for filament sliding in motility assays [86]. We refer to S1 as the "crossbridge".

orientation during contraction Cooke et al. [14]. The point of rotation "hinge" region is the location of a number of point mutations associated with Hypertrophic Cardiomyopathy (HCM) or Dilated Cardiomyopathy (DCM) [11, 31, 52].

Titin, the Third Sarcomere Filament Protein

Third filaments were reported in insect asynchronous flight muscles where they bridged the short gap between the ends of the thick filaments in the A band to the Z discs [3, 25, 66]. They were named "C" filaments and were considered at the time to be only a feature of highly specialized asynchronous insect flight muscle. There seemed to be no room for a third filament invertebrate muscle including cardiomyocytes. The extraction experiments described by Huxely and Hanson were interpreted as "denatured myosin and actin" and not as a bona fide sarcomeric proteins.

The number of thin filaments present in the sarcomere can be estimated by electron microscopy by counting filaments in serial transverse sections. In 1969 dos Remedios [16] reported there were 619 thin filaments in a single rabbit psoas muscle and found that, on average the A band has 2.0 thin filaments for every thick filament. However, in the I band instead of 2.0 the number reported was about 2.79, i.e. nearly 3. This result suggested that for every thick filament about 80% extended into the I band as an additional (non-actin) thin filament. They were named "residual" filaments and were directly visualized by electron microscopy in intact sarcomeres and were clearly imaged after myosin and actin extraction holding the Z discs together.

The history of the third "titin" filament was reviewed in detail [18, 19, 92], but more recently, Wolfgang Linke [50] published a comprehensive review of titin that summarizes our current understanding of its structure and function. Linke pointed out that the original two-filament hypothesis was unsatisfactory because, in the absence of a third filament, there would be nothing to stabilize sarcomere. The A bands could not be maintained in the middle of the sarcomere, and contraction of shorter sarcomeres (with more overlap of thick and thin filaments) would pull apart the longer sarcomeres where there was less overlap, especially in skeletal myocytes. Also, naturally occurring spontaneous oscillatory contractions (SPOCs) characterized by Shin'ichi Ishiwata and his colleagues [36] later demonstrated that the true functional unit of contraction is the *half* rather than a whole sarcomere. Telley et al. [84] raised the prospect of an enhanced 'residual' force that remained elevated after a quick stretch of activated single myofibrils. This force increased linearly with the degree of half-sarcomere length non-uniformities and was attributed to the passive force imposed by titin [68]. Studies measured as time-resolved displacement of the A band indicated that residual force was present irrespective of half-sarcomere non-uniformity [9, 68]. This raises the question of whether residual force enhancement primes the muscles for the next set of contractions, and thus formed the basis of the Frank-Starling Law?

Titin is the third most abundant protein (10% of the total muscle mass) and is also the largest protein ever recorded having nearly 36,000 amino acids [44]. Its molecular weight varies (3–4 MDa) depending on whether the isoform is N2B and N2BA. The shorter isoform was missing the N2A elastic elements. The N-terminus is anchored in the Z disc where it binds to the N-terminus of a titin in the next sarcomere (see titin at the N and C termini, Fig. 6.5). Its C-terminus stretches through half of the sarcomere to terminate at the M line where it binds to the C-terminus of the next titin, a distance over 1 μm. Thus, consecutive titins are interconnected along the entire length of the sarcomere with binding site along the thick and thin filaments as it continues to the intercalated discs at the cardiomyocyte ends. Titin is therefore a prime target for stretch-activation of the heart in the Frank-Starling Law. The expression level of the small titin isoform correlates with its contribution to overall muscle stiffness, suggesting that differential expression of large (e.g., N2BA) and small (e.g., N2B) titin isoforms is an effective means to modulate the filling performance of the heart [94].

In the Z disc: Titin contains up to seven Z-repeats that interact with α-actinin and cross-link the barbed ends of actin filaments. The first two Ig domains of two titin filaments bind to the stretch sensor TCAP (telethonin) and they are also the substrate of titin kinase that regulates the anti-parallel assembly of titin in the Z disc [87]. Mutations in TCAP were identified in patients with hypertrophic cardiomyopathy (HCM) and

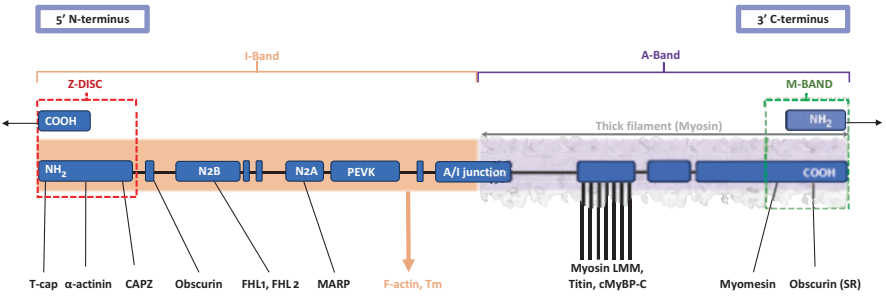

Fig. 6.5 Schematic illustration of the giant protein, titin. Its N-terminus is in the Z disc and it extends through the I band and half the A band where its C-terminus reaches the M in the middle of the sarcomere, a distance of over 1 μm. Titin has many binding partners discussed in detail in the text. In the I band it has a highly extensible PEVK region that functions as an extensible entropic spring and is responsible for its unusual elastic properties. At least six titin molecules run along the surface of the thick filament. Titin plays a crucial role in the Frank-Starling Law.

dilated cardiomyopathy (DCM) [31]. Titin also binds to CAPZ, an actin-capping protein that anchors the actin filaments to the Z disc. It directly controls maximum isometric tension generation by the sarcomere. It is important for anchoring PKC-β(II) and reducing myofilament sensitivity to Ca^{2+} [67].

In the I band: Obscurin (OBSCN) is another giant (721 kDa) protein located in the I band close to the Z disc (Fig. 6.5). Like titin, it has multiple immunoglobulin (Ig) and some fibronectin (Fn) domains that bind to a smaller isoform of titin called Novex-3. Obscurin has also been reported at the M line [45] (see below). As the titin sequence extends into the I band, one report [70] suggests it binds to the thin filament protein, tropomyosin. This connection with the thin filament might explain the reduced thin filament count in the I band described above [15]. Nearby the sequence has two splice isoforms, N2B and N2A, both present in cardiac titin filaments. Cardiac N2B binds to the FHL1 while the N2A splice isoform acts as an entropic spring element that can unfold with persistent force modulated by Ankrd [88]. FHL1 and Ankrd are mechanosensors that modulate muscle-specific signalling and growth. C-terminal to the N2A is the well-studied highly extensible PEVK domain that functions as an extensible "spring". Atomic force microscopy of the PEVK region strongly suggests it binds to F-actin. These data strongly implicate the binding of titin to F-actin [48]. Very recently Squarci et al. [77] used small angle X-ray diffraction and reported that PEVK can switch from a sarcomere length (SL)-dependent "OFF-state" to a SL-independent rectifier "ON-state". This allows free shortening of titin while resisting a quick stretch (the stiffness was ~3pN per half sarcomere per half thick filament) and enables the I band section of titin to transmit a suddenly increased load to the thick filament. In the "ON-state" the periodic interactions of A band titin with the myosin alter their "disposition" in a load-dependent manner. No doubt future investigations will resolve whether cMyBPC plays a role here. The PEVK region is also associated with other proteins

including Four and a Half LIM domain 2 (FHL2) that regulates heart development, and Muscle Ankyrin Repeat Protein (MARP) involved in the stretch-response of muscle.

In the A band: A total of six titins are located on the surface of the thick filament and bind to the LMM region of myosin (see "Turnover" discussion below). This is also seen as 9–11 stripes of cMyBP-C associated with 49 levels of myosin heads in each half sarcomere (Fig 6.5). In this way titin acts as "blueprint for the thick filament architecture [7]. This was earlier demonstrated using selective deletions of titin's C-zone super-repeats where the results showed not only a reduction of cMyBP-C stripes but also a reduction in overall thick filament length and resulting force production [85]. Alignment of the myosin motor domains in the thick filament produce the well-known myosin crossbridge periodicity which in turn mandate the binding of cMyBP-C as 7–9 stripes A band (Fig. 6.5).

In the M line: The main structural component is myomesin which strongly cross-links the thick filaments and therefore maintains the alignment of the thick filaments. The M line is also where the C-terminal sequences of two titins overlap and bind strongly to each other, effectively maintaining the continuity of the titin filament along the sarcomere. The myomesin also binds to obscurin [65] suggesting it makes a close connection to the lateral cisternae, the main Ca^{2+} storage-release site in adjacent to the myofibrils (see I band discussion above) thereby implicating it in the Ca^{2+}-induced Ca^{2+}-release mechanism.

Turnover Rate of Titin: Experiments evaluating the half-lives of sarcomeric proteins in the adult rat heart have yielded varying results. Older reports suggested that about 50% of myosin heavy chains turn over in 5.4 days [23], while half of troponin I turns over in 3.2 days [53]. We now know that the huge size and functional complexity of titin as a stress-sensor makes it a target gene in cardiomyopathies [49]. Recent studies, employing more sophisticated techniques have demonstrated much faster exchange times or accelerated turnover rates [71]. Da Silva Lopes et al. [15] inserted a GFP probe into the C-terminus of the TTN gene that carried an endogenous promoter using cultured beating embryonic cardiomyocytes using Fluorescence Recovery After Photobleaching (FRAP). They took several precautions including: (1) Minimising phototoxicity by observing FRAP from a single sarcomere; (2) Eliminating the contribution of de novo synthesis of TTN using cycloheximide that blocks translation elongation; (3) Using knock-in TTN-eGFP cardiomyocytes to increase the signal-to-noise ratio; (4) Following the fluorescence for 14 h to achieve a steady-state determination of the half-life of fluorescence recovery; and finally (5) Ensuring their measurements were not confounded by excluding the short Novex isoforms of titin. The fluorescent tag represented <1% of the molecular weight of titin. They measured FRAP using embryonic cardiomyocytes at normal, high, and low Ca^{2+} levels, in the presence of BDM (an inhibitor of myosin ATPase) using neonatal cardiomyocytes. Unexpectedly, they found the exchange half-lives from 1.7 h to 3.7 h with an average half-life of only 2.2 h. Movement of titin was largely unrestricted and high Ca^{2+} levels also inhibited its mobility. They concluded that the slow rate was attributable to the movement of existing titin within the sarcomere. These rapid turnover rates of titin have been validated by recent studies also using FRAP, revealing a surprisingly fast half-life for titin of 1.2 h [8]. These findings align with studies that showed even faster half-lives for cardiac troponins, which occur in the order of minutes in mouse embryonic cardiomyocytes also measured by

FRAP. Titin visualization in real time revealed an unexpected level of mobility within and between sarcomeres. Da Silva Lopes et al. [15] argued that movement along the fibril would require a tumbling motion but lateral movement between filaments would not, and their observations were consistent with titin movement that was equally efficient along and between fibrils. They concluded that titin is much more dynamic than expected and that it can detach from the M line and Z disc and move freely in either direction before it re-attaching.

Cardiac Myosin Binding Protein C

As its name suggests, cMyBP-C is a protein associated with the thick filament. It was first reported by Gerald Offer and his colleagues at King's College Biophysics [58] as the third largest protein contaminant (140.5 kDa) of skeletal myosin preparations. However, it was some time before the importance of cMyBP-C was more fully understood [6]. Cardiac cMyBP-C differs from its skeletal muscle isoform by the addition of a C0 domain at its N-terminus, thus placing it close to the motor domain of myosin including the Regulatory Light Chain (RLC) [69]. Immunohistochemistry revealed that cMyBP-C is in the A band as 7–9 stripes [62] with a spacing corresponding to the myosin crossbridge repeat of 43 nm [6] (lower panel in Fig. 6.6). Protein Kinase A (PKA)-catalysed phosphorylation of both cMyBP-C and cardiac troponin I (cTnI) decreases the Ca^{2+} sensitivity of force and accelerates the rate of crossbridge cycling [80].

Of the N-terminal domains, C0 is expressed in the heart but is not expressed in skeletal muscle [72]. The first three domains bind to either the base of the myosin S1 crossbridge (RLC) and S2 (Fig. 6.6) or they bind to the actin filament. Domains C3–C6 have no known binding partners and we have assigned them to the interfilament space. The four C-terminal domains, C7–C10, were reported to bind to titin, and C9–C10 domains bind to LMM in the thick filament [63, 85]. However, just-published super-resolution cryo-electron microscopy of fully relaxed cardiac muscle [20, 83] describe a clear physical separation between cMyBP-C and titin in the thick filament. Unfortunately, there are currently no cryo-EM data on activated thick filaments to determine whether this cMyBP-C/titin separation is maintained when the thick filaments are activated as proposed under the Frank-Starling Law.

Domains C0, C1 and C2 of cMyBP-C bind to F-actin but when they are stretched, they detach from actin and bind to myosin S2. The N-terminal C0 domains binds to the regulatory light chain (RLC) of myosin and together with C1 and C2 activate the myosin crossbridges to interact strongly with F-actin [62]. The functional importance of this complex is that cMyBP-C regulates whether myosin crossbridges in the C zone are shifted from the super relaxed state (see below) into the activated state that produces the enhanced force characteristic of the Frank-Starling Law.

The C3–C6 domains leave the thick filament and extend out into the gap between thin and thick filaments. This region of cMyBP-C also contacts actin filaments as shown in Fig. 6.8 [78]. Strangely, although cMyBP-C appears to be critical for the function of cardiac muscle, when the gene was introduced into skeletal muscle, it did not appear to make the critical connection between titin and the myosin crossbridge needed to explain the Frank-Starling Law [73].

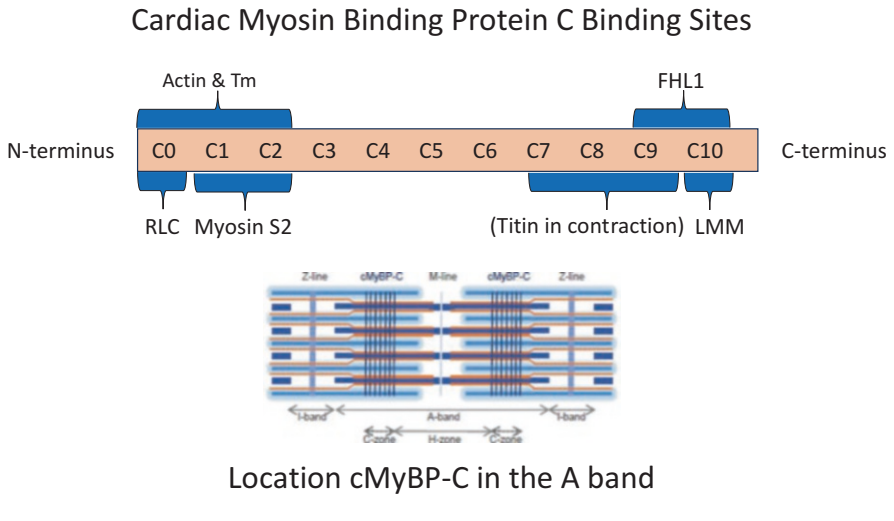

Fig. 6.6 The structure, localization and binding partners of cardiac myosin binding protein C (cMyBP-C). The 11 immunoglobulin/fibronectin domains of cMyBP-C are numbered C0-C10 from N- to C-terminus (top figure). Of the N-terminal domains, C0 is only expressed in the heart and is absent in skeletal muscle. The first three domains bind to either the base of the myosin crossbridge (RLC and S2) (Fig. 6.4) or they bind to actin in the thin filament. The middle four domains have no specific binding partners and have been assigned to the interfilament space. Of the C-terminal four domains, C10 binds to LMM in the thick filament, but recent super-resolution cryo-electron microscopy of relaxed cardiac muscle report a clear physical separation between cMyBP-C and titin. There are currently no cryo-EM data on whether this separation also changes during activation and contraction as suggested by historical reports. C9 and C10 are binding sites for FHLIM1, a biosensor known to bind to titin. The bottom figure shows cMyBP-C is limited to 7–9 distinct stripes in the A band.

cMyBP-C's role in disease. A mouse homozygous knockout of cMyBP-C resulted in development of hypertrophic cardiomyopathy, but the heterozygotes were unaffected. In humans, mutations in the MYBPC3 and the MHH7 genes are more frequent than other sarcomeric genes and represent about half of all HCM patients and in one Icelandic population they accounted for >90% of HCM [1]. cMyBPC is inherited an autosomal dominant and mainly affects diastolic dysfunction. Mutations can also lead to DCM. Formin Homology 2 domain-containing 3 (Fhod3) is abundantly expressed in the heart and plays an essential role in the regulation of actin assembly [56]. Its N-terminal region is cardiospecific (Fig. 6.6) and is responsible for the localisation of cMyBP-C to the C zone. Mutations in Fhod3 result in defective binding of cMyBPC3 to actin and contribute to the pathogenesis of cMyBP-C-related cardiomyopathy. The precise mechanism for the above remains a mystery. Phosphorylation (PKA) of cMyBP-C reduces its Ca^{2+} sensitivity of myosin crossbridge cycling [12].

Cardiac MyBP-C Plays a Significant Role in Modulating Crossbridge Activity. Under low Ca^{2+} conditions achieved during relaxation, cMyBP-C primes thick filaments by synergistically shifting tropomyosin to the closed rather than off state [47]. Previs et al. [64] showed that eccentric release of Ca^{2+} from the SR at the Z disc predict-

ably resulted in non-uniform activation on a beat-to-beat timescale if it were not for cMyBP-C that "exquisitely" sensitises the unbalanced release of Ca^{2+} so it is perfectly counterbalanced the Ca^{2+} diffusion in the half-sarcomere. More recently, Hanft et al. [29] reported that phosphorylation of cMyBP-C regulates sarcomere length-dependence of cardiac myofilaments. Thus, cMyBP-C affects the Frank-Starling Law by regulating sarcomere length-dependence of myofibrillar power and controls the beat-to-beat performance of the heart.

cMyBP-C Has Emerged as a Major Controller of Crossbridge Activity. The N terminus of cMyBP-C can bind to either the thin filament or the myosin crossbridge while its C terminus is anchored to LMM in the thick filament (see Fig. 6.6), and during sarcomere activation also appears to bind to titin. As Heling et al. [32] concluded, cMyBP-C is indeed one protein to govern them all.

Super-Relaxation Crossbridge Kinetics

Before 2010 myosin crossbridges were thought to be in one of two states. They were either in the actin-bound state (Fig. 6.7a) where ATP is rapidly hydrolysed (Fig. 6.7b) or they were completely detached (Fig. 6.7a Disordered Relaxed (DRX) state) where crossbridge ATP hydrolysis is slow (Fig. 6.7b). However, in 2010 Roger Cooke and his colleagues reported that the ATPase activity of myosin in free solution was significantly faster than when it was measured in muscle fibers where myosin heads were constrained to the thick filaments [81]. They concluded that myosin crossbridges that were not attached to the thick filaments could be either in the DRX state or in a new state they named the Super Relaxed (SRX) state. Here the myosin crossbridge was closely aligned along the thick filament where either it had some degree of movement (the non-IHM state) or one head was folded back and immobilised on the second head aligned along the rod (the interacting heads motif, IHM). These crossbridges were temporarily unavailable for force development but could be rapidly "activated" and contribute to force development. The SRX state can be quickly converted to the DRX state (Fig. 6.7) when there is a sustained demand for increased power.

Attachment of the myosin crossbridges described by AF Huxley and Niedergerke and Huxley and Hanson in 1954 provided little or no detail of the nature of the attached crossbridges. By 1969 Hugh Huxley [35] used electron microscopy to show that contraction involved an interdigitation of myosin and actin filaments produced by a cyclic attachment of crossbridges. These crossbridges were projections that attached to the thin filaments. Three years later dos Remedios et al. [17] used fluorescence polarization of tryptophan intrinsic to the S1 portion of the myosin crossbridges to show that there was little or no rotation of S1 probably occurred in the S2 portion of myosin. This conclusion was subsequently confirmed using electron paramagnetic resonance spectroscopy of myosin extrinsically labelled light chains [96]. In 2004 the dos Remedios et al. publication was selected in a list of the 50 most important in the history of muscle contraction and myosin motility [59].

In November 2023 two important papers were published back-to-back in Nature [20, 83] that used single-particle 3D reconstructions (cryo-electron microscopy) and

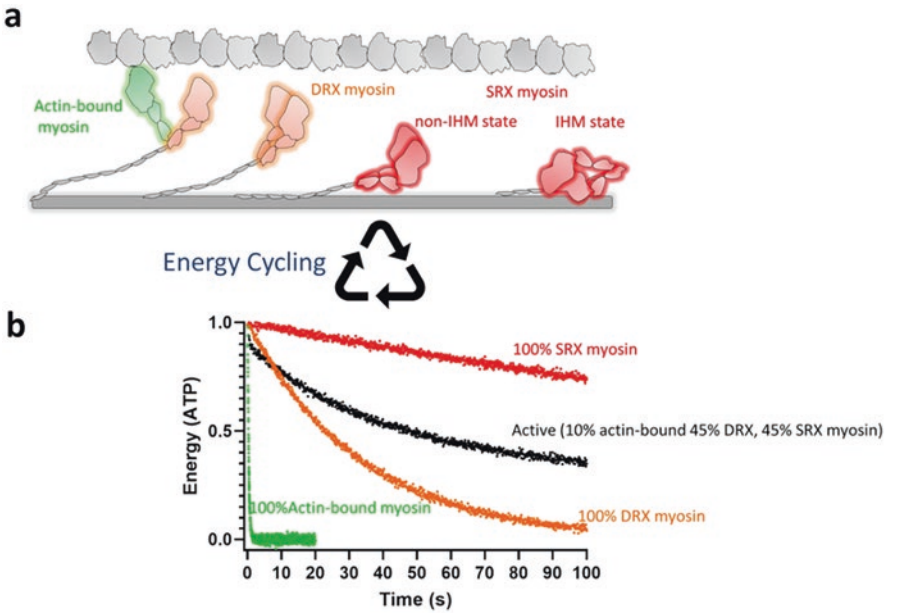

Fig. 6.7a Illustrates the possible functional energetic states of myosin filaments where the crossbridges in: (1) actin-bound (green) state (grey, top); (2) a Disordered Relaxed (DRX) state (orange) with both crossbridges unattached; and (3) the Super-Relaxed (SRX) state, where the two myosin crossbridges either do not interact (non-IHM state) or where one crossbridge is bound to the other head (IHM state). **Fig. 6.7b** plots the simulated myosin ATPase rates for: (1) actin-bound myosin (3 s-1) (green); (2) relaxed crossbridges in the DRX state (0.03 s-1) (orange); and (3) the SRX state (0.003/sec) (red). The black curve simulates the ATPase rate in actively contracting cardiomyocytes assuming 45% of the crossbridges are in SRX, 45% are in DRX and 10% are bound to actin. This figure is reproduced from Nag and Trivedi [57] under the Creative Commons licensing agreement.

achieved an unprecedented resolution of 4-6Å using images of the C zone region of native single myosin thick filaments isolated from frozen cardiomyocytes from human donors [20] or from human and mouse hearts [83]. This achievement required the actin filaments were removed by dissolving them with gelsolin. They reported that myosin crossbridges exhibit three configurations where crossbridges interact with: (1) each other; (2) specific domains on titin; and (3) with cMyBP-C. They also suggested a mechanism to explain how mutations in cMyBP-C might disrupt crossbridge function. In these preparations crossbridges were stabilised in the DRX and IHM-SRX states using mavacampten, a drug that strongly inhibits ATP-hydrolysis (see Fig. 6.6a). However, they found no evidence for the non-interactive (folded back) IHM head proposed by Nag et al. [57]. Furthermore, their reconstructions supported a strong interaction between titin on the surface of the thick filament and LMM and myosin heads, but that titin was clearly separated from C-terminal cMyBP-C within the thick filaments, at least when the of thin filaments were removed. It remains to be seen whether the lack of titin-cMyBP-C interaction in relaxation changes during rapid force increase that

characterizes the Frank-Starling Law since this change cannot be attributed increased thick-thin filament overlap.

In Fig. 6.8 we propose that the Frank-Starling stretch-activation mechanism involves both titin (blue) and cMyBP-C (green). In the relaxed state the N-terminus of cMyBP-C binds to the actin thin filament and the C-terminal domains bind to LMM in the thick filament, without contacting titin (consistent with [20, 83]). As the heart chamber is filled during diastole, titin is stretched which tenses the thick filament, causing a conformational change in cMyBP-C in the C zone. This causes it to release its attachment to the thin filament so its C0-C2 domains can now attach to a crossbridge at the point where crossbridge rotations [17] (indicated by the dashed red line). These changes allow the C-terminal domains (C8-C10) of cMyBP-C to attach more extensively to the surface of the thick filament and possibly allow it to change its attachment to titin provided it is phosphorylated [4, 12, 29, 57, 80].

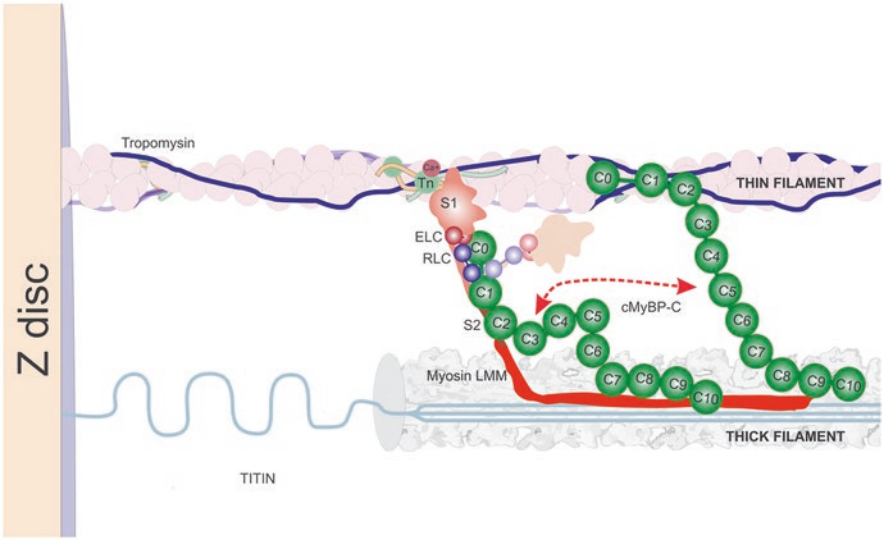

Fig. 6.8 Schematic representation of the interactions of cMyBP-C (green) with the thick (grey) and thin (pink) actin filaments in the C-zone of the sarcomere A band. Here cMyBP-C is shown in two alternative locations. In the relaxed (DRX, see Fig. 6.7) state shown on the right, its C-terminal domain (C10) binds to the LMM portion of myosin (grey). Historical evidence suggests that C7-C8-C9 bind to titin but his was recently disputed by cyro-electron microscopy. The middle domains (C3-C6) lie in the space between the thick and thin filaments, and the N-terminal domains (C0, C1, C2) bind to the nearest thin filament. When the relaxed heart chamber is stretched by increased filling, it is sensed by titin which binds cMyBP-C causing it to leave its attachment to actin and attaches to the myosin crossbridge (via the RLC and S2), thereby shifting it from the SRX state to the actin-bound state (Fig. 6.7). Note, for contraction to occur, Ca^{2+} must be released from the sarcoplasmic reticulum causing a chain of events that expose the myosin-binding sites on F-actin. Thus, crossbridge activity is regulated by cMyBP-C (green) and titin (blue-grey line).

Computational Analysis of the Frank-Starling Law and Length Dependent Activation

The super-relaxed state provides significant insights into Length Dependent Activation (LDA) and the Frank Starling Law [51]. Stretched mammalian cardiomyocytes exhibit an increased Ca^{2+}-sensitivity and maximum Ca^{2+}-activated force. Stretch increases the rate of diastolic ATP hydrolysis, and recruits reserve myosin heads from the SRX state. X-ray diffraction studies of relaxed muscle have confirmed there is less helical order in the thick filament when stretch is applied, concomitant with a displacement of myosin heads towards actin, which facilitates crossbridge formation upon activation. Mavacamten (a drug that shifts actin-bound myosin towards the SRX state) prevents other structural changes associated with LDA in the sarcomere, such as decreased lattice spacing or troponin-displacement. This strongly indicates LDA and its systolic consequences are dependent on the population of myosin heads competent to form crossbridges and involves the recruitment of myosin heads from the reserve pool of SRX state during diastole [7, 39, 76].

The shorter I band in cardiac muscle (compared to skeletal muscle) means that at normal working sarcomere lengths (cardiac curve in Fig. 6.1) the overlap of thick and thin filaments does not need to change. Instead, LDA is achieved by progressively recruiting myosin crossbridges from the pool of super relaxed crossbridges along the thick filaments until greater force is required and SRX heads are progressively recruited.

LDA is required to observe both the Frank-Starling mechanism and a positive response to high vascular fillings. Kosta and Dauby [41] pointed out that a dynamic length dependent activation-driven response to changes in preload involves interactions between the cellular, ventricular, and cardiovascular levels, and therefore fundamentally involves multiscale behaviour. They concluded it is challenging to study multiscale phenomena experimentally, and that computational approach contributes to a more comprehensive knowledge of the sophisticated length-dependent properties of cardiac muscle.

Similarly, Campbell et al. [10] presented a mathematical model of muscle contraction that was developed to investigate this hypothesis. It included myosin heads that transitioned between an off state that could not interact with actin (SRX), a DRX state that could bind to actin (DRX), and a single actin-bound state (see Fig. 6.7). Simulations were limited to experimental data using multidimensional parameter optimization. Statistical analysis showed that a model in which the rate of the off-to-on transition increased linearly with force reproduced the LDA behaviour using chemically permeabilized myocardium better than a model with a constant off-to-on transition rate (F-test, $p < 0.001$). Their result suggests that the thick filament transitions are modulated by force, and that a model incorporating a mechanosensitive thick filament also reproduced twitch responses measured in trabeculae stretched to different lengths. A final set of simulations predicted how reducing passive stiffness impacts the length dependence of Ca^{2+} sensitivity of contractile force. These results suggest that force-dependent recruitment of myosin heads from the thick-filament off state contributes to length-dependent activation and the Frank-Starling relationship [10].

A New Approach: Is PIEZO1 Is Involved in the Frank-Starling Law?

Here we propose novel molecular pathways that may help to explain the Frank-Starling Law. The discovery of the mechanosensitive receptors PIEZO1 and PIEZO2 was recently recognised by the 2021 Nobel Prize [54]. However, since PIEZO2 is not expressed in the cardiomyocytes, our focus is on PIEZO1.

PIEZO1 is a transmembrane homotrimer ion channel protein that non-specifically conducts Ca^{2+}. It is expressed at relatively low levels in the healthy heart, but it is up regulated in cardiomyopathies [37, 82]. It is expressed in the T tubules that invaginate the sarcolemma and penetrate deep within the cell [95]. T tubules make close contact with the lateral cisternae of the sarcoplasmic reticulum (SR), forming the triad junction that controls the activation of cardiomyocytes [27, 37] and initiates hypertrophy [94].

PIEZO1 Is Expressed in the T Tubules

The Frank-Starling Law states that when a heart chamber is suddenly filled by an increased volume of venous blood, the cardiomyocytes are stretched and quickly generate action potentials that travel along the sarcolemma and into the T tubules (Fig. 6.9, yellow). There, the PIEZO1 channel (purple) senses the force but cannot itself generate action potentials, which are produced in concert with the adjacent protein, Transient Receptor Potential cation channel from subfamily M (TRPM4) shown in blue in Fig. 6.9. Limited quantities of Ca^{2+} are conducted through the PIEZO1 channel which may induce a larger release of Ca^{2+} from the lateral cisternae of the sarcoplasmic reticulum (not shown in Fig. 6.9). This Ca^{2+}-induced Ca^{2+}-release binds to troponin C on the thin filaments (see Fig. 6.8), activating the movement of tropomyosin thereby allowing the myosin crossbridges to generate force. The whole process is then rapidly reversed when the longitudinal tubules of the SR sequester Ca^{2+} and return it to the lateral cisternae.

PIEZO1 Is Also Expressed in the Intercalated Discs

The desmosome-like intercalated discs (ICDs) structurally and functionally interconnect cardiomyocytes (CMs). They not only allow the action potentials to travel between CMs, they also provide strong mechanical connections and even the passage of small molecules such as peptides [7]. Structurally, the Transitional Junction [6] component of the ICD replaces the last Z disc of the sarcomere (Fig. 6.8). Like the Z disc, it therefore provides attachment for both the actin (pink) and titin (blue) filaments (Fig. 6.10). PIEZO1 channels are also expressed in the ICD [46, 82] but unlike the T tubules

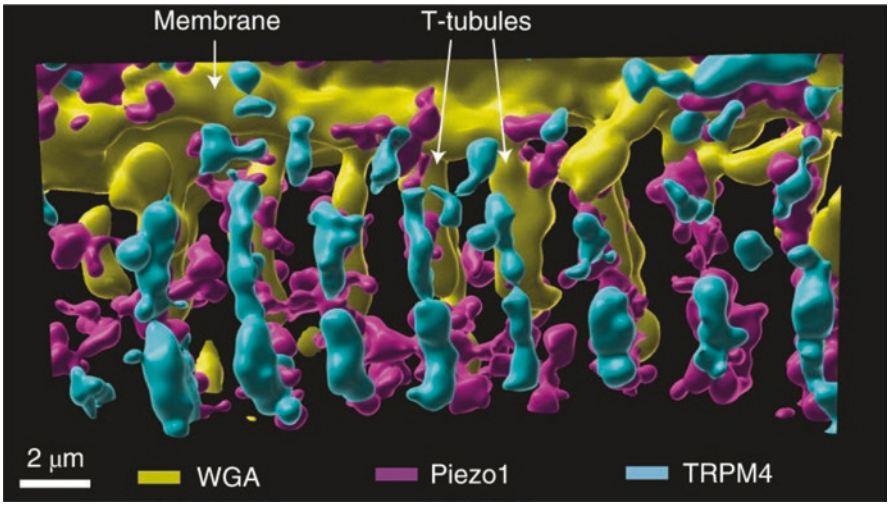

Fig. 6.9 A fluorescence micrograph of a rat ventricular cardiomyocyte showing that PIEZO1 (purple) is strongly associated with T tubules that invaginate the sarcolemma and travel deep within the cardiomyocyte. PIEZO1 can sense an applied stretch and release Ca2+, which binds and activates an adjacent TRPM4 receptor (blue), thereby initiating an action potential that activates the release of Ca2+ from the sarcoplasmic reticulum lateral cisternae. (Reproduced from Yu et al. [95] under the Creative Commons Attribution License).

described above, they do not express TRPM4 and therefore cannot generate action potentials [63]. We reported that forces arising from actin-mediated contractility as well as within the membrane lipid bilayer act synergistically to regulate PIEZO1 activation by stimuli applied at contacts between cells [5]. PIEZO1 channels in the ICD membrane are tethered to actin filaments via cadherin and β-catenin [91] which are also known components of the ICD [89]. Titin is known to bind to α-actinin in the Z disc (discussed in Fig. 6.5) and is also a well-established component of the ICD [22]. These observations are summarized in Fig. 6.10 showing PIEZO1 adjacent to both actin filaments (pink) and the I band titin filament (blue curving line). Its expression is upregulated in the ICDs in hypertrophic cardiomyopathy [95]. This increase would enhance its physical connection with the sarcomeric proteins. In Fig. 6.10 we summarise the above reports and propose that under Frank-Starling conditions stretch activates PIEZO1 in the Transitional Junction and increases the strain on an attached titin (curving blue line) which is then transmitted along the myosin thick filament to the C zone where it can then bind to the C-terminal domains of cMyBP-C. This strain causes cMyBP-C to release its attachment from the actin filament and so it can attach to the myosin cross-bridge as illustrated in Fig. 6.8.

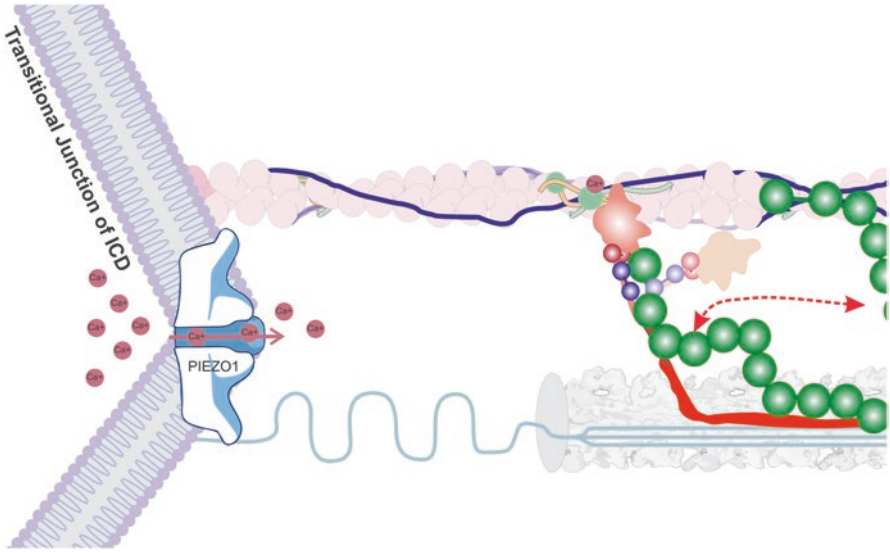

Fig. 6.10 A schematic representation of the Transitional Junction membrane portion of the intercalated discs. PIEZO1 is placed here because it is known to bind to actin and titin filaments. Rapid filling of the heart stretches the cardiomyocytes, which is sensed by PIEZO1. Thus, apart from its expression in the T tubules (Fig. 6.9), PIEZO1 is also a known component of the ICDs and is probably located in the Transitional Junction described in detail by Bennett et al. [6]. Stretch activates PIEZO1 causing a transient release of extracellular Ca2+ required for the activation of processes other than the activation of actomyosin. Stretch also tenses titins (pale blue-grey lines) which are continuously linked through the cardiomyocyte. connection through successive sarcomeres (myofibrils) to reach the ICD the surface of the thick filament the end of the thick filament (grey) to the Transitional Junction membrane and by cMyBP-C (Fig. 6.8).

Concluding Remarks

Role of Contractile Proteins in the Frank-Starling Law Although the Frank-Staring Law remains to be fully explained, this review suggest how it might be achieved. Like previous reviews we agree that titin is an important component of the Law as assessed by the effects of mutations and other reports, and despite early proposals there were only two types of filaments in muscle, there is now general acceptance that titin is an essential component of the sarcomere in transmitting a stretch force to the contractile proteins [2, 48, 75]. However, in fully relaxed cardiomyocytes while titin binds to the surface of the myosin thick filaments, it is not directly connected to the myosin cross-bridges. cMyBP- C is limited to the C zone of the A band but appears to have a major influence on contraction. The historical literature [4, 93] suggest that cMyBP- C binds to actin, the myosin cross-bridge and to titin, but recent cryo-electron microscopy reveals that cMyBP-C is clearly separated from titin in the fully relaxed (mavacampten) state. However, by cryo-EM imaging is difficult if not impossible to capture activated thick filament structure under Frank-Starling Law dynamic and therefore unstable conditions. We therefore raise the possibility that in the thick filament during force devel-

opment, cMyBP-C comes to the rescue and makes direct contact with titin under Frank-Starling Law conditions. Sequeira and van der Velden [75] suggest that Super Relaxation plays an important reserve source of crossbridges in the Frank-Starling Law [41] and that cMyBP-C plays a major role. Expression of PIEZO1 is emerging as a major component of cardiomyocytes. In the mouse [37] deletion of this mechanoreceptor reduces Ca2+ release from cardiomyocyte T tubules by failing to activate adjacent TRPM4, by reducing the generation of reactive oxygen species (ROS) and LV ejection fraction. PIEZO1 is also expressed in the ICDs and here we suggest a possible pathway whereby increased expression might directly contribute to sarcomere contractility without the involvement of the SR. It seems we are getting ever closer to a molecular understanding of the Frank-Starling Law.

Acknowledgements The authors are grateful to the following funding sources: National Health and Medical Research Council of Australia, the Australian Research Council, Medical Advances Without Animals (MAWA) and Mr. Andrew Lockie for his generous donation to this research.

References

1. Adalsteinsdottir B, Burke M, Maron BJ, Danielsen R, Lopez B, Diez J, Jarolim P, Seidman J, Seidman CE, Ho CY et al (2020) Hypertrophic cardiomyopathy in myosin–binding protein C (MYBPC3) Icelandic founder mutation carriers. Open Heart 7(1):e001220. https://doi.org/10.1136/openhrt-2019-001220. From NLM
2. Ait-Mou Y, Hsu K, Farman GP, Kumar M, Greaser ML, Irving TC, de Tombe PP (2016) Titin strain contributes to the Frank–Starling law of the heart by structural rearrangements of both thin- and thick-filament proteins. Proc Natl Acad Sci 113(8):2306–2311. https://doi.org/10.1073/pnas.1516732113
3. Auber J (1967) Remarks on the structure of fibrils of the muscles of the insect wing, at the level of the M streak. C R Acad Hebd Seances Acad Sci D 264(25):2916–2918. From NLM
4. Barefield D, Sadayappan S (2010) Phosphorylation and function of cardiac myosin binding protein–C in health and disease. J Mol Cell Cardiol 48(5):866–875. https://doi.org/10.1016/j.yjmcc.2009.11.014. From NLM
5. Bavi N, Richardson J, Heu C, Martinac B, Poole K (2019) PIEZO1-mediated currents are modulated by substrate mechanics. ACS Nano 13(11):13545–13559. https://doi.org/10.1021/acsnano.9b07499
6. Bennett PM, Maggs AM, Baines AJ, Pinder JC (2006) The transitional junction: a new functional subcellular domain at the intercalated disc. Mol Biol Cell 17(4):2091–2100. https://doi.org/10.1091/mbc.E05-12-1109
7. Bennett PM (2018) Riding the waves of the intercalated disc of the heart. Biophys Rev 10(4):955–959. https://doi.org/10.1007/s12551-018-0438-z
8. Cadar AG, Feaster TK, Bersell KR, Wang L, Hong T, Balsamo JA, Zhang Z, Chun YW, Nam YJ, Gotthardt M et al (2020) Real-time visualization of titin dynamics reveals extensive reversible photobleaching in human induced pluripotent stem cell-derived cardiomyocytes. Am J Physiol Cell Physiol 318(1):C163–c173. https://doi.org/10.1152/ajpcell.00107.2019. From NLM
9. Campbell KS (2009) Interactions between connected half-sarcomeres produce emergent mechanical behavior in a mathematical model of muscle. PLoS Comput Biol 5(11):e1000560. https://doi.org/10.1371/journal.pcbi.1000560. From NLM

10. Campbell KS, Janssen PML, Campbell SG (2018) Force-dependent recruitment from the myosin off state contributes to length-dependent activation. Biophys J 115(3):543–553. https://doi.org/10.1016/j.bpj.2018.07.006. From NLM
11. Catrina BI, Batar F, Baltat G, Bitea CI, Puia A, Stoia O, Fleacă SR, Teodoru M (2023) A family with Myh7 mutation and different forms of Cardiomyopathies. Biomedicines 11(7). https://doi.org/10.3390/biomedicines11072065. From NLM
12. Chen PP, Patel JR, Rybakova IN, Walker JW, Moss RL (2010) Protein kinase A-induced myofilament desensitization to Ca(2+) as a result of phosphorylation of cardiac myosin–binding protein C. J Gen Physiol 136(6):615–627. https://doi.org/10.1085/jgp.201010448. From NLM
13. Cingolani HE, Pérez NG, Cingolani OH, Ennis IL (2013) The Anrep effect: 100 years later. Am J Physiol Heart Circ Physiol 304(2):H175–H182. https://doi.org/10.1152/ajpheart.00508.2012. From NLM
14. Cooke R, Crowder MS, Thomas DD (1982) Orientation of spin labels attached to cross-bridges in contracting muscle fibres. Nature 300(5894):776–778. https://doi.org/10.1038/300776a0. From NLM
15. da Silva Lopes K, Pietas A, Radke MH, Gotthardt M (2011) Titin visualization in real time reveals an unexpected level of mobility within and between sarcomeres. J Cell Biol 193(4):785–798. https://doi.org/10.1083/jcb.201010099. From NLM
16. Dos Remedios CG (1969) Comparative studies of striated muscle. PhD thesis, University of Sydney
17. Dos Remedios CG, Millikan RG, Morales MF (1972) Polarization of tryptophan fluorescence from single striated muscle fibers. A molecular probe of contractile state. J Gen Physiol 59(1):103–120. https://doi.org/10.1085/jgp.59.1.103. From NLM
18. dos Remedios C, Gilmour D (2017) An historical perspective of the discovery of titin filaments. Biophys Rev 9(3):179–188. https://doi.org/10.1007/s12551-017-0269-3
19. dos Remedios CG (2018) An historical perspective of the discovery of titin filaments –Part 2. Biophys Rev 10(4):1201–1203. https://doi.org/10.1007/s12551-017-0393-0
20. Dutta D, Nguyen V, Campbell KS, Padrón R, Craig R (2023) Cryo-EM structure of the human cardiac myosin filament. Nature (London) 623(7988):853–862. https://doi.org/10.1038/s41586-023-06691-4
21. Engelhardt V, Ljubimova M, Meitina R (1941) Chemistry and mechanics of the muscle studied on myosin threads. Compt Rend De l'acad Sci de l'USSR 30:644
22. Estigoy CB, Pontén F, Odeberg J, Herbert B, Guilhaus M, Charleston M, Ho JWK, Cameron D, dos Remedios CG (2009) Intercalated discs: multiple proteins perform multiple functions in non-failing and failing human hearts. Biophys Rev 1(1):43–49. https://doi.org/10.1007/s12551-008-0007-y
23. Everett AW, Prior G, Zak R (1981) Equilibration of leucine between the plasma compartment and leucyl-tRNA in the heart, and turnover of cardiac myosin heavy chain. Biochem J 194(1):365–368. https://doi.org/10.1042/bj1940365. From NLM
24. Frank O (1959) On the dynamics of cardiac muscle. Am Heart J 58(2):282–317
25. Garamvölgyi N (1965) The arrangement of the myofilaments in the insect flight muscle. II J Ultrastruct Res 13(5):425–434. https://doi.org/10.1016/s0022-5320(65)90005-5. From NLM
26. Gergely J (1953) Studies on myosin-Adenosinetriphosphatase. J Biol Chem 200(2):543–550. https://doi.org/10.1016/S0021-9258(18)71399-9
27. Guo Y, Yu Z-Y, Wu J, Gong H, Kesteven S, Iismaa SE, Chan AY, Holman S, Pinto S, Pironet A et al (2021) The Ca2+−activated cation channel TRPM4 is a positive regulator of pressure overload-induced cardiac hypertrophy. eLife 10:e66582. https://doi.org/10.7554/eLife.66582
28. Han J-C, Loiselle D, Taberner A, Tran K (2021) Re-visiting the Frank-Starling nexus. Prog Biophys Mol Biol 159:10–21. https://doi.org/10.1016/j.pbiomolbio.2020.04.003

29. Hanft LM, Fitzsimons DP, Hacker TA, Moss RL, McDonald KS (2021) Cardiac MyBP-C phosphorylation regulates the Frank-Starling relationship in murine hearts. J Gen Physiol 153(7). https://doi.org/10.1085/jgp.202012770. From NLM
30. Hanson J, Huxley HE (1953) Structural basis of the cross-striations in muscle. Nature 172(4377):530–532. https://doi.org/10.1038/172530b0
31. Hayashi T, Arimura T, Itoh-Satoh M (2005) Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy. ACC Curr J Rev 14(4):62–63. https://doi.org/10.1016/j.accreview.2005.03.035
32. Heling LWHJ, Geeves MA, Kad NM (2020) MyBP-C: one protein to govern them all. J Muscle Res Cell Motil 41(1):91–101. https://doi.org/10.1007/s10974-019-09567-1
33. Huxley AF, Niedergerke R (1954) Structural changes in muscle during contraction; interference microscopy of living muscle fibres. Nature 173(4412):971–973. https://doi.org/10.1038/173971a0. From NLM
34. Huxley H, Hanson J (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature 173(4412):973–976. https://doi.org/10.1038/173973a0
35. Huxley HE (1969) The mechanism of muscular contraction: recent structural studies suggest a revealing model for cross-bridge action at variable filament spacing. Science 164(3886):1356–1366
36. Ishiwata SI, Anazawa T, Fujita T, Fukuda N, Shimizu H, Yasuda K (1993) Spontaneous tension oscillation (SPOC) of muscle fibers and myofibrils minimum requirements for SPOC. In: Sugi H, Pollack GH (eds) Mechanism of myofilament sliding in muscle contraction. Springer, Boston, pp 545–556
37. Jiang F, Yin K, Wu K, Zhang M, Wang S, Cheng H, Zhou Z, Xiao B (2021) The mechanosensitive Piezo1 channel mediates heart mechano-chemo transduction. Nat Commun 12(1):869. https://doi.org/10.1038/s41467-021-21178-4
38. Katz AM (2002) Ernest Henry Starling, his predecessors, and the law of the heart. Circulation (New York, NY) 106(23):2986–2992. https://doi.org/10.1161/01.CIR.0000040594.96123.55
39. Kawai M, Jin J-P (2021) Mechanisms of Frank-Starling law of the heart and stretch activation in striated muscles may have a common molecular origin. J Muscle Res Cell Motil 42(2):355–366. https://doi.org/10.1007/s10974-020-09595-2
40. Kobirumaki-Shimozawa F, Inoue T, Shintani SA, Oyama K, Terui T, Minamisawa S, Ishiwata SI, Fukuda N (2014) Cardiac thin filament regulation and the Frank–Starling mechanism. J Physiol Sci 64(4):221–232. https://doi.org/10.1007/s12576-014-0314-y
41. Kosta S, Dauby PC (2021) Frank-Starling mechanism, fluid responsiveness, and length–dependent activation: unravelling the multiscale behaviors with an in silico analysis. PLoS Comput Biol 17(10):e1009469
42. Kühne W (1864) Untersuchungen über das Protoplasma und die Contractilität. W. Engelmann
43. Kuhtz-Buschbeck JP, Drake-Holland A, Noble MIM, Lohff B, Schaefer J (2018) Rediscovery of Otto Frank's contribution to science. J Mol Cell Cardiol 119:96–103. https://doi.org/10.1016/j.yjmcc.2018.04.017
44. Labeit S, Kolmerer B, Linke WA (1997) The Giant protein Titin: emerging roles in physiology and pathophysiology. Circ Res 80(2):290–294. https://doi.org/10.1161/01.RES.80.2.290
45. Lange S, Pinotsis N, Agarkova I, Ehler E (2020) The M-band: the underestimated part of the sarcomere. Biochim Biophys Acta, Mol Cell Res 1867(3):118440. https://doi.org/10.1016/j.bbamcr.2019.02.003. From NLM
46. Liang J, Huang B, Yuan G, Chen Y, Liang F, Zeng H, Zheng S, Cao L, Geng D, Zhou S (2017) Stretch-activated channel Piezo1 is up-regulated in failure heart and cardiomyocyte stimulated by AngII. Am J Transl Res 9(6):2945–2955. From NLM

47. Lin BL, Li A, Mun JY, Previs MJ, Previs SB, Campbell SG, dos Remedios CG, Tombe P d P, Craig R, Warshaw DM et al (2018) Skeletal myosin binding protein-C isoforms regulate thin filament activity in a Ca2+–dependent manner. Sci Rep 8(1):2604. https://doi.org/10.1038/s41598-018-21053-1
48. Linke WA, Kulke M, Li H, Fujita-Becker S, Neagoe C, Manstein DJ, Gautel M, Fernandez JM (2002) PEVK domain of Titin: an entropic spring with actin-binding properties. J Struct Biol 137(1):194–205. https://doi.org/10.1006/jsbi.2002.4468
49. Linke WA (2008) Sense and stretchability: the role of titin and titin-associated proteins in myocardial stress-sensing and mechanical dysfunction. Cardiovasc Res 77(4):637–648. https://doi.org/10.1016/j.cardiores.2007.03.029. From NLM
50. Linke WA (2023) Stretching the story of titin and muscle function. J Biomech 152:111553. https://doi.org/10.1016/j.jbiomech.2023.111553
51. Ma W, Henze M, Anderson RL, Gong H, Wong FL, Del Rio CL, Irving T (2021) The super-relaxed state and length dependent activation in porcine myocardium. Circ Res 129(6):617–630. https://doi.org/10.1161/circresaha.120.318647. From NLM
52. Marston S, Montgiraud C, Munster AB, Copeland ON, Choi O, dos Remedios C, Messer AE, Ehler E, Knöll R (2015) OBSCN mutations associated with dilated cardiomyopathy and Haploinsufficiency. PLoS One 10(9):e0138568. https://doi.org/10.1371/journal.pone.0138568
53. Martin AF (1981) Turnover of cardiac troponin subunits. Kinetic evidence for a precursor pool of troponin-I. J Biol Chem 256(2):964–968. From NLM
54. Martinac B (2022) 2021 Nobel Prize for mechanosensory transduction. Biophys Rev 14(1):15–20. https://doi.org/10.1007/s12551-022-00935-9. From NLM
55. Maruyama K, Natori R, Nonomura Y (1976) New elastic protein from muscle. Nature 262(5563):58–60. https://doi.org/10.1038/262058a0
56. Matsuyama S, Kage Y, Fujimoto N, Ushijima T, Tsuruda T, Kitamura K, Shiose A, Asada Y, Sumimoto H, Takeya R (2018) Interaction between cardiac myosin-binding protein C and formin Fhod3. Proc Natl Acad Sci PNAS 115(19):E4386–E4395. https://doi.org/10.1073/pnas.1716498115
57. Nag S, Trivedi DV (2021) To lie or to lie: super-relaxing with myosins. eLife 10:e63703. https://doi.org/10.7554/eLife.63703
58. Offer G, Moos C, Starr R (1973) A new protein of the thick filaments of vertebrate skeletal myofibrils. Extractions, purification and characterization. J Mol Biol 74(4):653–676. https://doi.org/10.1016/0022-2836(73)90055-7. From NLM
59. Ostap EM, Franzini-Armstrong C, Goldman YE, Pollard TD, Sellers JR, Sweeney HL, Szent-Györgyi A (2004) Fifty important papers in the history of muscle contraction and myosin motility. J Muscle Res Cell Motil 25:483–487
60. Patterson SW, Starling EH (1914) On the mechanical factors which determine the output of the ventricles. J Physiol 48(5):357–379. https://doi.org/10.1113/jphysiol.1914.sp001669
61. Peng Y, Gregorich ZR, Valeja SG, Zhang H, Cai W, Chen YC, Guner H, Chen AJ, Schwahn DJ, Hacker TA et al (2014) Top-down proteomics reveals concerted reductions in myofilament and Z-disc protein phosphorylation after acute myocardial infarction. Mol Cell Proteomics 13(10):2752–2764. https://doi.org/10.1074/mcp.M114.040675. From NLM
62. Pfuhl M, Gautel M (2012) Structure, interactions and function of the N-terminus of cardiac myosin binding protein C (MyBP-C): who does what, with what, and to whom? J Muscle Res Cell Motil 33(1):83–94. https://doi.org/10.1007/s10974-012-9291-z. From NLM
63. Piao H, Takahashi K, Yamaguchi Y, Wang C, Liu K, Naruse K (2015) Transient receptor potential melastatin-4 is involved in hypoxia-reoxygenation injury in the cardiomyocytes. PLoS One 10(4):e0121703–e0121703. https://doi.org/10.1371/journal.pone.0121703

64. Previs MJ, Prosser BL, Mun JY, Previs SB, Gulick J, Lee K, Robbins J, Craig R, Lederer WJ, Warshaw DM (2015) Myosin-binding protein C corrects an intrinsic inhomogeneity in cardiac excitation-contraction coupling. Sci Adv 1(1). https://doi.org/10.1126/sciadv.1400205
65. Prill K, Carlisle C, Stannard M, Windsor Reid PJ, Pilgrim DB (2019) Myomesin is part of an integrity pathway that responds to sarcomere damage and disease. PLoS One 14(10):e0224206–e0224206. https://doi.org/10.1371/journal.pone.0224206
66. Pringle JW (1967) Evidence from insect fibrillar muscle about the elementary contractile process. J Gen Physiol 50(6)., Suppl:139–156. https://doi.org/10.1085/jgp.50.6.139
67. Pyle WG, La Rotta G, de Tombe PP, Sumandea MP, Solaro RJ (2006) Control of cardiac myofilament activation and PKC-betaII signaling through the actin capping protein. CapZ J Mol Cell Cardiol 41(3):537–543. https://doi.org/10.1016/j.yjmcc.2006.06.006. From NLM
68. Rassier DE, Pavlov I (2012) Force produced by isolated sarcomeres and half-sarcomeres after an imposed stretch. Am J Physiol Cell Physiol 302(1):C240–C248. https://doi.org/10.1152/ajpcell.00208.2011. From NLM
69. Ratti J, Rostkova E, Gautel M, Pfuhl M (2011) Structure and interactions of myosin-binding protein C domain C0: cardiac-specific regulation of myosin at its neck? J Biol Chem 286(14):12650–12658. https://doi.org/10.1074/jbc.M110.156646
70. Raynaud F, Astier C, Benyamin Y (2004) Evidence for a direct but sequential binding of titin to tropomyosin and actin filaments. Biochimica et biophysica acta. Proteins Proteomics 1700(2):171–178. https://doi.org/10.1016/j.bbapap.2004.05.001
71. Reil JC, Reil GH, Kovács Á, Sequeira V, Waddingham MT, Lodi M, Herwig M, Ghaderi S, Kreusser MM, Papp Z et al (2020) CaMKII activity contributes to homeometric autoregulation of the heart: A novel mechanism for the Anrep effect. J Physiol 598(15):3129–3153, 3043–3307. https://doi.org/10.1113/jp279607. From NLM
72. Rybakova IN, Greaser ML, Moss RL (2011) Myosin binding protein C interaction with actin: characterization and mapping of the binding site. J Biol Chem 286(3):2008–2016. https://doi.org/10.1074/jbc.M110.170605
73. Sadayappan S, de Tombe PP (2012) Cardiac myosin binding protein-C: redefining its structure and function. Biophys Rev 4(2):93–106. https://doi.org/10.1007/s12551-012-0067-x
74. Sagawa K, Lie RK, Schaefer J (1990) Translation of Otto Frank's paper "Die Grundform des Arteriellen Pulses" Zeitschrift für Biologie 37: 483–526 (1899). J Mol Cell Cardiol 22(3):253–254. https://doi.org/10.1016/0022-2828(90)91459-k. From NLM
75. Sequeira V, van der Velden J (2017) The Frank–Starling Law: a jigsaw of titin proportions. Biophys Rev 9(3):259–267. https://doi.org/10.1007/s12551-017-0272-8
76. Sequeira V, Maack C, Reil G-H, Reil J-C (2024) Exploring the connection between relaxed myosin states and the Anrep effect. Circ Res 134(1):117–134. https://doi.org/10.1161/CIRCRESAHA.123.323173
77. Squarci C, Bianco P, Reconditi M, Pertici I, Caremani M, Narayanan T, Horváth ÁI, Málnási-Csizmadia A, Linari M, Lombardi V et al (2023) Titin activates myosin filaments in skeletal muscle by switching from an extensible spring to a mechanical rectifier. Proc Natl Acad Sci PNAS 120(9):e2219346120–e2219346120. https://doi.org/10.1073/pnas.2219346120
78. Squire JM, Luther PK, Knupp C (2003) Structural evidence for the interaction of C-protein (MyBP-C) with actin and sequence identification of a possible actin-binding domain. J Mol Biol 331(3):713–724. https://doi.org/10.1016/S0022-2836(03)00781-2
79. Starling EH (1918) The Linacre lecture on the law of the heart. Longmans, Green, & Company, London
80. Stelzer JE, Patel JR, Walker JW, Moss RL (2007) Differential roles of cardiac myosin–binding protein C and cardiac troponin I in the myofibrillar force responses to protein kinase A phosphorylation. Circ Res 101(5):503–511. https://doi.org/10.1161/circresaha.107.153650. From NLM

81. Stewart MA, Franks-Skiba K, Chen S, Cooke R (2010) Myosin ATP turnover rate is a mechanism involved in thermogenesis in resting skeletal muscle fibers. Proc Natl Acad Sci USA 107(1):430–435. https://doi.org/10.1073/pnas.0909468107. From NLM
82. Su SA, Zhang Y, Li W, Xi Y, Lu Y, Shen J, Ma Y, Wang Y, Shen Y, Xie L et al (2023) Cardiac Piezo1 exacerbates lethal ventricular Arrhythmogenesis by linking mechanical stress with Ca(2+) handling after myocardial infarction. Research (Wash D C) 6:0165. https://doi.org/10.34133/research.0165. From NLM
83. Tamborrini D, Wang Z, Wagner T, Tacke S, Stabrin M, Grange M, Kho AL, Rees M, Bennett P, Gautel M et al (2023) Structure of the native myosin filament in the relaxed cardiac sarcomere. Nature 623(7988):863–871. https://doi.org/10.1038/s41586-023-06690-5
84. Telley IA, Denoth J, Stüssi E, Pfitzer G, Stehle R (2006) Half-sarcomere dynamics in myofibrils during activation and relaxation studied by tracking fluorescent markers. Biophys J 90(2):514–530. https://doi.org/10.1529/biophysj.105.070334
85. Tonino P, Kiss B, Strom J, Methawasin M, Smith JE, Kolb J, Labeit S, Granzier H (2017) The giant protein titin regulates the length of the striated muscle thick filament. Nat Commun 8(1):1041. https://doi.org/10.1038/s41467-017-01144-9
86. Toyoshima YY, Kron SJ, McNally EM, Niebling KR, Toyoshima C, Spudich JA (1987) Myosin subfragment-1 is sufficient to move actin filaments in vitro. Nature 328(6130):536–539. https://doi.org/10.1038/328536a0. From NLM
87. Valle G, Faulkner G, De Antoni A, Pacchioni B, Pallavicini A, Pandolfo D, Tiso N, Toppo S, Trevisan S, Lanfranchi G (1997) Telethonin, a novel sarcomeric protein of heart and skeletal muscle. FEBS Lett 415(2):163–168. https://doi.org/10.1016/S0014-5793(97)01108-3
88. van der Pijl RJ, Domenighetti AA, Sheikh F, Ehler E, Ottenheijm CAC, Lange S (2021) The titin N2B and N2A regions: biomechanical and metabolic signaling hubs in cross-striated muscles. Biophys Rev 13(5):653–677. https://doi.org/10.1007/s12551-021-00836-3
89. Vite A, Radice GL (2014) N-Cadherin/Catenin complex as a master regulator of intercalated disc function. Cell Commun Adhes 21(3):169–179. https://doi.org/10.3109/15419061.2014.908853
90. Wang K, McClure J, Tu A (1979) Titin: major myofibrillar components of striated muscle. Proc Natl Acad Sci USA 76(8):3698–3702. https://doi.org/10.1073/pnas.76.8.3698. From NLM
91. Wang J, Jiang J, Yang X, Zhou G, Wang L, Xiao B (2022) Tethering Piezo channels to the actin cytoskeleton for mechanogating via the cadherin-↓-catenin mechanotransduction complex. Cell Rep 38(6):110342. https://doi.org/10.1016/j.celrep.2022.110342. From NLM
92. Weiwad WK, Linke WA, Wussling MH (2000) Sarcomere length–tension relationship of rat cardiac myocytes at lengths greater than optimum. J Mol Cell Cardiol 32(2):247–259
93. Winegrad S (1999) Cardiac myosin binding protein C. Circ Res 84(10):1117–1126
94. Wu Y, Cazorla O, Labeit D, Labeit S, Granzier H (2000) Changes in titin and collagen underlie diastolic stiffness diversity of cardiac muscle. J Mol Cell Cardiol 32(12):2151–2161
95. Yu Z, Gong H, Kesteven S, Guo Y, Wu J, Li J, Cheng D, Zhou Z, Iismaa S, Kaidonis X (2022) Piezo1 is the cardiac mechanosensor that initiates the cardiomyocyte hypertrophic response to pressure overload in adult mice. Nat Cardiovasc Res 1:577–591
96. Zhao L, Pate E, Baker AJ, Cooke R (1995) The myosin catalytic domain does not rotate during the working power stroke. Biophys J 69(3):994–999