Sarcomere dynamics during muscular contraction and their implications to muscle function

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REVIEW PAPER

Sarcomere dynamics during muscular contraction and their implications to muscle function

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Abstract This article attempts to identify the key aspects of sarcomere inhomogeneity and the dynamics of sarcomere length changes in muscle contraction experiments and focuses on understanding the mechanics of myofibrils or muscle fibres when viewed as independent units of biological motors (the half-sarcomeres) connected in series. Muscle force generation has been interpreted traditionally on the basis of the kinetics of crossbridge cycling, i.e. binding of myosin heads to actin and consecutive force generating conformational change of the head, under controlled conditions and assuming uniformity of sarcomere or half-sarcomere behaviour. However, several studies have shown that re-distribution of internal strain within myofibrils and muscle fibres may be a key player, particularly, during stretch or relaxation so that force kinetics parameters are strongly affected by sarcomere dynamics. Here, we aim to shed light on how force generation, crossbridge kinetics, and the complex sarcomere movements are to be linked and which mechanical concepts are necessary to develop a comprehensive contraction model of a myofibril.

Keywords Inhomogeneity · Myofibril mechanics · Half-sarcomere · Relaxation

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Introduction

One of the intriguing features of the 'motor construct' in vertebrate heart and skeletal muscle is its hierarchical and highly organised structure, from the whole organ down to the molecular level. Unlike industrial motors, which have only up to a dozen combustion units, nature's design, the biological machine, consists of thousands of microscopic motor units, the sarcomeres containing the molecular motors, regularly aligned to constitute myofibrils and muscle fibres; their parallel arrangement builds a muscle that is able to produce a force of thousands of Newton. In fact, the functional motor unit is the half-sarcomere and the term 'motor unit' will be used in this sense throughout the paper. From a physiological point of view, such architecture has the advantage that failure of some motor units is easily compensated and repaired by means of efficient cell biological reorganisation (Goldspink 2005; Hill et al. 2003). From a mechanical-engineering point of view, however, its stability and regulation would demand a sophisticated control system; for example, a single strand with N biological force generators (motor units, e.g. half-sarcomeres in a myofibril) connected in series up to constant total length (a) is no stronger than the weakest motor unit, and (b) has N-1 degrees of freedom. The first property is deduced from the *action-reaction* law in mechanics, hence that in-series segmental forces are equal. The second property implies that despite constant total length the motor units may change their length dynamically, which in the case of a muscle-like motor system would change the actively generated and passively developed force (Fig. 1). Moreover, in a 3D network of sarcomeres, such as the muscle fibre, the forces would also be transmitted laterally (radially) and hence, despite the increased number of degrees of freedom and the higher complexity, the system has

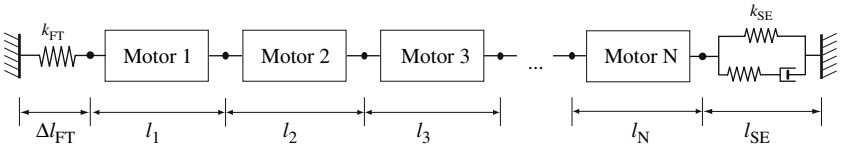

Fig. 1 Schematic representation of a linear chain of N biological motor units connected to the ends via a general viscoelastic element (with elasticity k_{SE}, representative for both ends) and in series with a force transducer element with spring constant k_{FT}. The total length $l_{tot} = \sum l_i + l_{SE} + \Delta l_{FT}$ is either held constant ('end-held' measurement) or undergoes an externally controlled length change, e.g. ramp stretch or shortening. Generally, force measurement is nothing else than acquiring the deflection Δl_{FT} of a calibrated linear transducer element and transforming the values with the formula $F = k_{FT} \cdot \Delta l_{FT}$. A requirement of the force transducer is a high stiffness to reduce the deflection to a minimum while guaranteeing enough resolution. Importantly, this deflection, which allows the sample to shorten, is generally known. On the other hand, the 'end compliance' or 'series element' (SE) of the system, which is in most cases a complex, non-linear viscoelastic element, is generally not well characterised. The motors can generate 'active' force, which basically means that they can increase force by keeping the same length, which also implies a change in their stiffness. Muscle sarcomeres can, additionally, shorten while maintaining the high force generation, proving that they cannot be represented by simple springs that switch between two stiffness levels. Note that each of the motor units in series has to bear the same force. The dynamics of each motor unit is then mathematically coupled to all others via l_{tot}

more boundary conditions and is generally more stabilised, i.e. has less internal dynamics than a single strand such as the myofibril.

For length measurement and modelling force generation, the sarcomere network is often simplified as a large crystal-like system of repeating structural unit cells (sarcomeres) on the basis of homogeneity assumptions (Rudel and Zite-Ferenczy 1979). However, this is unreliable with such small biological systems because irregularities are common, and force response cannot be interpreted as response of a specific molecular motor (i.e. myosin) in the system, which prevents valid mechanical characterisation. Taking the sarcomere as the structural unit cell of the system, it is unlikely that all the sarcomeres in a muscle fibre should have exactly the same characteristics, e.g. strength, number of potential crossbridges or sensitivity to $[Ca^{2+}]$. Past experiments have revealed that inhomogeneous sarcomere length changes develop predominantly during isometric tension rise on activation and tension relaxation on deactivation (Edman and Flitney 1982; Edman and Reggiani 1984a; Mutungi and Ranatunga 2000; Stehle et al. 2002a; Telley et al. 2006a) in end-held contraction experiments. Experimental (Edman and Reggiani 1984b) and theoretical (Denoth et al. 2002) studies have pointed out that sarcomere dynamics is slow ('creeping'). Consequently, results from experimental protocols involving fast perturbation such as T-jumps (Coupland and Ranatunga 2003), length steps (Bagni et al. 2005) and fast sinusoidal perturbation (Kawai and Brandt 1980) are presumably less affected by the internal dynamics, although all these protocols involve initial activation of the preparation. Moreover, techniques have been developed to reduce inhomogeneities by controlling the striation of a small fibre segment (see later in this article). With respect to ramp stretches, it has been suggested for a long time that rapid overextension of some sarcomeres (i.e. 'popping') may occur (Morgan 1990), but we and others have recently shown that such extremes are not observed (Rassier et al. 2003a; Telley et al. 2006b).

Three important issues must be addressed when investigating muscle cell or myofibril mechanics. First, at the level of a single half-sarcomere, any change in length produces a characteristic response from crossbridge kinetics as expected from strain dependent features, exemplified by the response to a step length change (Huxley and Simmons 1973). Second, the study of mechanical properties such as length-dependent force of a whole muscle cell or myofibril needs to be extended to their internal dynamics for the proper description of a multi-sarcomeric system. Third, in a multi-dimensional system dictated by dynamic and biochemical processes, an apparent steady-state observed over the time of a tetanus may actually be a transient response over a longer time scale. We will attempt to outline these issues that relate to sarcomere dynamics and have not been considered adequately until recently. Indeed, Sugi and Tsuchiya (1998) have pointed out that, despite tremendous technical advances allowing high temporal and spatial resolution, sarcomere length inhomogeneity prevents interpretation of experimental data at the level of elementary crossbridge events. In today's research such interpretations are still made although the dynamics of lengths during contraction is neither controlled nor monitored (e.g. Peterson et al. 2004).

The present article gives an overview of the most relevant literature in the field of sarcomere inhomogeneity and reviews recent findings from sarcomere dynamics that impact on the interpretation of force kinetics.

Problems of segment length measurement and control

Single intact and skinned muscle fibre experiments are commonly used by researchers to unravel the molecular events during contraction. On the other hand, a single fibre

is a complex compound of different filaments with an ordered alignment, forming thousands of sarcomeres in series and parallel, presumably operating as more or less independent force generators. Insofar, the force as system response is a sophisticated convolution of the dynamics of all functional units (Denoth et al. 2002). In principle, an interpretation of force recordings down to the level of molecules implicitly assumes that all half-sarcomeres are operating identically (homogeneity assumption). However, early observations of sarcomere length inhomogeneity (Edman et al. 1982; Edman and Flitney 1982; Edman and Reggiani 1984b, 1987; Gordon et al. 1966; Julian and Morgan 1979; Morgan and Proske 1984; Saldana and Smith 1991) have let to the idea that a biological variability among sarcomeres exists, i.e. that differences up to 10% in force generation capacity or passive elasticity of sarcomeres might cause the irregular striation pattern obtained from microscopic images (Hill 1953, 1970).

Sarcomere length measurements during experiments are usually provided by laser light diffraction (Rudel and Zite-Ferenczy 1979). Roughly, the first order peak of the diffraction pattern is inversely proportional to the mean periodicity of the striation, the regular repeat of A- and I-bands from sarcomeres at the spot of the laser. It has been observed that the left and right diffraction lines generally display differences in intensity, and that the intensity is dependent on the incident angle of the laser beam. This has led to the conclusion that the diffractive lines are caused by a large number of sarcomeres of myofibrillar planes that fulfil the Bragg condition and give an average estimate for the sarcomere length. Hereby, small deviations from the ideal volume grating cause blurring in, or fragmentation of, the first order peak of the diffraction pattern. The *origin*, however, and the amount of irregularities are not detected. Edman and Flitney (1982) suggested that such 'diffraction splitting' could be caused either by different populations of sarcomere behaviour or by out-of-plane movement of the different myofibrils. However, they emphasised that the interpretation of force is critically dependent on the existence of non-uniformity and not on the exact nature of mechanisms leading to the differences in sarcomere behaviour. A direct interpretation of filament overlap dependent force and crossbridge kinetics from such myofibres is not possible because of the non-steady situation and the intrinsic variability. The mechanism itself can only be studied by means of a more realistic model that takes account of many half-sarcomeres operating non-uniformly in a network.

Several modifications of the light diffraction technique have been elaborated. It has been incorporated in a feedback system in which mean sarcomere length of a very short fibre segment is *clamped* by keeping the distance between the zero and first order peak constant (Bagni et al. 1988), but the basic difficulties of laser diffraction remain the same. A different approach is the 'spot follower' technique in which several markers are put on the fibre surface and their position is observed by a photo-electric device (Gordon et al. 1966). The length of two markers can also be held constant ('length clamp') with a feedback system and a servomotor that pulls one fibre end. By doing so, sarcomere length is measured as an average of all sarcomeres, in the range of 10–50 sarcomeres, lying between the markers, and sarcomere non-uniformity is averaged out. The potential to assess and compensate the effect of sarcomere dynamics on force generation has been demonstrated by Edman and Reggiani (1984a). They clamped a short segment (0.5–0.8 mm, 230–360 sarcomeres) of a frog muscle fibre during activation and force development and showed that the early depression of force during rise ('tension creep') vanished and that the rate of force development is much faster. They pointed out that the force derived from such measurements is probably very close to the sarcomeric *isometric* force development in the observed segment. The fact that shortening sarcomeres produce less force than isometric ones (Huxley 1957) provides a reasonable explanation for early force depression. While filaments slide past each other during shortening, the initially strained, force-producing crossbridges that have just attached and undergone the power stroke lose their force generation and detach quickly, or may even be strained negatively. Thus, the duty ratio is generally lower, and force development of a population of crossbridges (in a half-sarcomere) is slower. We will outline this issue later in the article.

A neat technique of controlling sarcomere (striation) uniformity in fibres is the use of a 'striation follower'. It is an integral technique based on laser light diffraction by which a fibre region is monitored for striation uniformity (Huxley et al. 1981). In principle, at two positions of the fibre, at a distance of 1–3 mm, the zero- and first-order diffraction pattern is reconstructed to form a sinusoidal intensity distribution of the image of about ten consecutive sarcomeres. The pattern is optically averaged to form a single-cycle (i.e. one period of) sinusoidal light intensity distribution that is projected on an array of five photodiodes. The signal of these photodiodes is combined to give the fibre displacement in terms of sarcomere length, therefore counting the number of sarcomeres entering and leaving the optical field. The sarcomere length in the fibre segment is clamped by driving the motor such that the number of sarcomeres entering and leaving the segment on each side is identical. The method has been successfully used in numerous studies, most recently e.g. in Decostre et al. (2005), Bagni et al. (2005), Mantovani et al. (2001), and is more reliable compared to laser diffraction alone. However, despite accurate measurements of the mean

sarcomere length in the fibre segment, information about possible non-uniformity within the segment is effectively averaged out in length recordings and cannot be acquired.

Myofibrillar recordings make single sarcomere mechanics tangible

The single myofibril is the smallest regulated, functional experimental model of the muscle, since it retains the fundamental sarcomeric structure and has many technical advantages over muscle fibres (Lionne et al. 2003). Lately, mechanical studies on single myofibrils or bundles have become possible with the advent of sophisticated force measurement techniques with glass micro-needles (Anazawa et al. 1992; Colomo et al. 1997), miniature platinum wire loops in a magnetic field (Friedman and Goldman 1996; Iwazumi 1987), optical fibres with known compliance (Bartoo et al. 1993) or commercial atomic force cantilevers (Stehle et al. 2002b). Similar to single fibre experiments, myofibrils have been attached between the transducer and a motor driven micro-tool using adhesive coating or adhesion forces emerging between glass and the myofibril. All laboratories concluded that the physiological properties of myofibrils do not differ significantly from those of fibres; because of their small width (the diffusion distances are less than 2 μm), however, single myofibrils equilibrate with bathing solutions in less than a millisecond providing a powerful method to study Ca^{2+} activation and relaxation kinetics (Stehle et al. 2002a; Tesi et al. 2002b) and to perform chemical perturbation experiments, e.g. with ATPase products (Piroddi et al. 2006; Tesi et al. 2002a). Another important advantage of myofibrils is that they consist of a limited number of sarcomeres, which facilitates the observation of single sarcomeric behaviour during contraction.

An important issue still under discussion is whether muscle fibre mechanical properties estimated from force measurement during perturbation are a simple summation of the properties of its myofibrils. Cell biological studies have reported that intermediate filaments (e.g. desmin) connect neighbouring myofibrils (Wang and Ramirez-Mitchell 1983). It has also been suggested that these connections transmit force laterally towards the fibre membrane and stabilize the sarcomeric network (Shah et al. 2002). Friedman and Goldman (1996) reported that, in bundles of 2–3 myofibrils, a myofibril only partly attached to the mechanical setup showed restrained shortening of sarcomeres due to the lateral connections compared to single myofibrils. However, they found that these lateral connections do not influence markedly basic contractile properties, such as isometric force, force-velocity relation and response to quickstep perturbation (Huxley and Simmons 1971), but rather reduce local non-uniform length changes of sarcomeres. Colomo et al. (1997) compared their passive myofibril data with those in fibre studies, which would potentially lead to a mechanical (component) dissection of membranous and lateral non-membranous forces from pure (myofibrillar) axial forces originating from actomyosin interaction and cytoskeletal structures such as titin.

First quantitative analyses of individual sarcomere lengths in relaxed myofibrils were presented in the early nineties (Bartoo et al. 1993; Linke et al. 1994). Briefly, sarcomere lengths were determined by observing or analysing the band pattern of the myofibril, originating from consecutive A- and I-bands, and calculating the distance between peaks of the intensity (profile analysis). Semi-quantitative analysis of sarcomere lengths from image profiles of Ca^{2+}-activated myofibrils was performed by Anazawa et al. (1992) focusing on spontaneous oscillatory contractions (SPOC). First length traces of individual myofibrillar sarcomeres in cardiac myofibrils during the relaxation phase were presented by Stehle et al. (2002a); they showed that during the fast phase of force relaxation sarcomeres exhibit spatially and temporally organised but non-uniform behaviour. In another study by Rassier et al. (2003a, b) the dynamics of sarcomeres during ramp stretch was shown to be non-uniform, but sarcomeres remained more stable than assumed in earlier years based on modelling considerations (Morgan 1990, Morgan et al. 1982).

The afore-mentioned image profile analysis, however, is not valid in terms of what is measured and how it is interpreted. The principle of sarcomere length measurement is to have a quantitative estimate for the overlap between thin and thick filaments, and hence for the potential force generated from the actomyosin interactions (Gordon et al. 1966). However, a sarcomere has an overlap zone in each of the two halves; a difference in force-generating properties can cause larger overlap in one half compared to the other, i.e. an asymmetry in the sarcomere (Horowits and Podolsky 1987). As has been outlined in one of our recent studies (Telley et al. 2006a), the distance between consecutive A-bands or I-bands does not provide a valid measure of filament overlap because sarcomeric asymmetry is averaged out and non-uniformity on the half-sarcomere level is generally underestimated.

Half-sarcomere dynamics examined using fluorescence labelling and computer vision

The most prominent boundary in the muscle sarcomere is the Z-line that anchors the actin filaments; the connections are formed by α-actinin linking anti-parallel actin filaments

from adjacent half-sarcomeres (Luther et al. 2003). The high concentration of α-actinin localised in the Z-line has been used for sarcomere boundary tagging (Littlefield and Fowler 2002). The M-band is a less prominent boundary structure that divides a sarcomere into two halves and cross-links the myosin filaments, and it varies much in its structural arrangement among species (Agarkova and Perriard 2005). The occurrence of several auxiliary proteins (e.g. M-protein, myomesin) has promoted the development of markers for specific or unspecific M-band protein detection (Agarkova et al. 2000; Grove et al. 1984; Obermann et al. 1996). The two structures, M-band and Z-line, define a spatial measure for the overlap zone between actin and myosin in a single half-sarcomere. Their proper localisation circumvents potential problems when analysing the intensity profile of the striation bands in phase contrast images where sarcomere asymmetry may occur (see previous section).

Recently, immuno-fluorescence labelling of myomesin and α-actinin has facilitated the study of half-sarcomere behaviour in fully functional, contracting myofibrils (Telley et al. 2006a, b). This technique facilitates the accurate measurement of changes in the length of individual half-sarcomeres and, hence, the elementary filament sliding mechanism in them. Despite the limited resolution capacity of light microscopy (Rayleigh limit), it has become possible to determine length changes in the nanometre scale via model-based post-processing. The fluorescently labelled areas corresponding to the Z-line and M-band were tracked frame by frame on the basis of a radiometric and geometric transformation model and by stochastic noise analysis, which yields the position of the signal pattern in sub-pixel resolution (see Fig. 2); this method is similar to the *FIONA* technique developed to track the position of fluorescent molecules, in principle by performing single point source deconvolution with a Gaussian approximation (Yildiz and Selvin 2005). Importantly, our technique does not only work for point-shaped signal patterns as required with FIONA. In both techniques the resulting positional accuracy, however, is strongly dependent on the signal-to-noise ratio of the image and thus on the number of acquired photons per frame; a compromise between temporal and spatial resolution is inevitable.

The most significant findings from our recent studies of Telley et al. (2006a, b) were threefold. First, in cardiac myofibrils the elongation of sarcomeres after Ca^{2+} removal (Stehle et al. 2002a) is sequential at the half-sarcomere level; this wave-like process seems to involve different coupling mechanisms in M-bands and Z-lines. Second, independent of the experimental protocol, a whole sarcomere may not operate symmetrically so that (the myosin containing) A-band may shift towards one end (Horowits and Podolsky 1987); this asymmetry arises concomitant with the force rise during activation and with amplitudes up to 100 nm. In fact, this value highlights the general observation that filament sliding in contracting half-sarcomeres presumably involves several crossbridge attachment-detachment cycles. Third, contrary to earlier predictions (Morgan 1990) a sarcomere exhibits increased stability during stretch and no sarcomere 'popping' is observed. The occurrence of A-band displacement, the second of the three findings, is far easier to explain with current models than the other two. An imbalance in force generating crossbridges in the two halves is counterbalanced by extending the (non-crossbridge) passive structures, i.e. titin filaments in the (weaker) lengthening half of the sarcomere until a steady-state with no further length change emerges. However, in order to explain the sarcomere stability during stretch, additional assumptions have to be made for the crossbridge kinetics, such as catch-bonds (Guo and Guilford 2006) or for non-crossbridge interactions between filaments (Kellermayer and Granzier 1996) that might be enhanced during stretch. It is generally accepted that kinetic models of crossbridge action alone cannot account for these features, and the use of rigorous mechanical modelling, probably in more than one dimension, combined with multi-state kinetics would be necessary to fully understand the underlying processes. A priori, it is also not clear if the introduction and tuning of simple viscoelastic passive structures parallel to actomyosin interaction in a multi-segmental model is capable of explaining the homogenisation during stretch. Further studies have to be performed to elucidate these issues.

Force kinetics and (half-) sarcomere dynamics during relaxation

The tension relaxation process plays a key role in the function of striated muscles. In the heart it is involved in rapid activation-deactivation cycles e.g. during each diastole, and in skeletal muscle it is important during dynamic contractions e.g. while running. The process itself is governed by two distinct kinetic systems; the thin filament regulation mediated by free cytoplasmic Ca^{2+} whose concentration is controlled by gated Ca^{2+} channels and membrane-bound Ca^{2+} pumps, and the cyclic interaction of myosin S1 with the regulated thin filaments (crossbridge turnover). A decrease of cytoplasmic $[Ca^{2+}]$ causes Ca^{2+} dissociation from troponin C on the thin filament, which in turn inhibits actomyosin interaction (see e.g. Gordon et al. 2000).

To date, it is still unresolved which one of the kinetic processes determines the characteristics of relaxation and hence rate-limits the relaxation process. A striated muscle

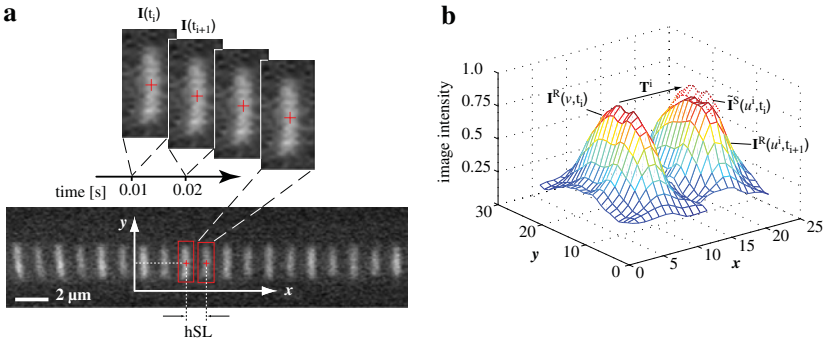

Fig. 2 Illustration of the pattern tracking process used to measure half-sarcomere lengths from video streams. (**a**) Video frame of a fluorescently labelled myofibril showing the Z-line and M-band patterns (Telley et al. 2006a). Around each pattern a region of interest (ROI, red box) is defined in the very first frame. The distance between two neighbouring patterns, hence the half-sarcomere length, is computed by calculating the vector difference between the positions (red cross) of the two ROIs following the matching procedure; this is repeated for all frames $I(t_i)$, i = 1…n on the discrete time scale. (**b**) The aim of the matching procedure is to find the pattern of interest (Z-line or M-band signals) in the search image which corresponds to the template according to an objective function (e.g. sum of squares of differences). The minimisation of the objective function drives the determination of a set of parameters of a *radiometric* and a *geometric* transformation between the Reference (template, time t_i with intensity I^R) and the Search image (time t_{i+1} with intensity I^S):$I^S(v) \underset{T}{\mapsto} \tilde{I}^S(u^i) \underset{R+e}{\mapsto} I^R(u^i)$. The geometric transformation T deals with image changes due to displacement, rotation and deformation and involves interpolation of intensity values for the transformed coordinate system $v = Tu^i$ from the original coordinate system u^i. The radiometric transformation R models intensity changes due to temporal variation in illumination and reflection characteristics, and is assumed to be linear. The intensity signal is perturbed by noise e modelled as zero mean normally distributed, independent and additive for each sample. The combination of the two transformations and the noise model yield an error equation for every pixel, of which the summed square is then argument-minimised and yields the transformation parameters. The solution of this least squares problem involving stochastic data is sought on the basis of the Gauss-Markov model, which delivers an a posteriori estimate of the image noise; it allows to calculate an estimate of accuracy (defined as standard deviation σ) of the positional changes. This estimate is based on the assumption that image noise is zero-mean, normally distributed and uncorrelated. For details see Danuser (1997)

relaxes in two phases: a slow phase 1 (0.1–2.0 s^{-1} depending on the species and muscle, see Poggesi et al. 2005) accompanied by almost linear force decay and practically isometric sarcomeres, and a faster phase 2 exhibiting exponential force decay (roughly ten times faster than in phase 1) and large, non-uniform changes in sarcomere length (Edman and Flitney 1982; Stehle et al. 2002a). Phase 1 has been probed with different interventions (P_i, MgADP) to elucidate the turnover of detaching crossbridges. Flash photolysis in skinned trabeculae (Mulligan et al. 1999; Simnett et al. 1998) and solution switching in myofibrils (Stehle et al. 2002a; Tesi et al. 2002b) circumvent the kinetics of cytoplasmic Ca^{2+} sequestration, which may play a role in the early stage of relaxation. The studies confirmed that MgADP decelerates early relaxation kinetics whereas P_i accelerates it in cardiac (Stehle et al. 2002a) and mammalian skeletal muscle (Tesi et al. 2002b) but not in frog muscle as shown by Simnett et al. (1998) and Mulligan et al. (1999). However, the findings basically support the idea that crossbridges can leave force-generating states either by completing the cycle (which is slowed by MgADP) or via reverse stroke and detachment (accelerated by P_i). Mulligan et al. (1999) suggested from their flash photolysis experiments with frog fibres that phase 1 is governed by the rate of Ca^{2+} removal from troponin C. Furthermore, they proposed that the slowing of phase 2 at higher [P_i] in frog fibres is caused by an increase in a strongly-bound low-force P_i state. On the other hand, Tesi et al. (2002b) concluded from experiments with fast switching between different [Ca^{2+}] that full deactivation of the regulatory system is fast, and that the observed slow linear phase is predominantly determined by the crossbridge detachment kinetics. This is in accordance with the short off-time of thin filament deactivation probed with stopped-flow techniques (Shitaka et al. 2004; Stehle et al. 2006) and is refuting the early idea that sarcomere length changes in phase 2 are due to variations in activity along a preparation (Edman and Flitney 1982).

The circumstance of sarcomere dynamics in phase 2 prevents straightforward interpretation of force in terms of crossbridge kinetics. Nevertheless, of particular interest is the cause for the transition from phase 1 to 2, which occurs contemporaneously with the initiation of sarcomere dynamics. Data from Poggesi et al. (2005) reveals that the decrease in active force of phase 1, in fraction of maximum force P_0, is 22% for frog skeletal, 10–12% for mammalian skeletal and 3–8% for mammalian cardiac muscle. Specifically, in case of cardiac myofibrils from guinea pig the critical force decay is (mean ± SE) 7.6 ± 0.8% P_0 at 10°C and is enhanced slightly (~10%) for elevated [P_i] and higher temperatures (Stehle et al. 2002a). On the other hand, numbers of Tesi et al. (2002b) show that in psoas myofibrils at 5°C the same value is almost tripled from 10.8 ± 0.6% P_0 to 29.2 ± 3.4% P_0 when introducing 5 mM

P_i but is unaltered at higher temperatures and at elevated [MgADP]. However, since it is a relative value and force is suppressed by 30% at 5 mM P_i it should not be overvalued. The phosphate and temperature dependence suggests that the critical force level is linked to the occupation of force-generating states, possibly to an absolute critical number of still attached crossbridges that have to bear the remaining force and, thus, are strained to a higher extent. To further understand the mechanics in this situation, we consider, among N other half-sarcomeres in a myofibril, the 'weakest' half-sarcomere that is just about to relax at the end of phase 1. The crossbridges in a cross-sectional unit cell of this half-sarcomere (one myosin plus two actin filaments, see Fig. 3a) operate in parallel and are attached to an elastic spring. This spring represents several components (in the same cross-section along the myofibril): (a) all elastic structures in series to the crossbridges of the particular half-sarcomere considered, i.e. the filaments in the non-overlap zone, the Z-line and M-band, and (b) all half-sarcomeres in series, *each* involving filament compliance and the sum of still attached crossbridges. A simple mechanical calculation reveals that, regardless of the crossbridge detachment kinetics, if the stiffness of one crossbridge is in the same range as the series elasticity (i.e. stiffness ratio ~1) almost 2/3 of the attached heads have to dissociate to reach a critical force level of ~92% P_0 (for cardiac preparations); the remaining crossbridges are then strained by additional 6 nm besides the strain at maximum isometric force (~2 nm, see Piazzesi et al. 2002). Stiffness ratio 1 can be attained when the half-sarcomeres in series are still much stiffer (roughly of the order N on average) than the weakest, or if there is a compliant component in series to crossbridges in half-sarcomeres (e.g. filaments). Assuming 60 of 150 potential crossbridges initially attached in a cross-sectional unit cell (~40% occupation, Linari et al. 1998), only 15 crossbridges would bear the force at the transition from phase 1 to phase 2. The large crossbridge strain at this point might accelerate considerably the detachment rate (Veigel et al. 2003) such that these strained crossbridges finally detach within microseconds and the (weak) half-sarcomere elongates until force is borne by passive structures. However, we point out that these results depend on the ratio between crossbridge stiffness and the stiffness of structures in series to them (filaments, attachment, etc). Our theoretical considerations further show that with much stiffer series elasticity less

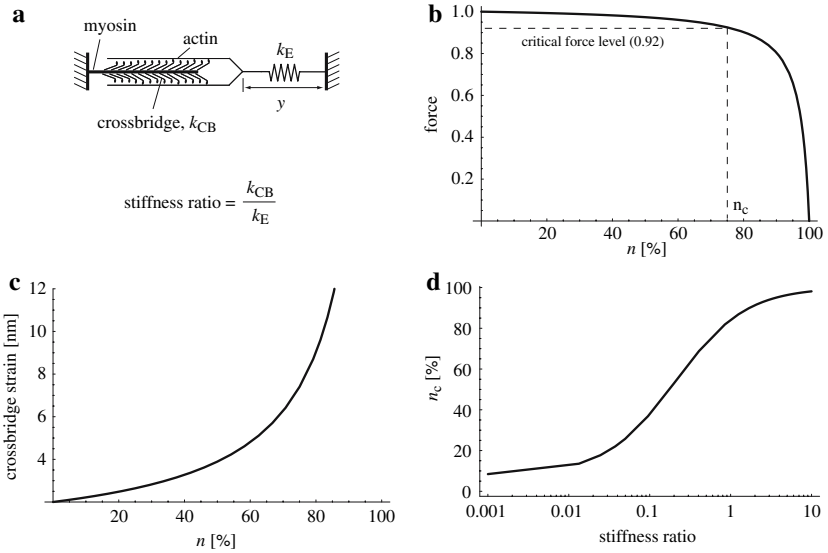

Fig. 3 Theoretical considerations on relaxation as a pure mechanical process of discrete detachment of myosin heads independent of the kinetics of detachment. (**a**) Model of an actomyosin unit cell (in a half-sarcomere) connected to a linear elastic spring (k_E). Each unit cell involves a myosin filament, two actin filaments and ~150 potential crossbridges with a linear spring constant k_{CB} ~5 pN/nm, x = 2 nm initial maximal isometric strain, and ~40% occupation during maximal isometric force generation (Linari et al. 1998; Piazzesi et al. 2002). No reattachment is assumed during relaxation. Then, the force of the initially N attached heads equals the force of the external spring; for n detached heads the remaining are strained by an additional amount Δx, and the force equation is: $(N-n)k_{CB}(x+\Delta x) = k_E(y-\Delta x)$. This equation yields a functional relation between n and Δx. (**b**) Relationship between normalised force (1.0 for isometric maximum, P_0) and fraction n of detached heads for a stiffness ratio 0.6. At a critical force level of 0.92 P_0 (end of phase 1 during relaxation, see text) the critical fraction n_c of detached heads is ~75%. (**c**) Relationship between crossbridge strain and fraction n of detached heads. At the end of phase 1 the remaining crossbridges would have a strain of ~8 nm. (**d**) The critical fraction of detached heads depends on the ratio of external to crossbridge elasticity. If crossbridges and other structures have roughly the same elasticity (Huxley and Tideswell 1996; Linari et al. 1998), then 60–80% are predicted to detach in order to reach the force level where the remaining crossbridges dissociate and the contractile structure collapses (transition of phase 1 to phase 2 in force)

than 50% of the crossbridges have to detach to reach the critical force level (see Fig. 3d).

The sequential relaxation of half-sarcomeres in cardiac myofibrils during phase 2 cannot be understood without taking into account the structural mechanics. Two relevant issues / findings have to be addressed when postulating a mechanism: (a) Relaxation is not only sequential in time (one half-sarcomere after another) but also in space (from one to a neighbour only) which demands for a mechanical coupling that is attributed to structures localised at the interface between neighbouring half-sarcomeres. (b) The transfer time, as defined in an earlier paper (Telley et al. 2006a), is too long (40–80 ms) to be explained by simple material wave propagation; hence, a rather slow biochemical process must be involved in the relaxation mechanism of phase 2. It has been proposed earlier (Stehle et al. 2002a) that the strain from a relaxing, lengthening sarcomere taken up from still short sarcomeres enhances detachment and promotes a next sarcomere to relax. This statement is equivalent to the mechanical law of force identity in a series connected system (i.e. axial force is the coupling parameter). However, it only explains sequential behaviour *in time* without telling where the next half-sarcomere to relax is localised in the myofibril. Thus, the question remains what causes the weakening of the neighbouring half-sarcomere only, e.g. by enhancing crossbridge detachment in it. We recently proposed a theory that involves the filament spacing, its propagation and effect on the interaction of crossbridges (Telley et al. 2006a, see also Fig. 4).

The phase 2 of relaxing skeletal muscle exhibits a faster sarcomere dynamics than in cardiac muscle, and it is not clear so far whether the same mechanism causes a sequential relaxation but much faster than can be acquired by means of current techniques. We suggest that the propagating process of sarcomeric relaxation plays a relevant physiological role in daily life. High heart rates (up to 180 s^{-1}) and fast skeletal movements (running, eyelid batting) would probably not be possible without structurally enhanced relaxation processes as found exemplarily in cardiac and skeletal myofibrils.

Sarcomere shortening during activation

The sliding filament theory and the crossbridge theory initially introduced by Huxley (1957) foresee that force production is depressed in shortening muscle, which is reflected by the force-velocity curve for steady-state shortening. Many past studies have revealed that sarcomeres shorten during force development to tetanus or twitch contraction in intact preparations (Cecchi et al. 1991; Edman et al. 1993; Edman and Flitney 1982; Julian and Morgan 1979; Mutungi and Ranatunga 2000; Piazzesi et al. 1992; Piazzesi et al. 2003), in maximally Ca^{2+} activated skinned preparations (Brenner and Eisenberg 1986; Hilber and Galler 1998) and recently also in myofibrils (Telley et al. 2006a). Series elasticity might explain the slower force rise achieved in experiments than predicted from crossbridge modelling. The contractile elements have to lengthen this elastic component in order for tension to be transmitted to the attachment sites. Edman and Reggiani (1984a) showed that by clamping a small segment, i.e. by lengthening the muscle fibre to prevent early shortening in a segment accelerates force development upon activation. In their X-ray diffraction study Cecchi et al. (1991) provided evidence that sarcomere shortening during tetanus rise produce changes in lattice spacing and reduce force development, but these effects could be minimised up to 90% when initial sarcomere shortening was reduced to <0.3% during length-clamping. Since in clamp experiments equatorials and stiffness had a similar time course but led tension, it is reasonable to conclude that, despite early stiffness increase, some structure in series to the crossbridges has to be elongated as to transmit the force to the transducer where it is recorded. Julian (1969) added a series elastic element to the 1957 Huxley crossbridge formalism to simulate this internal shortening during the rise of force and its effect on force development in end-held contracting fibres. Studies investigating force and stiffness rise during activation have revealed that force development is slower than stiffness development in the early phase of activation (see Bagni et al. 1988 and references therein). Although several of these studies have proposed new crossbridge states or cooperativity of actomyosin interaction to explain this time lag, internal shortening due to series elasticity may provide the simplest mechanical explanation. Luo et al. (1994) explored the temporal evolvement of stiffness and force in a crossbridge model connected to a series elasticity and demonstrated that this model could explain (a) the time lag between force and stiffness, and (b) the slower force development compared to that from attachment rates used in Huxely's model in a strictly isometric contraction. This shows that the problem of internal dynamics during activation has been recognised for a long time. Nevertheless, parameters are extracted from the kinetics of force development to check muscle functionality, and are then translated into biochemical kinetics underlying muscle contraction in a more or less straightforward manner. These parameters are surprisingly reproducible for myofibrils from different species (Poggesi et al. 2005). However, a myofibril set up in a testing device underlies the laws of mechanics (force transmission, load-dependent strains, series connection), and myofibrillar force measurement is not a mere problem of biochemical kinetics any longer (see also next section). In fact, a mere

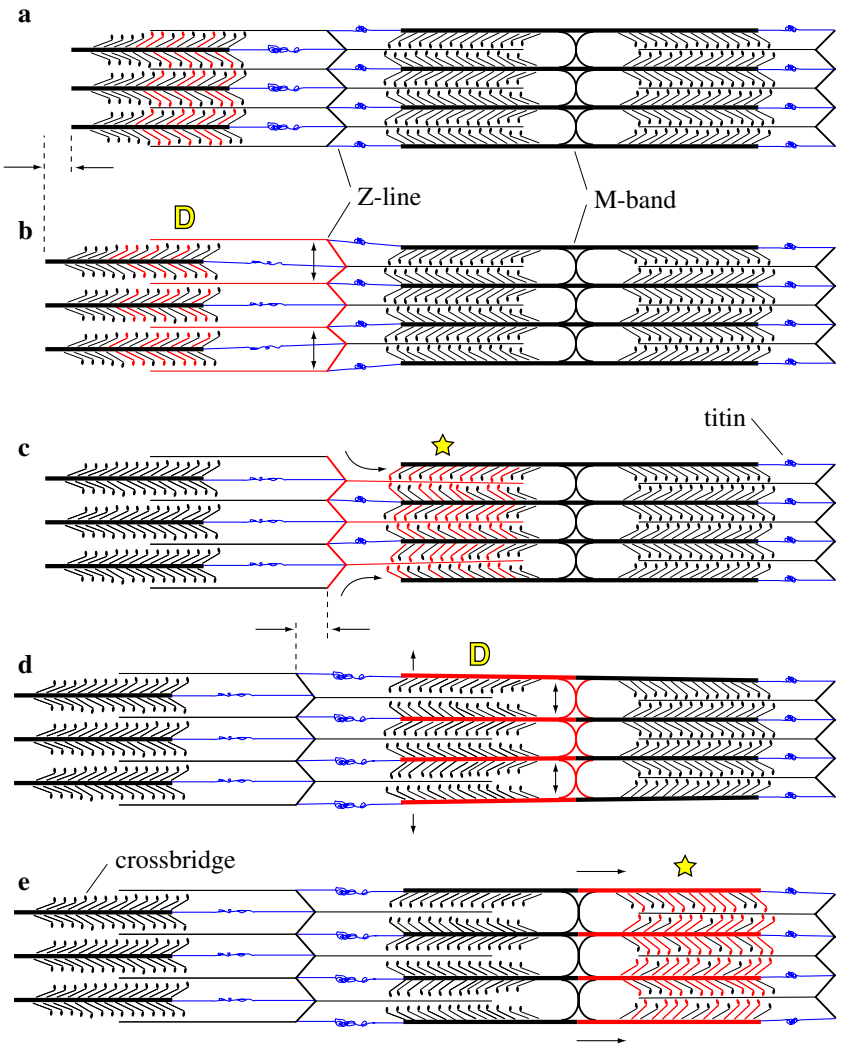

Fig. 4 Scheme of the sequential relaxation process in a cardiac myofibril. (**a**) In a longer half-sarcomere with, consequently, small filament overlap the few remaining attached crossbridges are highly strained so that they leave their force-generating state and detach rapidly (illustrated by the red heads). (**b**) Complete detachment ('D') causes this particular half-sarcomere to lengthen (horizontal arrows) until, in a steady state, titin bears the force. Crossbridge detachment causes a disturbance in the lattice distance of the actin filaments (red). The result is a lateral strain in the Z-line. (**c**) Since actin filaments do not penetrate the Z-line structure and extend into the adjacent half-sarcomere, the lateral strain in the network structure of the Z-line affects weakly the lattice in the adjacent half-sarcomere (low coupling). Thin filaments are moved from their preceding position in active state; this affects the affinity of the bound heads by shifting the free energy potential (marked with a star). The rigidity of the crossbridge in lateral direction is assumed to be higher than in axial direction, which promotes detachment. (**d**) The accelerated dissociation of myosin heads allow this half-sarcomere to elongate (horizontal arrows, as in b). Crossbridge dissociation also alters the lateral force equilibrium, causing an increase in the distance between myosin filaments, which is taken up partly by the lateral structures in the M-band (red). (**e**) Since M-band structures are moderately stiff and myosin filaments extend to the neighbouring half-sarcomere, the disturbance in the lattice is transmitted largely (high coupling) to the right and the attached myosin heads there are strained laterally; this promotes rapid detachment (star)

binding-unbinding model to characterize the kinetics would not explain force generation of crossbridges without any strain involved. Importantly, taking into account the findings from above mentioned studies, one should reconsider the significance (and validity) of such *functional indicators* first of all regarding current model thinking and also in terms of actual crossbridge kinetics. We acknowledge the power of these parameters as empirical descriptors, but have reservations about how they are interpreted in terms of mechanokinetics. Therefore, the relationships between kinetic parameters, series elasticity and perturbation from internal shortening have to be further elucidated.

Here, we revaluate briefly the recently observed shortening dynamics (Telley et al. 2006a) with regard to force development kinetics by considering a contractile model connected to a series elastic element (Fig. 3a). The contractile model obeys the basic rules from the Huxley (1957) formalism in which crossbridges are either attached or

detached and act as linear springs; it also implies crossbridge strain during force generation and, hence, the force is dependent on filament sliding (and suppressed by shortening). We neglect a crossbridge conformational change because this implies a transition from a lower to a higher force per crossbridge while undergoing the cycle, and would not alter significantly the general idea of the following theoretical considerations. In particular, all kinetic parameters are set such that the steady state force-velocity relationship of rabbit psoas muscle at 10 °C is reproduced (see Fig. 5 for details). In a first, rather simple simulation the spring is neglected and the contractile model is allowed to shorten transiently at a range of velocities up to the maximum unloaded shortening velocity. The simulation shows that in a strictly isometric contraction the rate of Ca^{2+} induced force development k_{ACT} is in the range of 40 s^{-1}. On the other hand, if the contractile model is allowed to shorten upon activation, this rate drops substantially to similar values as measured experimentally (5–8 s^{-1}, see Tesi et al. 2002 or Telley et al. 2006a). As a consequence, k_{ACT} is insensitive to sarcomere dynamics if the series elasticity of the system is rather compliant – which is the case in most experiments – but, as a kinetic indicator, it underestimates the isometric crossbridge turnover kinetics by a factor 5–8. In a second, more realistic simulation the force generated by the contractile model stretches the elastic spring, causing a negative (mechanical) feedback via filament sliding (shortening) and force suppression in the contractile model. In this case the force development kinetics involves more than one exponential, one being most prominent for lower spring stiffness. We find that the rate of force development depends approximately linearly on the stiffness of the spring (Fig. 6). Again, with a more compliant spring and, thus, with more internal shortening the rate of force development decreases to ~7 s^{-1}. Overall, these findings emphasize the importance of acquiring length changes of not only each half-sarcomere but also the compliant structures in series when interpreting force data on the basis of crossbridge attachment-detachment kinetics.

The mechanics of an in series-connected motor system

A single muscle fibre is a three dimensional network of linearly aligned and interconnected sarcomere strings, the myofibrils. The mechanical representation of a myofibril is a series arrangement of sarcomere models, each consisting of two mirrored force-generator models for the two halves of a sarcomere. Only few (dynamic) multi-segmental models have been proposed in the past, involving contractile, elastic and viscous elements for each sarcomere (Denoth et al. 2002; Yaniv et al. 2006; Zahalak 1997) or half-sarcomere model (Nishiyama 1984; Telley et al. 2003). Some studies have included only static considerations or have neglected active muscle generation by developing a simple spring-dashpot framework.

In a first approach one can assume no direct interaction via mechanical or chemo-mechanical coupling mechanism between neighbouring segments. Then, one of the laws of nature tells us that the force is identical in all segments in series (Fig. 7a). Consequently, in length-controlled experiments, such as end-held or segment length-clamped measurements, the force becomes the determining (coupling) parameter of the system (Denoth et al. 2002). The

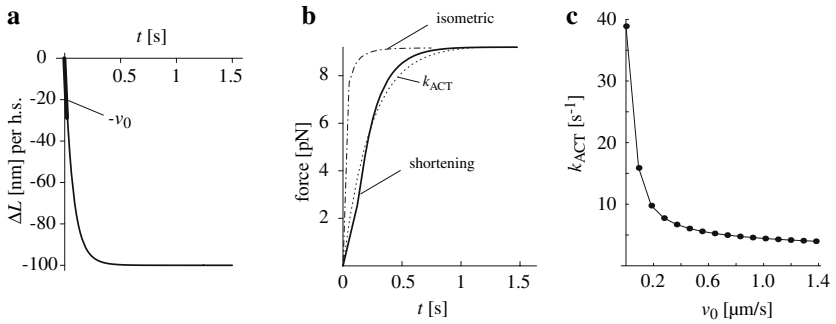

Fig. 5 Simulation of force development after activation of a 2-state model (representing a half-sarcomere) according to the Huxley (1957) formalism in a strictly isometric case and during transient shortening. The kinetic parameters f and g as well as crossbridge stiffness are tuned, by simulating ramp shortening (not shown here), to reproduce the force velocity curve of rabbit psoas muscle at 10°C (Sun et al. 2001), while hydrolysing one ATP molecule (83 pnJ) per crossbridge cycle (Cooke 1997). (**a**) Shortening profile applied to the crossbridge model, resembling the experimental findings of Telley et al (2006a). The initial (maximum) velocity v_0 ranges from zero to maximum unloaded shortening velocity (1.2 L_0/s for rabbit psoas). (**b**) Force (per crossbridge) during activation for the isometric case (dash-dotted) and for transient shortening (outlined); force rise is slowed during shortening, as expected from the Huxley formalism. The curves are fitted with a single exponential function to determine the rate of (Ca^{2+} induced) force development k_{ACT}. (**c**) Dependence of k_{ACT} on the initial shortening velocity v_0. The true kinetics is reflected by the (undisturbed) isometric force development (~39 s^{-1}). At higher velocities k_{ACT} is more or less insensitive with values of 5–8 s^{-1} as experimentally found at these temperatures

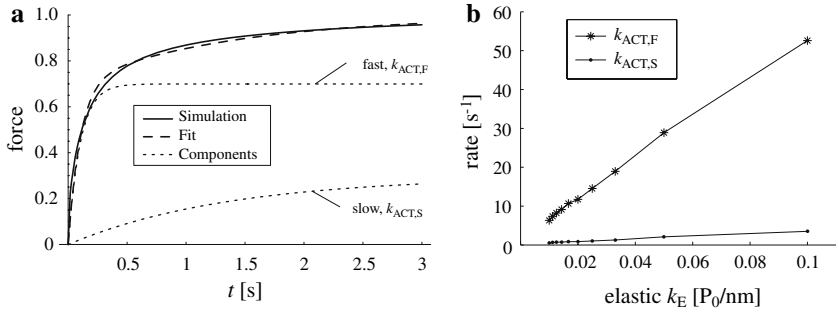

Fig. 6 Simulation of force development of a contractile (actomyosin) model connected to a linear elastic spring in series, as depicted in Fig. 3a (formalism and kinetic parameters as in Fig. 5). The total length is held constant, and the internal degree of freedom allows the contractile element to shorten upon activation. (**a**) Force development curve (normalised to the strictly isometric maximal force) of the spring-actomyosin system after activation, and the bi-exponential fit (dashed) with the fast component (rate $k_{ACT,F}$) and slow component (rate $k_{ACT,S}$). The series elasticity k_E in this example is 0.017 P$_0$/nm and the contractile model shortens 60 nm. (**b**) Relationship between the serial elastic spring constant k_E and the rates of force development $k_{ACT,F}$ and $k_{ACT,S}$ (fast and slow components). For more compliant springs (low k_E) the internal movement is larger, allowing the actomyosin contractile element to shorten more, which suppresses the force development and causes smaller rates. Note that for a low stiffness around 0.01 P$_0$/nm the internal shortening is ~100 nm, and the rate of force development is ~6 s^{-1}, similar to values found experimentally (Telley et al. 2006a)

simple physical law of force identity has several far-reaching consequences: (1) In length-controlled experiments force response can not be attributed to one (half-) sarcomere and hence to crossbridges but is the complex convolving response of the whole system, regardless of whether or not a small segment was clamped. (2) Hence, the dynamics of each half-sarcomere depends on the dynamics of all others. (3) Only force-controlled, especially isotonic (iso | tonic = unchanging tension) experiments decouple the system such that half-sarcomeres are operating independently of each other. (4) Force-clamped experiments with rather slow (>1 ms) feedback control do not provide *true* isotonic conditions because correcting any undesired change in the force at a finite temporal resolution introduces very short length-controlled phases. (5) The only length controlled protocol that allows direct interpretation of force and kinetics could be facilitated (in theory) by clamping the length of a single half-sarcomere. (6) Force response depends largely on the 'end compliance', i.e. on the element that couples the preparation to the measurement device; this element is generally not well characterised in mechanical terms. (7) Relationships such as the length dependent steady-state force (of the whole preparation, fibre segment, etc.) do not directly correspond to (changes in) filament overlap of a half-sarcomere and cannot be translated unconditionally. (8) Variability phenomena and interpretation of sarcomere non-uniformity can neither be explained by, nor approximated by, nor used for predictions with only *one* simple steady-state relationship of force, length and velocity, as often done in the past (see e.g. Herzog 2005 or Morgan 1994). Instead, a relationship representing the filament overlap and the force

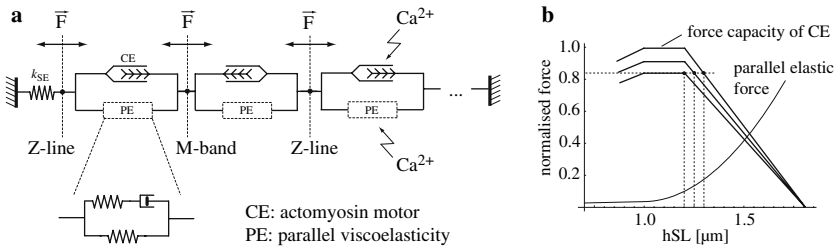

Fig. 7 (**a**) Schematic representation of a myofibril model as a series of half-sarcomere models fixed to the measurement setup via an elastic spring (the three dots on the right denote additional half-sarcomere models). Each half-sarcomere model consists of an actomyosin contractile element (CE) and a parallel element (PE). The parallel element is a general viscoelastic spring-damper system. The force F is identical in each node (Z-line or M-band) between the models. Both contractile and parallel element may change their mechanical properties with addition of Ca^{2+}. (**b**) The maximum steady-state force of each half-sarcomere depends on its filament overlap, thus the half-sarcomere length. The plateau level is the *force capacity* of the half-sarcomere, possibly a measure of the number of potential crossbridge bindings. We propose variability in this number to explain the non-uniform half-sarcomere behaviour, otherwise half-sarcomeres would behave identically; thus the plateau level of different half-sarcomeres may vary (here three levels). In case of a steady state (no movements) the forces equal in each one (horizontal dashed line) and half-sarcomeres can never operate at the same length (vertical dashed lines). However, the reader is alerted that this is a special case during steady state which is rather artificial; the general situation involves dynamic force components from half-sarcomere movement

potential has to be considered for *each* half-sarcomere. We suggest that half-sarcomeres do not have equal numbers of potential crossbridge bonds and hence different force levels at optimal length, i.e. variable force capacities (strength). With this and the requirement of force identity along series-connected half-sarcomeres the statement that they can operate at the same length is ultimately refuted (Fig. 7b). We point out, however, that this is merely a static contemplation and is more complicated in real dynamic situation.

The active contractile force is generally correlated to the stiffness or the compliance of crossbridges, filaments and cytoskeletal structures in muscle mechanical studies. Stiffness is the inverse of the compliance and equal to the force-extension quotient. In a series arrangement of mechanical segments, such as half-sarcomeres in a myofibril, the compliances c_i are summed up and result in the compliance c of the whole system; the sum of all inverted stiffnesses k_i equals the inverse of the stiffness k of the whole system:

$$c = \sum_i c_i \quad \frac{1}{k} = \sum_i \frac{1}{k_i}$$

In a real experimental situation a linear system of individual segments, such as in a myofibril with its half-sarcomeres, includes the (usually unknown) end 'compliance', a mechanical component that is not only elastic but also generally viscous/viscoelastic (see also Fig. 1). Therefore, measuring systemic stiffness by applying rapid stretches has two consequences: (1) the risk of underestimating the unknown end compliance in the force response if it is comparable to the elasticity of all other segments (the half-sarcomeres), and (2) by necessity it involves a velocity-dependent (viscous) component that leads to an overestimation of the real stiffness. Therefore, it is inevitable to observe and measure all segments (half-sarcomeres) and components (transducer, attachment, see Fig. 1) of a linear system for correct interpretation of motor data into function.

Changes in mechanical properties during Ca^{2+}-activation and their implications

Several studies have shown or hypothesised that the force contribution lying outside of the crossbridges may change with the level of [Ca^{2+}]. Kellermayer and Granzier (1996) demonstrated by means of an in vitro motility assay that titin interacts with actin filaments and reconstituted thin filaments, and that this interaction is dependent on the calcium concentration. Linke et al. (1997) showed that extraction of actin or myosin filaments in isolated cardiac myofibrils decreases the passive stiffness by more than half. Tatsumi et al. (2001) demonstrated the binding affinity of titin for Ca^{2+} ions on a molecular basis and localised the binding site. Bagni et al. (2004) showed in single fibres that when inhibiting crossbridge formation a stiffness component is increased by Ca^{2+} despite crossbridge force inhibition. The time course of the stiffness was found to be similar to that of the intracellular [Ca^{2+}]. Labeit et al. (2003) provided evidence that the extensibility of the PEVK segment of titin, which is assumed to be the main reason for passive force, is decreased and the persistence length increased by free calcium. Also, in experiments in which actomyosin interaction was inhibited by BDM, the force response to stretch of skinned fibres was consistent with their molecular findings. Finally, Pinniger et al. (2006) revealed that the persisting force increase after stretch ('force enhancement') can not be accounted by crossbridges, instead other candidates residing in the myofibril, e.g. titin or C-protein interacting with actin, need to be investigated.

Pinniger et al. (2006) also stated that the interpretation of force in terms of crossbridge and non-crossbridge components is complicated by the above findings. From a classic modelling point of view, it is often assumed that the 'passive', i.e. the non-crossbridge component is independent of activation. However, the above-mentioned studies provide strong evidence that this is not the case. Consequently, the total force upon activation is not the summation of passive (relaxed state) force and (active) crossbridge force; hence, crossbridge force cannot be achieved by deducing relaxed from total active force (see e.g. Schachar et al. 2002). On the contrary, we suggest that force must be interpreted as a sum of actomyosin (crossbridge) and other force generating components that, a priori, do not directly correlate with the activation state (Fig. 8). Then, the actomyosin component obeys the rules of a cyclic pathway, whereas the non-crossbridge component might be almost static but, nevertheless, be dependent on [Ca^{2+}]. Therefore, terms such as passive and active *force components* should be avoided and not be confused with passive and active *force*. From an experimental point of view, we suggest to apply methods that dissect the structurally related mechanical components from each other e.g. by means of inhibitors like BTS (Cheung et al. 2002; Pinniger et al. 2005; Shaw et al. 2003) or BDM (Bagni et al. 2004; Galler et al. 2005; Rassier and Herzog 2004; Tesi et al. 2002a) or by protein extraction (Granzier et al. 1997; Kulke et al. 2001), independent of the activation state. Additionally, one has to keep in mind that these inhibitors may have unspecific effects that complicate the component dissection (Ostap 2002). The challenge for the future will be to determine the dependence of the elastic and viscoelastic non-crossbridge components on intracellular

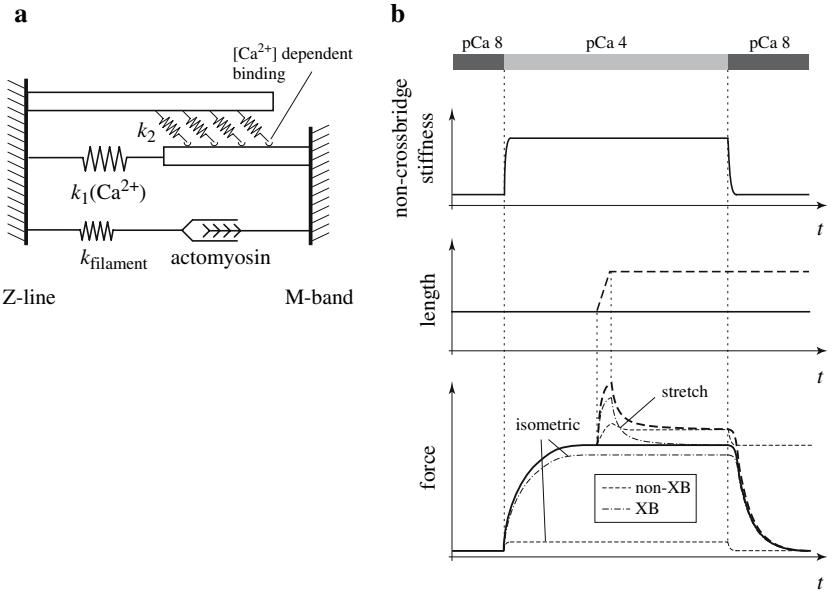

Fig. 8 Hypothetical model of force generating elements in a half-sarcomere. (**a**) The model shows two separate components, the non-crossbridge (top strand) and the crossbridge (actomyosin, bottom strand) based element. Both strands are linear systems and transmit force from Z-line to M-band only. The non-crossbridge component is modelled with a general Ca-dependent spring (k_1), such as titin may be, and additional elements (k_2) that can bind to the strand and increase the stiffness (e.g. actin–C-protein bonds). Any viscoelastic effects can be included in the model but will not be discussed any further here. In a first approach the filament stiffness in the actomyosin strand is Ca-independent. (**b**) Illustration of non-crossbridge stiffness (top), external length (middle) and force responses (bottom) of the half-sarcomere model during activation and relaxation (pCa 4 ↔ 8). The thick dashed curve corresponds to the stretch protocol shown in the middle. In the simplest case the non-crossbridge stiffness increases linearly with pCa via additional bonds (top). We assume a simple actomyosin pathway (attachment – power stroke – detachment) and optimal actomyosin filament overlap in both isometric and stretch protocols. Presumably, the force in a strictly isometric protocol is governed mainly by cycling crossbridges, and a small portion of force is generated from increasing non-crossbridge stiffness; this is mainly because altered stiffness generally implies a change in the equilibrium length of the elastic element. The componential separation of force is illustrated in the lower graph by the thin curves (labelled 'isometric'). However, during stretch the non-cycling, non-crossbridge component (thin dashed curve, labelled 'stretch') contributes to force increase and remains at a higher level while the force from cycling crossbridges (dash-dotted) approaches the isometric level again. Calcium removal and, hence, deactivation causes the non-crossbridge force response from preceding stretch to vanish. This hypothetical model could, at least in part, explain the force enhancement after stretch observed in many experiments. Yet, such a componential separation of force from *fibre* experiments is, apart from being disturbed by sarcomere dynamics, a non-trivial problem and most often not performed

$[Ca^{2+}]$ and to localize the responsible structures and interactions.

Concluding remarks

The occurrence of sarcomere dynamics and inhomogeneity has been a well-recognised problem in muscle contraction experiments and is particularly enhanced at physiological temperatures. Although irregularities in $[Ca^{2+}]$ along the fibre can play a role in intact and large skinned muscle preparations, ion-controlled experiments with single myofibrils suggest that non-uniform sarcomere behaviour is mainly caused by a (biological) variability in the strength of the half-sarcomeres, e.g. the number of potential force-generating crossbridges, and thus has more profound reasons to be sought in the structure. The main difficulty of internal dynamics is the lack of knowledge how inhomogeneity affects the force response in terms of crossbridge turnover. We believe that, at present, the combined technique of microscopic imaging and mechanical measurement in single myofibrils is the most appropriate systems biology approach to study the mechanisms of dynamic force generation and unitary filament sliding, the impact of stretch on half-sarcomere dynamics and force, and the mechanisms of relaxation and its impairment due to diseases.

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