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Phase transitions and the molecular mechanism of contraction

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Abstract

In this paper, the rotating cross-bridge mechanism for muscle contraction is discussed and much contradictory evidence is put forward. As an alternative, a model is given in which the motor of muscle contraction is placed in the myosin-rod hinge and/or in the actin filament. No definite choice for one of the proposed models can be made yet, although it is clear that some kind of phase transition plays an important role in the mechanism.

Keywords: Muscle contraction; Myosin; Actin; Cross-bridge mechanism; Phase transitions; Hinge melting

1. Introduction

The muscle contraction model brought to prominence by Bill Harrington, to whom this volume is dedicated, grew out of the work of eminents such as Paul Flory, Leo Mandelkern and Aaron Katchalsky. These investigators held that contraction of muscle arose out of a molecular phase transition: a gross structural change triggered by a subtle shift in environment. Those long enough in the field will, for example, remember Katchalsky's contraction machine built of a loop of collagen, immersed alternately in a salt bath and in distilled water [1]. By alternate "contraction" and "relaxation" of the belt, the machine was able to generate power quantitatively similar to that of the frog's sartorius muscle. Underlying the operation of this "perpetual motion" machine was a structural transition, or melt, of the belt of collagen.

Harrington's work emphasized the role of myosin melting. He focussed on the region of the myosin rod lying between HMM and LMM, commonly known as the "hinge" (see Fig. 1). The central theme was that a helix–coil transition in the hinge was the generator of contraction [2].

In the specific scheme brought forth by Harrington, the cross-bridge first swings out and attaches to actin. Then the hinge melts. Melting shortens the hinge, and thus, brings about translation of thin filaments past thick ones. If the filaments are not permitted to slide, melting results in the development of tension [3]. Thus, the helix–coil transition in the hinge region is viewed as the source of the power stroke. The scheme could account for elementary ATPase properties of myosin, was consistent with a host of biophysical observations, and was shown to explain basic mechanical features of contraction [4]. It also prompted the execution of a string of follow-up

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studies (see below), many of which have served to solidify the hypothesis.

2. Demise of the melting hypothesis?

Meanwhile, the proposal of hinge-region involvement was dealt a sudden blow with the advent of the in vitro motility assay [5,6]. It soon became clear that relative sliding of the actin filament past myosin could occur perfectly well even when the hinge region was not included in the assay. To many, the implication of this observation seemed compellingly straightforward: hinge melting could not be the central element of contraction. Supportive evidence notwithstanding, the model quickly lost its appeal.

On the other hand, it has been pointed out that the in vitro motility assay evidence is not necessarily conclusive on this issue. While velocities measured in the assay are normal, the force is argued to be substantially lower than the force (per molecule) measured in intact specimens [7]. Thus, the assay may be missing a force-producing component of the contractile mechanism. At this stage, not much can be made of any such force deficiency, since different instrumentation and assumptions can lead to different conclusions about the level of measured force [8–10]. However, it is impressive that in the hands of the same (Yanagida) group, using the same instrumentation, the standard assay with myosin head alone produces 1.5 pN [11], whereas the assay containing whole myosin linked to the thick filament backbone produces 5.3 pN [12]. This difference, 3.5 times, is not discussed by the authors, but one is led to wonder whether the higher value is caused by the inclusion of the hinge, in its near-natural configuration. Hinge melting could, in theory, generate an appropriate magnitude of force [4], on the order of 5–10 pN [13].

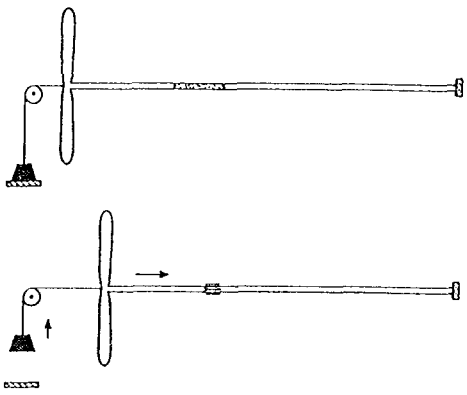

Fig. 1. Hinge-melting mechanism. A two-headed myosin molecule is shown schematically, with load attached. The hinge region (stippled) undergoes a transition from helix to random coil, and in doing so, is able to shorten and lift the load.

At face value, then, the motility-assay observation could be taken to imply that hinge melting is unnecessary for motility and should be discarded. The considerations above, however, imply that a component of the natural contractile mechanism may be missing in the standard motility assay, and that this missing component could well be hinge melting. If so, the hinge-melting hypothesis would remain very much alive, although it could not, as implied in the Harrington scheme, be the sole mechanism of contraction.

In the pages following, I argue that Harrington's hinge-melting hypothesis remains intact – and possibly correct – although I believe it is manifested differently from the way Harrington envisaged. I preface this offering by first considering the adequacy of the current swinging cross-bridge theory, where the "motor" lies not in the hinge region, but in the myosin head. I then proceed to hinge melting, and show that this mechanism is supported by too much evidence for its relevance to be easily dismissed. I go on to consider an additional structural transition that may complement hinge melting, one that occurs in the actin filament. I show that this latter mechanism is also supported by substantial experimental evidence, and can explain the filament translation seen in the in vitro motility assay. Finally, I point out that these two structural transitions, taken together, explain a broad array of basic features of the contractile process.

3. The model of the day and its problems

The swinging cross-bridge theory has remained the field's prevailing paradigm for almost 40 years. The theory prevails not only because it is intuitive and elegant, but also because the volume of evidence consistent with the theory is appreciable. Particularly

compelling among these pieces of evidence are the following: the theory's structural underpinning is well supported by X-ray diffraction evidence [14]; the myosin head contains the expected actomyosin-ATPase site [15]; a scheme coupling actomyosin ATPase with a plausible mechanical cycle can be formulated from biochemical kinetic evidence [16]; a basis for the cross-bridge stroke is implicit in crystallographic evidence for the structures of actin and myosin [17–20]; many mechanical measurements, as well as in vitro motility-assay observations, are interpretable within the framework of the theory ([21],

Fig. 2. Cross-section of frog semitendinosus muscle. Muscle was stretched to approximately 2.8 μm sarcomere length prior to fixation. Section grazes the thin filament tips, some of which can be seen among the array of thick filaments. Thus, the section is far from the M-region. Note bridges between adjacent thick filaments. V. Popov and the author, unpublished.

part a); in particular, actin–filament translation step size measured by optical trapping is in good accord with theoretical expectations [9]. This sketchy summary is obviously not meant to be comprehensive, and the uninformed reader is commended to any of a number of excellent reviews for further detail [14,16,22]. In this short space, I cannot reasonably consider all supporting evidence.

On the other hand, there is no shortage of evidence that has been claimed to be in conflict with this theory. In a recent monograph [13], nine potentially serious issues are considered. Here, I will briefly consider several of the ones that seem particularly serious, including two that have gotten a fair amount of press lately.

One important issue is the inability to find clear evidence for a decisive cross-bridge stroke. Numerous attempts have been made to detect anticipated bridge-angle changes associated with the power stroke, but, except for a 3° tilt of the light chain ([21], part b), recent experiments have proved consistently negative [22–27]. The failure to find clear evidence of substantial bridge-angle change appears to be of concern in the field, even, it seems, to some of the theory's long-term advocates [28,29].

One reason for this failure may be that the structure of the bridge is different from the textbook view of structure. While the myosin head is viewed as free to rotate, evidence has been presented that the bridges are instead static elements that link adjacent thick filaments in rung-like manner. This feature is illustrated in the electron micrograph shown in Fig. 2. The figure shows cross-links between thick filaments, much like those known to exist in the M-region. If bridges do interconnect thick filaments to one another, as published evidence implies (for review, see [13]), it is understandable why bridge rotation might not be observed. Such links do not necessarily imply that acto–myosin interactions do not occur (see below); they merely question the idea of head rotation.

A second area of doubt centers around the "step-size", i.e., the amount of filament sliding powered by hydrolysis of a single molecule of ATP ([21], part a). An early result seemed in accord with the theory's expectation, i.e., a step size of 10–20 nm [30]. Thus, a single burst of energy could power a stroke whose size was in reasonable accord with the bridge's molecular dimension. But with increasing consistency, experimental results have implied unexpectedly large steps, up to several hundred nanometers [31–38]. To explain the large size of the step, investigators have been forced to presume either that the bridge can somehow stroke well beyond its own size, or more commonly, that the stroke is of normal size, but ATP's energy is released fractionally, over many strokes. Although partitioning of energy liberation is invoked rather casually, there is concern whether the implied fractionation of energy of a single molecular bond could be theoretically sound [39]. Even if it were, the concept of energy partitioning certainly detracts from the elegance of the originally conceived one-stroke/one-ATP textbook model.

A third, and long-standing, issue concerns the length–tension relation. The cross-bridge theory predicts that active tension should vary directly with the number of available cross-bridges. As the sarcomere is stretched to reduce thick–thin filament overlap, tension should fall proportionately. Textbooks faithfully report the confirmatory classical result [40], but do not cite the results that are in conflict [41]. More than a half-dozen studies now report that as overlap is decreased, tension remains considerably higher than expected, and in some cases there is no appreciable fall-off of active tension until overlap is decreased to 50% of maximal. Such results are occasionally implied to be technically inept [42,43], or, more often, they are ignored. A serious attempt to reconcile these discrepant results with the classical one has recently been published [44,45]. These papers offer an explanation. They bring to the surface the question of whether the agreement between the shape of the length–tension relation and the prediction of the cross-bridge theory is as secure as once believed.

Also problematical in the length–tension domain are some results at short sarcomere length – on the so-called "ascending limb". Several reports in both cardiac and skeletal muscle show little or no ascending limb [46,47]. These almost flat length–tension relations are found mainly in the thinnest of preparations, where the results might be thought to be most reliable. Even in thicker preparations, if driven to maximal activation by sufficiently rapid stimulation or by activation-enhancing agents, the ascending limb rises practically to flatness [48–50]. The mechanism

by which the sarcomere could continue to generate full tension while short enough to have compressed the thick filament, seems difficult to envision within the cross-bridge framework.

Which brings me to the fourth issue: do thick filaments, or A-bands, really remain at constant length during contraction? I speak here not of the special case in which the sarcomere shortens enough to have compressed the thick filament, but of the longer, more physiological sarcomere length range. In this range, the textbook view implies filament length constancy. The original papers, however, are mixed. In unactivated sarcomeres, manually stretched or released, the absence of any filament length change is reported consistently. The evidence is unchallenged. In activated sarcomeres undergoing shortening, however, thick filament (or A-band) shortening of some magnitude is reported in numerous other papers [41]. These include the classical studies which are frequently cited as showing the opposite [51,52]. The papers that show A-band shortening are rarely quoted, perhaps because accepted theory has become so entrenched that any such "discrepant" result, viewed in isolation, could not be expected to be sound. Indeed, the authors themselves on occasion seem reluctant to accept their findings in light of the (presumed) body of evidence to the contrary.

To this author, these results present a rather consistent picture, which X-ray diffraction evidence does not necessarily contradict ([13], pp. 94–96). Except for a single paper published by H.E. Huxley and colleagues recently, in which a quick-freeze, electron micrographic method was used [53], I know of not a single structural study published during the entire century that shows systematically that A-bands or thick filaments remain at constant length as the sarcomere shortens actively from long to short sarcomere length. Huxley's recent result is at odds with numerous others employing different techniques, and also with the results of an earlier, though less comprehensive, study using the same technique [54]. The interested reader is invited to peruse this collection of papers and decide independently whether the issue of constancy of filament length is really closed, or whether the earlier view that A-bands shorten is alive and kicking.

From the evidence presented above, it seems clear that the swinging cross-bridge theory is not without contradictions. Although the theory remains the dominant paradigm of the day, some rather basic inconsistencies, four of which were discussed here, remain to be addressed. Bluntly stated, the theory needs to step aside for a time, to allow space for fresh thinking.

In the space below, I will consider some alternative ideas. I will focus on two models. In these models, the motor mechanism – i.e., the conformational change that drives contraction – lies at sites different from the myosin head. In the first model, the motor lies in the hinge region along the myosin rod. This had been proposed by Harrington, but I will suggest a modification of Harrington's original scheme. In the second model, although involvement of myosin is not denied, the motor itself is postulated to lie within the actin filament. Both mechanisms are based on phase transitions, which result in sharp changes of structure in one or the other of the muscle's two major proteins. As I will show, both the myosin-rod motor and the actin-filament motor are mechanisms supported by substantial bodies of evidence, although, like the cross-bridge model, neither one alone provides a complete picture.

4. A myosin-rod motor?

Early evidence for hinge melting has been reviewed fairly recently [13], and I need not detail it here. This evidence derives from biophysical methods such as optical rotatory dispersion, and from structural methods including high-resolution electron microscopy. Among these results, the following has been shown: the melt zone lies in the region of the molecule depicted in Fig. 1; myosin rods subjected to an increase in temperature or an increase in pH are up to 20 nm shorter than those observed under control conditions; and rod shortening can be elicited at physiological temperatures [55–59]. A gallery of myosin molecules showing the melted region is shown in Fig. 3.

Before proceeding with more up-to-date evidence, it should be noted that hinge melting could be manifested in at least two ways. The distinction depends on whether the head–rod junction is, or is not, bound to the thick filament backbone as the molecule melts. If the head–rod junction is not bound, then melting

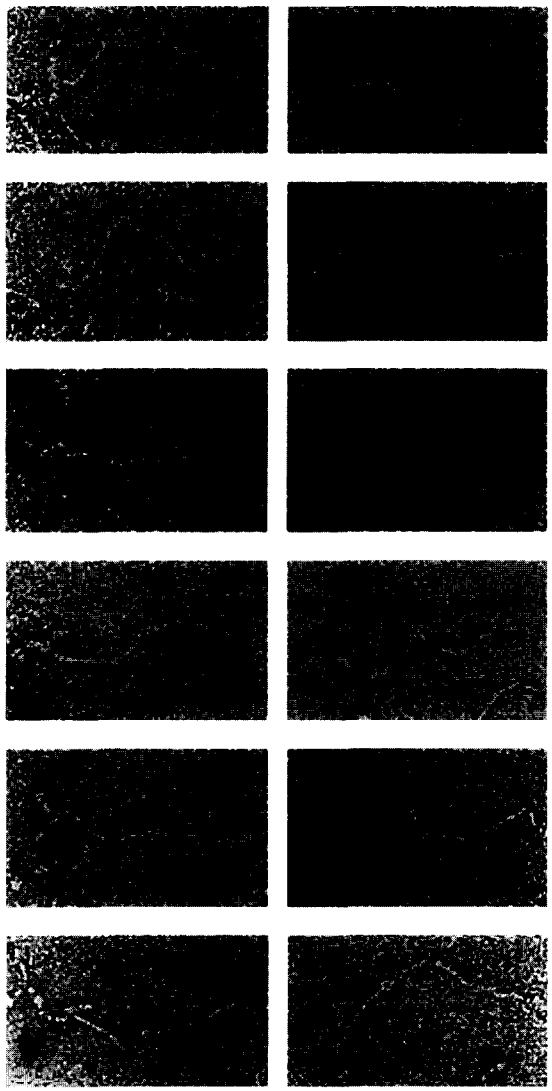

Fig. 3. Gallery of myosin molecules subjected to conditions that favor myosin-rod shortening. Note melting of rod at a consistent locus 30–40% of the way along rod from head to tail, on each image. From Walker and Trinick [59].

can exert its force directly on the thin filament, as in the original Harrington scheme. If the junction is bound, melt force is instead exerted on an adjacent myosin in the backbone, and the rod of that adjacent molecule is driven past the one that melts. The net result is a small amount of localized thick filament shortening. If additional molecules melt, the thick filament shortens more. Thus, a paradigm distinct from the Harrington scheme can be built on the helix–coil principle [13].

In the framework of such a thick filament shortening model, a preliminary result obtained with the environmental electron microscope is worth mentioning [60]. Sugi and colleagues placed nanogold-labelled antibody markers on myosin heads in reconstituted thick filaments. Movements of these gold labels were tracked during activation. This movement had a magnitude of 20 nm typically, and was directed purely along the filament axis. Magnitude and direction are in excellent agreement with the thick filament shortening model driven by hinge melting [13].

Hinge-region involvement is also implied in studies in which antibodies were used as focal inhibitors of molecular action. In skinned rabbit psoas fibers, polyclonal antibodies directed against myosin S2 had little effect on shortening velocity, but isometric force was depressed [61,62]. Although such antibody experiments should be interpreted with caution, the result was inferred by the authors to suggest that unloaded shortening involved S1 alone, but active force also involved S2, including the hinge. Similarly in the in vitro motility assay, Margossian et al. [63] reported that motility was suppressed by a polyclonal antibody directed against a 20-residue peptide of the cardiac myosin hinge. A role of the hinge was again implicated.

Recent molecular biological evidence also points strongly to hinge involvement. In the *Drosophila* myosin heavy chain gene, exons 15a and 15b encode the central region of the hinge. Interestingly, the patterns of usage of these exons – both stage-specific and tissue-specific – correlate with contractile properties [64,65]. In muscles that contract slowly (larval and adult body wall) only exon 15b is used. In muscles that contract frequently with a large delayed tension response (indirect flight muscle), or generate high levels of steady-state force (jump muscles), only exon 15a is used. In muscles that contract at intermediate rates (leg, proboscis) some transcripts contain exon 15a while others contain 15b. Other regions along the rods of these muscles are invariant [64,66]. Thus, the *Drosophila* evidence implies a central role of the hinge.

The same appears true in other species. In rat cardiac muscle, the alpha and beta myosin heavy

chains are highly conserved, but the hinge regions show a cluster of differences. These differences are thought to underlie the observed differences of function ([67], part a). In the scallop, the myosin heavy chain gene was recently found to encode alternative forms of the same hinge region ([67], part b). One type of hinge is used in striated muscle, the other in smooth and catch muscle – again emphasizing the correlation between contractile properties and hinge type.

In summary, hinge involvement in the contractile process is supported by a body of evidence that seems difficult to dismiss as coincidental. This includes not only the earlier biophysical and structural evidence reviewed elsewhere, but also recent studies, including those in the realm of molecular biology. Melting is now also under consideration as part of the motility mechanism in the kinesin–tubulin system [68]. The oft-posed argument that hinge-melting can be ruled out by in vitro assay observations is of uncertain validity: while it is true that translational motion can occur in the absence of the hinge, full force potential may not be realizable unless the hinge is present. Hinge melting, in other words, is certainly not the sole agent of in vitro motility, but could well be a component of an overall contractile mechanism. In the intact sarcomere, hinge melting could underlie the thick filament shortening that is frequently observed but infrequently discussed.

5. An actin-filament motor?

With hinge melting as a putative contractile mechanism component, the question arises: what might be another component? And might this complementary component also involve a phase transition similar to hinge melting?

An option worthy of exploration is a structural transition in the actin filament. Actin's involvement in force generation was first implied long ago by the studies of Oosawa and colleagues, who noted an array of factors that could induce structural transitions in actin [69–72]. On the basis of Oosawa's and other observations, a generic model was put forth several years ago in which the crawling motion seen in the in vitro motility assay could be explained by the propagation of these structural transitions along the actin filament [13].

Several more detailed actin-based models have been advanced since then. Among them are the so-called "thermal ratchet" mechanisms [73,74]. In much the same way as A.F. Huxley's 1957 theory [75] proposed that thermal energy could induce motion in the myosin head, these newer thermal mechanisms posit that the motion can be induced in the actin (or tubulin) filament. Through splitting of ATP, this motion is then rectified, and hence made unidirectional. In another version, the actin filament undergoes transition from a ribbon configuration to a helix configuration [39,76]. The rationale for such a transition comes not only from the work of Oosawa and colleagues, but from crystallographic evidence [76,77] showing two states of actin packing, one longer, the other shorter. Thus, length transitions propagating along the filament could drive actin past myosin in much the same way as a caterpillar propels itself along a tree branch.

To those accustomed to thinking of the actin filament as a more or less passive element, such proposals will seem radical. However, they are based on a growing body of evidence implicating the actin filament in an active role. Summarized next, this evidence falls naturally into two categories, the first implying that actin has two conformational states, the second indicating that any transition from one state to the other likely propagates along the filament.

5.1. Evidence for functional transitions in the actin filament

That F-actin subunits undergo conformational change during contraction has been reported in a series of studies carried out over two decades [78–80], although the change is not picked up by all methods [81,82]. These conformational changes are detected mainly through optical measurements on actin-based probes.

X-ray diffraction methods have also detected changes in actin conformation. Among relaxed, activated, and rigor states, X-ray intensity distributions differ substantially. Those differences deriving from the thin filament were claimed to be inexplicable on the basis of tropomyosin shift or myosin-head movement [83]. The authors conclude that a conforma-

tional change in actin itself must occur as the muscle passes from rest to contraction.

A change in actin is also picked up by phosphorescence anisotropy measurements [84]. When F-actin and myosin S1 were activated by exposure to ATP, phosphorescence probes on actin picked up a significant structural change. Large-scale conformational fluctuations were found to occur along the full length of the filament.

Yet another indication of conformational change comes from recent measurements made using the fluorescence resonance energy transfer method to report actin-subdomain spacing [85]. Oriented about 30° off the actin-filament axis, the probes used in this study gave an inter-subdomain spacing of 2.22 nm. When myosin S1 was added, this spacing increased by 0.38 nm, a change of about 17%.

The nature of actin's conformational change has been given definition through studies of actin-containing crystals. While only a single conformation is reported in crystals of actin-DNAase [17], crystals of actin-profilin pack in two interconvertible ways [76,77]. In one arrangement, actin subunits are arranged in the well-known helical configuration with ca. 38 nm repeat. In the other arrangement, packing is ribbon-like, the same actin subunits now spanning 43 nm. This result is taken by the authors to show that the actin filament has both short and long states, which are interconvertible. The spacing difference is ca. 15%. The fact that the spacing of one of the two actin repeats is commensurate with the myosin repeat (43 nm) could evidently have profound mechanistic consequence.

Dual spacing implied by these crystal studies can also be found in electron micrographic images of the intact sarcomere. The "fine periodicity" observed along the I-band is typically 38 nm [86]. Under some conditions, however, this periodicity is measured to be just short of 43 nm [87], or precisely 43 nm [88]. These values of 38 nm and 43 nm correspond rather strikingly to those found in the crystal studies. A structural transition is also noted in isolated actin filaments. Although the conventional spacing is detected most generally in these isolated filaments, when the filaments are exposed to physiologically natural ligands such as phosphofructokinase, the conventional actin spacing is converted to one that is myosin-based [89].

Two filament states are also implied in bridge-angle studies. In rigorized insect flight muscle allowed to shorten during the induction of rigor, bridges are found not only at the well-known 45° rigor angle, but also at the 135° anti-rigor angle [90–92]. The bridge angle found in rigor is thus multi-valued, not single-valued, as generally thought. Except for rare cases [93], bridge angles associated with a given actin filament do not mix; bridges are segregated either at 45° or at 135° [90,91]. The fact that the bridge angle associated with each actin filament is so consistently one or the other, implies that the angle is set not by the bridge, but by some feature of the filament. Two angles would then imply two actin-filament states.

Finally, actin polymerization studies also report dual statehood, at least under certain experimental conditions [94], though not all [95]. When actin filaments in the former study were polymerized with ADP, the filament was found to be convoluted and flexible. But when polymerization took place in the presence of ATP, the filaments were rod-like. The former state could be converted to the latter state merely by exposing the ADP filaments to ATP.

It is worth noting that there is good precedent for the existence of structural transition in at least one other fibrous protein. In elastin, transition from a long state to a shorter, condensed state has been found to occur, and this fact has been exploited to design an artificial model muscle [96]. Polymers built of the most prominently repeating pentapeptide (VPGVG) found in natural elastin can be triggered to contract reversibly by change of any number of parameters including: temperature, pH, ion composition, and serine residue phosphorylation. The power generated during contraction is comparable to that of an activated muscle of similar size. Considering that the phase-transition principle has been used successfully by engineers to create an artificial muscle, the idea that the celestial engineers may have accomplished a similar feat sometime earlier in the design of natural muscle, can hardly be dismissed as far-fetched.

5.2. Evidence that state transitions propagate along the actin filament

Viewed in the optical microscope, fluorescently labelled actin filaments are frequently described as

wiggly, or undulatory, or reptational [97–101]. While such dynamics are generally ascribed to thermally induced Brownian movements, the evidence below seems equally compatible with the possibility that the wiggles are manifestations of phase transitions propagating along the filament.

In F-actin in suspension, the presence of snake-like wiggling motions has long been known [72,102,103]. Those who have seen cinemicrographic recordings of suspended actin filaments will not likely forget their vivid reptilian dance. Because such movements are decisively influenced by exposure to myosin heads and ATP, they have been presumed related to the contractile process [71]. Reptational motion has also been detected by the stop-action feature of cryo-electron microscopy. Actin filaments exposed to myosin heads, and activated by photocleavage of ATP analogs are observed to undergo millisecond-scale transition – from a configuration that is rod-like, to one that is snake-like [104]. Thus, the optical microscopic method and the cryo method give similar results.

Snake-like movements are even more clearly observed in the in vitro motility assay. As the fluorescently labelled actin filaments translate over a lawn of myosin, they do not move linearly; they undulate [100]. Undulatory components of motion become particularly apparent as ionic strength is elevated to levels approaching those in vivo [101], where the wiggling component becomes increasingly prominent. The visual impression is that translation and undulation are intimately linked – as they are in the snake that propels itself along.

Measurements of actin-filament velocity support the visual impression. In the in vitro motility assay, systematic analysis of the dynamics of motion show that actin-filament translation velocity has a prominent periodic component [99,100,105]. This periodicity is unlikely to arise from synchrony of cross-bridge strokes, since myosins are randomly dispersed and have no obvious way of communicating with one another to confer this periodicity. A more plausible explanation is that some molecular transition propagates along the actin filament, at regularly repeating intervals – similar in principle to what may happen in cilia and flagella. As the transitions propagate, they generate the observed undulations and create the speed variation. The fact that the frequency of undulation is the same as the frequency of speed variation [100], lends credence to this supposition.

A few general conclusions seem to emerge from this collection of evidence. First, the actin filament appears to have two states. The states differ in their physical properties, and possibly also in their subunit-packing arrangements. There is good evidence for an activation-based transition from one state to the other. Second, the prominence of reptational motion implies that whatever transition takes place may propagate along the filament. Some experiments imply a wave-like process, not unlike that proposed

Fig. 4. Actin-filament motor mechanism. Actin subunits indicated by contiguous rectangles, myosins by triangles. Time series is shown, from i to vi. Structural transitions from long to short, and from short to long, propagate rightward, and in doing so, move the filament toward the right, in caterpillar-like fashion. Diagram is highly schematic, and not to scale.

by Schutt and colleagues [76], but perhaps less complex in detail.

6. Actin-motor specifics

A simple scheme that seems compatible with these observations is the one shown in Fig. 4. In this scheme, myosin heads do not rotate. The heads form static attachment points, to which the actin filament can anchor. In the sarcomere, these anchor points are regularly arrayed along the thick filament, while in the in vitro motility assay they are randomly distributed on the assay surface. (Head distribution is not a particularly critical consideration in this mechanism.) Filament translation is generated as actin packing undergoes transition from a long to a short state, and as this transition propagates along the filament. To make the scheme work, it is further assumed (consistent with observation [85]), that in its long state, actin's affinity for myosin is high, while in its short state its affinity is low.

For illustrative purposes, the entire actin-filament segment is assumed to lie initially in its long state (panel i). In this state, affinity for myosin is high, and all myosins in the vicinity of an attachment site will tend to be attached. The active process begins at the trailing (left) end of the actin filament, where long segments undergo transition to short (panel ii). The transition propagates toward the filament's leading (right) end. As the wave reaches the first myosin, affinity is diminished, and the myosin detaches (panel ii, arrow). Detachment may involve ATP hydrolysis. The transition then progresses toward the next bound myosin (panel iii), whereupon myosin detaches (panel iii, arrow), and the process continues (panel iv). Except for periods of pause required for ATP hydrolysis, the tail of the actin filament continues to advance rightward.

The filament then begins recovering toward its long state. The simplest way this could occur is if a lengthening (reverse) transition begins where the shortening transition began – at the filament's trailing end. Reversal could be triggered by any of a number of agents: by the binding of ATP to the filament's trailing (barbed) end, by an ATP-induced or calcium-induced change of vicinal water structure [106], or by phosphorylation of a nearby residue. The reverse transition progresses rightward (panel v), whereupon myosins properly juxtaposed can rebind (panel v, arrow), although rebinding is not obligatory for recovery wave progression. Both forward and reverse transitions thus propagate rightward along the filament at the same time, producing a caterpillar-like wave (panel vi).

Several features of this model are worthy of comment. First, tail velocity is a global phenomenon, whose magnitude depends on what is happening along the entire filament. In panels i–iv, tail velocity is high, since the only action along the filament is shortening. Between panels iv and v, because lengthening and shortening occur simultaneously, tail velocity is diminished. With initiation of the next shortening wave (not shown) velocity returns to a high value. Thus, tail velocity is expected to oscillate between high and low values [99,100]. Eventually, the wavefront reaches the leading end of the filament, where again periods of high and low velocity alternate. Because of the finite propagation speed, there is a time delay between velocity oscillations at the front and rear ends of the filament [100].

A second feature implicit in the model of Fig. 4 is the expectation of reptational motion. Imagine a filament segment transiently constrained at either end by attachment to myosin. In panel vi, for example, an attachment point may be envisioned off to the right. The segment straddled by these attachment points contains both long and short segments. Any transient increase in the fraction of long units will induce segment buckling. Buckling – or, reptational motion – is therefore a natural consequence of this kind of mechanism. In the motility assay, where myosins are irregularly disposed and long stretches of bare actin are anticipated, reptation should be particularly evident [97,98]. In shorter stretches, the phenomenon should be less obvious, but with appropriate resolution, it has evidently proved detectable [104].

A third feature of this mechanism relates to the directionality of filament translation. Experimentally, actin filaments are capable of translating not only in zones in which myosin is correctly polarized relative to actin, but also in zones in which myosins are reverse oriented. This anomaly is seen not only with translation over thick filaments [107,108], but also with translation over isolated myosin heads planted on a substrate in oriented fashion [109]. With the

cross-bridge theory, translation in reverse is explained by 180° bridge twist: properly oriented relative to actin, the bridge then takes its stroke. In the case of the planted myosins, filament velocity is the same in either direction, normal or reverse. Thus, bridges would need to stroke with consistent dynamics whether untwisted or twisted. With the actin-based mechanism under consideration, myosin-head orientation is not an issue: so long as myosin and actin can bind, the mechanism can work.

At this early stage, the model does not specify the source of energy. Energy could come from splitting of ATP by myosin, conferring directionality to the motion, as in thermal ratchet models [73,74]. Or, it could come from ATP hydrolysis at the barbed end of the actin filament. One ATP per oscillation would explain the relatively low consumption of ATP during unloaded sliding [110]. Other, less conventional sources could involve ATP-driven changes of water hydration layers, which impact protein dynamics [106]. The point, however, is that several ATP-mediated sources of energy are potentially available to support this kind of motion.

The manner in which such a scheme could operate in the physiologically activated sarcomere has been sketched out [13]. The actin filament resides in the lattice surrounded by three myosin filaments. These three filaments form a cage, within which the actin filament is free to reptate. The reptational process can be initiated by the action potential, travelling along the transverse tubule and reaching the junctional SR, the latter closely juxtaposed to the site of initiation of the wave. If each stimulus pulse gave rise to a single transition wave, and if the maximum filament length change is on the order of 15% (38–43 nm), translation of several tenths of a micrometer per sarcomere would be anticipated from this mechanism.

With actin's central involvement in the production of filament sliding, several otherwise anomalous experimental observations fall readily into place. For example, consider the actin residue E316 in insect flight muscle. Even though this residue is far from the myosin-docking site, mutation of this single residue (E to K) has a decisive impact on mechanics [111]. This impact would not be anticipated if actin's role were merely passive. It is simpler to interpret if the contractile process involves the actin filament in a more central role. Similarly, subtilisin-induced cleavage of a single bond within the actin subunit has unexpectedly profound and non-stoichiometric influence on mechanics, even though maximum activation of actomyosin ATPase remains unchanged [112]. This result, again, is not immediately reconcilable with a cross-bridge-based motor, but is consistent with an actin-based mechanism. Finally, several of the conflicts with the cross-bridge model that were discussed earlier are no longer conflicts. To wit: the absence of substantial bridge rotation and the absence of 1:1 correlation between splitting of an ATP and execution of the (putative) bridge stroke.

Also reconcilable with the actin-based mechanism is the observation that actin can split ATP [69,113–115]. A quarter-century ago, this had been an established fact. Later, as it became evident that ATP splitting was involved in the process of actin polymerization, the actin-based ATPase was assumed to arise exclusively from this process. With increasing focus on ATP splitting by myosin [116], any remaining interest in a functional actin ATPase was quickly snuffed out. But it is now clear that subsequent to polymerization, actin continues to split ATP [117]. Polymerization, therefore, is unlikely to be the full explanation of the actin ATPase. More work is needed on this potentially important subject. The expectation, however, that actin might split ATP, is by no means devoid of experimental support.

It is the kinds of evidence described in the paragraphs above that are responsible for the recent emergence of several actin-based theories [13,39,73,74]. But the idea that actin may take part in the contractile process is not new. Among many Japanese researchers, an actin-based contractile framework has been under active consideration for years [71]. And in his classic 1969 *Science* article, H.E. Huxley [118] also describes an actin-based mechanism as a possible fall-back alternative to the swinging cross-bridge mechanism. Thus, "...the cross bridges might remain rigidly fixed in position while repetitive internal changes in the actin filaments enabled them to crawl along the series of fixed points so provided". With great interest in actin from those outside the muscle field, and with new and additional evidence for conformational change in actin, it is clear why Huxley's fall-back proposal is now gaining increased attention.

7. Conclusion

To this observer, the rotating cross-bridge mechanism has been accorded more attention than is warranted. Supporters of the theory need to deal with the mounting pile of contradictory evidence, and weigh its impact on the theory's adequacy.

The mechanisms described herein deal constructively with this contradictory evidence. They place the motor elsewhere the sarcomeric protein complex–in the myosin-rod hinge, and/or in the actin filament. Both mechanisms are supported by substantial bodies of evidence, and as far as I am aware, there is no case against either one that can be regarded as definitive, although future experimentation could change that. Additional experiments are clearly warranted, particularly those that probe details of the proposed changes in conformation. The two mechanisms may appear different, but both are based on structural transitions, one propagating along the thick filament, the other propagating along the thin filament. Thus, Harrington's view of a contractile mechanism based on orthodox physicochemical phenomena (such as a phase transition) is well supported by these concepts.

A possibility worth considering is that the myosin and actin mechanisms work together, in a complementary way. The actin-motor mechanism could be a primitive process designed to effect the largely unloaded motions characteristic of motile cells and organisms including those lower on the phylogenetic scale. Hinge-melting could be a more highly evolved process developed by specialized cells such as muscle to produce large forces. In muscle, the two mechanisms would then work in concert to confer the broad range of performance capability in the realms of both shortening and force [119]. To achieve its well-recognized versatility, muscle would thus exploit the strengths of each of these two mechanisms.

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