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MUSCLE PHYSIOLOGY

Insights into the kinetics of Ca^{2+}-regulated contraction and relaxation from myofibril studies

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Abstract Muscle contraction results from force-generating interactions between myosin cross-bridges on the thick filament and actin on the thin filament. The force-generating interactions are regulated by Ca^{2+} via specialised proteins of the thin filament. It is controversial how the contractile and regulatory systems dynamically interact to determine the time course of muscle contraction and relaxation. Whereas kinetics of Ca^{2+}-induced thin-filament regulation is often investigated with isolated proteins, force kinetics is usually studied in muscle fibres. The gap between studies on isolated proteins and structured fibres is now bridged by recent techniques that analyse the chemical and mechanical kinetics of small components of a muscle fibre, subcellular myofibrils isolated from skeletal and cardiac muscle. Formed of serially arranged repeating units called sarcomeres, myofibrils have a complete fully structured ensemble of contractile and Ca^{2+} regulatory proteins. The small diameter of myofibrils (few micrometres) facilitates analysis of the kinetics of sarcomere contraction and relaxation induced by rapid changes of [ATP] or [Ca^{2+}]. Among the processes studied on myofibrils are: (1) the Ca^{2+}-regulated switch on/off of the troponin complex, (2) the chemical steps in the cross-bridge adenosine triphosphatase cycle, (3) the mechanics of force generation and (4) the length dynamics of individual sarcomeres. These studies give new insights into the kinetics of thin-filament regulation and of cross-bridge turnover, how cross-bridges transform chemical energy into mechanical work, and suggest that the cross-bridge ensembles of each half-sarcomere cooperate with each other across the half-sarcomere borders. Additionally, we now have a better understanding of muscle relaxation and its impairment in certain muscle diseases.

Keywords Muscle contraction · Muscle relaxation · Myocardial contraction · Myocardial relaxation · Myofibrils · Sarcomeres · Calcium · Thin-filament regulation · Cross-bridge kinetics · Relaxation · Cross-bridge · Muscle mechanics · Cardiac sarcomere · Cardiac muscle · Cardiac function · Caged calcium · Calcium regulation · Skinned fibre

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Myofibrils: a model to study kinetics of sarcomeric processes

Striated muscles have a hierarchically organized architecture. Skeletal muscles are large bundles of multinucleated cells, called fibres, aligned in parallel. Cardiac muscle consists of networks of branching single nucleated cells called myocytes. The cells contain, in turn, bundles of myofibrils that form the contractile machinery. Myofibrils are aligned in parallel within a skeletal muscle fibre or in

branching bundles within a myocyte. On the single myofibril level, skeletal and cardiac muscles have very similar substructures. Both types of myofibrils are constructed of stacks of short cylindrical repeating units called sarcomeres. The sarcomere is the most highly ordered structure of all cellular organelles. It has a bilateral symmetry, i.e. it consists of two antipodal halves, the half-sarcomeres. The sarcomere is constructed by two types of transversally oriented multi-protein scaffolds, the M-line and the Z-disc which anchor an ordered lattice of three types of axial filaments, called the thick, thin and titin filaments. Z-discs form the outer ends of a sarcomere and are shared with neighbouring sarcomeres (Fig. 1). The M-line is at the middle of the sarcomere and is shared by the two half-sarcomeres. The midpoint of the thick filaments are anchored in the M-line; they extend towards the Z-discs but, normally, do not touch it. In contrast, the thin filaments are anchored to the Z-lines but not to the M-line. The elastic titin filament is also attached to the Z-discs and is the only filament that forms a permanent connection between the Z-disc and the M-line; thereby, it determines the elastic properties of the relaxed sarcomere [47, 86]. The thin and thick filaments each contain a precise assembly of several proteins, which together forms the Ca^{2+}-regulated contractile aggregate. Thin filaments mainly consist of actin and associated regulatory proteins, the troponin complex (Tn) and tropomyosin (Tm). Thick filaments are mainly composed of myosin, myosin light chains and myosin binding protein C. Parts of the myosin, called heads or cross-bridges, protrude at regular intervals from the thick filament backbone towards the thin filaments.

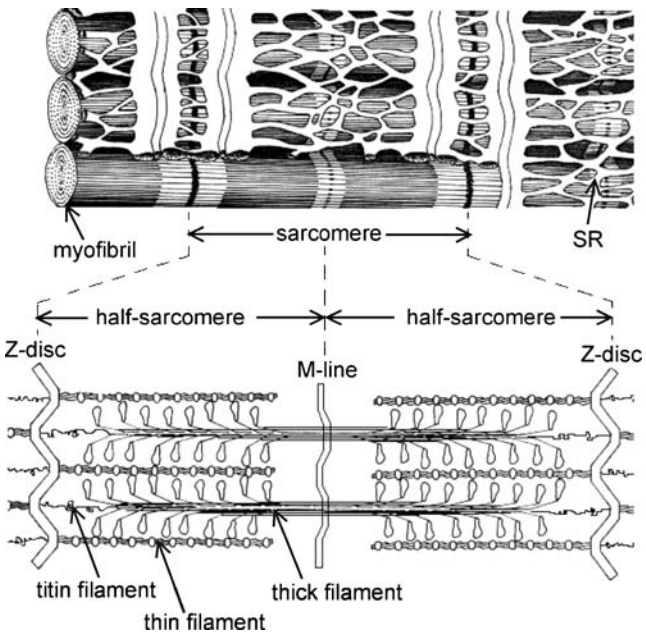

Fig. 1 Schematic drawing of a small section from a muscle cell showing some myofibrils and parts of the intracellular Ca^{2+} stores, the sarcoplasmic reticulum (*SR*). Excitation of the muscle cell leads to Ca^{2+} release from the SR into the cytoplasm of the sarcomere, the sarcoplasma. The thin diameter (≈1 μm) of the myofibril enables rapid diffusion and homogeneous distribution of Ca^{2+} within the sarcomere. Re-uptake of Ca^{2+} into the SR lowers the sarcoplasmic [Ca^{2+}] and leads to relaxation of the sarcomere. The length of a single relaxed sarcomere of vertebrates is typically ≈2.0 and 2.0–2.5 μm in cardiac and skeletal muscle, respectively. The architecture of the sarcomere is explained in the text

This structural hierarchy bundles contractile activity from the molecular level through the filament, the half-sarcomere, the myofibril, the cell and up to the muscle. The smallest complete contractile unit is the half-sarcomere of a single myofibril. The complete functional motor unit is a group of muscle fibres driven by a single motor neuron or a heart. At the molecular and filament level, contraction and relaxation are regulated by the Ca^{2+}-binding/dissociation to/from troponin C (TnC). Ca^{2+} binding results in a cascade of conformational changes involving TnC and the other thin-filament regulatory proteins, troponin I (TnI), troponin T (TnT), and Tm; this allows the cross-bridges to interact with actin [46, 75, 76]. Driven by their cyclic adenosine triphosphatase (ATPase) activity, cross-bridges exert a force on the thin filament which pulls the latter towards the sarcomere centre, the M-line; the half-sarcomere contracts and shortens [59, 61, 62]. Thereby, cross-bridge cycling kinetics determines the shortening dynamics of a particular half-sarcomere [27]. When the [Ca^{2+}] falls and Ca^{2+} dissociates from TnC, the thin filament inactivates; the force-generating interaction of cross-bridges ceases and the half-sarcomere returns to its relaxed length determined by the titin's elasticity.

If the ensemble of individual half-sarcomeres acted independently, the mechanism of striated muscle contraction would be defined by the characteristics of a single half-sarcomere. However, the kinetics of the cross-bridge cycle, in particular the transitions of cross-bridges through the force-generating states that determine the apparent rate of cross-bridge detachment from actin, depend themselves on the velocity of filament sliding [41, 59, 129]. This, in turn, depends on the overall force generated by all serially coupled half-sarcomeres. The intersarcomeric coupling of filament sliding and cross-bridge detachment becomes apparent when myofibrils relax after the end of contraction. Rapid relaxation occurs by sequential lengthening of individual half-sarcomeres which spatially propagates along the myofibril until all half-sarcomeres resume their relaxed length [135, 143]. This sequential process enables rapid release of mechanical strain and fast relaxation [117, 135, 147]. The arrangement of myofibrils in fibres and myocytes is optimized for the specific function of the organ. In skeletal muscles, the parallel alignment of myofibrils and fibres transmits their force directly to the tendons. In the heart, branched myocytes form cell bundles with a

preferred but not unidirectional orientation that wrap in helices around the cavities [109]. In both muscles, the cells are stabilised by extracellular collagen matrix, which protects them from damage by excessive stretch.

In summary, the mechanical performance of a muscle results from interactions occurring at many levels of organisation: intramolecular and intermolecular, between thick and thin filaments, between sarcomeres and between muscle cells and other structures. It is challenging, therefore, to gain insight into the many mechanisms behind the dynamics of a contraction–relaxation cycle. In principle, kinetic parameters can be obtained from preparations at all levels of the structural hierarchy, i.e. from the organ in vivo down to the isolated molecule. While the complete cellular physiology of muscle contraction and relaxation can be only studied in intact muscle preparations that comprise not only the sarcomere but also the intact Ca^{2+}-handling structures [5, 67], the gradual rise and fall of $[Ca^{2+}]$ in these preparations prevents exploration of the kinetic mechanisms which underlie the mechanical performance of the muscle. If we focus on the force-generating process and its regulation in the sarcomere, there are mainly two models for investigating their mechanisms.

The classical model for studying the Ca^{2+} regulation of contraction has been the 'demembranated' or 'skinned' muscle fibre preparation, where the cell membrane and the sarcoplasmic reticulum have been removed and the sarcoplasma is replaced by an external buffer. One limitation of this model is that diffusion of molecules, e.g. of Ca^{2+}, into the thick muscle fibres takes relatively long and it is not possible to obtain information of the fast kinetic processes in the sarcomere by changing the buffer. This problem can be overcome by loading fibres with photo-labile caged compounds that can rapidly release products, e.g. Ca^{2+} or Ca^{2+} chelators, by light flashes [4, 9, 30, 69, 93, 96, 100, 111, 149, 156]. However, the use of caged compounds has other drawbacks (discussed in [46, 117]). For these reasons, skinned fibres had been mostly used to investigate the mechanisms of Ca^{2+}-regulated contraction under steady $[Ca^{2+}]$ [16, 17, 21, 28, 34, 78, 120] and only few studies investigated the force kinetics induced by rapid changes in $[Ca^{2+}]$ (e.g. [9, 100, 111, 149]). Several studies that investigate force kinetics in intact and skinned fibres induce force transients by small sinusoidal or step-shaped length changes (e.g. [2, 3, 6, 20, 31, 63, 70, 71, 84, 118]; reviewed in [27] and [46]). These investigations give straightforward insights into cross-bridge kinetics near steady-state conditions but do not include the dynamics of Ca^{2+}-induced activation and inactivation of the thin filament that is an integral part of a physiological contraction–relaxation cycle. In contrast to cross-bridge kinetics that have been mainly studied in fibres, the kinetics of thin-filament activation and inactivation have been mainly studied, with few exceptions [9, 17] in non-contractile preparations, either with Tn alone or with reconstituted thin filaments (Tn·Tm·actin) interacting with isolated myosin subfragment S1 (e.g. [32, 33, 37, 102, 122–124]). Not only our knowledge of thin-filament regulation but also our understanding of the chemical intermediates in the cross-bridge ATPase cycle is mainly based on studies on isolated actomyosin (reviewed in [44]). This is because chemical intermediates are difficult to control and quantify in skinned fibres. Even though isolated proteins do not resemble the structured sarcomere, most theories about the molecular mechanisms of thin-filament regulation and chemomechanical energy conversion are based on a composite of isolated protein and fibre studies. In effect, the great differences in structural complexity and mechanical constraints between the two models are implicitly neglected. To at least partially bridge this gap, physiologists currently investigate the kinetic mechanisms of striated muscle regulation and contractility in single myofibrils composed of relatively few sarcomeres.

Though myofibrils were used in structural experiments that led to the sliding filament hypothesis of muscle contraction [61] and in biochemical experiments that led to the discovery of the role of Ca^{2+} for contraction regulation [151], mechanical and kinetic investigations in myofibrils started later than the pathbreaking fibre and solution studies that put the basis for classical models of cross-bridge cycle and its regulation [38, 54, 63, 97]. Major reasons why myofibrils became established as a model for mechanical studies much later than fibres are their apparent fragility and very low force levels. Because a fibre contains 100–1,000 myofibrils, the force of a single myofibril is less than 1 μN, i.e. 100–1,000 times smaller than the force developed by a single fibre. The power output of a single contracting myofibril is about the same as the one generated by a single opened ion channel. Therefore, experimental setups to measure myofibril mechanics were considered rather demanding and only few laboratories have so far developed myofibril setups that are relatively easy to handle.

Myofibrils have some definite advantages over skinned muscle fibres. They are short and thin enough to monitor the length changes of all their sarcomeres under high resolution with a compound microscope [1, 85, 119, 135, 143, 144]. Intriguingly, the collective dynamic behaviour of these serially arranged and mechanically coupled sarcomeres, the 'intersarcomere dynamics', appears to be highly organised both temporally and spatially [1, 85, 135, 143, 154]. The impact of sarcomere dynamics on the systemic response of a muscle is usually ignored [36] because, in solution studies with isolated proteins, the sarcomere structure is lacking and, in fibres, lengths of individual sarcomeres are difficult to resolve. The organised sarcomere dynamics detected in myofibrils, however, demonstrates that the physiology of

muscle contraction and in particular that of muscle relaxation remains incompletely understood until all levels of structural organisation are functionally analysed.

The most significant value of myofibrils over skinned fibres is their fast equilibration with the bathing solution. The longer diffusion distances in skinned fibres cause concentration gradients of substrates (ATP) and products (P_i, adenosine diphosphate (ADP)) during contraction [28]. In the few-micrometre-thin myofibrils, the solvent environment is absolutely defined by the applied buffer. This enables investigators to determine precisely defined *steady-state* force–[P_i] and force–[ATP] relations [145, 146, 150]. Mixing myofibrils with Ca^{2+}-rich solution induces conformational changes in cardiac TnC (cTnC) with rates as fast as ~2,000 s^{-1} [132] proving that equilibration by diffusion takes place within 1 ms. The short diffusion distance makes the myofibril an exceptionally suited and powerful model for kinetic analysis of the functional response of proteins, within a structured sarcomere, to rapid and defined changes in concentration of activators, inhibitors, substrates or products.

Two techniques have been used to rapidly change the concentration of compounds in myofibrils: (1) rapid mixing of myofibril suspensions with buffers in a reaction chamber by stopped flow or quench flow to study the transient kinetics of myofibril ATPase and myofibril Ca^{2+} regulation [7, 8, 58, 64, 88, 92, 98, 132, 134] and (2) rapid switching between two laminar flows of solution ejected by a double-channel micropipette to induce force kinetics and sarcomere dynamics in single myofibrils mounted in a force-recording apparatus [26]. The principle of this technique is illustrated in Fig. 2a. These technical developments on myofibrils make them a favourite tool to test the transferability of kinetic mechanisms proposed from protein studies to the structured muscle.

Overview of kinetic mechanisms investigated in myofibrils

A number of issues have been addressed using myofibrils as an experimental model.

Troponin regulatory kinetics The switch kinetics of muscle regulatory proteins have been, with few exceptions [9, 17], mostly studied on isolated Tn, its subunits or reconstituted thin filaments [33, 37, 102, 124]. Stopped-flow techniques applied to myofibril suspensions enables comparison

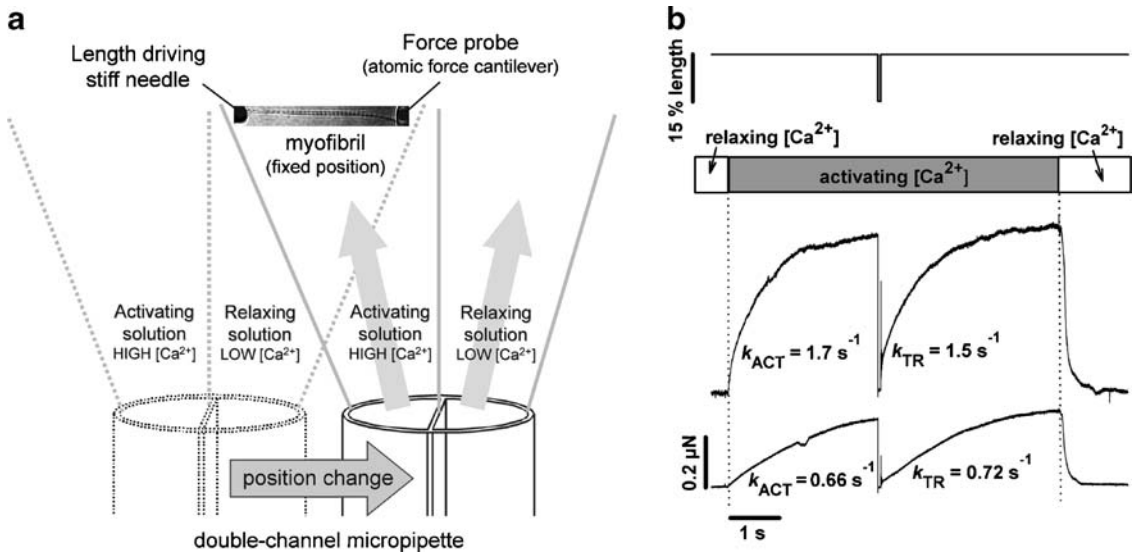

Fig. 2 Measurement of myofibril force kinetics. **a** Experimental setup. The myofibril is mounted within a chamber filled with relaxing solution to the tip of a stiff needle and the tip of an atomic force cantilever. A double-channel micropipette continuously ejects two laminar streams of solution and the myofibril is always exposed to one. By changing the position of the micropipette with a piezo actuator, the myofibril is exposed to the other solution within ≈10 ms. To induce Ca^{2+}-activated force development, the pipette is moved from the left position (flow configuration drawn in *dashed lines*) to the right (*solid lines*). To induce relaxation, the movement is reversed. To study mechanically induced force kinetics, the needle holding the myofibril on the left can be moved by a piezo actuator. **b** Typical force transients obtained from left ventricular myofibrils of guinea pig under full and partial Ca^{2+} activation at 10°C. At the time indicated by the *left vertical dashed line*, the solution was switched from relaxing pCa 7.5 to fully activating pCa 4.5 (upper transient) or to partially activating pCa 5.64 (lower transient). Following Ca^{2+} application, force develops exponentially with a rate constant k_{ACT}. During contraction at steady [Ca^{2+}], a large (15% of myofibril length) release–restretch is applied to the myofibril. Upon the release, the myofibril slackens and actively shortens at zero external load. Rapid stretch to the original length quickly returns the myofibril to isometric conditions whereupon force re-develops exponentially with a rate constant k_{TR}. At a given Ca^{2+}-activated steady-state force, the kinetics of force development by Ca^{2+} or by stretch are the same ($k_{ACT} \approx k_{TR}$). These two kinetic parameters increase in parallel with increase in the Ca^{2+} activated force (see also Fig. 5)

between the Ca^{2+}-induced switch kinetics of the isolated fluorescently labelled cardiac troponin complex (cTn) before and after it is incorporated into the sarcomere. This reveals that the kinetics of the switch on and switch off in the structured sarcomere are faster than those in solution [132].

Steady-state and transient kinetics of the cross-bridge ATPase cycle The ATPase rate and the kinetic mechanism of the initial steps in the cross-bridge ATPase cycle have been analysed by quench flow. Using this method, myofibril suspensions are rapidly mixed with ATP and then quenched at different times with acid to determine the time course of $[P_i]$ produced by ATP hydrolysis [57, 90, 92, 98].

Rapid mixing techniques are required to measure the true rate of myofibril ATPase and its Ca^{2+} dependence [57]. When Ca^{2+} activated, unloaded myofibrils irreversibly over-contract within less than a second to unphysiologically short sarcomere lengths [7, 90]. Only the first few 100 ms yield meaningful ATPase rates with structural preservation of the filament lattice [57]. Thereafter, the ATPase rate is reduced about fivefold [90]. Therefore, conventional long-term measurements greatly underestimate the true ATPase of structurally intact unloaded myofibrils.

Though the overall mechanism of the cross-bridge ATPase cycle in myofibrils is similar to the one established by studies on isolated unregulated and regulated acto-S1, myofibril studies revealed some significant differences concerning the initial steps of the cycle. While in myofibrils the cross-bridges bind tightly the ATP before they detach from the thin filament [134], the sequence of the two events in acto-S1 seems to be different [12, 104]. The size of the so-called P_i-burst indicates that the equilibrium constant of the ATP hydrolysis step is about one order of magnitude higher for myofibrils [52, 53, 58, 89, 98] than that reported for unregulated and regulated acto-S1 [50, 153]. It is therefore unlikely that the hydrolysis step modulates the rate of ATPase of a muscle as implied by studies of isolated acto-S1.

The force-generating step in the cross-bridge ATPase cycle, the so-called power stroke, is either directly or at least closely associated with the release of phosphate (P_i) from the active site of myosin [30, 44]. The introduction of a phosphate-binding protein assay [19] enables investigators to directly probe the kinetics of P_i release from Ca^{2+}-activated myofibrils [7, 64, 89, 91, 92] and leads to straightforward conclusions about chemical states. The P_i transient induced by mixing myofibrils with ATP exhibits a lag when myofibrils shorten in the absence of external load; thus, during active unloaded shortening, most cross-bridges are in ADP·P_i states [89]. When the cross-bridges are covalently cross-linked to the actin to prevent shortening of the myofibrils and mimic isometric contraction, the lag in the P_i transient disappears but a rapid initial rise of free P_i is not observed [91]. Hence, increasing the load during contraction shifts some cross-bridges from ADP·P_i-binding to ADP-binding states but the ADP-binding states are not predominant.

The rates of the late steps in the cross-bridge ATPase cycle associated with the ADP release have been explored by Chaen and coworkers [22, 128] in isometrically contracting myofibrils using fluorescent nucleotide analogues. The concept that the rate of muscle shortening is determined by steps rate-limiting cross-bridge detachment, i.e., by the late steps in the cross-bridge cycle, is a basic element of cross-bridge models [59, 114]. Correlations of the rate of ADP release from isolated acto-S1 with rates of muscle shortening suggest that shortening is rate-limited by an isomerisation closely coupled to ADP release [129, 152]. However, the studies by Chaen and coworkers [22, 128] in myofibrils were the first to prove this mechano-chemical coupling by measuring the kinetics of nucleotide release in a contracting muscle preparation.

Force kinetics during a contraction–relaxation cycle By switching rapidly between two solutions of different $[Ca^{2+}]$, kinetics of force activation and relaxation can be determined (Fig. 2). Both the initial and the final $[Ca^{2+}]$ can be specified using conventional high-affinity Ca^{2+} buffers like ethylene glycol tetraacetic acid. This technique circumvents the limitations of caged Ca^{2+} or caged Ca^{2+} chelators which have to be used in the diffusion-limited skinned fibres for similar kinetic studies. For example, the final $[Ca^{2+}]$ reached after the photolysis of caged compounds is not clearly defined nor is the homogeneity of the product within the fibre. Also, the affinities of caged Ca^{2+} chelators required to induce relaxation kinetics are too low to switch from full activation to full relaxation [46]. Therefore, the myofibril technique holds clear advantages in the investigation of relaxation kinetics.

Figure 2b shows typical force recordings of a cardiac myofibril activated and relaxed by rapidly increasing and decreasing the $[Ca^{2+}]$. While Ca^{2+}-induced force development is described by a single exponential with a rate constant k_{ACT}, relaxation induced by rapid reduction in $[Ca^{2+}]$ is biphasic [117, 135, 136, 147] as illustrated in Fig. 4: relaxation starts with a slow, seemingly linear, force decline with a rate constant k_{LIN}. This phase lasts for the time t_{LIN}, then force decays rapidly and exponentially with a rate constant k_{REL}, typically ten to 20-fold faster than k_{LIN}. Accordingly, a slightly different terminology is used by Poggesi's group [147] to indicate the rate constants of the two relaxation phases: slow k_{REL} and fast k_{REL} [147] instead of k_{LIN} and k_{REL} [136]. The meaning of these kinetic parameters is discussed in the section "Cross-bridge kinetics and thin-filament inactivation during relaxation".

Sarcomere dynamics in myofibrils Using video microscopy, length changes of individual sarcomeres in myofibrils have been analysed during contraction and relaxation leading to the discovery of highly organised 'sarcomere dynamics'. Spontaneous oscillatory contractions (SPOCs) characterised by alternated rapid lengthening and slow shortening of individual sarcomeres were analysed in skeletal and cardiac myofibrils by partially activating them by high [ADP] instead of Ca^{2+} (so-called ADP-SPOC) [1, 125, 154]. In cardiac myofibrils, SPOCs were shown to occur at low partial Ca^{2+} activation (Ca-SPOC) [85, 126] and also at full Ca^{2+} activation in millimolar [P_i] (P_i-SPOC) [143]. An intriguing feature demonstrated for all types of SPOCs is that the rapid lengthening propagates from one sarcomere to the next [1, 85, 143]. Cardiac myofibrils exhibit, therefore, inherent coordinated behaviours of their sarcomeric units which is likely present in the heart as well [125, 126].

A direct physiological manifestation of sarcomere dynamics appears to be the sequential rapid lengthening and (mechanical) relaxation of individual sarcomeres, evident at the onset of the fast phase of relaxation [135]. The pronounced filament sliding that occurs during the fast relaxation phase is very likely the reason for the fast rate of cross-bridge detachment, reflected in k_{REL} that is approximately ten to 20-fold faster than the isometric detachment rate, reflected by k_{LIN} [117, 135, 136, 147]. The breakdown of isometric conditions at the single sarcomere level is one of the most or perhaps the most important determinant for the rapidity of cardiac relaxation.

Recent technical developments that mark the half-sarcomere boundaries (Z-lines and M-bands) by fluorescent antibodies to track the length changes of individual half-sarcomeres have refined investigations of the relaxation process to the half-sarcomere level [143]. The observed dynamics of individual half-sarcomeres demonstrated that the half-sarcomere is the smallest functional unit in muscle contraction and relaxation [143] and enables us to adequately test elementary mechanisms of striated muscle function at the half-sarcomere level [144].

Dynamics of titin's mechanical function As soon as cross-bridges detach, the giant protein titin determines the diastolic mechanical properties of cardiac myocytes. Epitope labeling of different targets on the titin molecule has successfully elucidated titin mechanical properties within the myofibril [87]. Elastic recoil of titin can drive high-speed passive shortening of stretched myofibrils and support systole [110]. Alternatively, elastic recoil of titin can promote re-lengthening of shortened sarcomeres back to slack length and, thereby, aid early diastolic filling [51]. It remains to be discovered whether or not the viscoelastic properties of titin can significantly modulate the kinetics of myofibrillar force development and/or relaxation. These will strongly depend on the load and the sarcomere length changes during the contraction–relaxation cycle.

Comparative physiology Comparison of skeletal muscle fibres from different species, muscle or fibre type has increased understanding of structure–function relations in skeletal muscle contraction [13]. Additionally, myofibrils from skeletal and cardiac muscle with similar shape and sarcomere length can be compared. From this, cardiac-specific functional features can be extracted and assigned to cardiac-specific protein isoforms. Such mechanical investigations on single myofibrils have determined, for example, that the different passive stiffness of cardiac and skeletal sarcomeres arises from the titin isoforms expressed [86].

Kinetic properties intrinsic to myofibrils are a major determinant of cardiac dynamics. Contraction and relaxation kinetics of human atrial myofibrils are fourfold faster than those of human ventricular myofibrils [116]. This correlates with the higher expression of the α-myosin heavy chain isoform (α-MHC) in human atrial myofibrils. In addition, in ventricular myofibrils, there are species-specific differences in force kinetics and frequencies of SPOC oscillations which correlate with the species-specific heart rate [125, 126, 136]. It is noteworthy that large differences in myofibrillar force kinetics and sarcomere dynamics also exist among species which solely express β-MHC in the ventricle [125, 126, 136]. Hence, the α/β-MHC isoform ratio is not the only sarcomeric determinant of cardiac dynamics. Studies of stretch-induced force kinetics in skinned fibres from skeletal and cardiac muscles also suggest that myosin light chain (MLC) isoforms may underlie species-dependent variability in cross-bridge kinetics [2, 3]. The role of regulatory and essential MLCs on the kinetics of myofibril contraction and relaxation remains, however, to be tested.

Replacement of endogenous Tn and/or Tm with exogenous proteins can be used in myofibrils to investigate the role of thin-filament regulation (or of specific isoforms) on contraction–relaxation kinetics [35, 115, 131]. For example, skeletal myofibrils exchanged either with the fetal cardiac TnI isoform, i.e. slow skeletal TnI (ssTnI), or with the adult cardiac TnI isoform (cTnI) exhibit markedly different Ca^{2+} sensitivities but no change in the kinetics of Ca^{2+}-controlled force development and relaxation [35]. This is evidence that the intrinsic cross-bridge cycling rate is not directly altered by thin-filament regulation.

Altered myofibril function in cardiomyopathies Myofibril force kinetics have been shown to be altered by cardiomyopathy-related mutations or post-translational modifications of cTnI [65, 80, 107] and by mutations of myosin heavy chain [11]. In addition, passive mechanical properties of

myofibrils are altered by titin-dependent mechanisms in dilated and ischemic cardiomyopathy [99, 108].

Because the cardiac myofibril model is particularly useful for studying sarcomere relaxation kinetics and titin-dependent diastolic properties of cardiac muscle, it has great potential for the elucidation of the pathophysiological mechanisms of diastolic dysfunction at sarcomere level [65, 80, 107]. Diastolic dysfunction can lead to diastolic heart failure and is a highly prevalent feature in hypertrophic cardiomyopathy, diabetes and the ageing heart. This opens many options for future myofibril research especially because many myofibrils can be prepared and tested from a very small cardiac biopsy or ectomy [11, 99, 108]. Therefore, the myofibril model provides direct access to study disease-related mechanical and kinetic dysfunctions in human cardiac sarcomeres.

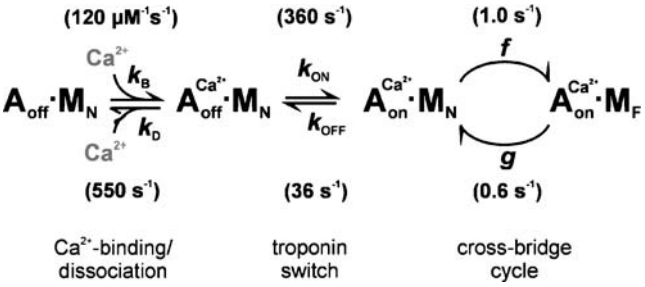

Fig. 3 Model of Ca^{2+}-regulated cross-bridge kinetics. *A* represents the regulatory unit consisting of Tn, Tm and seven associated actins and *M* the myosin cross-bridge. Ca^{2+} binding to and dissociation from TnC is in rapid equilibrium. Ca^{2+} binding is defined by the second-order rate constant k_B, Ca^{2+} dissociation by the first-order rate constant k_D, i.e., only the Ca^{2+}-binding reaction depends on the free [Ca^{2+}]. While binding Ca^{2+}, the regulatory unit is in a rapid equilibrium between switched-on (A_{on}) and switched-off (A_{off}) states. Cross-bridges next to a switched-on unit (A_{on}) cycle with constant rates *f* and *g* through force-generating (M_F) and non-force-generating (M_N) states. Cross-bridges next to a switched-off unit do not cycle and remain in non-force states. Values for rate constants are for ventricular myofibrils from guinea pig at 10°C; these are derived from kinetic data in [132, 136] and from Fig. 5 of this review

General implications of myofibril kinetics for contraction and relaxation

As summarized in the previous section, myofibrils are suitable for the basic kinetic analysis of a contraction–relaxation cycle. Determining the rate of Ca^{2+}-regulated thin-filament activation (and inactivation) and the rate of force development (and relaxation) in myofibrils aims to answer these fundamental questions: (1) which of the following processes rate-limits contraction and relaxation: the rates by which Ca^{2+} binds to and dissociates from TnC, the rates by which thin-filament regulatory proteins switch on and off or the turnover rates of cross-bridge attachment and detachment? (2) How do the kinetics of contraction and relaxation depend on [Ca^{2+}] and which mechanism can explain this dependence? (3) What fraction of cross-bridges can be estimated to cycle from the observed rates of thin-filament activation and inactivation? What fraction of the overall cycle time do cycling cross-bridges spend in force-generating states according to force kinetic parameters? (4) How do sarcomere dynamics affect cross-bridge turnover kinetics?

As described in the following sections, the essential kinetic features of Ca^{2+}-induced myofibrillar thin-filament activation and of force development can be expressed by a remarkably simple model (Fig. 3). In this model, the same kinetic parameters describing force activation also account for the rate of thin-filament inactivation as well as for the rate of the initial slow phase of relaxation.

Kinetics of myofibril Ca^{2+} activation and inactivation

Recently, it has been shown that the structural environment of cTn provided by myofibrils affects the rate of the Ca^{2+}-controlled thin-filament regulation. The kinetics of the switch on and switch off of cTn are faster when the complex is incorporated into cardiac myofibrils than for the isolated complex [132]. Additionally, studies on reconstituted thin filaments interacting with non-cycling rigor S1-heads suggest that actin, in particular, and strong-binding cross-bridges influence the kinetics of the cTn switch [33, 122, 123]. This model does not represent the full set of sarcomeric proteins in a myofibril nor the complete physiologic situation of cycling cross-bridges in a contracting or relaxing muscle.

Solzin et al. [132] show that Ca^{2+} induces two subsequent conformational changes in cTn. The first conformational change is so rapid that it approximates a diffusion-limited reaction ($k_B \sim 10^8$ M^{-1} s^{-1}) and the only plausible implication is that it represents the rapid binding of Ca^{2+} to cTnC. The second slower phase is probably the regulatory conformational change in TnC. This is corroborated by the findings of Bell et al. [9] on skinned fibres, who studied the kinetics of conformational changes of cTnC and of force development after flash-photolysis of caged Ca^{2+}. They also found two subsequent conformational changes. However, it is the fast phase in the study of Bell et al. [9] which kinetically correlates with Solzin's [132] slow phase. The very rapid Ca^{2+} binding was not detected in Bell's study, probably because of the insensitivity of their method. Bell et al. additionally detected a slow conformational change that correlated with the kinetics of force development and was not detected in the myofibril experiments by Solzin et al. This could arise from a contraction-induced torsion of the thin filament sensed by

their polarisation technique or reflect a feedback of force-generating cross-bridges on the switch on. In Solzin's experiments, the myofibrils contract freely in the absence of external load and, due to the low fraction of force-generating cross-bridges under such conditions, a feedback may have been missed. In summary, the two complementary experimental approaches by Bell and Solzin in studies of Ca^{2+}-induced changes of cTnC reveal three processes: first, the very rapid, possibly diffusion-limited, Ca^{2+} binding; second, the conformational change of cTn which induces contraction (termed here as the cTn switch); and third, a slow conformational change in cTn likely driven by the feedback from force-generating cross-bridges on the switch on.

As the cTn switch is more than ~20-fold faster than the rate of force development [9, 132] [132], it is not directly rate-limiting contraction. Furthermore, studies on intact cardiomyocytes (e.g., [5]) demonstrated that the force transient significantly lags the Ca^{2+} transient. The results of these three studies taken together argue that the sequence of events during activation is a rapid increase of intracellular Ca^{2+} followed by an even faster Ca^{2+} binding to cTnC that induces a fast cTn switch regulating the relatively slow turnover of cross-bridges. The last clearly rate-limits contraction.

On relaxation, rapid reduction of $[Ca^{2+}]$ in myofibrils induces an intermediately fast switch off of cTn (k_{OFF}~ 40 s^{-1}) which limits Ca^{2+} dissociation (k_D calculated to be ~550 s^{-1}) although it does not directly rate-limit force relaxation [132, 138]. Because the kinetics of force relaxation is biphasic, correlation of the switch-off kinetics with force is more complex than for Ca^{2+} activation (Fig. 4c). The biphasic kinetics consist of the initial, slow and linear force decay, probably when the thin filaments are switched off ([117, 135, 147]; this review), followed by the fast exponential phase when a single sarcomere rapidly lengthens and the rate of cross-bridge detachment via forward and backwards cycling increases (see section "Sarcomere dynamics during relaxation and its relation to cross-bridge kinetics"). Figure 4c shows that in cardiac myofibrils about half of the switch off occurs within the first 20 ms; this could determine the early part of the slow relaxation phase but unlikely its whole (~100 ms) duration. It remains to be tested whether the situation is similar in skeletal myofibrils. Interestingly, it has been recently shown that the duration of the slow relaxation phase is increased or decreased by few tens of millisecond in rabbit psoas myofibrils exchanged with fast skeletal TnC mutants that have a reduced or increased Ca^{2+} dissociation rate, respectively, while the rates of myofibril relaxation were unaffected [78]. Hence, although the present studies on cardiac myofibrils suggest no direct rate-limiting effect of cTn on the kinetics of force relaxation, skeletal muscle may be different.

Furthermore, in the study by Solzin et al. [132], the switch-off rate has been determined in myofibrils floating free in a suspension, obviously not under isometric conditions, and relaxation is likely initiated from very low numbers of force-generating cross-bridges. If cycling, force-generating cross-bridges inhibit the switch off as discussed ahead in the section "Cross-bridge kinetics and thin-filament inactivation during relaxation"; the switch off of cTn during a physiological relaxation under load might be slower. What happens during loaded relaxation, i.e.

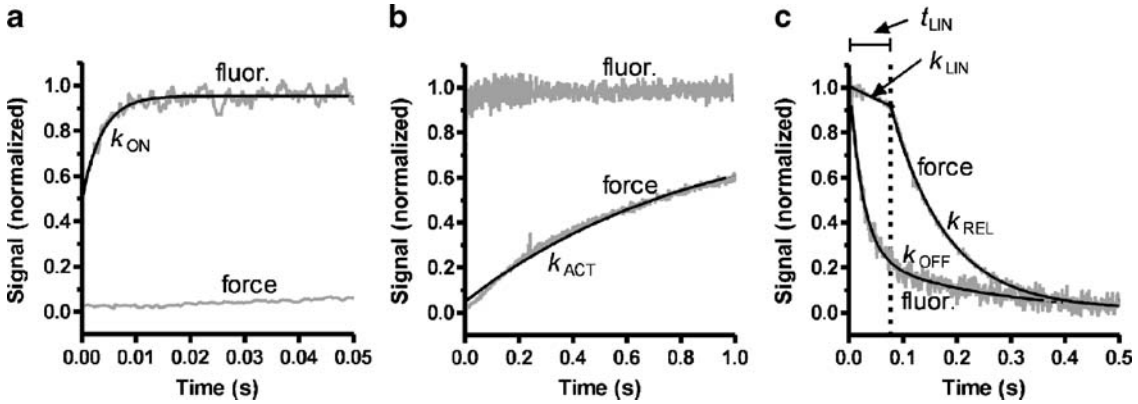

Fig. 4 Comparison of the time course of switch on and switch off of Tn with the time course of force development and relaxation (*left* to *right*). To measure the switch kinetics indicated by the fluorescence transients (*fluor.*), IANBD-labeled human $cTnC^{C35S}$ was incorporated into myofibrils, and the myofibrils were mixed at time=0 with Ca^{2+} (switch-on kinetics, **a** and **b**) or with the Ca^{2+} buffer BAPTA (switch-off kinetics, **c**) in a stopped-flow apparatus (SFM-400/S; Bio-Logic, Claix, France). Myofibril force transients (force) were measured in a mechanical setup using the rapid solution change technique depicted in Fig. 2. **a** Fluorescence and force transients during the first 50 ms following a rapid increase in $[Ca^{2+}]$ from pCa 7.5 to pCa 4.5. **b** Same as in **a** but on a 20-fold-longer timescale to show the much slower force development compared to the switch on of myofibril Tn. **c** Fluorescence and force transients induced by reducing $[Ca^{2+}]$ from pCa 4.5 to 7.5. Note that, during relaxation, the force decays biphasically: first, slowly and linearly with a rate constant k_{LIN} and then rapidly and exponentially with a rate constant k_{REL}. The *black lines* are fit curves yielding the kinetic parameters indicated in the graph

whether the switch off occurs before or concurrently with cross-bridge detachment, is not known yet.

Cross-bridge kinetics during force generation

Experiments with skeletal and cardiac myofibrils from different species reveal that force development kinetics are the same whether the force generation is induced by Ca^{2+} activation of the myofibril or by a large mechanical perturbation [116, 135, 136, 147, 148]. The latter, called force *re*-development, is induced by returning from a transient period of unloaded shortening back to isometric conditions and has been widely investigated in skinned fibres [16]; for a review, see [46]. The basic idea is that, when returning to isometric contraction, cross-bridges redistribute by cycling to the force-generating states; thus, the rate constant k_{TR} of the exponential transient of force re-development reflects the overall rate limit for the cross-bridge ATPase cycle [18]. The mechanical perturbation is applied to the preparation in steady Ca^{2+} activation with only minor changes in thin-filament activation [17]. Therefore, k_{TR} should be much less influenced by the switch on of the thin filament than k_{ACT}, the rate constant of Ca^{2+}-induced force development. The similarity of k_{ACT} and k_{TR}, illustrated in Fig. 2b, implies that the kinetics of the Ca^{2+}-induced force development k_{ACT} is not affected by the switch-on rate of the thin filament. This conclusion is in good agreement with that in the previous section that Ca^{2+}-induced thin-filament activation in cardiac myofibrils [132] and skinned fibres [9] is too fast to exert any rate-limiting effect on force development.

Regnier et al. [120] reported that k_{ACT} can be significantly slower than k_{TR} in skinned cardiac trabeculae. This is at variance with myofibril studies [116, 135, 136, 147, 148] as well as some fibre [149] and cardiac trabeculae studies [111]. The contrary result may be related to different experimental conditions including the progressive accumulation of P_i during contraction in the case of cardiac trabeculae and higher basal P_i levels: k_{TR} may have been measured at higher $[P_i]$ than k_{ACT} by Regnier et al. [120]. In addition, activation is a coupled process of thin-filament activation and cross-bridge binding and cycling, as described in the model reported below (see Fig. 3). Based on these coupled processes, one can predict that at high $[P_i]$ k_{TR} may increase more than k_{ACT} as $[P_i]$ increases the cross-bridge cycling rate and thin-filament activation may contribute limiting force generation during Ca^{2+} activation.

A basic feature of k_{ACT} and k_{TR} is that they both increase with increasing $[Ca^{2+}]$ (Fig. 5). This is a common finding in skeletal myofibrils and cardiac myofibrils [116, 136, 147] and has been previously shown in skinned cardiac fibres [111]. An increase of k_{ACT} with increasing $[Ca^{2+}]$, i.e. slower kinetics of force activation at low than at high $[Ca^{2+}]$, has been also shown for skinned myocytes [4] and skeletal fibres [149]. At first glance, this might appear to contradict the conclusion that Ca^{2+} activation is rapid and not rate limiting even at low $[Ca^{2+}]$. However, the so-called rate *modulation* of the *observed* rate constant of a slow process through the dynamic equilibrium of a much faster process is a common

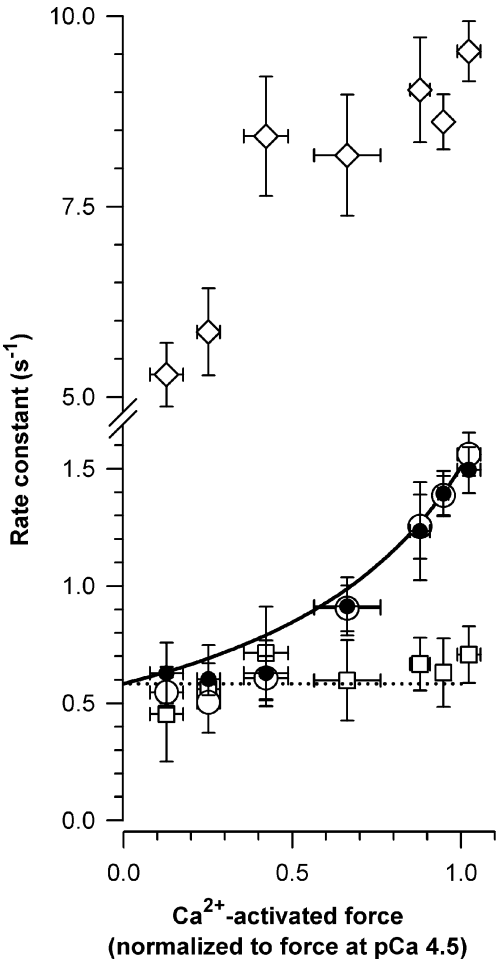

Fig. 5 Dependence of force kinetic parameters on Ca^{2+}-activated force. Cardiac myofibrils from guinea pig were activated at different pCas, mechanically perturbed, and afterwards relaxed by switching to pCa 7.5 (for experimental protocol and force transients, see Fig. 2b). Rate constants of Ca^{2+}-induced force development (k_{ACT}, *open circles*) and of mechanically induced force re-development (k_{TR}, *filled circles*) were obtained by fitting mono-exponential functions to force transients. Force decays following Ca^{2+} reduction were fitted by a biphasic linear–exponential function (see Fig. 4c) yielding the rate constant for the initial, slow, linear phase, k_{LIN} (*squares*) and the rate constant for the fast exponential phase of relaxation, k_{REL} (*diamonds*). Note the ten to 20-fold-higher values of k_{REL} (*break* in the *y*-axis) compared to k_{LIN}. The *solid* and *dotted lines* represent model calculations of the rate constant of the force development and that of force decay, respectively. The calculation was done based on the model in Fig. 3 using the values k_D=550 s^{-1}, k_B=120 μM^{-1} s^{-1}, k_{OFF}=36 s^{-1}, k_{ON}=360 s^{-1}, f=1.0 s^{-1}, and g=0.6 s^{-1}. As predicted by the model, the data of k_{LIN} are independent on the activating $[Ca^{2+}]$ and very similar to those of k_{ACT} and k_{REL} at low $[Ca^{2+}]$

feature of sequentially coupled reversible reactions. While the observed rate constant of force production ($k_{obs}=k_{ACT}=k_{TR}$) is not directly rate-limited by the fast switch (k_{ON}, $k_{OFF} \gg k_{obs}$), the value of k_{obs} varies between the rate constant of cross-bridge detachment (g) and the sum of the rate constants for attachment and detachment ($f+g$) depending on the position of the dynamic equilibrium, i.e. on the $[Ca^{2+}]$-dependent ratio of switched-on and switched-off states (see model and definition of transitions and states in Fig. 3). Note that, in the model, the switch on and off of the regulatory units and the two transitions in the cross-bridge cycle are assumed to be intrinsic $[Ca^{2+}]$-independent rates. Accordingly, k_{ON}, k_{OFF}, f and g are all treated as fixed rate constants that do not vary with $[Ca^{2+}]$. f is the slow intrinsic rate of the cross-bridge cycle while k_{ON} refers to a rapid regulatory conformational change in Tn that occurs *after* an even faster Ca^{2+}-binding reaction. Though only the extremely fast kinetics of Ca^{2+} binding are directly Ca^{2+} dependent, the observed rate constant of force kinetics is apparently $[Ca^{2+}]$ dependent. The minimum requirements for significant rate modulation of force kinetics by Ca^{2+} are $k_{ON} \geq k_{OFF}$ and $f \geq g$. These two requirements have to be fulfilled anyway for efficient activation (high fraction of switched-on actins) and force generation (high fraction of force-generating states) at high $[Ca^{2+}]$.

When $[Ca^{2+}]$ increases, in the model in Fig. 3, the rate of Ca^{2+} binding increases according to $k_B[Ca^{2+}]$ while the rate of Ca^{2+} dissociation k_D remains unaffected [37, 132]. At very low $[Ca^{2+}]$, when $k_B[Ca^{2+}] \ll k_D$, most regulatory units are switched off. At very high $[Ca^{2+}]$, when $k_B[Ca^{2+}] \gg k_D$, the fraction of switched-on units approximates $k_{ON}/(k_{OFF}+k_{ON})$ whereby the values of k_{OFF} and k_{ON} can be estimated from the observed $[Ca^{2+}]$ dependence of the switch kinetics [132]. As the fraction of switched-on regulatory units increases, more cross-bridges in non-force states are able to interact with a switched-on unit. Rate modulation of force kinetics (change in k_{ACT} or k_{TR}) is generated by the relative fluxes between states. Flux through f starts from the $A_{on}{}^{Ca2+}\cdot M_N$ state in Fig. 3 and therefore competes with the flux of this state to switched off states while flux through g is unaffected. At low $[Ca^{2+}]$, the equilibria are shifted towards off states and flux through f ceases. In the other extreme, when all regulatory units are turned on, all cross-bridges cycle like in a closed loop via f and g and the observed overall rate constant becomes the sum of f and g according to the classical situation of a reversible reaction among two states. Shift in rapid equilibria towards the on state, therefore, modulates the observed rate constant k_{ACT} and k_{TR}, from values similar to g at low $[Ca^{2+}]$ to a theoretical maximum of $f+g$, reached when all regulatory units are switched on. Realistically, even at saturating $[Ca^{2+}]$, some regulatory units will be switched off and their fraction is given by $k_{OFF}/(k_{ON}+k_{OFF})$. The more units remain switched off, the more the intrinsic cross-bridge cycling rates ($f+g$) are underestimated by the values of k_{ACT} and k_{TR} at high $[Ca^{2+}]$.

The relative increase of k_{TR} and k_{ACT} between low and high $[Ca^{2+}]$ is typically smaller in slow than in fast skeletal and cardiac muscle [103, 111, 117] (see also data in Fig. 5). In the current model, this could result either from a lower fraction of switched-on regulatory units (lower k_{ON} to k_{OFF} ratio) or from a lower duty ratio of cycling cross-bridges (lower f to g ratio). In the slow ventricular myofibrils of the guinea pig, however, k_{on}/k_{off} is already high (i.e., ≥10) [132]. It seems therefore unlikely that the weaker Ca^{2+} dependence of k_{TR} and k_{ACT} in slow muscles results from incomplete activation at high $[Ca^{2+}]$. This conclusion is in agreement with the previous interpretation that fast muscles have a higher f to g ratio [103]. Nevertheless, in slow skeletal and cardiac muscles, k_{TR} and k_{ACT} increase at least ≈3-fold with $[Ca^{2+}]$, indicating that f is at least as large as g. In the example reported in Fig. 5 for ventricular myofibrils from guinea pig (Fig. 5), k_{ACT} and k_{TR} increase from 0.6 s^{-1} at low $[Ca^{2+}]$ to 1.6 s^{-1} at high $[Ca^{2+}]$.

The rate modulation of k_{ACT} and k_{TR} predicted by the model in Fig. 3 is similar to the one expected from Brenner's model [16]. Though the latter model does not include Ca^{2+}-dependent switch kinetics, taking an appropriate value for the apparent rate constant f_{app} at maximum force leads to a very similar-shaped k_{TR}–force relation as that in Fig. 5. Complete identity of the two relations is reached for the limiting case of infinite k_{OFF}. Nevertheless, as $k_{OFF} \gg f$, the deviation between the two relations is negligible. Accordingly, the significance of the Ca^{2+}-independent apparent rate constant g_{app} in Brenner's model [16] is essentially the same as the one of g in our model (Fig. 3): while the Brenner model predicts that at zero force $k_{TR}=g_{app}$, in analogy, our model predicts that at zero $[Ca^{2+}]$ $k_{TR}=g$. The latter identity is only strictly correct for infinite high k_{OFF}, but again, as long as $k_{OFF} \gg f$, any deviation of k_{TR} from g can be neglected.

From the rate constants k_{ON}, k_{OFF}, f and g given in Fig. 3, we can calculate the fractional occupancy of cross-bridge states under isometric conditions. At high $[Ca^{2+}]$ (pCa 4.5), most (96%) of the regulatory units are switched on, thereby allowing 96% of cross-bridges in the actin–myosin overlap region to cycle. Only 4% of cross-bridges are next to an A_{off} state and are thus currently not recruited (non-cycling fraction). About 60% of all cross-bridges occupy the force-generating state and about 36% belong to the currently cycling but non-force-generating fraction. The fraction of cycling cross-bridges in force-generating states (62%) is called the duty ratio; it is $f/(f+g)$ in the model (Fig. 3). An almost identical duty ratio (~60%) and a slightly lower fraction of force-generating cross-bridges (~50%) has been previously reported from measurements of force re-development kinetics and ATPase of skinned rat

cardiac trabeculae [34]. The values of 50% and 60% for force-generating cross-bridges estimated from kinetic studies on cardiac fibres and myofibrils are in the upper range of those estimated from structural and mechanical studies on fast skeletal muscle fibres (for a review, see [27]). X-ray diffraction studies estimate 50–75% [77] in agreement with the kinetic studies. On the other hand, recent fluorescence polarisation studies indicate that only 10–35% of heads are susceptible to stretch-induced changes in orientation [56]. Stiffness measurements which take into account filament compliance suggest that 33% of heads generate force [84]. This is about half the 60% estimated here for cardiac myofibrils. It is unlikely that the difference is due to muscle type because the rate modulation by Ca^{2+} of k_{TR} and k_{ACT} is larger in fast skeletal myofibrils and fibres [16, 117, 147] than in slow cardiac myofibrils ([136] (data in Fig. 5 and for fibres [111]). Hence, kinetic data of fast skeletal muscle fibres would place the duty ratio even higher than 60%. It has been argued that the second head of a myosin molecule binds only under external stretch [20]. If only one of the two heads cycles during an isometric contraction, i.e. if half of the heads are prevented from cycling and do not participate in rate modulation of force kinetics, then the value of 62% estimated from the $[Ca^{2+}]$ dependences of k_{ON} [132] and the k_{TR} data (Fig. 5) would be twofold too high. However, the value of 50% estimated by de Tombe and Stienen [34] would be unaffected by non-cycling cross-bridges because it is based on the ATPase rate.

In summary, modelling based on kinetic studies on cardiac and fast skeletal muscles predict that ≥50% of cross-bridges occupy force-generating states during maximum Ca^{2+}-activated contraction. This implies that both muscles maximise isometric force by making use of most of its motors. In contrast to this conclusion, a low fraction of force-generating cross-bridges was proposed to protect the skeletal muscle under stretch by enabling stretch-induced binding of the second head of the myosin to actin [20]. However, mechanical studies indicate that stretch increases the fraction of force-generating states only by about 15% [25, 94]. The same relative increase (≈15%) is obtained by slowing down the transition of cross-bridges through force-generating states with high [ADP] [95]. Such limited further accumulation of cross-bridges in force states during maximal isometric contraction can be simply explained if most cross-bridges are already in force states.

Cross-bridge kinetics and thin-filament inactivation during relaxation

The rapid kinetics of cTnC, isolated cTn and cTn incorporated in myofibrils confirm the idea that, in the steady state, Ca^{2+} binds and dissociates continuously and rapidly from cTnC [32, 132]. When $[Ca^{2+}]$ falls, the rate constant of Ca^{2+} dissociation is unchanged, but the second-order rate constant of Ca^{2+} binding decreases. According to the model and rate constants for cardiac myofibrils in Fig. 3, cross-bridges detach via g but have little chance to re-enter force-generating states via f because the regulatory unit switch off is ~40-fold faster than f. Thus, flux via f becomes negligible on Ca^{2+} removal. Because $k_{OFF} \gg g$, the rate of force relaxation following Ca^{2+} removal is expected to be almost solely rate-limited by g. In fact, this agrees with experimental findings for cardiac and skeletal myofibrils of different species. The rate constant k_{LIN} of the initial slow relaxation phase is generally similar to those of force development and re-development at low $[Ca^{2+}]$ [116, 117, 135, 136, 147], which reflects g [16]. As outlined in the previous chapter, g can be estimated from the value of either k_{ACT} or k_{TR} at very low Ca^{2+} activation or, more exactly, from their values extrapolated to zero $[Ca^{2+}]$ or zero Ca^{2+}-activated force. Both g values estimated from k_{ACT} and from k_{TR} at zero force are in agreement with that estimated from k_{LIN} (Fig. 5). Similar agreements between these parameters were found in several types of skeletal and cardiac myofibrils [117, 136, 147]. This striking symmetry in kinetics of relaxation and contraction led to the conception that k_{LIN} is determined by the same transitions as the ones defining the kinetics of isometric force development at low $[Ca^{2+}]$. The latter had been shown by Brenner [16] to be governed by 'g_{app}', the apparent Ca^{2+}-independent rate constant by which cross-bridges leave force-generating states. In this context 'g_{app}' is equivalent to the 'isometric g' in the classic cross-bridge model of Sir AF Huxley [59] and to the g in our model (Fig. 3). Therefore, k_{LIN} can be interpreted to reflect an intrinsic kinetic property of cross-bridges that cycle under isometric conditions; in short, $k_{LIN} \approx g$ [117, 135, 148].

Relaxation transients of skeletal and cardiac myofibrils reveal that the value of k_{REL} is typically ten to 20-fold higher than k_{LIN} [117, 135, 136, 148]. If force is assumed to change in proportion with the number of force-generating cross-bridges, this indicates that cross-bridge detachment becomes ten to 20-fold faster with the beginning of the fast relaxation phase. Consistent with this assumption, force was found to change in proportion with stiffness which is thought to measure cross-bridge attachment [16]. However, recent investigations refining the force–stiffness relation reveal significant non-linearities [84]and non-proportional changes of force and stiffness accompanying force activation and force relaxation in fibres [6, 100]. Whether or not these findings imply a significant non-proportional behaviour of force states depends in turn on the proportionality and nature of the stiffness. Proposed complications of the interpretation of force transients are strain changes in force-generating cross-bridges during a force transient [6], strain

dependence of transition rates [21], end compliance [142] and filament compliance [101]. However, the relative error indicated by the non-linear behaviour of stiffness during the time course of relaxation [6] compared to the ten to 20-fold increase in force kinetics during rapid relaxation is small. Moreover, myofibril stiffness, which depends on the cross-bridge attachment, is similar if measured during force development or force relaxation under similar force levels [138]. Hence, there should be no greater restrictions in the evaluation of cross-bridge kinetics from force relaxation as evaluation of them from force development transients; it seems safe to state that the rate of cross-bridge detachment increases by an order of magnitude during the fast relaxation phase. The only way to successfully simulate the whole force decay during relaxation by the model in Fig. 3 is to assume that in the transition from the slow to the fast relaxation phase g increases ~10-fold.

The integration of myofibril relaxation kinetics with the mechanistic models of thin-filament regulation is not trivial [139]. The well-known steric model of thin-filament regulation explains contraction–regulation coupling by a Ca^{2+}-induced conformational change of the regulatory Tn–Tm unit that unblocks a strong binding site on actin for myosin [49, 60, 113]. Major support for the model comes from 3D reconstruction of electron micrographs from reconstituted thin filaments decorated with isolated nucleotide-free myosin heads [82]. These heads act as allosteric activators by stabilising the Tn–Tm unit in the on position [15, 83, 102, 140]. This effect is so powerful that, once ≈30% of actin sites are occupied by nucleotide-free heads, the regulatory system is fully activated even in the absence of Ca^{2+} [15, 48, 102].

One idea developed as an extrapolation of thin-filament activation by rigor heads is the proposal that force-generating cross-bridges can also contribute to activation. However, such a mechanism for activation introduces a problem into muscle relaxation: how can cross-bridges be prevented from continuing to cycle when Ca^{2+} is removed, if they keep the thin filament activated independent of Ca^{2+} [14]? With 60% of cross-bridges residing in force-generating states during a maximum Ca^{2+} activation, as outlined in the previous chapter, on average about two heads would be bound to a regulatory unit formed by seven actins and the Tn·Tm complex. When Ca^{2+} is removed and one head detaches via g, the other head would still allosterically inhibit the switch off and keep the Tm·Tn switched on; hence, the heads next to this unit would still enter force-generating states via f. The more new force-generating interactions are formed after Ca^{2+} removal, the more would relaxation be delayed. Thus, the kinetics of relaxation should be very sensitive to the amount of acto-myosin interactions. To demonstrate this sensitivity, addition of NEM-S1 to skinned fibres, a treatment that traps S1 in a rigor-like state, slows down relaxation kinetics [42]. In contrast, the fraction of cycling force-generating cross-bridges does not significantly affect relaxation kinetics [117, 137, 147]. Thus, relaxation kinetics are independent on contraction history suggesting that neither the fraction of force-generating cross-bridges nor the level of Ca^{2+} activation prior to Ca^{2+} removal affects relaxation kinetics [117, 137, 147]. Hence, the extent of the feedback between cycling force-generating cross-bridges and thin-filament activation seems to be negligible or at least much less than that exerted by nucleotide-free heads. This point of view is also supported by X-ray diffraction studies showing that the structural features of cross-bridges present during contraction are different from those of nucleotide-free cross-bridges present under rigor conditions [77].

If force-generating cross-bridges do not sterically interfere with the switching off of the regulatory strand, as might be expected in the steric blocking model of regulation, how might relaxation be indifferent to their presence? There are alternative regulatory mechanisms. Instead of directly regulating actin–myosin interaction by blocking or unblocking binding sites, the state of the thin filament may allosterically control the transition of cross-bridges to force states [23, 24, 43, 121]. To control the transition instead of the binding would be advantageous for muscles, especially for the myocardium, enabling a fast reversible relaxation that is not counteracted by the cross-bridges formed during contraction. This is of special importance under high stress when the heart has to work at both higher loads and higher relaxation rates [112].

Sarcomere dynamics during relaxation and its relation to cross-bridge kinetics

The transition from the initial slow force decline to the rapid force decay occurs when a single mechanically weak sarcomere in the myofibril rapidly lengthens [135, 143]. Starting from this sarcomere, the rapid elongation propagates to the adjacent sarcomere [117, 135] or more exactly to the adjacent *half*-sarcomere [143]. Quantitative modeling of serial half-sarcomere dynamics based on spatially compressed models that implicate unidirectional strain in the axial direction cannot describe this spatially organised behaviour. It has been proposed, therefore, that the sequential relaxation of half-sarcomeres along the myofibril is mediated by non-axial structures like the Z-line and the M-band [142, 143]. Due to their transverse stiffness, these structures transmit the changes of the filament lattice that accompanies cross-bridge detachment in a given half-sarcomere to the adjacent half-sarcomere only, thereby promoting cross-bridge detachment in it [142, 143]. Also, changes in filament lattice spacing have been also proposed to modulate the sarcomere dynamics observed in SPOCs

[127]. Consistent with transverse structures determining the spatial propagation of sarcomere dynamics is the observation that the propagation across the stiffer Z-disc takes longer than it takes across the less stiff M-band [143]. Nevertheless, in principle, all non-axially arranged structures that favour cross-bridge detachment, like the helical arrangement of filamentous proteins that produce short-ranged perturbations along the thick and thin filaments, could contribute to the spatial propagation of sarcomere dynamics.

Stretch accelerates relaxation by initiating the fast relaxation phase [135, 147] and the sequential relaxation of sarcomeres [138]. This indicates that increasing the strain in the cross-bridge after fall in [Ca^{2+}] promotes their detachment. More recent evidence shows that, following Ca^{2+} removal, the minimum time required to initiate rapid relaxation by external stretch correlates with the time taken to switch off Tn [138]. Without external stretch, the detachment of highly strained cross-bridges in the weakest sarcomere is spontaneous and irrevocably initiates rapid relaxation. The weakest is frequently the sarcomere that became the longest in the preceding activation [135, 138]. These findings reveal that fast detachment of highly strained cross-bridges and the prevention of their reattachment by an early switch off of the thin filament are the underlying mechanisms for rapid muscle relaxation.

Inorganic phosphate (P_i) accelerates relaxation in a manner strikingly similar to stretch, i.e., by promoting the fast relaxation phase [135, 148]. This supports the concept that increasing strain in the cross-bridges during lengthening of the half-sarcomere favours reversal of the power stroke. Since the power stroke is known to be coupled to the release of P_i and the release of P_i is known to be reversible (for review, see [46]), reversal of the power stroke during relaxation might be coupled to rebinding of P_i by the cross-bridge [135, 148]. This concerted backward kinetics of cross-bridges when the sarcomere lengthens would conserve energy. Thus, by reforming a high-chemical-energy ADP·P_i state, the cross-bridges would conserve energy for the next cycle without having to bind 'fresh' ATP (see right half of Fig. 6). During the fast relaxation phase, only a few sarcomeres lengthen and may transform mechanical to chemical energy by stretch while strain in cross-bridges of other sarcomeres is released. Release of cross-bridge strain accelerates the release of ADP and thereby their detachment from actin according to kinetic studies of nucleotide exchange rates in shortening myofibrils [128]. ADP slows down force relaxation of skeletal and cardiac myofibrils and fibres which proves cross-bridge detachment via ADP release to be an important pathway rate-limiting relaxation kinetics [93, 130, 137, 147].

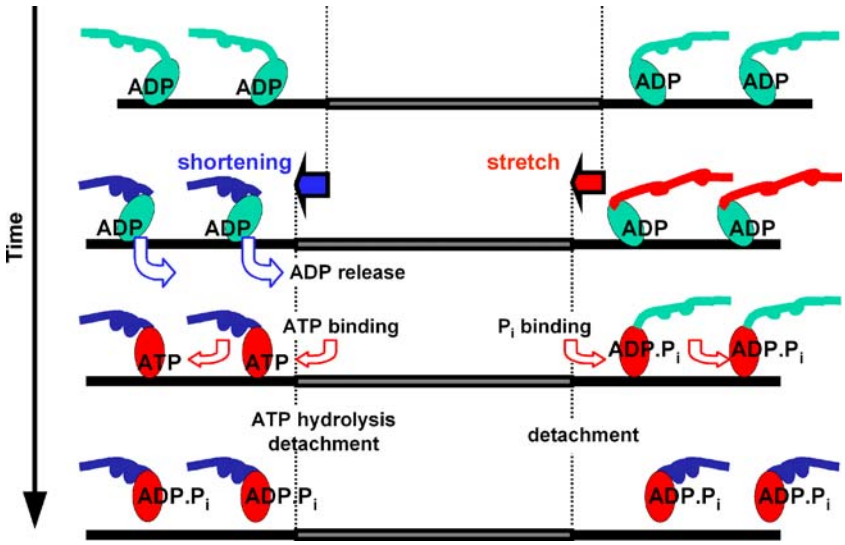

Fig. 6 Model of cross-bridge detachment during rapid muscle relaxation. Two Z-disc-separated half-sarcomeres (Z-disc not drawn) are shown. The colors assigned to the heads and springs indicate the chemical and the mechanical energy stored in a cross-bridge, respectively. *Blue* marks low energy; *green* marks medium energy and *red* marks high energy. Over time (from *top* to *bottom*), cross-bridge detachment proceeds with similar rates in each half-sarcomere but it occurs via different pathways in currently shortening (*left side*) or currently lengthening (*right side*) half-sarcomeres. *Left*: as long as a half-sarcomere still contracts during relaxation, the ongoing decay in force releases the strain in the cross-bridge. This leads to the release of ADP from the cross-bridge whereupon it detaches by completing the ATPase cycle. *Right*: when the half-sarcomere initiates to lengthen, the elastic element in the cross-bridge becomes additionally strained whereupon the cross-bridge transforms the added mechanical energy (strain) into chemical energy (binding of P_i). Because this reversal of the power stroke regenerates chemical energy without ATP consumption, it must be driven by the ATP consumption in the contracting half-sarcomeres. Energy transfer from contracting to lengthening half-sarcomeres occurs via the transmission of strain through the thin filaments (depicted by the *dashed line*) at the Z-disc or through thick filaments at the M-line

Figure 6 depicts how the chemical and the mechanical energy entering or leaving the cross-bridges in the two detachment processes could, in a thermodynamic sense, neutralize each other. We assume that during the rapid sequential sarcomere relaxation the two paths of cross-bridge detachment, backwards and forwards transitions, are coupled by the strain transmitted via the thick and thin filaments. The path which is taken by the cross-bridges depends on the current length change of the half-sarcomere. The forward path is taken within a shortening half-sarcomere until it lengthens (right half-sarcomere in Fig. 6): the release of strain during relaxation accelerates the rapid forward detachment of cross-bridges via ADP release and MgATP binding. When the half-sarcomere starts to lengthen, cross-bridges become strained (right half-sarcomere in Fig. 6): this promotes their backwards detachment via rebinding of P_i and the regeneration of chemical energy.

Assuming that force-generating cross-bridges sterically hinder regulatory units from switching off, the strain-based mechanism of rapid cross-bridge detachment during the fast relaxation phase could allow the regulatory units to escape from persistent cross-bridge activation. The ten to 20-fold increase in the rate constant of cross-bridge detachment which occurs at the onset of the fast relaxation phase is sufficient to efficiently release the regulatory units from any cross-bridge influence whereupon the units will be free to switch off. Indeed, whether the units do not switch off until the detachment of cross-bridges or whether they can switch off before cross-bridge detachment remains to be determined. To differentiate between these two scenarios provides a critical test for models of thin-filament regulation, i.e. whether or not force-generating cross-bridges interfere with the regulatory switch.

Sarcomeric mechanism of cardiomyopathy-related diastolic and systolic dysfunction

Several studies suggest that the dynamics of heart systole and diastole are rate-limited by kinetic processes intrinsic to the sarcomere [5, 55, 67, 117, 136]. Impairment of these processes, therefore, can directly result in systolic and diastolic dysfunction. To date, only a few myofibril studies elucidate the consequences of cardiomyopathies on the kinetics of the Ca^{2+}-controlled contraction and relaxation of the sarcomere. Some of these studies focused on the impact of altered cTnI structure on myofibril contraction and relaxation kinetics [65, 80, 107] and one of them used cardiac myofibrils from patients affected by familial hypertrophic cardiomyopathy (FHC) to investigate the impact of myofilament protein mutations on cardiac sarcomere kinetics [11]. In addition, human cardiac myofibrils have become an important model to elucidate the role of titin isoform changes on diastolic function in different types of cardiac disease [99, 108].

cTnI has a central regulatory role in myocardial contraction and relaxation. Phosphorylation of cTnI by PKA accelerates the kinetics of force decay and might be responsible for faster relaxation of the heart, i.e. the positive lusitropic effect of β-adrenergic stimulation [73, 81, 156]. Thus, cTnI-based mechanisms are important modulators of dynamic heart function [55, 75, 76, 155].

Currently, the proteins of the troponin complex are among the few proteins that can be exchanged in the intact sarcomere structure. The endogenous complex can be replaced by an exogenously added purified or reconstituted complex and, afterwards, the myofibril function can be analysed [17, 79, 115]. This is a tool for structure–function studies of cTnI in myofibrils [35, 80, 107]. A complementary approach is to prepare transgenic animals, which overexpress the mutated sarcomeric protein. Each of the two approaches has its benefits and limitations: transgenic animals allow study of whole-heart function and of isolated myofibrils whereas the exchange approach is restricted to functional analysis of myofibrils and skinned fibres. Myofibrils prepared from transgenic animals consist of thin filaments completely assembled in vivo, but any primary effects of the mutation on systolic and diastolic function might be confused by secondary adaptations, isoform shifts and/or phosphorylation of sarcomeric proteins. Such secondary effects are excluded from myofibrils exchanged with exogenous Tn. Both approaches together provide complementary information about the dysfunction at the sarcomere level induced by the disease and the molecular mechanism of the dysfunction.

Using the two complementary approaches, Krüger et al. [80] demonstrated that the mutation R145G ($cTnI^{R145G}$) linked to FHC [74] slows the kinetics of Ca^{2+}-induced force development and that of the force relaxation following Ca^{2+} removal. The mutation delayed relaxation by prolonging the time of the initial slow linear relaxation phase (t_{LIN}) and by decreasing the rate constant of the fast exponential phase (k_{REL}). The same effects on the two relaxation phases, i.e. a prolongation of t_{LIN} and reduction of k_{REL}, are found in cardiac myofibrils prepared from transgenic mice that over-express the lysine deletion ΔK183 in cTnI ($cTnI^{\Delta K183}$) associated with FHC [65] and in human cardiac myofibrils exchanged with $cTnI_{1–192}$ [107], a C-terminal-truncated cTnI corresponding to the proteolytic degradation product of cTnI from stunned myocardium. None of the three cTnI modifications ($cTnI^{R145G}$, $cTnI^{\Delta K183}$ or $cTnI_{1–192}$) affected the rate constant of the slow linear relaxation phase (k_{LIN}) reflecting g. This is consistent with the notion that intrinsic kinetic properties of acto-myosin dissociation are not altered. Altogether, these findings suggest that structural alterations

in cTnI related to different types of cardiomyopathies induce similar diastolic dysfunctions at the sarcomere level.

The mutation cTnI$^{\Delta K183}$ [65] significantly reduces the rate constant of the switch off (k_{OFF}) of cTn in myofibrils while it does not affect k_{ON}. The slowdown of the switch off does not occur in the isolated cTn [65]. Thus, the effect on the switch off requires the interaction of cTnI with the thin filament. This observation resembles the finding that the truncated cTnI$_{1–192}$ enhances the Ca^{2+}-binding affinity of cTn within reconstituted thin filaments but not in solution [141].

In contrast to the slowing of cardiac myofibrillar relaxation by pathologically modified cTnI structure, incorporation of wild-type TnI isoforms, either cardiac or slow skeletal, which have different off rates [40] into skeletal myofibrils does not alter the kinetics of force relaxation [35]. The fact that the nature of functional wild-type TnI does not directly determine the relaxation rate is in line with the finding that cTn switch kinetics *per se* are too fast to directly rate limit the rate of myofibrillar relaxation [132]. As long as TnI preserves its intact regulatory capacity, the dynamics of contraction and relaxation appear to be determined by cross-bridges kinetics [35]. Therefore, the impaired relaxation kinetics induced by FHC-linked mutations (cTnI^{R145G}, cTnI$^{\Delta K183}$) [65, 80] or truncation (cTnI$_{1–192}$) [107] in cTnI must be indirect effects on cross-bridge cycling. These are likely mediated by lack of full inhibition of acto-myosin interaction at diastolic [Ca^{2+}].

Several findings suggest that lack of full inhibition of thin-filament activation at nM [Ca^{2+}] strongly affects cross-bridge kinetics during relaxation. Both cardiac myofibrils carrying cTnI^{R145G} [80] or cTnI$^{\Delta K183}$ [65] and skeletal myofibrils replaced with cTnI$_{1–192}$ [10] showed an elevated Ca^{2+}-independent force that can be diminished using the inhibitor 2,3-butanedione-2-monoxime (BDM) by trapping cross-bridges in pre-power stroke non-force-generating states [148]. It was further shown that BDM reverses the effect of the cTnI$^{\Delta K183}$ mutation on relaxation kinetics [65]. This implies that the impaired relaxation kinetics results from the formation of force-generating cross-bridges at diastolic [Ca^{2+}]. The mechanism might be the same as the one responsible for the significant slowdown of force decay of fully activated wild-type myofibrils relaxed in [Ca^{2}] just above contraction threshold instead of complete Ca^{2+} removal. This effect was demonstrated for skeletal myofibrils [147] and is shown for cardiac myofibrils in Fig. 7. Incomplete Ca^{2+} removal not only reduces k_{REL} but also prolongs t_{LIN}, the duration of the initial slow relaxation phase. This seems analogous to the effects of pathologically altered cTnI proteins (cTnI^{R145G}, cTnI$^{\Delta K183}$ and cTnI$_{1–192}$) on relaxation parameters. These results suggest that, if there is any incomplete inactivation (by residual Ca^{2+} binding or intrinsic structural alterations in cTnI), it feeds back strongly on relaxation kinetics. All three pathological cTnI proteins (cTnI^{R145G} [80], cTnI$^{\Delta K183}$ [65] and cTnI$_{1–192}$

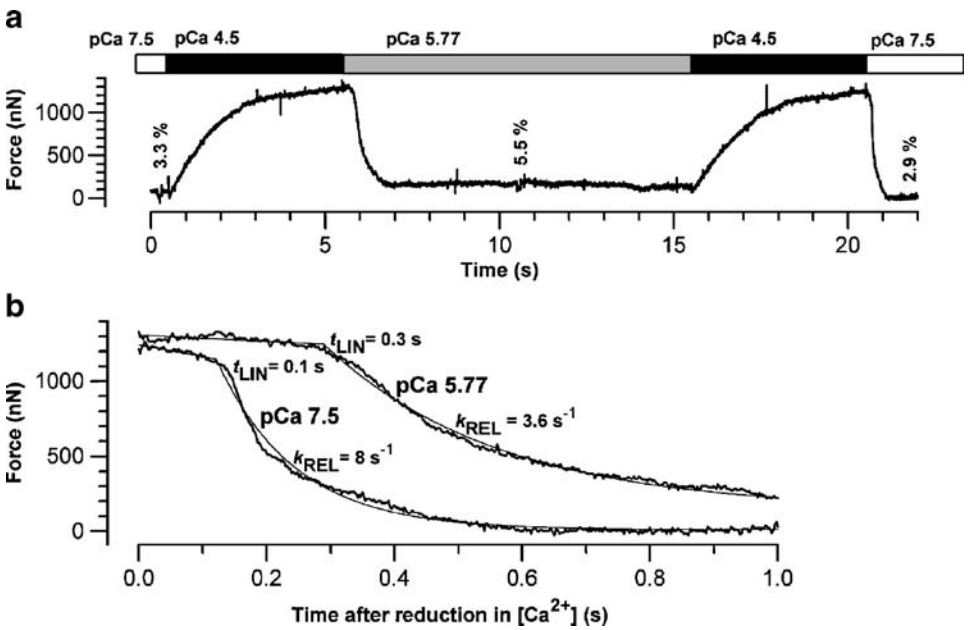

Fig. 7 Effect of a slight residual activation on cardiac myofibrillar relaxation kinetics. **a** Force transient illustrating the complete protocol: the myofibril was activated for two times by using the same activating solution (pCa 4.5). After the first activation, the myofibril was slightly incompletely relaxed by reducing the [Ca^{2+}] to pCa 5.77 whereupon force decayed to a final steady state which was 5.5% of the maximum Ca^{2+}-activated force (F_{MAX}). After the second activation, the myofibril was fully relaxed by reducing [Ca^{2+}] to pCa 7.5 which led to a final force of 2.9% of F_{MAX}. The force levels were determined by slackening the myofibril. **b** The two force decays of the transient illustrated in **a** are plotted together on an expanded timescale to illustrate the strong slowdown of relaxation kinetics in the slightly incomplete (pCa 5.77) compared to the full relaxation (pCa 7.5)

[10]) exert two common effects: they elevate cross-bridge-dependent force at low [Ca^{2+}] and they slow down force relaxation kinetics. Hence, they impair the extent as well as the rate by which cross-bridge formation is inhibited at diastolic [Ca^{2+}]. These two impaired functions could directly relate to the impaired dynamics of isovolumic relaxation (reduced $-dP/dt$, increased τ_{REL}) and the elevated basal mechanical tone (increased pressure during diastolic filling) found in working hearts from transgenic mice expressing murine $cTnI^{R146G}$ [66].

Despite their similar effects on the kinetics of force relaxation, $cTnI^{R145G}$ and $cTnI^{\Delta K183}$ induce subtly different effects on the kinetics of Ca^{2+}-induced force development. Mutation in the inhibitory region of cTnI ($cTnI^{R145G}$) impairs contraction in cTn-replaced myofibrils by depressing both k_{ACT} and F_{MAX} at maximum Ca^{2+} activation [80]. This suggests that structural modifications in the conserved inhibitory region of cTnI impairs systolic function by restricting the rate modulation of Ca^{2+}-induced force development at high [Ca^{2+}], most likely by weakening a Ca^{2+}-dependent cTnI–cTnC interaction [80]. In contrast, the transgenic expression of $cTnI^{\Delta K183}$ that is lacking a lysine in the mobile C terminus domain of cTnI does not alter myofibril k_{ACT} and slightly enhances F_{MAX} [65]. The effects of the two FHC-related single-site mutations are consistent with the concept that in the presence of Ca^{2+} the inhibitory region but not the C terminus domain of cTnI interacts with troponin C [105].

Open questions remain concerning the effect of the large C-terminal truncation of cTnI represented by $cTnI_{1-192}$ on sarcomeric function during Ca^{2+} activation. Because it did not affect F_{MAX} and only slightly decreased k_{ACT} in human cardiac myofibrils replaced with recombinant human cTn complexes, Narolska et al. [107] concluded that it does not impair systolic function. Recently, the effects of the cTnI truncation has been also studied by Tachampa et al. [141] in skinned rat trabeculae by using recombinant murine cTnI from different species. Force at high [Ca^{2+}] was depressed while the rate constant of force re-development (k_{TR}) and the fibre ATPase were increased. These authors [141] proposed that the truncated cTnI causes depressed myofilament function and increased energy cost, which could be related to the systolic dysfunction and its manifestation in the dilated heart phenotype of transgenic mice overexpressing the truncated cTnI [106]. As discussed by Tachampa et al. [141], it remains to be discovered whether or not the contrasting effects on systolic parameters, in the study of Narolska et al. and their study, result from the different models used in the two studies.

The functional impact of cardiomyopathy-related modifications of sarcomeric proteins in humans is still weakly documented because it is difficult to obtain consistent measurements of functionally relevant parameters from patient cardiac tissue. At variance with traditional multicellular skinned cardiac preparations, myofibrils can be isolated in large amounts even from small, fresh or frozen, human cardiac samples and offer a significant advance in our ability to directly measure changes in mechanics and kinetics of human cardiac sarcomeres [39, 99, 107, 108, 116]. A recent myofibril study [11] is the first to directly examine the mechanical kinetics of cardiac sarcomeres from a patient affected by FHC. The patient carried the severe R403Q mutation in the β-MHC, the first mutation to be identified as responsible for FHC [45]. The observation that both the kinetics of tension activation and the rate constant k_{LIN} of the initial slow isometric phase of relaxation were markedly increased in the R403Q myofibrils compared to controls is rather striking and shows that the mutation increases g, the rate of dissociation of myosin from actin. Consistent with a wide range of biochemical and nuclear-magnetic-resonance-based studies in both FHC patients and animal models of the disease, this would be expected to greatly increase the overall tension cost in the FHC heart [29, 68, 72, 133]. This myofibril study on an FHC patient represents an important advance both with respect to the conclusions on the observed effects of a specific myosin mutation at the level of the cardiac cross-bridge and as a methodology that may provide a unique and more biophysically proximal phenotypic characterisation of FHC. Finally, the accelerating effects of the R403Q mutation in β-MHC on myofibril force kinetics corroborate the general conclusion that the intrinsic kinetic properties of cycling cross-bridges rather than thin-filament activation/inactivation rate limit cardiac myofibril contraction and relaxation.

Concluding remarks

Single or small bundles of myofibrils are highly useful in studies of the kinetic mechanisms of thin-filament regulation, acto-myosin ATPase, passive viscoelasticity, cross-bridge kinetics and sarcomere dynamics. Even though these individual mechanisms are fairly well characterised, the dynamic coupling between them is limited or controversial. Elucidation of these couplings is necessary to completely understand the dynamic interactions within and among the sarcomere(s) and their impairment under pathophysiological conditions.

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References

1. Anazawa T, Yasuda K, Ishiwata S (1992) Spontaneous oscillation of tension and sarcomere length in skeletal myofibrils. Microscopic measurement and analysis. Biophys J 61:1099–1108
2. Andruchov O, Andruchova O, Galler S (2006) Fine-tuning of cross-bridge kinetics in cardiac muscle of rat and mouse by myosin light chain isoforms. Pflugers Arch 452:667–673
3. Andruchov O, Andruchova O, Wang Y, Galler S (2006) Dependence of cross-bridge kinetics on myosin light chain isoforms in rabbit and rat skeletal muscle fibres. J Physiol 571:231–242
4. Araujo A, Walker JW (1994) Kinetics of tension development in skinned cardiac myocytes measured by photo release of Ca^{2+}. Am J Physiol 267:H1643–H1653
5. Backx PH, Gao WD, Azan-Backx MD, Marban E (1995) The relationship between contractile force and intracellular [Ca^{2+}] in intact rat cardiac trabeculae. J Gen Physiol 105:1–19
6. Bagni MA, Cecchi G, Colombini B (2005) Cross bridge properties investigated by fast ramp stretching of activated frog muscle fibres. J Physiol 565:261–268
7. Barman T, Brune M, Lionne C, Piroddi N, Poggesi C, Stehle R, Tesi C, Travers F, Webb MR (1998) ATPase and shortening rates in frog fast skeletal myofibrils by time-resolved measurements of protein-bound and free Pi. Biophys J 74:3120–3130
8. Barman TE, Bellamy SR, Gutfreund H, Halford SE, Lionne C (2006) The identification of chemical intermediates in enzyme catalysis by the rapid quench-flow technique. Cell Mol Life Sci 63:2571–2583
9. Bell MG, Lankford EB, Gonye GE, Ellis-Davies GC, Martyn DA, Regnier M, Barsotti RJ (2006) Kinetics of cardiac thin-filament activation probed by fluorescence polarization of rhodamine-labeled troponin C in skinned guinea pig trabeculae. Biophys J 90:531–543
10. Belus A, Narolska NA, Piroddi N, Scellini B, Deppermann S, Jaquet K, Foster DB, van Eyk J, van der Velden J, Tesi C, Stienen GJ, Poggesi C (2007) Human C-terminal truncated cardiac troponin I exchanged into rabbit psoas myofibrils is unable to fully inhibit acto-myosin interaction in the absence of Ca^{2+}. Biophys J 92:629a
11. Belus A, Piroddi N, Scellini B, Tesi C, Amati GD, Girolami F, Yacoub M, Cecchi F, Olivotto I, Poggesi C (2008) The familial hypertrophic cardiomyopathy-associated myosin mutation R403Q accelerates tension generation and relaxation of human cardiac myofibrils. J Physiol 586:3639–3644
12. Biosca JA, Barman TE, Travers F (1984) Transient kinetics of the binding of ATP to actomyosin subfragment 1: evidence that the dissociation of actomyosin subfragment 1 by ATP leads to a new conformation of subfragment 1. Biochemistry 23:2428–2436
13. Bottinelli R (2001) Functional heterogeneity of mammalian single muscle fibres: do myosin isoforms tell the whole story? Pflugers Arch 443:6–17
14. Brandt PW, Schachat FH (1997) Troponin C modulates the activation of thin filaments by rigor cross-bridges. Biophys J 72:2262–2267
15. Bremel RD, Weber A (1972) Cooperation within actin filament in vertebrate skeletal muscle. Nat New Biol 238:97–101
16. Brenner B (1988) Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibers: implications for regulation of muscle contraction. Proc Natl Acad Sci USA 85:3265–3269
17. Brenner B, Chalovich JM (1999) Kinetics of thin filament activation probed by fluorescence of *N*-((2-(iodoacetoxy)ethyl)-*N*-methyl)amino-7-nitrobenz-2-oxa-1, 3-diazole-labeled troponin I incorporated into skinned fibers of rabbit psoas muscle: implications for regulation of muscle contraction. Biophys J 77:2692–2708
18. Brenner B, Eisenberg E (1987) The mechanism of muscle contraction. Biochemical, mechanical, and structural approaches to elucidate cross-bridge action in muscle. Basic Res Cardiol 82 (Suppl 2):3–16
19. Brune M, Hunter JL, Corrie JE, Webb MR (1994) Direct, real-time measurement of rapid inorganic phosphate release using a novel fluorescent probe and its application to actomyosin subfragment 1 ATPase. Biochemistry 33:8262–8271
20. Brunello E, Reconditi M, Elangovan R, Linari M, Sun YB, Narayanan T, Panine P, Piazzesi G, Irving M, Lombardi V (2007) Skeletal muscle resists stretch by rapid binding of the second motor domain of myosin to actin. Proc Natl Acad Sci USA 104:20114–20119
21. Burton K, Simmons RM, Sleep J, Smith DA (2006) Kinetics of force recovery following length changes in active skinned single fibres from rabbit psoas muscle: analysis and modelling of the late recovery phase. J Physiol 573:305–328
22. Chaen S, Shirakawa I, Bagshaw CR, Sugi H (1997) Measurement of nucleotide release kinetics in single skeletal muscle myofibrils during isometric and isovelocity contractions using fluorescence microscopy. Biophys J 73:2033–2042
23. Chalovich JM, Eisenberg E (1982) Inhibition of actomyosin ATPase activity by troponin–tropomyosin without blocking the binding of myosin to actin. J Biol Chem 257:2432–2437
24. Chalovich JM, Chock PB, Eisenberg E (1981) Mechanism of action of troponin tropomyosin. Inhibition of actomyosin ATPase activity without inhibition of myosin binding to actin. J Biol Chem 256:575–578
25. Colombini B, Nocella M, Benelli G, Cecchi G, Bagni MA (2007) Cross bridge properties during force enhancement by slow stretching in single intact frog muscle fibres. J Physiol 585:607–615
26. Colomo F, Nencini S, Piroddi N, Poggesi C, Tesi C (1998) Calcium dependence of the apparent rate of force generation in single striated muscle myofibrils activated by rapid solution changes. Adv Exp Med Biol 453:373–381
27. Cooke R (1997) Actomyosin interaction in striated muscle. Physiol Rev 77:671–697
28. Cooke R, Pate E (1985) The effects of ADP and phosphate on the contraction of muscle fibers. Biophys J 48:789–798
29. Crilley JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P, McKenna WJ, Ostman-Smith I, Clarke K, Watkins H (2003) Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. J Am Coll Cardiol 41:1776–1782
30. Dantzig JA, Goldman YE, Millar NC, Lacktis J, Homsher E (1992) Reversal of the cross-bridge force-generating transition by photogeneration of phosphate in rabbit psoas muscle fibres. J Physiol 451:247–278
31. Davis JS, Harrington WF (1993) A single order–disorder transition generates tension during the Huxley-Simmons phase 2 in muscle. Biophys J 65:1886–1898
32. Davis JP, Tikunova SB (2008) Ca^{2+} exchange with troponin C and cardiac muscle dynamics. Cardiovasc Res 77:619–626
33. Davis JP, Norman C, Kobayashi T, Solaro RJ, Swartz DR, Tikunova SB (2007) Effects of thin and thick filament proteins on calcium binding and exchange with cardiac troponin C. Biophys J 92:3195–3206
34. de Tombe PP, Stienen GJ (2007) Impact of temperature on cross-bridge cycling kinetics in rat myocardium. J Physiol 584:591–600
35. de Tombe PP, Belus A, Piroddi N, Scellini B, Walker JS, Martin AF, Tesi C, Poggesi C (2007) Myofilament calcium sensitivity

does not affect cross-bridge activation–relaxation kinetics. Am J Physiol Regul Integr Comp Physiol 292:R1129–R1136
36. Denoth J, Stussi E, Csucs G, Danuser G (2002) Single muscle fiber contraction is dictated by inter-sarcomere dynamics. J Theor Biol 216:101–122
37. Dong WJ, Wang CK, Gordon AM, Rosenfeld SS, Cheung HC (1997) A kinetic model for the binding of Ca^{2+} to the regulatory site of troponin from cardiac muscle. J Biol Chem 272:19229–19235
38. Eisenberg E, Greene LE (1980) The relation of muscle biochemistry to muscle physiology. Annu Rev Physiol 42:293–309
39. Elhamine F, Stehle R, Brockmeier K, Bennink G, Emmel M, Raji R, Pfitzer G (2006) Force kinetics and Ca^{2+}-sensitivity of myofibrils isolated from right ventricles of patients with tetralogy of Fallot. J Muscle Res Cell Motil 27:481–544
40. Engel PL, Kobayashi T, Biesiadecki B, Davis J, Tikunova S, Wu S, Solaro RJ (2007) Identification of a region of troponin I important in signaling cross-bridge-dependent activation of cardiac myofilaments. J Biol Chem 282:183–193
41. Fenn WO (1924) The relation between the work performed and the energy liberated in muscular contraction. J Physiol 58:373–395
42. Fitzsimons DP, Patel JR, Moss RL (2001) Cross-bridge interaction kinetics in rat myocardium are accelerated by strong binding of myosin to the thin filament. J Physiol 530:263–272
43. Galinska-Rakoczy A, Engel P, Xu C, Jung H, Craig R, Tobacman LS, Lehman W (2008) Structural basis for the regulation of muscle contraction by troponin and tropomyosin. J Mol Biol 379:929–935
44. Geeves MA, Holmes KC (1999) Structural mechanism of muscle contraction. Annu Rev Biochem. 68:687–728
45. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, Seidman JG (1990) A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell 62:999–1006
46. Gordon AM, Homsher E, Regnier M (2000) Regulation of contraction in striated muscle. Physiol Rev 80:853–924
47. Granzier H, Labeit S (2002) Cardiac titin: an adjustable multifunctional spring. J Physiol 541:335–342
48. Greene LE, Eisenberg E (1980) Cooperative binding of myosin subfragment-1 to the actin-troponin–tropomyosin complex. Proc Natl Acad Sci USA 77:2616–2620
49. Haselgrove JC, Huxley HE (1973) X-ray evidence for radial cross-bridge movement and for the sliding filament model in actively contracting skeletal muscle. J Mol Biol 77:549–568
50. Heeley DH, Belknap B, White HD (2002) Mechanism of regulation of phosphate dissociation from actomyosin-ADP-P_i by thin filament proteins. Proc Natl Acad Sci USA 99:16731–16736
51. Helmes M, Trombitas K, Centner T, Kellermayer M, Labeit S, Linke WA, Granzier H (1999) Mechanically driven contour-length adjustment in rat cardiac titin's unique N2B sequence: titin is an adjustable spring. Circ Res 84:1339–1352
52. Herrmann C, Houadjeto M, Travers F, Barman T (1992) Early steps of the Mg2+-ATPase of relaxed myofibrils. A comparison with Ca^{2+}-activated myofibrils and myosin subfragment 1. Biochemistry 31:8036–8042
53. Herrmann C, Sleep J, Chaussepied P, Travers F, Barman T (1993) A structural and kinetic study on myofibrils prevented from shortening by chemical cross-linking. Biochemistry 32:7255–7263
54. Hill TL, Eisenberg E, Greene L (1980) Theoretical model for the cooperative equilibrium binding of myosin subfragment 1 to the actin-troponin-tropomyosin complex. Proc Natl Acad Sci USA 77:3186–3190
55. Hinken AC, Solaro RJ (2007) A dominant role of cardiac molecular motors in the intrinsic regulation of ventricular ejection and relaxation. Physiology (Bethesda) 22:73–80
56. Hopkins SC, Sabido-David C, van der Heide UA, Ferguson RE, Brandmeier BD, Dale RE, Kendrick-Jones J, Corrie JE, Trentham DR, Irving M, Goldman YE (2002) Orientation changes of the myosin light chain domain during filament sliding in active and rigor muscle. J Mol Biol 318:1275–1291
57. Houadjeto M, Barman T, Travers F (1991) What is the true ATPase activity of contracting myofibrils? FEBS Lett 281:105–107
58. Houadjeto M, Travers F, Barman T (1992) Ca^{2+}-activated myofibrillar ATPase: transient kinetics and the titration of its active sites. Biochemistry 31:1564–1569
59. Huxley AF (1957) Muscle structure and theories of contraction. Prog Biophys Biophys Chem 7:255–318
60. Huxley HE (1973) Muscular contraction and cell motility. Nature 243:445–449
61. Huxley H, Hanson J (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature 173:973–976
62. Huxley AF, Niedergerke R (1954) Structural changes in muscle during contraction; interference microscopy of living muscle fibres. Nature 173:971–973
63. Huxley AF, Simmons RM (1971) Proposed mechanism of force generation in striated muscle. Nature 233:533–538
64. Iorga B, Candau R, Travers F, Barman T, Lionne C (2004) Does phosphate release limit the ATPases of soleus myofibrils? Evidence that (A)M·ADP·P_i states predominate on the cross-bridge cycle. J Muscle Res Cell Motil 25:367–378
65. Iorga B, Blaudeck N, Solzin J, Neulen A, Stehle I, Lopez Davila AJ, Pfitzer G, Stehle R (2008) Lys184 deletion in troponin I impairs relaxation kinetics and induces hypercontractility in murine cardiac myofibrils. Cardiovasc Res 77:676–686
66. James J, Zhang Y, Osinska H, Sanbe A, Klevitsky R, Hewett TE, Robbins J (2000) Transgenic modeling of a cardiac troponin I mutation linked to familial hypertrophic cardiomyopathy. Circ Res 87:805–811
67. Janssen PM, Stull LB, Marban E (2002) Myofilament properties comprise the rate-limiting step for cardiac relaxation at body temperature in the rat. Am J Physiol Heart Circ Physiol 282: H499–H507
68. Javadpour MM, Tardiff JC, Pinz I, Ingwall JS (2003) Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. J Clin Invest 112:768–775
69. Johns EC, Simnett SJ, Mulligan IP, Ashley CC (1997) Troponin I phosphorylation does not increase the rate of relaxation following laser flash photolysis of diazo-2 in guinea-pig skinned trabeculae. Pflugers Arch 433:842–844
70. Kawai M, Halvorson HR (1991) Two step mechanism of phosphate release and the mechanism of force generation in chemically skinned fibers of rabbit psoas muscle. Biophys J 59:329–342
71. Kawai M, Saeki Y, Zhao Y (1993) Cross bridge scheme and the kinetic constants of elementary steps deduced from chemically skinned papillary and trabecular muscles of the ferret. Circ Res 73:35–50
72. Keller DI, Coirault C, Rau T, Cheav T, Weyand M, Amann K, Lecarpentier Y, Richard P, Eschenhagen T, Carrier L (2004) Human homozygous R403W mutant cardiac myosin presents disproportionate enhancement of mechanical and enzymatic properties. J Mol Cell Cardiol 36:355–362
73. Kentish JC, McCloskey DT, Layland J, Palmer S, Leiden JM, Martin AF, Solaro RJ (2001) Phosphorylation of troponin I by protein kinase A accelerates relaxation and cross-bridge cycle kinetics in mouse ventricular muscle. Circ Res 88:1059–1065
74. Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M, Hiroi S, Sasaoka T, Ohbuchi N, Nakamura T, Koyanagi T, Hwang TH, Choo JA, Chung KS, Hasegawa A, Nagai R,

Okazaki O, Nakamura H, Matsuzaki M, Sakamoto T, Toshima H, Koga Y, Imaizumi T, Sasazuki T (1997) Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. Nat Genet 16:379–382
75. Kobayashi T, Solaro RJ (2005) Calcium, thin filaments, and the integrative biology of cardiac contractility. Annu Rev Physiol 67:39–67
76. Kobayashi T, Jin L, de Tombe PP (2008) Cardiac thin filament regulation. Pflugers Arch 457:37–46
77. Kraft T, Mattei T, Radocaj A, Piep B, Nocula C, Furch M, Brenner B (2002) Structural features of cross-bridges in isometrically contracting skeletal muscle. Biophys J 82:2536–2547
78. Kreutziger KL, Piroddi N, Scellini B, Tesi C, Poggesi C, Regnier M (2008) Thin filament Ca^{2+} binding properties and regulatory unit interactions alter kinetics of tension development and relaxation in rabbit skeletal muscle. J Physiol 586:3683–3700
79. Kruger M, Pfitzer G, Stehle R (2003) Expression and purification of human cardiac troponin subunits and their functional incorporation into isolated cardiac mouse myofibrils. J Chromatogr B Analyt Technol Biomed Life Sci 786:287–296
80. Kruger M, Zittrich S, Redwood C, Blaudeck N, James J, Robbins J, Pfitzer G, Stehle R (2005) Effects of the mutation R145G in human cardiac troponin I on the kinetics of the contraction–relaxation cycle in isolated cardiac myofibrils. J Physiol 564:347–357
81. Layland J, Solaro RJ, Shah AM (2005) Regulation of cardiac contractile function by troponin I phosphorylation. Cardiovasc Res 66:12–21
82. Lehman W, Craig R, Vibert P (1994) Ca^{2+}-induced tropomyosin movement in Limulus thin filaments revealed by three-dimensional reconstruction. Nature 368:65–67
83. Lehrer SS, Geeves MA (1998) The muscle thin filament as a classical cooperative/allosteric regulatory system. J Mol Biol 277:1081–1089
84. Linari M, Caremani M, Piperio C, Brandt P, Lombardi V (2007) Stiffness and fraction of myosin motors responsible for active force in permeabilized muscle fibers from rabbit psoas. Biophys J 92:2476–2490
85. Linke WA, Bartoo ML, Pollack GH (1993) Spontaneous sarcomeric oscillations at intermediate activation levels in single isolated cardiac myofibrils. Circ Res 73:724–734
86. Linke WA, Ivemeyer M, Olivieri N, Kolmerer B, Ruegg JC, Labeit S (1996) Towards a molecular understanding of the elasticity of titin. J Mol Biol 261:62–71
87. Linke WA, Ivemeyer M, Mundel P, Stockmeier MR, Kolmerer B (1998) Nature of PEVK-titin elasticity in skeletal muscle. Proc Natl Acad Sci USA 95:8052–8057
88. Lionne C, Herrmann C, Travers F, Barman T (1995) The myofibril as a model for muscle fiber ATPase. Biophys J 68:217s
89. Lionne C, Brune M, Webb MR, Travers F, Barman T (1995) Time resolved measurements show that phosphate release is the rate limiting step on myofibrillar ATPases. FEBS Lett 364:59–62
90. Lionne C, Travers F, Barman T (1996) Mechanochemical coupling in muscle: attempts to measure simultaneously shortening and ATPase rates in myofibrils. Biophys J 70:887–895
91. Lionne C, Iorga B, Candau R, Piroddi N, Webb MR, Belus A, Travers F, Barman T (2002) Evidence that phosphate release is the rate-limiting step on the overall ATPase of psoas myofibrils prevented from shortening by chemical cross-linking. Biochemistry 41:13297–13308
92. Lionne C, Iorga B, Candau R, Travers F (2003) Why choose myofibrils to study muscle myosin ATPase? J. Muscle Res Cell Motil 24:139–148
93. Lipscomb S, Palmer RE, Li Q, Allhouse LD, Miller T, Potter JD, Ashley CC (1999) A diazo-2 study of relaxation mechanisms in frog and barnacle muscle fibres: effects of pH, MgADP, and inorganic phosphate. Pflugers Arch 437:204–212
94. Lombardi V, Piazzesi G (1990) The contractile response during steady lengthening of stimulated frog muscle fibres. J Physiol 431:141–171
95. Lu Z, Swartz DR, Metzger JM, Moss RL, Walker JW (2001) Regulation of force development studied by photolysis of caged ADP in rabbit skinned psoas fibers. Biophys J 81:334–344
96. Luo Y, Davis JP, Smillie LB, Rall JA (2002) Determinants of relaxation rate in rabbit skinned skeletal muscle fibres. J Physiol 545:887–901
97. Lymn RW, Taylor EW (1971) Mechanism of adenosine triphosphate hydrolysis by actomyosin. Biochemistry 10:4617–4624
98. Ma YZ, Taylor EW (1994) Kinetic mechanism of myofibril ATPase. Biophys J 66:1542–1553
99. Makarenko I, Opitz CA, Leake MC, Neagoe C, Kulke M, Gwathmey JK, del Monte F, Hajjar RJ, Linke WA (2004) Passive stiffness changes caused by upregulation of compliant titin isoforms in human dilated cardiomyopathy hearts. Circ Res 95:708–716
100. Martin H, Bell MG, Ellis-Davies GC, Barsotti RJ (2004) Activation kinetics of skinned cardiac muscle by laser photolysis of nitrophenyl-EGTA. Biophys J 86:978–990
101. Martyn DA, Chase PB, Regnier M, Gordon AM (2002) A simple model with myofilament compliance predicts activation-dependent cross bridge kinetics in skinned skeletal fibers. Biophys J 83:3425–3434
102. McKillop DF, Geeves MA (1993) Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament. Biophys J 65:693–701
103. Metzger JM, Moss RL (1990) Calcium-sensitive cross-bridge transitions in mammalian fast and slow skeletal muscle fibers. Science 247:1088–1090
104. Millar NC, Geeves MA (1988) Protein fluorescence changes associated with ATP and adenosine 5′-[gamma-thio]triphosphate binding to skeletal muscle myosin subfragment 1 and actomyosin subfragment 1. Biochem J 249:735–743
105. Murakami K, Yumoto F, Ohki SY, Yasunaga T, Tanokura M, Wakabayashi T (2005) Structural basis for Ca^{2+}-regulated muscle relaxation at interaction sites of troponin with actin and tropomyosin. J Mol Biol 352:178–201
106. Murphy AM, Kogler H, Georgakopoulos D, McDonough JL, Kass DA, Van Eyk JE, Marban E (2000) Transgenic mouse model of stunned myocardium. Science 287:488–491
107. Narolska NA, Piroddi N, Belus A, Boontje NM, Scellini B, Deppermann S, Zaremba R, Musters RJ, dos Remedios C, Jaquet K, Foster DB, Murphy AM, van Eyk JE, Tesi C, Poggesi C, van der Velden J, Stienen GJ (2006) Impaired diastolic function after exchange of endogenous troponin I with C-terminal truncated troponin I in human cardiac muscle. Circ Res 99:1012–1020
108. Neagoe C, Kulke M, del Monte F, Gwathmey JK, de Tombe PP, Hajjar RJ, Linke WA (2002) Titin isoform switch in ischemic human heart disease. Circulation 106:1333–1341
109. Nikolic SD, Feneley MP, Pajaro OE, Rankin JS, Yellin EL (1995) Origin of regional pressure gradients in the left ventricle during early diastole. Am J Physiol 268:H550–H557
110. Opitz CA, Kulke M, Leake MC, Neagoe C, Hinssen H, Hajjar RJ, Linke WA (2003) Damped elastic recoil of the titin spring in myofibrils of human myocardium. Proc Natl Acad Sci USA 100:12688–12693
111. Palmer S, Kentish JC (1998) Roles of Ca^{2+} and cross bridge kinetics in determining the maximum rates of Ca^{2+} activation and

relaxation in rat and guinea pig skinned trabeculae. Circ Res 83:179–186
112. Parker JD, Landzberg JS, Bittl JA, Mirsky I, Colucci WS (1991) Effects of beta-adrenergic stimulation with dobutamine on isovolumic relaxation in the normal and failing human left ventricle. Circulation 84:1040–1048
113. Parry DA, Squire JM (1973) Structural role of tropomyosin in muscle regulation: analysis of the X-ray diffraction patterns from relaxed and contracting muscles. J Mol Biol 75:33–55
114. Pate E, Franks-Skiba K, White HD, Cooke R (1993) The use of differing nucleotides to investigate cross-bridge kinetics. J Biol Chem 268:10046–10053
115. Piroddi N, Tesi C, Pellegrino MA, Tobacman LS, Homsher E, Poggesi C (2003) Contractile effects of the exchange of cardiac troponin for fast skeletal troponin in rabbit psoas single myofibrils. J Physiol 552:917–931
116. Piroddi N, Belus A, Scellini B, Tesi C, Giunti G, Cerbai E, Mugelli A, Poggesi C (2007) Tension generation and relaxation in single myofibrils from human atrial and ventricular myocardium. Pflugers Arch 454:63–73
117. Poggesi C, Tesi C, Stehle R (2005) Sarcomeric determinants of striated muscle relaxation kinetics. Pflugers Arch 449:505–517
118. Ranatunga KW, Coupland ME, Mutungi G (2002) An asymmetry in the phosphate dependence of tension transients induced by length perturbation in mammalian (rabbit psoas) muscle fibres. J Physiol 542:899–910
119. Rassier DE, Herzog W, Pollack GH (2003) Dynamics of individual sarcomeres during and after stretch in activated single myofibrils. Proc Biol Sci 270:1735–1740
120. Regnier M, Martin H, Barsotti RJ, Rivera AJ, Martyn DA, Clemmens E (2004) Cross-bridge versus thin filament contributions to the level and rate of force development in cardiac muscle. Biophys J 87:1815–1824
121. Resetar AM, Stephens JM, Chalovich JM (2002) Troponin–tropomyosin: an allosteric switch or a steric blocker? Biophys J 83:1039–1049
122. Rosenfeld SS, Taylor EW (1985) Kinetic studies of calcium and magnesium binding to troponin C. J Biol Chem 260:242–251
123. Rosenfeld SS, Taylor EW (1985) Kinetic studies of calcium binding to regulatory complexes from skeletal muscle. J Biol Chem 260:252–261
124. Rosenfeld SS, Taylor EW (1987) The mechanism of regulation of actomyosin subfragment 1 ATPase. J Biol Chem 262:9984–9993
125. Sasaki D, Fujita H, Fukuda N, Kurihara S, Ishiwata S (2005) Auto-oscillations of skinned myocardium correlating with heartbeat. J Muscle Res Cell Motil 26:93–101
126. Sasaki D, Fukuda N, Ishiwata S (2006) Myocardial sarcomeres spontaneously oscillate with the period of heartbeat under physiological conditions. Biochem Biophys Res Commun 343:1146–1152
127. Shimamoto Y, Suzuki M, Ishiwata S (2008) Length-dependent activation and auto-oscillation in skeletal myofibrils at partial activation by Ca^{2+}. Biochem Biophys Res Commun 366:233–238
128. Shirakawa I, Chaen S, Bagshaw CR, Sugi H (2000) Measurement of nucleotide exchange rate constants in single rabbit soleus myofibrils during shortening and lengthening using a fluorescent ATP analog. Biophys J 78:918–926
129. Siemankowski RF, Wiseman MO, White HD (1985) ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. Proc Natl Acad Sci USA 82:658–662
130. Simnett SJ, Johns EC, Lipscomb S, Mulligan IP, Ashley CC (1998) Effect of pH, phosphate, and ADP on relaxation of myocardium after photolysis of diazo 2. Am J Physiol 275: H951–H960
131. Siththanandan V, Tobacman LS, Homsher E (2007) Force production by single myofibrils to full and partial activation following replacement of endogenous tropomyosin. Biophys J 92:626a
132. Solzin J, Iorga B, Sierakowski E, Gomez Alcazar DP, Ruess DF, Kubacki T, Zittrich S, Blaudeck N, Pfitzer G, Stehle R (2007) Kinetic mechanism of the Ca^{2+}-dependent switch-on and switch-off of cardiac troponin in myofibrils. Biophys J 93:3917–3931
133. Spindler M, Saupe KW, Christe ME, Sweeney HL, Seidman CE, Seidman JG, Ingwall JS (1998) Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. J Clin Invest 101:1775–1783
134. Stehle R, Lionne C, Travers F, Barman T (2000) Kinetics of the initial steps of rabbit psoas myofibrillar ATPases studied by tryptophan and pyrene fluorescence stopped-flow and rapid flow-quench. Evidence that cross-bridge detachment is slower than ATP binding. Biochemistry 39:7508–7520
135. Stehle R, Kruger M, Pfitzer G (2002) Force kinetics and individual sarcomere dynamics in cardiac myofibrils after rapid Ca^{2+} changes. Biophys J 83:2152–2161
136. Stehle R, Kruger M, Scherer P, Brixius K, Schwinger RH, Pfitzer G (2002) Isometric force kinetics upon rapid activation and relaxation of mouse, guinea pig and human heart muscle studied on the subcellular myofibrillar level. Basic Res Cardiol 97(Suppl 1):I127–I135
137. Stehle R, Kruger M, Pfitzer G (2003) Does cross-bridge activation determine the time course of myofibrillar relaxation? Adv Exp Med Biol 538:469–479
138. Stehle R, Solzin J, Iorga B, Gomez D, Blaudeck N, Pfitzer G (2006) Mechanical properties of sarcomeres during cardiac myofibrillar relaxation: stretch-induced cross-bridge detachment contributes to early diastolic filling. J Muscle Res Cell Motil 27:423–434
139. Stehle R, Iorga B, Pfitzer G (2007) Calcium regulation of troponin and its role in the dynamics of contraction and relaxation. Am J Physiol Regul Integr Comp Physiol 292: R1125–R1128
140. Swartz DR, Yang Z, Sen A, Tikunova SB, Davis JP (2006) Myofibrillar troponin exists in three states and there is signal transduction along skeletal myofibrillar thin filaments. J Mol Biol 361:420–435
141. Tachampa K, Kobayashi T, Wang H, Martin AF, Biesiadecki BJ, Solaro RJ, de Tombe PP (2008) Increased cross-bridge cycling kinetics after exchange of C-terminal truncated troponin I in skinned rat cardiac muscle. J Biol Chem 283:15114–15121
142. Telley IA, Denoth J (2007) Sarcomere dynamics during muscular contraction and their implications to muscle function. J Muscle Res Cell Motil 28:89–104
143. Telley IA, Denoth J, Stussi E, Pfitzer G, Stehle R (2006) Half-sarcomere dynamics in myofibrils during activation and relaxation studied by tracking fluorescent markers. Biophys J 90:514–530
144. Telley IA, Stehle R, Ranatunga KW, Pfitzer G, Stussi E, Denoth J (2006) Dynamic behaviour of half-sarcomeres during and after stretch in activated rabbit psoas myofibrils: sarcomere asymmetry but no 'sarcomere popping'. J Physiol 573:173–185
145. Tesi C, Colomo F, Nencini S, Piroddi N, Poggesi C (1999) Modulation by substrate concentration of maximal shortening velocity and isometric force in single myofibrils from frog and rabbit fast skeletal muscle. J Physiol 516(Pt 3):847–853
146. Tesi C, Colomo F, Nencini S, Piroddi N, Poggesi C (2000) The effect of inorganic phosphate on force generation in single myofibrils from rabbit skeletal muscle. Biophys J 78:3081–3092
147. Tesi C, Piroddi N, Colomo F, Poggesi C (2002) Relaxation kinetics following sudden Ca^{2+} reduction in single myofibrils from skeletal muscle. Biophys J 83:2142–2151

148. Tesi C, Colomo F, Piroddi N, Poggesi C (2002) Characterization of the cross-bridge force-generating step using inorganic phosphate and BDM in myofibrils from rabbit skeletal muscles. J Physiol 541:187–199
149. Wahr PA, Rall JA (1997) Role of calcium and cross bridges in determining rate of force development in frog muscle fibers. Am J Physiol 272:C1664–C1671
150. Wakayama J, Yamada T (2000) Contractility of single myofibrils of rabbit skeletal muscle studied at various MgATP concentrations. Jpn J Physiol 50:533–542
151. Weber A, Herz R (1963) The binding of calcium to actomyosin systems in relation to their biological activity. J Biol Chem 238:599–605
152. White HD, Belknap B, Jiang W (1993) Kinetics of binding and hydrolysis of a series of nucleoside triphosphatase by actomyosin-S1. J Biol Chem 268:10039–10045
153. White HD, Belknap B, Webb MR (1997) Kinetics of nucleoside triphosphate cleavage and phosphate release steps by associated rabbit skeletal actomyosin, measured using a novel fluorescent probe for phosphate. Biochemistry 36:11828–11836
154. Yasuda K, Shindo Y, Ishiwata S (1996) Synchronous behavior of spontaneous oscillations of sarcomeres in skeletal myofibrils under isotonic conditions. Biophys J 70:1823–1829
155. Yasuda S, Coutu P, Sadayappan S, Robbins J, Metzger JM (2007) Cardiac transgenic and gene transfer strategies converge to support an important role for troponin I in regulating relaxation in cardiac myocytes. Circ Res 101:377–386
156. Zhang R, Zhao J, Mandveno A, Potter JD (1995) Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation. Circ Res 76:1028–1035