Minireview

A new perspective on muscle contraction

Clarence E. Schutt[a] and Uno Lindberg[b]

[a]*The Henry H. Hoyt Laboratory, Washington Road, Princeton University, Princeton, NJ 08544, USA* and [b]*Zoological Cell Biology, WGI, Arrhenius Laboratories for Natural Sciences, Stockholm University, S-10691 Stockholm, Sweden*

Recent experimental findings suggest that the myosin cross-bridge theory may no longer be adequate to account for certain basic facts concerning muscle contraction. A newly-proposed mechanism based on length changes in actin filaments might be the basis for a simpler explanation for how the free energy of ATP hydrolysis can be transduced into work by muscle fibers.

1. THE THEORY OF INDEPENDENT FORCE-GENERATORS

The sliding filament hypothesis in its simplest form states that muscle contraction is to be understood in terms of actin filaments moving along myosin filaments [1,2]. It is a *two-filament model* for how sarcomeres, the functional units of striated muscle fibers, shorten during a contraction. It is almost universally accepted that the myosin cross-bridges spanning the gaps between actin thin filaments and the myosin thick filaments are the motors that pull passive actin filaments toward the center of the sarcomere [3]. In solution, myosin S1-heads have an ATPase activity that is activated by the presence of actin [4]. Since the linear dimensions ($\sim$ 150 Å) of the S1-fragment of myosin [5] containing the ATPase are far smaller than the relative sliding of the filaments (5,000 Å), it is natural to suppose that multiple cycles of attachment and detachment between myosin heads and actin filaments are a plausible basis for force production.

There are, of course, other good reasons for believing in a myosin 'power-stroke' of about 100 Å in length, accompanied by the hydrolysis of one molecule of ATP. Collectively, they are known as the 'independent generator hypothesis', a self-consistent interpretation of four kinds of observations on the mechanics of contracting muscle [6]. The crucial assumption made in this analysis is that actin filaments are virtually inextensible. Given this assumption, it can be concluded that muscle fibers consist of a linear array of motors that possess two properties: (i) an active *force generator* that can develop an approximately constant force over a distance of

Correspondence address. C.E. Schutt, The Henry H. Hoyt Laboratory, Washington Road, Princeton University, Princeton, NJ 08544, USA.

about 100 Å; and (ii) a passive *instantaneous hookean element* of length 31–39 Å which can store elastic energy which can be converted to work. If actin filaments have any significant elasticity, this model could not work, since the motors would locally stretch the tension-bearing element and destroy the independence of the generators.

2. THE ACTIN POWER-STROKE THEORY

As long as actin is considered to be a passive cable upon which myosin motors pull, it is difficult to imagine a better theory than the moving cross-bridge theory. However, contrary to what is widely supposed, the cross-bridge mechanism is not the only one that can account for the essential facts concerning muscle. Indeed, as we have previously shown, the four points of Huxley's independent generator hypothesis can be explained by a mechanism involving length changes in actin, as long as tropomyosin is given the role of integrating the forces developed by the contraction of independent segments of actin filaments [7].

At first sight, especially when local steps in the mechanism are examined, a *three-filament model* might seem too complicated to survive 'Occam's razor' test of competing theories: *Entia non sunt multiplicanda praeter necessitatem.* However, viewed globally, the net effect of the local length changes in moving actin filaments is the production of 'contractile' waves travelling toward the center of sarcomeres. The mechanical energy in these waves is transferred to tropomyosin filaments which transmit the forces to the Z-disc. The waves arise in consequence of the *commensurability* of a stretched form of actin [8] with the spacings of successive 'crowns' of myosin heads on the thick filaments.

The binding of myosin heads to subunits on the actin

filament induces the cooperative extension of the actin filament toward the next crown of myosin heads nearer the center of the sarcomere. The extended segment of actin anchors to this next layer of heads, while its trailing end retracts, pulling on the parallel tropomyosin filament. A constant force is maintained as each successive actin monomer contracts to the helical ground state. The myosin heads bear the tension developed by the actin force generators. Thus, when bound to actin, they serve as the hookean elements in Huxley's independent generator theory. When reached by the helicalizing actin wave-front, the bound myosin head detaches, binds ATP, and awaits the timed arrival of the next actin wave-front.

The total force developed is simply proportional to the number of waves moving toward the centre of the sarcomere. In a fuller treatment of this theory ("The Energetics of the Actin Power-Stroke Model of Muscle Contraction", Schutt, C. and Lindberg, U., to be submitted), we show that this model can account for observed rates of ATP hydrolysis for muscles performing a given amount of work while contracting at a fixed velocity.

X-ray crystallographic studies on polyhedral viruses support the idea that an extensible polymer can be constructed from protein subunits [9]. The structural principle behind the construction of expandable viral shells is that at least one strong set of inter-subunit bonds is maintained while the subunits undergo rotations about internal hinge points. This concept of 'conserved contacts and variable linkages' can be applied to the actin monomer, now known to consist of subdomains connected by short stretches of polypeptide chain that are good candidates for hinge points [10]. The high resolution structure of the crystalline form of profilin:actin clearly shows that the 'ribbon' form of actin is held together by strong inter-subunit contacts (to be published).

3. THE FENN EFFECT

Any theory of muscle contraction must account for the striking fact that muscle fibers shortening against a load (and thereby performing work) are able to draw from biochemical sources significantly greater amounts of free energy than equivalent isometrically-contracting fibers. Originally discovered by W.O. Fenn [11], these observations imply that a muscle is not a spring that converts potential energy into mechanical work, but is rather a device in which mechanical events in the contracting fiber control the rates of the biochemical reactions that provide the energy used by the fiber to perform work.

If ATP hydrolysis on an actin subunit is associated with the transition from the extended ribbon state to the helical ground state, then a contracting actin segment can be thought of as a *linear motor* developing a con-

stant force between two points of contact, tropomyosin and the myosin head to which it is anchored. (The role of the myosin ATPase is simply to activate the formation of ribbon segments.) The concept of linear motors readily explains the Fenn effect, since isometrically-contracting muscle fibers, when allowed to shorten, can initiate more wave-fronts per unit time and heavier loads can be balanced by more wave-fronts per actin filament. Tropomyosin is required to explain the Fenn effect since it provides a means for integrating the forces developed by independent contracting segments of actin along one thin filament [9]. However, actin filaments can still move without tropomyosin in in vitro assays [22–24] according to this model, but the maximum force is limited to that produced by a single actin subunit owing to the lack of independence of the force generators.

4. A CHALLENGE TO THE CROSS-BRIDGE THEORY

The question of whether an actin ATPase is active during muscle contraction remains open, but our analysis suggests that its inclusion can resolve many of the conceptual problems now facing the field. To understand the seriousness of these new questions it is necessary to review the cross-bridge theory of chemomechanical transduction. There are, in principle, many types of cross-bridge mechanisms, but we will focus on the Huxley-Simmons model [12] because it accords so well with the requirements of the independent force generator hypothesis. The basic feature of the model is that myosin heads are capable of attaching to actin filaments at a succession of sites of increasing binding energy. As the head moves through these binding states, perhaps by tilting, it transmits a force proportional to the gradient of the binding energy to an elastic element situated somewhere in the cross-bridge. The stretched elastic element can then pull on the attached actin filament as elastic energy is converted into the work of moving a load. The Huxley-Simmons proposal has been analyzed in great detail [13]. It would appear from this analysis that the Fenn effect can be explained by this model as long as a myosin head remains attached while the hookean element discharges its stored energy.

A cross-bridge theory must also explain how ATP hydrolysis is coupled to force generation. The Lymn-Taylor model (steps 2,3,4 in Fig. 1), based on solution studies showing that actin stimulates the release of inorganic phosphate from myosin S1-heads [4], is a *tightly coupled model* in which P_i is released as an S1-head goes through its power-stroke. At the end of the power-stroke, myosin rebinds ATP and retains the products of hydrolysis until a new set of sites is found further along the moving actin filament. The discovery that ATP can be hydrolyzed by a bound complex of actin and myosin under nonphysiological-salt conditions (steps 3a in Fig.
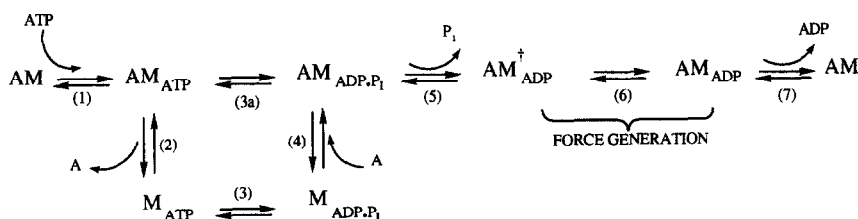
Fig. 1. The actomyosin ATPase cycle. The currently accepted scheme for the coupling of ATP hydrolysis to the actomyosin interaction. The classical Lymn-Taylor scheme [4] is initiated by the binding of ATP to the rigor-complex AM in step 1. The binding of ATP is accompanied by the dissociation of the AM complex, followed by the hydrolysis of ATP on the detached myosin head in step 3. The detached myosin head retains the bound products of hydrolysis until it binds actin in step 4. In the original scheme, steps 5–7 represented the power-stroke. Step 3a, hydrolysis of ATP in the bound state AM, occurs in solution only under non-physiological (low-salt) conditions but it is generally incorporated in models of muscle fiber contraction [13,14]. The kinetics of inorganic phosphate binding imply a non force-producing, but tension-bearing state, represented by the isomerization of the AM complex during phosphate release. Force is generated in step 6 during a reorganization within the bound AM complex. This scheme is consistent with the Fenn effect as long as steps 5–7 allow for strain in the bound molecular complex AM. In the actin power-stroke theory, steps 1–5 are involved with the activation of the actin motors along thin filaments. Force is developed in the filaments as actin subunits progressively contract to the helical state. It is a two ATPase mechanism, so that P_i release from actin subunits (not shown here) must also be involved in step 6. (More details are presented in "Energetics of the Actin Power-Stroke Theory of Muscle Contraction", by Schutt, C. and Lindberg, U., to be submitted).

1) has added the complexity of multiple equilibria between 'weak' and 'strong' actin binding states to the cross-bridge theory [13,14]. Even the phosphate release step is no longer thought to be simple [15–17]. Kinetic differences in the rates of tension development compared with increases of stiffness have been interpreted in terms of an 'isomerization' of the actomyosin complex during phosphate release [18].

The recent challenge to the cross-bridge theory comes from a number of experiments indicating that the distance through which an actin filament moves per ATP molecule hydrolyzed is at least 400 Å and may be over 1,000 Å [19–21]. Thus, the myosin power-stroke is longer than twice the physical length of the head so that, even lying on its back and transforming 180° to a position on its stomach, it would still fall far short of delivering its punch. One resolution to the problem appears to be to postulate long 'drag' tethers for each head [21]. The mechanical properties for these tethers have not been calculated so it remains to be seen whether they are compatible with principles of protein structure or the requirements of the independent generator hypothesis. Such tethers have not been observed by electron microscopy. In vitro 'motility assays' offer the possibility of sharpening the argument and somewhat shorter myosin step lengths have been reported [22,23], but the measurements are controversial [25], and movement does not seem to require the S2-linker of the myosin molecule [26], a structural element invoked to explain the length of the power-stroke.

Although multiple attachment/detachment cycles have also been proposed as a way out of this power-stroke paradox [19,21,22,24,27,28], no consideration appears to have been given to the necessity of accounting for the Fenn effect, except for an indirect reference by Huxley [29]. The idea of 'fractionating' the free energy of hydrolysis from one ATP molecule in order to make multiple, but shorter, power-strokes is implausi-
ble. Such a mechanism would require that a *detached* head hold its state of strain even though the energy barriers between states are much shallower than brownian fluctuations would allow. Again, the Fenn effect is without an explanation in terms of the cross-bridge theory, unless some element of protein structure is discovered that can deliver the energy equivalent of about six hydrogen bonds (i.e. 10 kcal per mole of hydrolyzed ATP) in discrete packets over long distances. For comparison, a ten residue polypeptide chain can contract from a length of 38 Å to a 15 Å long α-helix with the formation of six hydrogen bonds.

5. A PREDICTION

The concept of linear actin motors not only explains the Fenn effect, but does so with a set of structural biological principles that are well-established from X-ray crystallography. Since actin is the force generating component of the system and myosin heads in the bound state are the hookean elements, the development of force and stiffness need no longer be strictly in phase, thus offering a means of explaining the effect of phosphate on the *complex* stiffness [18]. The essence of the problem is to reconcile in vitro studies of the actomyosin ATPase, in which random brownian fluctuations drive the transition, with the organized forces present in the lattice. In other words, since the Fenn effect has no counterpart in solution, it is not surprising that the actin ATPase has been so difficult to detect, except in situations where the organized structure is maintained [30–32].

The 'actin power-stroke' model predicts that *actin filaments hydrolyze ATP when muscle fibers are producing work*. The release of inorganic phosphate from actin is tightly coupled to the force-producing length changes in actin subunits as they bind to tropomyosin.

The field of muscle contraction is in a state of crisis.

The prevailing paradigm, the rotating cross-bridge theory, which has served so well to guide the design of experiments, seems less credible than it did just a few years ago [33,34]. This crisis comes at a time when X-ray crystallography is revealing images of the force-producing molecules at atomic resolution, and in vitro reconstitution systems and genetic engineering are providing the means to test the principal tenets of the theory [35,36]. We believe that the source of the difficulty is that the mechanical role of tropomyosin, the third filament system comprising sarcomeres, has not been properly understood, nor has the actin ATPase been appreciated as a source of Gibbs free energy for muscle fibers performing work.

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